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DESCRIPTION

[0001] The present invention relates to the field of vaccination, in particular to cancer vaccines.

[0002] The immune system can recognize and to some extent eliminate tumor cells, however, this anti-tumor response is often of low amplitude and inefficient. Boosting this weak anti-tumor response with therapeutic vaccination has been a long sought goal for cancer therapy. Modulating the immune system to enhance immune responses has thus become a promising therapeutic approach in oncology as it can be combined with standard of care treatments. Promising preclinical data and advances in clinical trials, show that active immunization is a safe and feasible treatment modality for certain cancer types. Induction of tumor-specific cytotoxic T lymphocytes (CTLs) mediated immune responses has been reported using different approaches including modified tumor cell vaccines, peptide vaccines, recombinant viral vectors, DNA, protein, or dendritic cell vaccines. However, the anti-tumoral immunity mediated by CTLs only occasionally correlates with tumor regression and only a few projects have reached the phase III clinical stage.

[0003] Overall, cancer vaccines showed very limited clinical efficacy so far. Indeed, at the end of 2011, amongst the 300 hundred ongoing cancer vaccine clinical trials, only 19 phase III trials were reported (*globaldata, 2012*). Amongst them, there are NeuVax, a peptide vaccine for breast cancer, Stimuvax, a liposome based vaccine for Non-Small Cell Lung Carcinoma (NSCLC) and breast cancer, TG4010, a vaccinia-based vaccine for NSCLC and GSK1572932A, an adjuvanted liposome for NSCLC. These four cancer vaccines are based on different technologies and have in common that they are targeting one single antigen.

[0004] Therapeutic cancer vaccines can be divided into two principal categories: personalized (autologous) and standardized vaccines, and further classified depending on the technology platform. Current personalized vaccines include tumor lysate vaccines as well as dendritic cells based vaccine (hereinafter cell based). For the latter, antigen loading can occur either with a pulse using tumor lysates, or transfection with RNA extracted from the tumors. In this case, the antigens are tumor specific or associated, but are not clearly defined. Dendritic cells can also be loaded with defined antigens either with peptide pulse or using a protein such as the Prostatic Acid Phosphatase (PAP) used to engineer the Provenge[®] vaccine. However, the manufacturing process of these cell-based therapies is time-consuming and labor-intensive while quality standards are difficult to reach and maintain. Immunomonitoring creates further complications. Moreover, the majority of the autologous cancer vaccines do not allow the identities or quantities of antigens used to be controlled, unlike defined and standardized vaccines.

[0005] In contrast to cell-based therapy (APCs, T cells, CARs, lysates), subunits vaccines (protein or peptides) allow the development of a standardized vaccine with an easier production and significantly better batch to batch reproducibility that can be administrated to a broad range of patients. Furthermore, the antigens are fully defined allowing for better

immune-monitoring and reducing the risk of unwanted effects of vaccine component.

[0006] The different approaches which were evaluated in pre-clinical and clinical development include short peptide vaccines (Slingluff CL, Jr. The present and future of peptide vaccines for cancer: single or multiple, long or short, alone or in combination? *Cancer journal* 2011;17(5):343-50), long-peptide vaccines (Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. *Nature reviews Cancer* 2008;8(5):351-60) and proteins. In contrast to long peptide and protein vaccines, short peptide vaccines have a very short half-life and can have negative consequences on the immune response.

[0007] A therapeutic cancer vaccine is administrated to cancer patients to strengthen the capability of their immune system to recognize and kill the tumor cells. The main goal of a therapeutic cancer vaccine is to generate killer T cells (also called cytotoxic T lymphocytes) specific for the tumor cells. To this end and to achieve a potent immune response, the vaccine must contain an antigen or an antigenic epitope that is also present in the tumor and that needs to be delivered to Antigen Presenting Cells (APCs), in particular dendritic cells (DCs), to initiate cancer immunity. The DCs process these tumor antigens into small peptides that are presented on cell surface expressed MHC class I or MHC class II molecules to T cells. Peptides that are then recognized by T cells and thereby induce their stimulation are called epitopes. Presentation by MHC class I and MHC class II molecules allows activation of two classes of T cells, CD8⁺ cytotoxic T lymphocytes (CTLs) and CD4⁺ helper T (T_h) cells, respectively. In addition, to become fully activated, beside antigen recognition T cells require a second signal, the co-stimulatory signal, which is antigen non-specific and is provided by the interaction between co-stimulatory molecules expressed on the surface of APCs and the T cell. Therefore two major requirements for an efficient therapeutic cancer vaccine are (i) the specificity of the tumor antigens and (ii) the ability to deliver them efficiently to DCs.

[0008] Taken together, induction of a tumor specific immune response thus requires three main steps: (i) an antigen must be delivered to dendritic cells, which will process it into epitopes, (ii) dendritic cells should receive a suitable activation signal, and (iii) activated tumor antigen-loaded dendritic cells must generate T-cell mediated immune responses in the lymphoid organs.

[0009] Since tumor cells can escape the immune system by down-regulating expression of individual antigens (passive immune escape), multi-epitopic antigen delivery provides an advantage. Indeed, protein based vaccines allow multi-epitopic antigen delivery to antigen presenting cells (APCs) such as dendritic cells (DCs) without the limitation of restriction to a single MHC allele. Another strength is long-lasting epitope presentation recently described in dendritic cells loaded with proteins (van Montfoort N, Camps MG, Khan S, Filippov DV, Weterings JJ, Griffith JM, et al. Antigen storage compartments in mature dendritic cells facilitate prolonged cytotoxic T lymphocyte cross-priming capacity. *Proceedings of the National Academy of Sciences of the United States of America* 2009;106(16):6730-5). Furthermore, proteins require uptake and processing by DCs to achieve MHC restricted presentation of their

constituent epitopes. This reduces the risk of inducing peripheral tolerance as shown after vaccination with short peptides that do not have such stringent processing requirements (Toes RE, Offringa R, Blom RJ, Melief CJ, Kast WM. Peptide vaccination can lead to enhanced tumor growth through specific T-cell tolerance induction. *Proceedings of the National Academy of Sciences of the United States of America* 1996;93(15):7855-60).

[0010] However, most soluble proteins are generally degraded in endolysosomes and are poorly cross-presented on MHC class I molecules and are therefore poorly immunogenic for CD8⁺ T cell responses (Rosalia RA, Quakkelaar ED, Redeker A, Khan S, Camps M, Drijfhout JW, et al. Dendritic cells process synthetic long peptides better than whole protein, improving antigen presentation and T-cell activation. *European journal of immunology* 2013;43(10):2554-65). Moreover, although mature DCs are more potent than immature DCs in priming and eliciting T-cell responses (Apetoh L, Locher C, Ghiringhelli F, Kroemer G, Zitvogel L. Harnessing dendritic cells in cancer. *Semin Immunol.* 2011; 23:42-49), they lose the ability to efficiently take up exogenous antigens, particularly for MHC class II restricted antigens (Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature.* 1998; 392:245-252). As a result, peptide-pulsed DCs as vaccines have several limitations. For example, peptide degradation, rapid MHC class I turnover, and the disassociation of peptide from MHC class I molecules during the preparation and injection of DC/peptides may result in short half-lives of MHC class I/peptide complexes on the DC surface, leading to weak T-cell responses.

[0011] To improve the efficacy of protein-based vaccine delivery, the use of cell penetrating peptides for intracellular delivery of cancer peptides into DCs was proposed (Wang RF, Wang HY. Enhancement of antitumor immunity by prolonging antigen presentation on dendritic cells. *Nat Biotechnol.* 2002; 20:149-156). Cell penetrating peptides (CPPs) are peptides of typically 8 to 40 residues that have the ability to cross the cell membrane and enter into most cell types (Copolovici DM, Langel K, Eriste E, Langel U. Cell-penetrating peptides: design, synthesis, and applications. *ACS nano* 2014;8(3):1972-94, Milletti F. Cell-penetrating peptides: classes, origin, and current landscape. *Drug Discov Today* 2012). Alternatively, they are also called protein transduction domain (PTDs) reflecting their origin as occurring in natural proteins. Several potent CPPs have been identified from proteins, including the Tat protein of human immunodeficiency virus, the VP22 protein of herpes simplex virus, and fibroblast growth factor (Berry CC. Intracellular delivery of nanoparticles via the HIV-1 tat peptide. *Nanomedicine.* 2008; 3:357-365; Deshayes S, Morris MC, Divita G, Heitz F. Cell-penetrating peptides: Tools for intracellular delivery of therapeutics. *Cell Mol Life Sci.* 2005; 62:1839-1849; Edenhofer F. Protein transduction revisited: Novel insights into the mechanism underlying intracellular delivery of proteins. *Curr Pharm Des.* 2008; 14:3628-3636; Gupta B, Levchenko TS, Torchilin VP. Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides. *Adv Drug Deliv Rev.* 2005; 57:637-651; Torchilin VP. Recent approaches to intracellular delivery of drugs and DNA and organelle targeting. *Annu Rev Biomed Eng.* 2006; 8:343-375). It was found that T-cell activity elicited by DC/TAT-TRP2 was 3- to 10-fold higher than that induced by DC/TRP2 (Wang HY, Fu T, Wang G, Gang Z, Donna MPL, Yang JC, Restifo NP, Hwu P, Wang RF. Induction of CD4⁺ T cell-dependent antitumor immunity by TAT-

mediated tumor antigen delivery into dendritic cells. *J Clin Invest.* 2002a; 109:1463-1470). WO 2014/041505 A1 describes fusion proteins of cell-penetrating peptides derived from the basic-leucine zipper (bZIP) transcriptional activator "ZEBRA" of Epstein-Barr virus (EBV) and cancer/tumor antigens.

[0012] In order to increase the level of co-stimulatory molecules on DCs and to augment the immune system's response to the target antigens, adjuvants may be used. Adjuvants may accomplish this task by mimicking conserved microbial components that are naturally recognized by the immune system. They include, for example, lipopolysaccharide (LPS), components of bacterial cell walls, and nucleic acids such as double-stranded RNA (dsRNA), single-stranded DNA (ssDNA), and unmethylated CpG dinucleotide-containing DNA. Their presence can increase the innate immune response to the antigen. Furthermore, this adjuvant should promote an adaptive immune response with CTLs and type polarized T_h1 rather than a humoral immune response resulting in antibody production. Different adjuvants have been evaluated, with a limited number having gained regulatory approval for human use. These include Alum, MPL (monophosphoryl lipid A) and ASO₄ (Alum and MPL) in the US, and MF59 (oil-in-water emulsion), ASO₄, liposomes in Europe (Lim, Y.T., Vaccine adjuvant materials for cancer immunotherapy and control of infectious disease. *Clin Exp Vaccine Res*, 2015. 4(1): p. 54-8).

[0013] Recently, Toll Like Receptor (TLR) ligands are emerging as promising class of adjuvants (Baxevas, C.N., I.F. Voutsas, and O.E. Tsitsilonis, Toll-like receptor agonists: current status and future perspective on their utility as adjuvants in improving anticancer vaccination strategies. *Immunotherapy*, 2013. 5(5): p. 497-511). A significant development of cancer vaccine studies was thus to include various TLR agonists to vaccine formulations, including TLR-3 (poly I:C), TLR-4 (monophosphoryl lipid A; MPL), TLR-5 (flagellin), TLR-7 (imiquimod), and TLR-9 (CpG) (Duthie MS, Windish HP, Fox CB, Reed SG. Use of defined TLR ligands as adjuvants within human vaccines. *Immunol Rev.* 2011; 239:178-196). The types of signaling and cytokines by immune cells after TLR stimulation control CD4⁺ T-cell differentiation into Th1, Th2, Th17, and Treg cells. Stimulation of immune cells such as DCs and T cells by most TLR-based adjuvants produces proinflammatory cytokines and promotes Th1 and CD8⁺ T responses (Manicassamy S, Pulendran B. Modulation of adaptive immunity with Toll-like receptors. *Semin Immunol.* 2009; 21:185-193).

[0014] Conjugating the vaccine to a TLR ligand is an attractive approach that offers several advantages over non-conjugated vaccines including (i) preferential uptake by the immune cells expressing the TLR, (ii) higher immune response and (iii) reduced risk of inducing peripheral tolerance. Indeed, all the antigen presenting cells loaded with the antigen will be simultaneously activated. Different groups explored this approach with various TLR ligands being mainly linked chemically to the peptide or protein vaccine (Zom GG, Khan S, Filippov DV, Ossendorp F. TLR ligand-peptide conjugate vaccines: toward clinical application. *Adv Immunol.* 2012;114:177-201). As the chemical linkage to peptide is easily performed, the most highly investigated TLR ligands for conjugate vaccine are the TLR2 agonist Pam2Cys and Pam3Cys (Fujita, Y. and H. Taguchi, Overview and outlook of Toll-like receptor ligand-antigen conjugate

vaccines. Ther Deliv, 2012. 3(6): p. 749-60). WO 2012/048190 A1 discloses annexin II or immunomodulatory fragment thereof as a TLR2 peptide agonist. EP 2 476 440 A1 discloses a TLR4 agonist comprising a fibronectin EDA domain.

[0015] Moreover, recently immune checkpoint modulators emerged as new targets for cancer immunotherapy, as described, for example, in Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012 Mar 22;12(4):252-64 and in WO 2015/103037 A2 and as shown by recent marketing approvals for Yervoy® (Ipilimumab; Bristol Myers Squibb), Opdivo® (Nivolumab; Bristol Myers Squibb) and Keytruda® (Pembrolizumab; Merck). Immune checkpoints are molecules in the immune system, in particular on certain immune cells, that need to be activated (stimulatory or costimulatory checkpoint molecules) or inactivated (inhibitory checkpoint molecules) to start an immune response. Many of the immune checkpoints are regulated by interactions between specific receptor and ligand pairs. Often cancers protect themselves from the immune system by using these checkpoints to avoid being attacked by the immune system.

[0016] In particular, the two checkpoint receptors CTLA-4 and PD-1 received a lot of attention. CTLA-4, PD-1 and their ligands are members of the CD28-B7 family of co-signaling checkpoint molecules that play important roles throughout all stages of T-cell function and other cell functions.

[0017] The PD-1 receptor is expressed on the surface of activated T cells and other immune cells, such as B cells. Its ligands (PD-L1 and PD-L2) are expressed on the surface of antigen-presenting cells, such as dendritic cells or macrophages, and other immune cells. Binding of PD-L1 or PD-L2 to PD1 triggers a signal in the T cell, which essentially switches the T cell off or inhibits it. Under non-pathological conditions, this interaction prevents T cells from attacking other cells in the body. However, cancer cells often take advantage of this system and express high levels of PD-L1 on their surface. Thereby, cancer cells are able to switch off T cells expressing PD-1 and, thus, to suppress the anticancer immune response. Inhibitors of PD1 and/or its ligands, such as inhibitory/antagonistic monoclonal antibodies directed to PD1 or to its ligands, can boost the immune response against cancer cells and are, thus, promising in treating cancers. Examples of inhibitory/antagonistic monoclonal antibodies against PD1, which are currently approved, include Opdivo® (Nivolumab; Bristol Myers Squibb) and Keytruda® (Pembrolizumab; Merck). Other inhibitors of the PD1 pathway, which are currently in clinical phase II and/or III include Pidilizumab (mAb inhibiting PD1; CureTech/Medivation), Durvalumab (mAb inhibiting PD-L1; MedImmune/AstraZeneca) and Atezolimab (mAb inhibiting PD-L1; Roche).

[0018] Yervoy® (Ipilimumab; Bristol Myers Squibb), another approved immune checkpoint modulator, is an inhibitory/antagonistic monoclonal antibody against cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). CTLA4 is also expressed on the surface of activated T cells and its ligands are expressed on the surface of professional antigen-presenting cells. CTLA-4 is thought to regulate T-cell proliferation early in an immune response, primarily in lymph

nodes and affects the functioning of regulatory T cells. Another inhibitor of CTLA-4, which is currently in clinical phase II, is, for example, Tremelimumab (MedImmune/AstraZeneca).

[0019] Cancer immunotherapies with checkpoint modulators, in particular with checkpoint inhibitors, were shown to be very effective at least in a subgroup of subjects ("responders"). However, many patients do not respond to checkpoint modulators and even in the responding population the response is not always complete or optimal.

[0020] Combination of inhibition of PD1- and CTLA4-pathways with the aim of increasing efficacy is thus desirable. Accordingly, combination therapy with Nivolumab and Ipilimumab was approved by the FDA in 2015 for the treatment of patients with BRAF V600 wild-type, unresectable or metastatic melanoma. In addition, a successful phase 1b study on the combination of Durvalumab and Tremelimumab in non-small cell lung cancer was recently reported (Antonia, Scott et al., 2016, Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study; *Lancet Oncol.* 2016 Feb 5. pii: S1470-2045(15)00544-6. doi: 10.1016/S1470-2045(15)00544-6. [Epub ahead of print]).

[0021] However, the combination of checkpoint modulators which each other only targets endogenous tumor-specific immunity, in particular since no tumor-specific antigens are provided. Another strategy for the treatment of cancer is to combine a checkpoint modulator with a vaccine containing an antigen or an antigenic epitope as described above, which provides specificity against a certain tumor. The combination of a checkpoint modulator and a vaccine containing an antigen or an antigenic epitope may enhance or prolong an anti-tumor response in a subject. Further, the combination of a vaccine containing an antigen or an antigenic epitope with a checkpoint modulator may enhance or prolong the effects of the checkpoint modulator, enable a subject to respond to a checkpoint modulator, or enable the reduction of the toxicity or the dose of a checkpoint modulator. For example, the checkpoint modulator may remove the "immune brake" before pressing the "treatment gas pedal" by vaccine administration.

[0022] Accordingly, there is a need to develop combination therapies to initiate or enhance the efficacy of checkpoint modulators in both, responders and non-responders. To this end, a combination of a checkpoint modulator with a potent vaccine is desired. However, to date the majority of cancer vaccines trials have shown only limited efficacy. One explanation is the lack of a therapy that can simultaneously (i) stimulate multi-epitopic cytotoxic T cell-mediated immunity, (ii) induce T_h cells and (iii) promote immunological memory. These three parameters are essential to generate potent, long lasting anti-tumor immunity. Indeed, CTLs specific for different epitopes will allow destruction of more cancer cells within a heterogeneous tumor mass and avoid the outgrowth of antigen-loss variants (tumor immune escape). T_h cells are involved in the maintenance of long-lasting cellular immunity and tumor infiltration by T_h cells is also an essential step for the recruitment and function of CD8⁺ CTLs. Immunological memory is essential to protect against tumor relapse.

[0023] In view of the above, it is the object of the present invention to overcome the drawbacks of current cancer vaccines outlined above and to provide a combination of an immune checkpoint modulator and a complex comprising a cell penetrating peptide, a cargo and a TLR agonist for cancer immunotherapy applications representing a more potent vaccine, in particular cancer vaccine, having improved anti-tumor activity. The present invention thus relates to an immune checkpoint modulator and a complex comprising a cell penetrating peptide, a cargo and a TLR agonist for use in a combination therapy to initiate, enable, enhance or improve an anti-tumor immune response, in particular which enables, enhances or improves the subject's or tumor response to checkpoint modulators.

[0024] This object is achieved by means of the subject-matter set out in the appended claims.

[0025] Although the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodologies, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

[0026] Throughout this specification and the claims which follow, unless the context requires otherwise, the term "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated member, integer or step but not the exclusion of any other non-stated member, integer or step. The term "consist of" is a particular embodiment of the term "comprise", wherein any other non-stated member, integer or step is excluded. In the context of the present invention, the term "comprise" encompasses the term "consist of". The term "comprising" thus encompasses "including" as well as "consisting" e.g., a composition "comprising" X may consist exclusively of X or may include something additional e.g., X + Y.

[0027] The terms "a" and "an" and "the" and similar reference used in the context of describing the invention (especially in the context of the claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0028] The word "substantially" does not exclude "completely" e.g., a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

[0029] The term "about" in relation to a numerical value x means $x \pm 10\%$.

Combination of a checkpoint modulator and a complex comprising a cell penetrating

peptide, at least one antigen or antigenic epitope and a TLR peptide agonist for use in medicine

[0030] In a first aspect the present invention provides a combination of

1. (i) an immune checkpoint modulator and
2. (ii) a complex comprising:
 1. a) a cell penetrating peptide;
 2. b) at least one antigen or antigenic epitope; and
 3. c) at least one TLR peptide agonist,

wherein the components a) - c) comprised by the complex (i.e. the cell penetrating peptide, the at least one antigen or antigenic epitope and the at least one TLR peptide agonist) are covalently linked for use in medicine.

[0031] In the following, the components of the combination for use according to the present invention, i.e. the immune checkpoint modulator and the complex comprising cell penetrating peptide, the at least one antigen or antigenic epitope and the at least one TLR peptide agonist, and preferred embodiments thereof, are described in detail. It is understood that (i) a preferred embodiment of the combination for use according to the present invention comprises a preferred embodiment of the immune checkpoint modulator; (ii) a preferred embodiment of the combination for use according to the present invention comprises a preferred embodiment of the complex comprising cell penetrating peptide, the at least one antigen or antigenic epitope and the at least one TLR peptide agonist; and (iii) a more preferred embodiment of the combination for use according to the present invention comprises a preferred embodiment of the immune checkpoint modulator and a preferred embodiment of the complex comprising cell penetrating peptide, the at least one antigen or antigenic epitope and the at least one TLR peptide agonist.

Immune checkpoint modulator

[0032] As used herein (i.e. throughout the present specification), the term "immune checkpoint modulator" (also referred to as "checkpoint modulator") refers to a molecule or to a compound that modulates (e.g., totally or partially reduces, inhibits, interferes with, activates, stimulates, increases, reinforces or supports) the function of one or more checkpoint molecules. Thus, an immune checkpoint modulator may be an "immune checkpoint inhibitor" (also referred to as "checkpoint inhibitor" or "inhibitor") or an "immune checkpoint activator" (also referred to as "checkpoint activator" or "activator"). An "immune checkpoint inhibitor" (also referred to as "checkpoint inhibitor" or "inhibitor") totally or partially reduces, inhibits, interferes with, or negatively modulates the function of one or more checkpoint molecules. An "immune checkpoint activator" (also referred to as "checkpoint activator" or "activator") totally or partially

activates, stimulates, increases, reinforces, supports or positively modulates the function of one or more checkpoint molecules. Immune checkpoint modulators are typically able to modulate (i) self-tolerance and/or (ii) the amplitude and/or the duration of the immune response. Preferably, the immune checkpoint modulator used according to the present invention modulates the function of one or more human checkpoint molecules and is, thus, a "human checkpoint modulator". Preferably, the immune checkpoint modulator is an activator or an inhibitor of one or more immune checkpoint molecule(s) selected from CD27, CD28, CD40, CD122, CD137, OX40, GITR, ICOS, A2AR, B7-H3, B7-H4, BTLA, CD40, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA, CEACAM1, GARP, PS, CSF1R, CD94/NKG2A, TDO, GITR, TNFR and/or FasR/DcR3; or an activator or an inhibitor of one or more ligands thereof.

[0033] Checkpoint molecules are molecules, such as proteins, are typically involved in immune pathways and, for example, regulate T-cell activation, T-cell proliferation and/or T-cell function. Accordingly, the function of checkpoint molecules, which is modulated (e.g., totally or partially reduced, inhibited, interfered with, activated, stimulated, increased, reinforced or supported) by checkpoint modulators, is typically the (regulation of) T-cell activation, T-cell proliferation and/or T cell function. Immune checkpoint molecules thus regulate and maintain self-tolerance and the duration and amplitude of physiological immune responses. Many of the immune checkpoint molecules belong to the B7:CD28 family or to the tumor necrosis factor receptor (TNFR) super family and, by the binding of specific ligands, activate signaling molecules that are recruited to the cytoplasmic domain (cf. Susumu Suzuki et al., 2016: Current status of immunotherapy. Japanese Journal of Clinical Oncology, 2016: doi: 10.1093/jjco/hyv201 [Epub ahead of print]; in particular Table 1).

[0034] Since a decade, the role of the B7 family was analyzed and new members and new functions of these important T cell regulator molecules were identified (Greenwald, R.J., G.J. Freeman, and A.H. Sharpe, The B7 family revisited. *Annu Rev Immunol*, 2005. 23: p. 515-48). Today, the B7:CD28 family comprises the most frequently used pathways in immune checkpoint research. The initial and complex mechanisms of CTLA-4 - B7-1/B7-2 were progressively seconded by the highly interesting PD-1 - B7-H1(PDL1)/B7-DC(PD-L2) couple that was shown to have an association with many immunological and clinical tumor microenvironments (Zou, W. and L. Chen, Inhibitory B7-family molecules in the tumour microenvironment. *Nat Rev Immunol*, 2008. 8(6): p. 467-77). Another member of this family is ICOS-ICOSL/B7-H2, which was identified at the same period. Other members of that family include B7-H3 (Chapoval, A.I., et al., B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production. *Nat Immunol*, 2001. 2(3): p. 269-74) and B7-H4 (Sica, G.L., et al., B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. *Immunity*, 2003. 18(6): p. 849-61), however, both ligands receptors' haven't yet been identified. However the role of B7-H3 in antitumor immunity (Loos, M., et al., B7-h3 and its role in antitumor immunity. *Clin Dev Immunol*, 2010. 2010: p. 683875) and its role in immune tumor evasion (Hofmeyer, K.A., A. Ray, and X. Zang, The contrasting role of B7-H3. *Proc Natl Acad Sci US A*, 2008.105(30): p. 10277-8) as well as its overexpression many advanced stage cancers (Zou, W. and L. Chen, Inhibitory B7-family molecules in the tumour microenvironment. *Nat Rev Immunol*, 2008. 8(6): p. 467-77), namely in colorectal cancer (Sun, J., et al., Clinical significance and regulation of

the costimulatory molecule B7-H3 in human colorectal carcinoma. *Cancer Immunol Immunother*, 2010. 59(8): p. 1163-71), suggest that research will be pursued in that direction. Similarly, B7-H4 recently entered in evaluation loop as possible agent acting on tumoral immune escape and antitumor response (Dangaj, D. and N. Scholler, Blocking the B7-H4 pathway with novel recombinant antibodies enhances T cell-mediated antitumor responses. *Oncoimmunology*, 2013. 2(8): p. e25913; Dangaj, D., et al., Novel recombinant human b7-h4 antibodies overcome tumoral immune escape to potentiate T-cell antitumor responses. *Cancer Res*, 2013. 73(15): p. 4820-9) or interacting with many kinase pathways (Wang, X., et al., B7-H4 Treatment o/TCells Inhibits ERK,JNK, p38, and AKT Activation. *PLoS One*, 2012. 7(1): p. e28232). The results of this interest is underlined by a publication on an anti-B7-H4 antibody-drug conjugate for the treatment of breast cancer (Leong, S.R., et al., An anti-b7-h4 antibody-drug conjugate for the treatment of breast cancer. *Mol Pharm*, 2015.12(6): p.1717-29).

[0035] CD28 is constitutively expressed on almost all human CD4+ T cells and on around half of all CD8 T cells. Binding with its two ligands are CD80 (B7-1) and CD86 (B7-2), expressed on dendritic cells, prompts T cell expansion. The co-stimulatory checkpoint molecule CD28 competes with the inhibitory checkpoint molecule CTLA4 for the same ligands, CD80 and CD86 (cf. Buchbinder E. I. and Desai A., 2016: CTLA-4 and PD-1 Pathways - Similarities, Differences and Implications of Their Inhibition; *American Journal of Clinical Oncology*, 39(1): 98-106).

[0036] Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA4; also known as CD152) is a CD28 homolog with much higher binding affinity for B7. The ligands of CTLA-4 are CD80 (B7-1) and CD86 (B7-2), similarly to CD28. However, unlike CD28, binding of CTLA4 to B7 does not produce a stimulatory signal, but prevents the co-stimulatory signal normally provided by CD28. Moreover, CTLA4 binding to B7 is assumed to even produce an inhibitory signal counteracting the stimulatory signals of CD28:B7 and TCR:MHC binding. CTLA-4 is considered the "leader" of the inhibitory immune checkpoints, as it stops potentially autoreactive T cells at the initial stage of naïve T-cell activation, typically in lymph nodes (Buchbinder E. I. and Desai A., 2016: CTLA-4 and PD-1 Pathways: Similarities, Differences and Implications of Their Inhibition; *American Journal of Clinical Oncology*, 39(1): 98-106). Preferred checkpoint inhibitors of CTLA4 include the monoclonal antibodies Yervoy® (Ipilimumab; Bristol Myers Squibb) and Tremelimumab (Pfizer/MedImmune). Further preferred CTLA-4 inhibitors include the anti-CTLA4 antibodies disclosed in WO 2001/014424, in WO 2004/035607, in US 2005/0201994, and in EP 1212422 B1. Additional preferred CTLA-4 antibodies are described in US 5,811,097, in US 5,855,887, in US 6,051,227, in US 6,984,720, in WO 01/14424 in WO 00/37504, in US 2002/0039581 and in US 2002/086014. Other preferred anti-CTLA-4 antibodies that can be used in the context of the present invention include, for example, those disclosed in WO 98/42752; US 6,682,736 and US 6,207,156; Hurwitz et al., *Proc. Natl. Acad. Sci. USA*, 95(17):10067-10071 (1998); Camacho et al., *J. Clin. Oncology*, 22(145):Abstract No. 2505 (2004) (antibody CP-675206); Mokyr et al., *Cancer Res.*, 58:5301-5304 (1998), in US 5,977,318, US 6,682,736, US 7,109,003, and in US 7,132,281.

[0037] Programmed Death 1 receptor (PD1) has two ligands, PD-L1 (also known as B7-H1

and CD274) and PD-L2 (also known as B7-DC and CD273). The PD1 pathway regulates previously activated T cells at the later stages of an immune response, primarily in peripheral tissues. An advantage of targeting PD1 is thus that it can restore immune function in the tumor microenvironment. Preferred inhibitors of the PD1 pathway include Opdivo® (Nivolumab; Bristol Myers Squibb), Keytruda® (Pembrolizumab; Merck), Durvalumab (MedImmune/AstraZeneca), MEDI4736 (AstraZeneca; cf. WO 2011/066389 A1), Atezolizumab (MPDL3280A, Roche/Genentech; cf. US 8,217,149 B2), Pidilizumab (CT-011; CureTech), MEDI0680 (AMP-514; AstraZeneca), Avelumab (Merck), MSB-0010718C (Merck), PDR001 (Novartis), BMS-936559 (Bristol Myers Squibb), REGN2810 (Regeneron Pharmaceuticals), MIH1 (Affymetrix), AMP-224 (Amplimmune, GSK), BGB-A317 (BeiGene) and Lambrolizumab (e.g. disclosed as hPD109A and its humanized derivatives h409A11, h409A16 and h409A17 in WO2008/156712; Hamid et al., 2013; N. Engl. J. Med. 369: 134-144).

[0038] Inducible T-cell costimulator (ICOS; also known as CD278) is expressed on activated T cells. Its ligand is ICOSL (B7-H2; CD275), expressed mainly on B cells and dendritic cells. The molecule seems to be important in T cell effector function.

[0039] B7-H3 (also known as CD276) was originally understood to be a co-stimulatory molecule but is now regarded as co-inhibitory. A preferred checkpoint inhibitor of B7-H3 is the Fc-optimized monoclonal antibody Enoblituzumab (MGA271; MacroGenics; cf. US 2012/0294796 A1).

[0040] B7-H4 (also known as VTCN1), is expressed by tumor cells and tumor-associated macrophages and plays a role in tumor escape. Preferred B7-H4 inhibitors are the antibodies described in Dangaj, D. et al., 2013; Cancer Research 73(15): 4820-9 and in Table 1 and the respective description of Jenessa B. Smith et al., 2014: B7-H4 as a potential target for immunotherapy for gynecologic cancers: A closer look. Gynecol Oncol 134(1): 181-189. Other preferred examples of B7-H4 inhibitors include antibodies to human B7-H4 as disclosed, e.g., in WO 2013/025779 A1 and in WO 2013/067492 A1 or soluble recombinant forms of B7-H4, such as disclosed in US 2012/0177645 A1.

[0041] The TNF superfamily comprises in particular 19 protein-ligands binding to 29 cytokine receptors. They are involved in many physiological responses such as apoptosis, inflammation or cell survival (Croft, M., C.A. Benedict, and C.F. Ware, Clinical targeting of the TNF and TNFR superfamilies. Nat Rev Drug Discov, 2013.12(2): p. 147-68). These commonly encountered effects make the TNF superfamily members highly attractive for drug development (more than 60 TNF family drugs in development in 2013 (Croft, M., C.A. Benedict, and C.F. Ware, Clinical targeting of the TNF and TNFR superfamilies. Nat Rev Drug Discov, 2013.12(2): p. 147-68)) but as well difficult to manage due to possible balancing counter-effects (Aggarwal, B.B., Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol, 2003. 3(9): p. 745-56). Croft, M., C.A. Benedict, and C.F. Ware, Clinical targeting of the TNF and TNFR superfamilies. Nat Rev Drug Discov, 2013.12(2): p. 147-68 suggest to prioritize nine receptors for cancer indications: TNFRSF4 (OX40/OX40L), TNFRSF5 (CD40L/CD40), TNFRSF7 (CD27 /CD70), TNFRSF8 (CD30/CD30L), TNFRSF9 (4-

1BB/4-1BBL), TNFRSF10 (TRAILR/TRAIL)), TNFRSF12 (FN14/TWEAK), TNFRSF13 (BAFFRTACI/APRIL-BAFF) and TNFRSF18 (GITR/GITRL) (Avogadri, F., et al., Modulation of CTLA-4 and CTR/or cancer immunotherapy. *Curr Top Microbiol Immunol*, 2011. 344: p. 211-44; Naidoo, J., D.B. Page, and J.D. Wolchok, Immune modulation for cancer therapy. *Br J Cancer*, 2014.111(12): p. 2214-9). This list is complemented for immuno-therapies by Bremer, E., Targeting of the tum or necrosis factor receptor superfamily for cancer immunotherapy. *ISRN Oncol*, 2013. 2013: p. 371854), adding Fas-Ligand and TNFRSF1 (TNF α /TNFR). Moreover, the B- and T-lymphocyte attenuator (BTLA) /herpes virus entry mediator (HVEM) pathway should be considered as target for enhancing immune responses, just like the CTLA-4 blockade (Croft, M., C.A. Benedict, and C.F. Ware, Clinical targeting of the TNF and TNFR superfamilies. *Nat Rev Drug Discov*, 2013.12(2): p. 147-68). Accordingly, in the context of the present invention such checkpoint modulators are preferred for the use in the treatment and/or prevention in cancer, which modulate one or more checkpoint molecules selected from TNFRSF4 (OX40/OX40L), TNFRSF5 (CD40L/CD40), TNFRSF7 (CD27 /CD70), , TNFRSF9 (4-1BB/4-1BBL), TNFRSF18 (GITR/GITRL), FasR/DcR3/Fas ligand, TNFRSF1 (TNF α /TNFR), BTLA/HVEM and CTLA4.

[0042] OX40 (also known as CD134 or TNFRSF4) promotes the expansion of effector and memory T cells, but it is also able to suppress the differentiation and activity of T-regulatory cells and to regulate cytokine production. The ligand of OX40 is OX40L (also known as TNFSF4 or CD252). OX40 is transiently expressed after T-cell receptor engagement and is only upregulated on the most recently antigen-activated T cells within inflammatory lesions. Preferred checkpoint modulators of OX40 include MEDI6469 (MedImmune/AstraZeneca), MEDI6383 (MedImmune/AstraZeneca), MEDI0562 (MedImmune/AstraZeneca), MOXR0916 (RG7888; Roche/Genentech) and GSK3174998 (GSK).

[0043] CD40 (also known as TNFRSF5) is expressed by a variety of immune system cells including antigen presenting cells. Its ligand is CD40L, also known as CD154 or TNFSF5, is transiently expressed on the surface of activated CD4+ T cells. CD40 signaling "licenses" dendritic cells to mature and thereby trigger T-cell activation and differentiation. However, CD40 can also be expressed by tumor cells. Thus, stimulation/activation of CD40 in cancer patients can be beneficial or deleterious. Accordingly, stimulatory and inhibitory modulators of this immune checkpoint were developed (Sufia Butt Hassan, Jesper Freddie Sorensen, Barbara Nicola Olsen and Anders Elm Pedersen, 2014: Anti-CD40-mediated cancer immunotherapy: an update of recent and ongoing clinical trials, *Immunopharmacology and Immunotoxicology*, 36:2, 96-104). Preferred examples of CD40 checkpoint modulators include (i) agonistic anti-CD antibodies as described in Sufia Butt Hassan, Jesper Freddie Sorensen, Barbara Nicola Olsen and Anders Elm Pedersen, 2014: Anti-CD40-mediated cancer immunotherapy: an update of recent and ongoing clinical trials, *Immunopharmacology and Immunotoxicology*, 36:2, 96-104, such as Dacetuzumab (SGN-40), CP-870893, FGK 4.5/FGK 45 and FGK115, preferably Dacetuzumab, and (ii) antagonistic anti-CD antibodies as described in Sufia Butt Hassan, Jesper Freddie Sorensen, Barbara Nicola Olsen and Anders Elm Pedersen, 2014: Anti-CD40-mediated cancer immunotherapy: an update of recent and ongoing clinical trials, *Immunopharmacology and Immunotoxicology*, 36:2, 96-104, such as

Lucatumumab (HCD122, CHIR-12.12). Further preferred immune checkpoint modulators of CD40 include SEA-CD40 (Seattle Genetics), ADC-1013 (Alligator Biosciences), APX005M (Apexigen Inc) and RO7009789 (Roche).

[0044] CD27 (also known as TNFRSF7) supports antigen-specific expansion of naïve T cells and plays an important role in the generation of T cell memory. CD27 is also a memory marker of B cells. The transient availability of its ligand, CD70 (also known as TNFSF7 or CD27L), on lymphocytes and dendritic cells regulates the activity of CD27. Moreover, CD27 co-stimulation is known to suppress Th17 effector cell function. A preferred immune checkpoint modulator of CD27 is Varlilumab (Celldex). Preferred immune checkpoint modulators of CD70 include ARGX-110 (arGEN-X) and SGN-CD70A (Seattle Genetics).

[0045] CD137 (also known as 4-1BB or TNFRSF9) is a member of the tumor necrosis factor (TNF) receptor family and is increasingly associated with costimulatory activity for activated T cells. In particular, CD137 signaling (via its ligand CD137L, also known as TNFSF9 or 4-1BBL) results in T-cell proliferation and protects T cells, in particular, CD8+ T cells, from activation-induced cell death. Preferred checkpoint modulators of CD137 include PF-05082566 (Pfizer) and Urelumab (BMS).

[0046] Glucocorticoid-Induced TNFR family Related gene (GITR, also known as TNFRSF18), prompts T cell expansion, including Treg expansion. The ligand for GITR (GITRL, TNFSF18) is mainly expressed on antigen presenting cells. Antibodies to GITR have been shown to promote an anti-tumor response through loss of Treg lineage stability. Preferred checkpoint modulators of GITR include BMS-986156 (Bristol Myers Squibb), TRX518 (GITR Inc) and MK-4166 (Merck).

[0047] Another preferred checkpoint molecule to be modulated is BTLA. B and T Lymphocyte Attenuator (BTLA; also known as CD272) is in particular expressed by CD8+ T cells, wherein surface expression of BTLA is gradually downregulated during differentiation of human CD8+ T cells from the naïve to effector cell phenotype. However, tumor-specific human CD8+ T cells express high levels of BTLA. BTLA expression is induced during activation of T cells, and BTLA remains expressed on Th1 cells but not Th2 cells. Like PD1 and CTLA4, BTLA interacts with a B7 homolog, B7H4. However, unlike PD-1 and CTLA-4, BTLA displays T-Cell inhibition via interaction with tumor necrosis family receptors (TNF-R), not just the B7 family of cell surface receptors. BTLA is a ligand for tumor necrosis factor (receptor) superfamily, member 14 (TNFRSF14), also known as herpes virus entry mediator (HVEM; Herpesvirus Entry Mediator, also known as CD270). BTLA-HVEM complexes negatively regulate T-cell immune responses. Preferred BTLA inhibitors are the antibodies described in Table 1 of Alison Crawford and E. John Wherry, 2009: Editorial: Therapeutic potential of targeting BTLA. *Journal of Leukocyte Biology* 86: 5-8, in particular the human antibodies thereof. Other preferred antibodies in this context, which block human BTLA interaction with its ligand are disclosed in WO 2011/014438, such as "4C7" as described in WO 2011/014438.

[0048] Another checkpoint molecule family includes checkpoint molecules related to the two

primary class of major histocompatibility complex (MHC) molecules (MHC class I and class II). Their function to trigger a response from the immune system through their respective pathways; killer Ig-like Receptor (KIR) for class I and lymphocyte activation gene-3 (LAG-3) for class II, seems opening novel immunotherapeutic strategies for cancer patients treatment (Hemon, P., et al., MHC class II engagement by its ligand LAG-3 (CD223) contributes to melanoma resistance to apoptosis. *J Immunol*, 2011. 186(9): p. 5173-83; Thielens, A., E. Vivier, and F. Romagne, NK cell MHC class I specific receptors (KIR): from biology to clinical intervention. *Curr Opin Immunol*, 2012. 24(2): p. 239-45).

[0049] Killer-cell Immunoglobulin-like Receptor (KIR) is a receptor for MHC Class I molecules on Natural Killer cells. An exemplary inhibitor of KIR is the monoclonal antibody Lirilumab (IPH 2102; Innate Pharma/BMS; cf. US 8,119,775 B2 and Benson et al., 2012, *Blood* 120:4324-4333).

[0050] Lymphocyte Activation Gene-3 (LAG3, also known as CD223) signaling leads to suppression of an immune response by action to Tregs as well as direct effects on CD8+ T cells. A preferred example of a LAG3 inhibitor is the anti-LAG3 monoclonal antibody BMS-986016 (Bristol-Myers Squibb). Other preferred examples of a LAG3 inhibitor include LAG525 (Novartis), IMP321 (Immutep) and LAG3-Ig as disclosed in WO 2009/044273 A2 and in Brignon et al., 2009, *Clin. Cancer Res.* 15: 6225-6231 as well as mouse or humanized antibodies blocking human LAG3 (e.g., IMP701 as described in WO 2008/132601 A1), or fully human antibodies blocking human LAG3 (such as disclosed in EP 2320940 A2).

[0051] Another checkpoint molecule pathway is the TIM-3/GAL9 pathway.). T-cell Immunoglobulin domain and Mucin domain 3 (TIM-3, also known as HAVcr-2) is expressed on activated human CD4+ T cells and regulates Th1 and Th17 cytokines. TIM-3 acts as a negative regulator of Th1/Tc1 function by triggering cell death upon interaction with its ligand, galectin-9 (GAL9). TIM-3 is a T helper type 1 specific cell surface molecule that is regulating the induction of peripheral tolerance. A recent study has indeed demonstrated that TIM-3 antibodies could significantly enhance antitumor immunity (Ngiow, S.F., et al., Anti-TIM3 antibody promotes T cell IFN-gamma-mediated antitumor immunity and suppresses established tumors. *Cancer Res*, 2011. 71(10): p. 3540-51). Preferred examples of TIM-3 inhibitors include antibodies targeting human TIM3 (e.g. as disclosed in WO 2013/006490 A2) or, in particular, the anti-human TIM3 blocking antibody F38-2E2 as disclosed by Jones et al., 2008, *J Exp Med.* 205 (12): 2763-79.

[0052] More recently a novel target named CEACAM1 (Carcinoembryonic antigen-related cell adhesion molecule 1) was suggested, as recent studies showed the role for members of the CEA CAM family in modulating the immune responses associated with cancer (Huang, Y.H., et al., CEACAM1 regulates TIM-3-mediated tolerance and exhaustion. *Nature*, 2015. 517(7534): p. 386-90; Gray-Owen, S.D. and R.S. Blumberg, CEACAM1: contact-dependent control of immunity. *Nat Rev Immunol*, 2006. 6(6): p. 433-46). A preferred checkpoint modulator of CEACAM1 is CM-24 (cCAM Biotherapeutics).

[0053] Another novel immune checkpoint molecule is GARP, which plays a role in the ability of tumors to escape the patient's immune system. Presently in clinical trials, the candidate (ARGX-115) seems demonstrating interesting effect. Accordingly, ARGX-115 is a preferred GARP checkpoint modulator.

[0054] Moreover, various research groups have demonstrated that another checkpoint molecule is phosphatidylserine (also referred to as "PS") may be targeted for cancer treatment (Creelan, B.C., Update on immune checkpoint inhibitors in lung cancer. *Cancer Control*, 2014. 21(1): p. 80-9; Yin, Y., et al., Phosphatidylserine-targeting antibody induces M1 macrophage polarization and promotes myeloid-derived suppressor cell differentiation. *Cancer Immunol Res*, 2013. 1(4): p. 256-68). A preferred checkpoint modulator of phosphatidylserine (PS) is Baviximab (Peregrine).

[0055] Another checkpoint pathway is CSF1/CSF1R (Zhu, Y., et al., CSF1/CSF1R Blockade Reprograms Tumor-Infiltrating Macrophages and Improves Response to T-cell Checkpoint Immunotherapy in Pancreatic Cancer Models. *Cancer Research*, 2014. 74(18): p. 5057-5069). Preferred checkpoint modulators of CSF1R include FPA008 (FivePrime), IMC-CS4 (Eli-Lilly), PLX3397 (Plexxicon) and RO5509554 (Roche).

[0056] Furthermore, the CD94/NKG2A natural killer cell receptor is evaluated for its role in cervical carcinoma (Sheu, B.C., et al., Up-regulation of inhibitory natural killer receptors CD94/NKG2A with suppressed intracellular perforin expression of tumor infiltrating CD8+ T lymphocytes in human cervical carcinoma. *Cancer Res*, 2005. 65(7): p. 2921-9) and in leukemia (Tanaka, J., et al., Cytolytic activity against primary leukemic cells by inhibitory NK cell receptor (CD94/NKG2A)-expressing T cells expanded from various sources of blood mononuclear cells. *Leukemia*, 2005. 19(3): p. 486-9). A preferred checkpoint modulator of NKG2A is IPH2201 (Innate Pharma).

[0057] Another preferred checkpoint molecule is IDO, the indoleamine 2,3-dioxygenase enzyme from the kynurenine pathway (Ball, H.J., et al., Indoleamine 2,3-dioxygenase-2; a new enzyme in the kynurenine pathway. *Int J Biochem Cell Biol*, 2009. 41(3): p. 467-71). Indoleamine 2,3-dioxygenase (IDO) is a tryptophan catabolic enzyme with immune-inhibitory properties. IDO is known to suppress T and NK cells, generate and activate Tregs and myeloid-derived suppressor cells, and promote tumour angiogenesis. IDO1 is overexpressed in many cancer and was shown to allow tumor cells escaping from the immune system (Liu, X., et al., Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. *Blood*, 2010. 115(17): p. 3520-30; Ino, K., et al., Inverse correlation between tumoral indoleamine 2,3-dioxygenase expression and tumor-infiltrating lymphocytes in endometrial cancer: its association with disease progression and survival. *Clin Cancer Res*, 2008. 14(8): p. 2310-7) and to facilitate chronic tumor progression when induced by local inflammation (Muller, A.J., et al., Chronic inflammation that facilitates tumor progression creates local immune suppression by inducing indoleamine 2,3 dioxygenase. *Proc Natl Acad Sci US A*, 2008. 105(44): p. 17073-8). Preferred IDO inhibitors include Exiguamine A, epacadostat (INCB024360; InCyte), Indoximod (NewLink Genetics), NLG919 (NewLink Genetics/Genentech), GDC-0919 (NewLink

Genetics/Genentech), F001287 (Flexus Biosciences/BMS) and small molecules such as 1-methyl-tryptophan, in particular 1-methyl-[D]-tryptophan and the IDO inhibitors listed in Table 1 of Sheridan C., 2015: IDO inhibitors move center stage in immune-oncology; *Nature Biotechnology* 33: 321-322.

[0058] Another preferred immune checkpoint molecule to be modulated is also a member of the kynurenine metabolic pathway: TDO (tryptophan-2,3-dioxygenase). Several studies already demonstrated the interest of TDO in cancer immunity and autoimmunity (Garber, K., Evading immunity: new enzyme implicated in cancer. *J Natl Cancer Inst*, 2012. 104(5): p. 349-52; Platten, M., W. Wick, and B.J. Van den Eynde, Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion. *Cancer Res*, 2012. 72(21): p. 5435-40; Platten, M., et al., Cancer Immunotherapy by Targeting IDO/TDO and Their Downstream Effectors. *Front Immunol*, 2014. 5: p. 673).

[0059] Another preferred immune checkpoint molecule to be modulated is A2AR. The Adenosine A2A receptor (A2AR) is regarded as an important checkpoint in cancer therapy because the tumor microenvironment has typically relatively high concentrations of adenosine, which is activating A2AR. Such signaling provides a negative immune feedback loop in the immune microenvironment (for review see Robert D. Leone et al., 2015: A2aR antagonists: Next generation checkpoint blockade for cancer immunotherapy. *Computational and Structural Biotechnology Journal* 13: 265-272). Preferred A2AR inhibitors include Istradefylline, PBS-509, ST1535, ST4206, Tozadenant, V81444, Preladenant, Vipadenant, SCH58261, SYN115, ZM241365 and FSPTP.

[0060] Another preferred immune checkpoint molecule to be modulated is VISTA. V-domain Ig suppressor of T cell activation (VISTA; also known as C10orf54) is primarily expressed on hematopoietic cells so that consistent expression of VISTA on leukocytes within tumors may allow VISTA blockade to be effective across a broad range of solid tumors. A preferred VISTA inhibitor is JNJ-61610588 (ImmuNext), an anti-VISTA antibody, which recently entered a phase 1 clinical trial.

[0061] Another immune checkpoint molecule is CD122. CD122 is the Interleukin-2 receptor beta sub-unit. CD122 increases proliferation of CD8+ effector T cells.

[0062] The most preferred examples of checkpoint molecules include the "CTLA4-pathway" and the "PD1-pathway" with CTLA4 and its ligands CD80 and CD86 as well as PD1 with its ligands PD-L1 and PD-L2 (more details on CTLA4 and PD-1 pathways as well as further participants are described in Buchbinder E. I. and Desai A., 2016: CTLA-4 and PD-1 Pathways - Similarities, Differences and Implications of Their Inhibition; *American Journal of Clinical Oncology*, 39(1): 98-106). In more general, preferred examples of checkpoint molecules include CD27, CD28, CD40, CD122, CD137, OX40, GITR, ICOS, A2AR, B7-H3, B7-H4, BTLA, CD40, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA, CEACAM1, GARP, PS, CSF1R, CD94/NKG2A, TDO, GITR, TNFR and/or FasR/DcR3 as well as, in particular, their ligands.

[0063] Immune checkpoint molecules are responsible for co-stimulatory or inhibitory interactions of T-cell responses. Accordingly, checkpoint molecules can be divided into (i) (co-)stimulatory checkpoint molecules and (ii) inhibitory checkpoint molecules. Typically, (co-)stimulatory checkpoint molecules act positively in concert with T-cell receptor (TCR) signaling induced by antigen stimulation and inhibitory checkpoint molecules negatively regulate TCR signaling. Examples of (co-)stimulatory checkpoint molecules include CD27, CD28, CD40, CD122, CD137, OX40, GITR and ICOS. Examples of inhibitory checkpoint molecules include CTLA4 as well as PD1 with its ligands PD-L1 and PD-L2; and A2AR, B7-H3, B7-H4, BTLA, IDO, KIR, LAG3, TIM-3, VISTA, CEACAM1, GARP, PS, CSF1R, CD94/NKG2A, TDO, TNFR and FasR/DcR3.

[0064] Preferably, the immune checkpoint modulator is an activator of a (co-)stimulatory checkpoint molecule or an inhibitor of an inhibitory checkpoint molecule or a combination thereof. Accordingly, the immune checkpoint modulator is more preferably (i) an activator of CD27, CD28, CD40, CD122, CD137, OX40, GITR and/or ICOS or (ii) an inhibitor of A2AR, B7-H3, B7-H4, BTLA, CD40, CTLA-4, IDO, KIR, LAG3, PD-1, PDL-1, PD-L2, TIM-3, VISTA, CEACAM1, GARP, PS, CSF1R, CD94/NKG2A, TDO, TNFR and/or FasR/DcR3.

[0065] As described above, a number of CD27, CD28, CD40, CD122, CD137, OX40, GITR, ICOS, A2AR, B7-H3, B7-H4, CTLA-4, PD1, PDL-1, PD-L2, IDO, LAG-3, BTLA, TIM3, VISTA, KIR, CEACAM1, GARP, PS, CSF1R, CD94/NKG2A, TDO, TNFR and/or FasR/DcR3 modulators (inhibitors/activators) are known and some of them are already in clinical trials or even approved. Based on these known immune checkpoint modulators, alternative immune checkpoint modulators may be developed in the (near) future. In particular, known modulators of the preferred immune checkpoint molecules may be used as such or analogues thereof may be used, in particular chimerized, humanized or human forms of antibodies.

[0066] More preferably, the immune checkpoint modulator is an inhibitor of an inhibitory checkpoint molecule (but preferably no inhibitor of a stimulatory checkpoint molecule). Accordingly, the immune checkpoint modulator is even more preferably an inhibitor of A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, PDL-1, PD-L2, TIM-3, VISTA, CEACAM1, GARP, PS, CSF1R, CD94/NKG2A, TDO, TNFR and/or DcR3 or of a ligand thereof.

[0067] It is also preferred that the immune checkpoint modulator is an activator of a stimulatory or costimulatory checkpoint molecule (but preferably no activator of an inhibitory checkpoint molecule). Accordingly, the immune checkpoint modulator is more preferably an activator of CD27, CD28, CD40, CD122, CD137, OX40, GITR and/or ICOS or of a ligand thereof.

[0068] It is even more preferred that the immune checkpoint modulator is a modulator of the CD40 pathway, of the IDO pathway, of the CTLA-4 pathway and/or of the PD-1 pathway. In particular, the immune checkpoint modulator is preferably a modulator of CD40, CTLA-4, PD-L1, PD-L2, PD-1 and/or IDO, more preferably the immune checkpoint modulator is an inhibitor of CTLA-4, PD-L1, PD-L2, PD-1 and/or IDO or an activator of CD40, even more preferably the immune checkpoint modulator is an inhibitor of CTLA-4, PD-L1, PD-1 and/or IDO and most

preferably the immune checkpoint modulator is an inhibitor of CTLA-4 and/or PD-1.

[0069] It is even more preferred that the immune checkpoint modulator is a modulator of the CD40 pathway, of the IDO pathway, of the LAG3 pathway, of the CTLA-4 pathway and/or of the PD-1 pathway. In particular, the immune checkpoint modulator is preferably a modulator of CD40, LAG3, CTLA-4, PD-L1, PD-L2, PD-1 and/or IDO, more preferably the immune checkpoint modulator is an inhibitor of CTLA-4, PD-L1, PD-L2, PD-1, LAG3, and/or IDO or an activator of CD40, even more preferably the immune checkpoint modulator is an inhibitor of CTLA-4, PD-L1, PD-1, LAG3 and/or IDO, even more preferably the immune checkpoint modulator is an inhibitor of LAG3, CTLA-4 and/or PD-1, and most preferably the immune checkpoint modulator is an inhibitor of CTLA-4 and/or PD-1.

[0070] Accordingly, the checkpoint modulator may be selected from known modulators of the CD40 pathway, the CTLA-4 pathway or the PD-1 pathway. Preferably, the checkpoint modulator may be selected from known modulators of the CD40 pathway, the LAG3 pathway, the CTLA-4 pathway or the PD-1 pathway. Preferred inhibitors of the CTLA-4 pathway and of the PD-1 pathway include the monoclonal antibodies Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and Tremelimumab (Pfizer/MedImmune) as well as Opdivo[®] (Nivolumab; Bristol Myers Squibb), Keytruda[®] (Pembrolizumab; Merck), Durvalumab (MedImmune/AstraZeneca), MEDI4736 (AstraZeneca; cf. WO 2011/066389 A1), MPDL3280A (Roche/Genentech; cf. US 8,217,149 B2), Pidilizumab (CT-011; CureTech), MEDI0680 (AMP-514; AstraZeneca), MSB-0010718C (Merck), MIH1 (Affymetrix) and Lambrolizumab (e.g. disclosed as hPD109A and its humanized derivatives h409A11, h409A16 and h409A17 in WO2008/156712; Hamid et al., 2013; N. Engl. J. Med. 369: 134-144). More preferred checkpoint inhibitors include the CTLA-4 inhibitors Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and Tremelimumab (Pfizer/MedImmune) as well as the PD-1 inhibitors Opdivo[®] (Nivolumab; Bristol Myers Squibb), Keytruda[®] (Pembrolizumab; Merck), Pidilizumab (CT-011; CureTech), MEDI0680 (AMP-514; AstraZeneca), AMP-224 and Lambrolizumab (e.g. disclosed as hPD109A and its humanized derivatives h409A11, h409A16 and h409A17 in WO2008/156712; Hamid O. et al., 2013; N. Engl. J. Med. 369: 134-144). As described above, a preferred example of a LAG3 inhibitor is the anti-LAG3 monoclonal antibody BMS-986016 (Bristol-Myers Squibb). Other preferred examples of a LAG3 inhibitor include LAG525 (Novartis), IMP321 (Immutep) and LAG3-Ig as disclosed in WO 2009/044273 A2 and in Brignon et al., 2009, Clin. Cancer Res. 15: 6225-6231 as well as mouse or humanized antibodies blocking human LAG3 (e.g., IMP701 as described in WO 2008/132601 A1), or fully human antibodies blocking human LAG3 (such as disclosed in EP 2320940 A2).

[0071] Preferably, the immune checkpoint modulator is not a modulator of CD40. In particular it is preferred that the immune checkpoint modulator is not a CD40 ligand. It is also preferred that the immune checkpoint modulator is not an anti-CD40 antibody.

[0072] In the context of the present invention it is preferred if more than one immune checkpoint modulator (e.g., checkpoint inhibitor) is used, in particular at least 2, 3, 4, 5, 6, 7, 8,

9 or 10 distinct immune checkpoint modulators (e.g., checkpoint inhibitors) are used, preferably 2, 3, 4 or 5 distinct immune checkpoint modulators (e.g., checkpoint inhibitors) are used, more preferably 2, 3 or 4 distinct immune checkpoint modulators (e.g., checkpoint inhibitors) are used, even more preferably 2 or 3 distinct immune checkpoint modulators (e.g., checkpoint inhibitors) are used and most preferably 2 distinct immune checkpoint modulators (e.g., checkpoint inhibitors) are used. Thereby, "distinct" immune checkpoint modulators (e.g., checkpoint inhibitors) means in particular that they modulate (e.g., inhibit) different checkpoint molecule pathways.

[0073] Preferably, an inhibitor of the PD-1 pathway is combined with an inhibitor of the CTLA-4 pathway. For example, as described above a combination therapy with Nivolumab (anti-PD1) and Ipilimumab (anti-CTLA4) was approved by the FDA in 2015 for the treatment of patients with BRAF V600 wild-type, unresectable or metastatic melanoma. In addition, a successful phase 1b study on the combination of Durvalumab (anti-PD-L1) and Tremelimumab (anti-CTLA4) in non-small cell lung cancer was recently reported (Antonia, Scott et al., 2016, Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study; Lancet Oncol. 2016 Feb 5. pii: S1470-2045(15)00544-6. doi: 10.1016/S1470-2045(15)00544-6. [Epub ahead of print]). Accordingly, preferred combinations of immune checkpoint modulators of the PD-1 pathway and of the CTLA-4 pathway are (i) Nivolumab (anti-PD1) and Ipilimumab (anti-CTLA4) or (ii) Durvalumab (MEDI4736; anti-PD-L1) and Tremelimumab (anti-CTLA4). Combinations thereof, e.g. Nivolumab (anti-PD1) and Tremelimumab (anti-CTLA4) or Durvalumab (MEDI4736; anti-PD-L1) and Ipilimumab (anti-CTLA4) are also preferred.

[0074] Other preferred combinations of at least two distinct immune checkpoint modulators in the context of the present invention may comprise a combination selected from (i) a combination of a KIR inhibitor and a CTLA-4 inhibitor, such as Lirilumab/Ipilimumab; (ii) a combination of a KIR inhibitor and an inhibitor of the PD-1 pathway, such as a PD-1 inhibitor, for example Lirilumab/Nivolumab; (iii) a combination of a LAG3 inhibitor and an inhibitor of the PD-1 pathway, such as a PD-1 inhibitor or a PD-L1 inhibitor, for example as described in Woo et al., 2012, Cancer Res. 72: 917-27 or in Butler N. S. et al., 2011, Nat Immunol. 13: 188-95) and preferred examples of such a combination include Novilumab/BMS-986016 and PDR001/LAG525; (iv) a combination of checkpoint modulators targeting ICOS and an inhibitor of the CTLA-4, for example as described in Fu et al., 2011, Cancer Res. 71: 5445-54; (v) a combination of checkpoint modulators modulating 4-1 BB and inhibitors of CTLA-4, such as described in Curran et al., 2011, PLoS One 6(4): e19499; (vi) a combination of checkpoint modulators targeting PD1 and CD27, such as Novilumab/Varlilumab and Atezolizumab/Varlilumab; (vii) a combination of checkpoint modulators targeting OX40 and CTLA-4, such as MEDI6469/Tremelimumab; (viii) a combination of checkpoint modulators targeting OX40 and PD-1, such as MEDI6469/MEDI4736, MOXR0916/MPDL3280A, MEDI6383/MEDI4736 and GSK3174998/Pembrolizumab; (ix) a combination of checkpoint modulators targeting PD-1 and 4-1BB, such as Novilumab/Urelumab, Pembrolizumab/PF-05082566 and Avelumab/PF-05082566; (x) a combination of checkpoint modulators targeting PD-1 and IDO, such as Ipilimumab/Indoximod, Pembrolizumab/INCB024360, MEDI4736/

INCB024360, MPDL3280A/GDC-0919 and Atezolizumab/INCB024360; (xi) a combination of checkpoint modulators targeting PD-1 and CSF1R, such as Pembrolizumab/PLX3397, Novilumab/FPA008 and MPDL3280A/RO5509554; (xii) a combination of checkpoint modulators targeting PD-1 and G1TR, such as Novilumab/BMS-986156 and Pembrolizumab/MK-4166; (xiii) a combination of checkpoint modulators targeting PD-1 and CD40, such as MPDL3280A/RO7009789; (xiv) a combination of checkpoint modulators targeting PD-1 and B7-H3, such as Pembrolizumab/MGA271; (xv) a combination of checkpoint modulators targeting CTLA-4 and B7-H3, such as Ipilimumab/MGA271 and (xvi) a combination of checkpoint modulators targeting KIR and 4-1BB, such as Lirilumab/Urelumab.

[0075] Most preferably, the combination of the immune checkpoint modulator and the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist for use according to the present invention comprises at least (i) an inhibitor of CTLA-4 and (ii) an inhibitor of PD-1, PD-L1 or PD-L2, preferably at least (i) an inhibitor of CTLA-4 and (ii) an inhibitor of PD-1. Examples of such a preferred combination include a combination of Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and Opdivo[®] (Nivolumab; Bristol Myers Squibb), a combination of Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and Keytruda[®] (Pembrolizumab; Merck), a combination of Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and Durvalumab (MedImmune/AstraZeneca), a combination of Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and MEDI4736 (AstraZeneca; cf. WO 2011/066389 A1), a combination of Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and MPDL3280A (Roche/Genentech; cf. US 8,217,149 B2), a combination of Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and Pidilizumab (CT-011; CureTech), a combination of Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and MEDI0680 (AMP-514; AstraZeneca), a combination of Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and MSB-0010718C (Merck), a combination of Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and MIH1 (Affymetrix), a combination of Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and AMP-224, a combination of Yervoy[®] (Ipilimumab; Bristol Myers Squibb) and Lambrolizumab, a combination of Tremelimumab (Pfizer/MedImmune) and Opdivo[®] (Nivolumab; Bristol Myers Squibb), a combination of Tremelimumab (Pfizer/MedImmune) and Keytruda[®] (Pembrolizumab; Merck), a combination of Tremelimumab (Pfizer/MedImmune) and Durvalumab (MedImmune/AstraZeneca), a combination of Tremelimumab (Pfizer/MedImmune) and MEDI4736 (AstraZeneca; cf. WO 2011/066389 A1), a combination of Tremelimumab (Pfizer/MedImmune) and MPDL3280A (Roche/Genentech; cf. US 8,217,149 B2), a combination of Tremelimumab (Pfizer/MedImmune) and Pidilizumab (CT-011; CureTech), a combination of Tremelimumab (Pfizer/MedImmune) and MEDI0680 (AMP-514; AstraZeneca), a combination of Tremelimumab (Pfizer/MedImmune) and MSB-0010718C (Merck), a combination of Tremelimumab (Pfizer/MedImmune) and MIH1 (Affymetrix), a combination of Tremelimumab (Pfizer/MedImmune) and AMP-224 and a combination of Tremelimumab (Pfizer/MedImmune) and Lambrolizumab.

[0076] In the context of the present invention it is also preferred if more than one immune

checkpoint modulator (e.g., checkpoint inhibitor) of the same checkpoint pathway is used, in particular at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 immune checkpoint modulators (e.g., checkpoint inhibitors) of the same checkpoint pathway are used, preferably 2, 3, 4 or 5 immune checkpoint modulators (e.g., checkpoint inhibitors) of the same checkpoint pathway are used, more preferably 2, 3 or 4 immune checkpoint modulators (e.g., checkpoint inhibitors) of the same checkpoint pathway are used, even more preferably 2 or 3 immune checkpoint modulators (e.g., checkpoint inhibitors) of the same checkpoint pathway are used and most preferably 2 immune checkpoint modulators (e.g., checkpoint inhibitors) of the same checkpoint pathway are used. Preferred checkpoint pathways to be modulated are the PD-1 pathway, the CTLA-4 pathway, the CD40 pathway or the IDO pathway, more preferably the PD-1 pathway, the CTLA-4 pathway or the CD40 pathway and even more preferably the PD-1 pathway or the CTLA-4 pathway. For example, a combination of MEDI4736 and MEDI0680 may be used to modulate, in particular to inhibit, the PD-1 pathway.

[0077] In the context of the present invention immune checkpoint modulators may be any kind of molecule or agent, as long as it totally or partially reduces, inhibits, interferes with, activates, stimulates, increases, reinforces or supports the function of one or more checkpoint molecules as described above. In particular, the immune checkpoint modulator binds to one or more checkpoint molecules, such as checkpoint proteins, or to its precursors, e.g. on DNA- or RNA-level, thereby modulating (e.g., totally or partially reducing, inhibiting, interfering with, activating, stimulating, increasing, reinforcing or supporting) the function of one or more checkpoint molecules as described above. Preferred immune checkpoint modulators are oligonucleotides, siRNA, shRNA, ribozymes, anti-sense RNA molecules, immunotoxins, small molecule inhibitors and (e.g., checkpoint molecule blocking, antagonist or agonist of checkpoint molecule) antibodies or antigen binding fragments thereof.

[0078] Preferably, the immune checkpoint modulator is an oligonucleotide. Such an oligonucleotide is preferably used to decrease protein expression, in particular to decrease the expression of a checkpoint protein, such as the checkpoint receptors or ligands described above. Oligonucleotides are short DNA or RNA molecules, typically comprising from 2 to 50 nucleotides, preferably from 3 to 40 nucleotides, more preferably from 4 to 30 nucleotides and even more preferably from 5 to 25 nucleotides, such as, for example 4, 5, 6, 7, 8, 9 or 10 nucleotides. Oligonucleotides are usually made in the laboratory by solid-phase chemical synthesis. Oligonucleotides maybe single-stranded or double-stranded, however, in the context of the present invention the oligonucleotide is preferably single-stranded. More preferably, the checkpoint modulator oligonucleotide is an antisense-oligonucleotide. Antisense-oligonucleotides are single strands of DNA or RNA that are complementary to a chosen sequence, in particular to a sequence chosen from the DNA or RNA sequence (or a fragment thereof) of a checkpoint protein. Antisense RNA is typically used to prevent protein translation of messenger RNA strands, e.g. of mRNA for a checkpoint protein, by binding to the mRNA. Antisense DNA is typically used to target a specific, complementary (coding or non-coding) RNA. If binding takes place, such a DNA/RNA hybrid can be degraded by the enzyme RNase H. Moreover, morpholino-antisense oligonucleotides can be used for gene knockdowns in vertebrates. For example, Kryczek et al., 2006 (Kryczek I, Zou L, Rodriguez P, Zhu G, Wei S,

Mottram P, et al. B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. *J Exp Med.* 2006; 203:871-81) designed a B7-H4-specific morpholino that specifically blocked B7-H4 expression in macrophages, resulting in increased T-cell proliferation and reduced tumor volumes in mice with tumor associated antigen (TAA)-specific T cells.

[0079] Preferably, the immune checkpoint modulator is an siRNA. Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNA molecules, which is typically 20-25 base pairs in length. In the RNA interference (RNAi) pathway, siRNA interferes with the expression of specific genes, such as genes coding for checkpoint proteins, with complementary nucleotide sequences. siRNA functions by causing mRNA to be broken down after transcription, resulting in no translation. Transfection of exogenous siRNA may be used for gene knockdown, however, the effect maybe only transient, especially in rapidly dividing cells. This may be overcome, for example, by RNA modification or by using an expression vector for the siRNA. The siRNA sequence may also be modified to introduce a short loop between the two strands. The resulting transcript is a short hairpin RNA (shRNA, also "small hairpin RNA"), which can be processed into a functional siRNA by Dicer in its usual fashion. shRNA is an advantageous mediator of RNAi in that it has a relatively low rate of degradation and turnover. Accordingly, the immune checkpoint modulator is preferably an shRNA. shRNA typically requires the use of an expression vector, e.g. a plasmid or a viral or bacterial vector.

[0080] Preferably, the immune checkpoint modulator is an immunotoxin. Immunotoxins are chimeric proteins that contain a targeting moiety (such as an antibody), which is typically targeting an antigen on a certain cell, such as a cancer cell, linked to a toxin. In the context of the present invention, an immunotoxin comprising a targeting moiety, which targets a checkpoint molecule, is preferred. When the immunotoxin binds to a cell carrying the antigen, e.g. the checkpoint molecule, it is taken in through endocytosis, and the toxin can then kill the cell. Immunotoxins preferably comprise a (modified) antibody or antibody fragment, linked to a (fragment of a) toxin. For linkage, methods are well known in the art and the same methods as described herein for linkage of the components of the complex may be used. The targeting portion of the immunotoxin typically comprises a Fab portion of an antibody that targets a specific cell type. The toxin is usually cytotoxic, such as a protein derived from a bacterial or plant protein, from which the natural binding domain has been removed so that the targeting moiety of the immunotoxin directs the toxin to the antigen on the target cell. However, immunotoxins can also comprise a targeting moiety other than an antibody or antibody fragment, such as a growth factor. For example, recombinant fusion proteins containing a toxin and a growth factor are also referred to as recombinant immunotoxins.

[0081] Preferably, the immune checkpoint modulator is a small molecule drug (also referred to as "small molecule inhibitor"). A small molecule drug is a low molecular weight (up to 900 daltons) organic compound that typically interacts with (the regulation of) a biological process. In the context of the present invention, a small molecule drug which is an immune checkpoint modulator, is an organic compound having a molecular weight of no more than 900 daltons,

which totally or partially reduces, inhibits, interferes with, or negatively modulates the function of one or more checkpoint molecules as described above. The upper molecular weight limit of 900 daltons allows for the possibility to rapidly diffuse across cell membranes and for oral bioavailability. More preferably, the molecular weight of the small molecule drug which is an immune checkpoint modulator, is no more than 500 daltons. For example, various A2AR antagonists known in the art are organic compounds having a molecular weight below 500 daltons.

[0082] Most preferably, the immune checkpoint modulator is an antibody or an antigen-binding fragment thereof, in particular antibodies, or antigen binding fragments thereof, that bind to immune checkpoint receptors or antibodies that bind to immune checkpoint receptor ligands. Preferably, such antibodies, or antigen binding fragments thereof, are agonists or antagonists of immune checkpoint receptors or of immune checkpoint receptor ligands. Examples of antibody-type checkpoint modulators include immune checkpoint modulators, which are currently approved as described above, namely, Yervoy[®] (Ipilimumab; Bristol Myers Squibb), Opdivo[®] (Nivolumab; Bristol Myers Squibb) and Keytruda[®] (Pembrolizumab; Merck) and further anti-checkpoint receptor antibodies or anti-checkpoint ligand antibodies as described above.

[0083] As used herein, the term "antibody" encompasses various forms of antibodies, preferably monoclonal antibodies, including whole antibodies, antibody fragments, human antibodies, chimeric antibodies, humanized antibodies and genetically engineered antibodies (variant or mutant antibodies) as long as the characteristic properties according to the invention are retained, which means that the antibody (or the antigen-binding fragment) modulates (e.g., totally or partially reduces, inhibits, interferes with, activates, stimulates, increases, reinforces or supports) the function of one or more checkpoint molecules as described above. In particular, the antibody (or the antigen-binding fragment) mediates this function by binding to a checkpoint molecule. Accordingly, the antibody (or the antigen-binding fragment) is preferably a "blocking" antibody (or the antigen-binding fragment), in particular an "antagonist" antibody (or the antigen-binding fragment), or an "agonist" antibody (or the antigen-binding fragment). In particular, the term "antibody" includes both, glycosylated and non- glycosylated immunoglobulins of any isotype or subclass (preferably IgG) or to an antigen-binding region thereof that competes with the intact antibody for specific binding, unless otherwise specified. Preferred examples of antibodies include monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies, antibody mimetics, chimeric antibodies, humanized antibodies, human antibodies, antibody fusions, antibody conjugates, single chain antibodies, antibody derivatives, antibody analogues and fragments thereof, respectively. Unless otherwise indicated, the term "antibody" includes, in addition to antibodies comprising two full-length heavy chains and two full-length light chains, derivatives, variants, and fragments thereof. In some instances an "antibody" may include fewer chains. Especially preferred are human or humanized monoclonal antibodies and/or recombinant antibodies, especially as recombinant human monoclonal antibodies. Particularly preferred are human IgG-type antibodies.

[0084] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germ line immunoglobulin sequences. Human antibodies are well-known in the state of the art (van Dijk, M. A., and van de Winkel, J. G., *Curr. Opin. Chem. Biol.* 5 (2001) 368-374). Human antibodies can also be produced in transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire or a selection of human antibodies in the absence of endogenous immunoglobulin production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, e.g., Jakobovits, A., et al., *Proc. Natl. Acad. Sci. USA* 90 (1993) 2551-2555; Jakobovits, A., et al., *Nature* 362 (1993) 255-258; Bruggemann, M., et al., *Year Immunol.* 7 (1993) 3340). Human antibodies can also be produced in phage display libraries (Hoogenboom, H. R., and Winter, G., *J. Mol. Biol.* 227 (1992) 381-388; Marks, J. D., et al., *J. Mol. Biol.* 222 (1991) 581-597). The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); and Boerner, P., et al., *J. Immunol.* 147 (1991) 86-95). The term "human antibody" as used herein also comprises such antibodies which are modified, e.g. in the variable region to generate the properties according to the invention.

[0085] As used herein, the term "recombinant antibody" is intended to include all antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from a host cell such as for example a CHO cell or from an animal (e.g. a mouse) that is transgenic for, e.g. human, immunoglobulin genes or antibodies expressed using a recombinant expression vector transfected into a host cell. Thus, recombinant antibodies are antibodies that are prepared, expressed, created or isolated by recombinant means, such as (i) antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes or a hybridoma prepared therefrom, (ii) antibodies isolated from a host cell transfected to express the antibody, e.g., from a transfectoma, (iii) antibodies isolated from a recombinant, combinatorial antibody library, and (iv) antibodies prepared, expressed, created or isolated by any other means that involve splicing of immunoglobulin gene sequences to other DNA sequences. Such antibodies may have variable and constant regions derived from germline immunoglobulin sequences of two distinct species of animals. Antibodies subjected to in vitro mutagenesis (or, when an animal transgenic for human immunoglobulin sequences is used, in vivo somatic mutagenesis) are also preferred, while the amino acid sequences of the V_H and V_L regions of such antibodies may be sequences that, while derived from and related to the germline V_H and V_L sequences of a particular species (e.g., human), may not naturally exist within that species' antibody germline repertoire in vivo. Recombinant antibodies may have variable and constant regions in a rearranged form and/or, for example, certain mutations, which do not occur in nature.

[0086] As used herein, the terms "antigen binding fragment," "fragment," and "antibody fragment" are used interchangeably to refer to any fragment of an antibody of the invention that retains the specific binding activity of the antibody according to the invention. Examples of antibody fragments include a single chain antibody, Fab, Fab', F(ab')₂, Fv or scFv. Fragments

of the antibodies of the invention can be obtained from the antibodies by methods that include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction. Alternatively, fragments of antibodies can be obtained by cloning and expression of part of the sequences of the heavy and/or light chains. "Fragments" include Fab, Fab', F(ab')₂ and Fv fragments. The invention also encompasses single-chain Fv fragments (scFv) derived from the heavy and light chains of an antibody of the invention. For example, the invention includes a scFv comprising the CDRs from an antibody of the invention. Also included are heavy or light chain monomers and dimers, single domain heavy chain antibodies, single domain light chain antibodies, as well as single chain antibodies, e.g., single chain Fv in which the heavy and light chain variable domains are joined by a peptide linker. Antibody fragments of the invention may impart monovalent or multivalent interactions and be contained in a variety of structures as described above. For instance, scFv molecules may be synthesized to create a trivalent "triabody" or a tetravalent "tetraabody." The scFv molecules may include a domain of the Fc region resulting in bivalent minibodies.

[0087] In addition, the sequences of the invention may be a component of multispecific molecules in which the sequences of the invention target the checkpoint molecule and other regions of the molecule bind to other targets. Exemplary molecules include bispecific Fab₂, trispecific Fab₃, bispecific scFv, and diabodies (Holliger and Hudson, 2005, Nature Biotechnology 9: 1126-1136). Although the specification, including the claims, may, in some places, refer explicitly to antigen binding fragment(s), antibody fragment(s), variant(s) and/or derivative(s) of antibodies, it is understood that the term "antibody" or "antibody of the invention" includes all categories of antibodies, namely, antigen binding fragment(s), antibody fragment(s), variant(s) and derivative(s) of antibodies. Further, the term "antibody" as used herein includes both antibodies and antigen binding fragments thereof.

[0088] Preferably, the immune checkpoint modulators in the combination used according to the present invention are antibodies or antigen-binding fragments that can partially or totally block the PD-1 pathway (e.g., they can be partial or full antagonists of the PD-1 pathway), in particular PD-1, PD-L1 or PD-L2, more preferably, the antibody can partially or totally block PD-1 (e.g., they can be partial or full antagonists of PD-1). Such antibodies or antigen-binding fragments include anti- PD-1 antibodies, human anti- PD-1 antibodies, mouse anti- PD-1 antibodies, mammalian anti- PD-1 antibodies, humanized anti- PD-1 antibodies, monoclonal anti- PD-1 antibodies, polyclonal anti- PD-1 antibodies, chimeric anti- PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti- PD-1 adnectins, anti- PD-1 domain antibodies, single chain anti- PD-1 fragments, heavy chain anti- PD-1 fragments, and light chain anti-PD-1 fragments. For example, the anti-PD-1 antibody may be an antigen-binding fragment. Preferably, the anti-PD-1 antibody is able to bind to human PD-1 and to partially or totally block the activity of PD-1 (e.g., they can be partial or full antagonists of PD-1), thereby in particular unleashing the function of immune cells expressing PD-1.

[0089] Preferably, the immune checkpoint modulators in the combination used according to the present invention are antibodies or antigen-binding fragments that can partially or totally block the CTLA-4 pathway (e.g., they can be partial or full antagonists of the CTLA-4 pathway). Such

antibodies or antigen-binding fragments include anti-CTLA4 antibodies, human anti-CTLA4 antibodies, mouse anti-CTLA4 antibodies, mammalian anti-CTLA4 antibodies, humanized anti-CTLA4 antibodies, monoclonal anti-CTLA4 antibodies, polyclonal anti-CTLA4 antibodies, chimeric anti-CTLA4 antibodies, MDX-010 (ipilimumab), tremelimumab, anti-CD28 antibodies, anti-CTLA4 adnectins, anti-CTLA4 domain antibodies, single chain anti-CTLA4 fragments, heavy chain anti-CTLA4 fragments, and light chain anti-CTLA4 fragments. For example, the anti-CTLA4 antibody may be an antigen-binding fragment. Preferably, the anti-CTLA4 antibody is able to bind to human CTLA4 and to partially or totally block the activity of CTLA4 (e.g., they can be partial or full antagonists of CTLA-4), thereby in particular unleashing the function of immune cells expressing CTLA4.

Complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope

[0090] In addition to the immune checkpoint modulator described above, the combination for use according to the present invention comprises a complex comprising:

1. a) a cell penetrating peptide;
2. b) at least one antigen or antigenic epitope; and
3. c) at least one TLR peptide agonist,

wherein the components a) - c), i.e. the cell penetrating peptide, the at least one antigen or antigenic epitope and the at least one TLR peptide agonist, are covalently linked. In the following, it is also referred to such a complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist, which are covalently linked, by using the term "the complex" or "the complex comprised by the combination for use according to the present invention".

[0091] Such a complex comprised by the combination for use according to the present invention provides simultaneous (i) stimulation of multi-epitopic cytotoxic T cell-mediated immunity, (ii) induction of T_H cells and (iii) promotion of immunological memory. Thereby, a complex comprised by the combination for use according to the present invention provides a potent vaccine, in particular having improved anti-tumor activity.

[0092] The complex comprised by the combination for use according to the present invention is a polypeptide or a protein, in particular a recombinant polypeptide or a recombinant protein, preferably a recombinant fusion protein or a recombinant fusion polypeptide. The term "recombinant" as used herein (i.e. throughout the specification) means that it (here: the polypeptide or the protein) does not occur naturally. Accordingly, the complex comprised by the combination for use according to the present invention, which is a recombinant polypeptide or a recombinant protein, typically comprises components a) to c), wherein components a) to c) are of different origins, i.e. do not naturally occur in this combination.

[0093] In the context of the present invention, i.e. throughout the present application, the terms "peptide", "polypeptide", "protein" and variations of these terms refer to peptide, oligopeptide, oligomer or protein including fusion protein, respectively, comprising at least two amino acids joined to each other, preferably by a normal peptide bond, or, alternatively, by a modified peptide bond, such as for example in the cases of isosteric peptides. A peptide, polypeptide or protein can be composed of L-amino acids and/or D-amino acids. Preferably, a peptide, polypeptide or protein is either (entirely) composed of L-amino acids or (entirely) of D-amino acids, thereby forming "retro-inverso peptide sequences". The term "retro-inverso (peptide) sequences" refers to an isomer of a linear peptide sequence in which the direction of the sequence is reversed and the chirality of each amino acid residue is inverted (see e.g. Jameson et al., *Nature*, 368,744-746 (1994); Brady et al., *Nature*, 368,692-693 (1994)). In particular, the terms "peptide", "polypeptide", "protein" also include "peptidomimetics" which are defined as peptide analogs containing non-peptidic structural elements, which peptides are capable of mimicking or antagonizing the biological action(s) of a natural parent peptide. A peptidomimetic lacks classical peptide characteristics such as enzymatically scissile peptide bonds. In particular, a peptide, polypeptide or protein can comprise amino acids other than the 20 amino acids defined by the genetic code in addition to these amino acids, or it can be composed of amino acids other than the 20 amino acids defined by the genetic code. In particular, a peptide, polypeptide or protein in the context of the present invention can equally be composed of amino acids modified by natural processes, such as post-translational maturation processes or by chemical processes, which are well known to a person skilled in the art. Such modifications are fully detailed in the literature. These modifications can appear anywhere in the polypeptide: in the peptide skeleton, in the amino acid chain or even at the carboxy- or amino-terminal ends. In particular, a peptide or polypeptide can be branched following an ubiquitination or be cyclic with or without branching. This type of modification can be the result of natural or synthetic post-translational processes that are well known to a person skilled in the art. The terms "peptide", "polypeptide", "protein" in the context of the present invention in particular also include modified peptides, polypeptides and proteins. For example, peptide, polypeptide or protein modifications can include acetylation, acylation, ADP-ribosylation, amidation, covalent fixation of a nucleotide or of a nucleotide derivative, covalent fixation of a lipid or of a lipidic derivative, the covalent fixation of a phosphatidylinositol, covalent or non-covalent crosslinking, cyclization, disulfide bond formation, demethylation, glycosylation including pegylation, hydroxylation, iodization, methylation, myristoylation, oxidation, proteolytic processes, phosphorylation, prenylation, racemization, seneloylation, sulfatation, amino acid addition such as arginylation or ubiquitination. Such modifications are fully detailed in the literature (*Proteins Structure and Molecular Properties* (1993) 2nd Ed., T. E. Creighton, New York ; *Post-translational Covalent Modifications of Proteins* (1983) B. C. Johnson, Ed., Academic Press, New York ; Seifter et al. (1990) *Analysis for protein modifications and nonprotein cofactors*, *Meth. Enzymol.* 182: 626-646 and Rattan et al., (1992) *Protein Synthesis: Post-translational Modifications and Aging*, *Ann NY Acad Sci*, 663: 48-62). Accordingly, the terms "peptide", "polypeptide", "protein" preferably include for example lipopeptides, lipoproteins, glycopeptides and glycoproteins.

[0094] However, in a particularly preferred embodiment, the complex as described herein is a

"classical" peptide, polypeptide or protein, whereby a "classical" peptide, polypeptide or protein is typically composed of amino acids selected from the 20 amino acids defined by the genetic code, linked to each other by a normal peptide bond.

[0095] If the complex comprised by the combination for use according to the present invention is a polypeptide or a protein, it is preferred that it comprises at least 50, at least 60, at least 70, preferably at least 80, at least 90, more preferably at least 100, at least 110, even more preferably at least 120, at least 130, particularly preferably at least 140, or most preferably at least 150 amino acid residues.

Component a) - Cell penetrating peptide

[0096] The CPP allows for efficient delivery, i.e. transport and loading, in particular of at least one antigen or antigenic epitope, into the antigen presenting cells (APCs), in particular into the dendritic cells (DCs) and thus to the dendritic cells' antigen processing machinery.

[0097] The term "cell penetrating peptides" ("CPPs") is generally used to designate short peptides that are able to transport different types of cargo molecules across plasma membrane, and, thus, facilitate cellular uptake of various molecular cargoes (from nanosize particles to small chemical molecules and large fragments of DNA). "Cellular internalization" of the cargo molecule linked to the cell penetrating peptide generally means transport of the cargo molecule across the plasma membrane and thus entry of the cargo molecule into the cell. Depending on the particular case, the cargo molecule can, then, be released in the cytoplasm, directed to an intracellular organelle, or further presented at the cell surface. Cell penetrating ability, or internalization, of the cell penetrating peptide or of the complex (comprising said cell penetrating peptide) comprised by the combination for use according to the invention can be checked by standard methods known to one skilled in the art, including flow cytometry or fluorescence microscopy of live and fixed cells, immunocytochemistry of cells transduced with said peptide or complex, and Western blot.

[0098] Cell penetrating peptides typically have an amino acid composition that either contains a high relative abundance of positively charged amino acids such as lysine or arginine or have a sequence that contains an alternating pattern of polar/charged amino acids and non-polar, hydrophobic amino acids. These two types of structures are referred to as polycationic or amphipathic, respectively. Cell-Penetrating peptides are of different sizes, amino acid sequences, and charges but all CPPs have a common characteristic that is the ability to translocate the plasma membrane and facilitate the delivery of various molecular cargoes to the cytoplasm or to an organelle of a cell. At present, the theories of CPP translocation distinguish three main entry mechanisms: direct penetration in the membrane, endocytosis-mediated entry, and translocation through the formation of a transitory structure. CPP transduction is an area of ongoing research. Cell-penetrating peptides have found numerous applications in medicine as drug delivery agents in the treatment of different diseases including cancer and virus inhibitors, as well as contrast agents for cell labeling and imaging.

[0099] Typically, cell penetrating peptides (CPPs) are peptides of 8 to 50 residues that have the ability to cross the cell membrane and enter into most cell types. Alternatively, they are also called protein transduction domain (PTDs) reflecting their origin as occurring in natural proteins. Frankel and Pabo simultaneously to Green and Lowenstein described the ability of the transactivating transcriptional activator from the human immunodeficiency virus 1 (HIV-TAT) to penetrate into cells (Frankel, A.D. and C.O. Pabo, Cellular uptake of the tat protein from human immunodeficiency virus. *Cell*, 1988. 55(6): p. 1189-93). In 1991, transduction into neural cells of the Antennapedia homeodomain (DNA-binding domain) from *Drosophila melanogaster* was described (Joliot, A., et al., Antennapedia homeobox peptide regulates neural morphogenesis. *Proc Natl Acad Sci U S A*, 1991. 88(5): p. 1864-8). In 1994, the first 16-mer peptide CPP called Penetratin, having the amino acid sequence RQIKIYFQNRRMKWKK (SEQ ID NO: 1) was characterized from the third helix of the homeodomain of Antennapedia (Derossi, D., et al., The third helix of the Antennapedia homeodomain translocates through biological membranes. *J Biol Chem*, 1994. 269(14): p. 10444-50), followed in 1998 by the identification of the minimal domain of TAT, having the amino acid sequence YGRKKRRQRRR (SEQ ID NO: 2) required for protein transduction (Vives, E., P. Brodin, and B. Lebleu, A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. *J Biol Chem*, 1997. 272(25): p. 16010-7). Over the past two decades, dozens of peptides were described from different origins including viral proteins, e.g. VP22 (Elliott, G. and P. O'Hare, Intercellular trafficking and protein delivery by a herpesvirus structural protein. *Cell*, 1997. 88(2): p. 223-33) and ZEBRA (Rothe, R., et al., Characterization of the cell-penetrating properties of the Epstein-Barr virus ZEBRA trans-activator. *J Biol Chem*, 2010. 285(26): p. 20224-33), or from venoms, e.g. melittin (Dempsey, C.E., The actions of melittin on membranes. *Biochim Biophys Acta*, 1990. 1031(2): p. 143-61), mastoporan (Konno, K., et al., Structure and biological activities of eumenine mastoparan-AF (EMP-AF), a new mast cell degranulating peptide in the venom of the solitary wasp (*Anterhynchium flavomarginatum micado*). *Toxicon*, 2000. 38(11): p. 1505-15), maurocalcin (Esteve, E., et al., Transduction of the scorpion toxin maurocalcine into cells. Evidence that the toxin crosses the plasma membrane. *J Biol Chem*, 2005. 280(13): p. 12833-9), crotamine (Nascimento, F.D., et al., Crotamine mediates gene delivery into cells through the binding to heparan sulfate proteoglycans. *J Biol Chem*, 2007. 282(29): p. 21349-60) or buforin (Kobayashi, S., et al., Membrane translocation mechanism of the antimicrobial peptide buforin 2. *Biochemistry*, 2004. 43(49): p. 15610-6). Synthetic CPPs were also designed including the poly-arginine (R8, R9, R10 and R12) (Futaki, S., et al., Arginine-rich peptides. An abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery. *J Biol Chem*, 2001. 276(8): p. 5836-40) or transportan (Pooga, M., et al., Cell penetration by transportan. *FASEB J*, 1998. 12(1): p. 67-77). Any of the above described CPPs may be used as cell penetrating peptide, i.e. as component a), in the complex comprised by the combination for use according to the present invention. In particular, the component a), i.e. the CPP, in the complex comprised by the combination for use according to the present invention may comprise the minimal domain of TAT, having the amino acid sequence YGRKKRRQRRR (SEQ ID NO: 2). In particular, the component a), i.e. the CPP, in the complex comprised by the combination for use according to the present invention may comprise

Penetratin having the amino acid sequence RQIKIYFQNRMRMKWKK (SEQ ID NO: 1).

[0100] Various CPPs, which can be used as cell penetrating peptide, i.e. as component a), in the complex comprised by the composition for use according to the present invention, are also disclosed in the review: Milletti, F., Cell-penetrating peptides: classes, origin, and current landscape. Drug Discov Today 17 (15-16): 850-60, 2012. In other words, the CPPs disclosed in Milletti, F., 2012, Cell-penetrating peptides: classes, origin, and current landscape. Drug Discov Today 17 (15-16): 850-60 can be used as cell penetrating peptide, i.e. as component a), in the complex comprised by the combination for use according to the present invention. This includes in particular cationic CPPs, amphipatic CPPs, and hydrophobic CPPs as well as CPPs derived from heparan-, RNA- and DNA-binding proteins (cf. Table 1 of Milletti, F., Cell-penetrating peptides: classes, origin, and current landscape. Drug Discov Today 17 (15-16): 850-60, 2012), CPPs derived from signal peptides (cf. Table 2 of Milletti, F., Cell-penetrating peptides: classes, origin, and current landscape. Drug Discov Today 17 (15-16): 850-60, 2012), CPPs derived from antimicrobial peptides (cf. Table 3 of Milletti, F., Cell-penetrating peptides: classes, origin, and current landscape. Drug Discov Today 17 (15-16): 850-60, 2012), CPPs derived from viral proteins (cf. Table 4 of Milletti, F., Cell-penetrating peptides: classes, origin, and current landscape. Drug Discov Today 17 (15-16): 850-60, 2012), CPPs derived from various natural proteins (cf. Table 5 of Milletti, F., Cell-penetrating peptides: classes, origin, and current landscape. Drug Discov Today 17 (15-16): 850-60, 2012), and Designed CPPs and CPPs derived from peptide libraries (cf. Table 6 of Milletti, F., Cell-penetrating peptides: classes, origin, and current landscape. Drug Discov Today 17 (15-16): 850-60, 2012).

[0101] Preferably, the cell penetrating peptide, which is comprised by the complex,

1. i) has a length of the amino acid sequence of said peptide of 5 to 50 amino acids in total, preferably of 10 to 45 amino acids in total, more preferably of 15 to 45 amino acids in total; and/or
2. ii) has an amino acid sequence comprising a fragment of the minimal domain of ZEBRA, said minimal domain extending from residue 170 to residue 220 of the ZEBRA amino acid sequence according to SEQ ID NO: 3, wherein, optionally, 1 , 2, 3, 4, or 5 amino acids have been substituted, deleted, and/or added without abrogating said peptide's cell penetrating ability, or a sequence variant of such a fragment.

[0102] Thereby, it is preferred that the cell penetrating peptide, which is comprised by the complex

1. i) has a length of the amino acid sequence of said peptide of 5 to 50 amino acids in total, preferably of 10 to 45 amino acids in total, more preferably of 15 to 45 amino acids in total; and
2. ii) has an amino acid sequence comprising a fragment of the minimal domain of ZEBRA, said minimal domain extending from residue 170 to residue 220 of the ZEBRA amino

acid sequence according to SEQ ID NO: 3, wherein, optionally, 1, 2, 3, 4, or 5 amino acids have been substituted, deleted, and/or added without abrogating said peptide's cell penetrating ability, or a sequence variant of such a fragment.

[0103] Such preferred CPPs are disclosed in WO 2014/041505.

[0104] The term "ZEBRA" (also known as Zta, Z, EB1, or BZLF1) generally means the basic-leucine zipper (bZIP) transcriptional activator of the Epstein-Barr virus (EBV). The minimal domain of ZEBRA, which exhibits cell penetrating properties, has been identified as spanning from residue 170 to residue 220 of ZEBRA. The amino acid sequence of ZEBRA is disclosed under NCBI accession number YP_401673 and comprises 245 amino acids represented in SEQ ID NO: 3:

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MMDPNSTSEDVKFTDPYQVPFVQAFDQATRVYQDLGGPSQAPLPCVLWPVLPEPLPQGQL
TAYHVVSTAPTGSWFSA PQPAPENAYQAYAAPQLFPVSDITQNNQQTNQAGGEAPQPGDNST
VQTAAAVVFACPGANQGQQLADIGVPQPAPVAAPARRTRKPQQPESLEECDSELEIKRYKNR
VASRKCRAKFKQLLQHYREVA AAKSSENDRLRLLLKQMCPSLDVDSIIPRTPDVLHEDLLNF
(SEQ ID NO: 3 - ZEBRA amino acid sequence (natural sequence from Epstein - Barr virus
(EBV)) (YP_401673))
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[0105] Recently, a CPP derived from the viral protein ZEBRA was described to transduce protein cargoes across biological membranes by both (i) direct translocation and (ii) lipid raft-mediated endocytosis (Rothe R, Liguori L, Villegas-Mendez A, Marques B, Grunwald D, Drouet E, et al. Characterization of the cell-penetrating properties of the Epstein-Barr virus ZEBRA trans-activator. The Journal of biological chemistry 2010;285(26):20224-33). The present inventors assume that these two mechanisms of entry should promote both MHC class I and II restricted presentation of cargo antigens to CD8⁺ and CD4⁺ T cells, respectively. Accordingly, such a CPP can deliver multi-epitopic peptides to dendritic cells (DCs), and subsequently to promote CTL and Th cell activation and anti-tumor function. Such a CPP can thus efficiently deliver the complex comprised by the combination for use according to the present invention to antigen presenting cells (APCs) and lead to multi-epitopic MHC class I and II restricted presentation.

[0106] In the context of the present invention, the term "MHC class I" designates one of the two primary classes of the Major Histocompatibility Complex molecules. The MHC class I (also noted "MHC I") molecules are found on every nucleated cell of the body. The function of MHC class I is to display an epitope to cytotoxic cells (CTLs). In humans, MHC class I molecules consist of two polypeptide chains, α - and β 2-microglobulin (b2m). Only the α chain is polymorphic and encoded by a HLA gene, while the b2m subunit is not polymorphic and encoded by the Beta-2 microglobulin gene. In the context of the present invention, the term "MHC class II" designates the other primary class of the Major Histocompatibility Complex molecules. The MHC class II (also noted "MHC II") molecules are found only on a few

specialized cell types, including macrophages, dendritic cells and B cells, all of which are dedicated antigen-presenting cells (APCs).

[0107] Preferably, the sequence variant of a fragment of the minimal domain of ZEBRA as described above shares, in particular over the whole length, at least 70%, at least 75%, preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, particularly preferably at least 95%, most preferably at least 99% amino acid sequence identity with the fragment of the minimal domain of ZEBRA as described above without abrogating the cell penetrating ability of the cell penetrating peptide. In particular, a "fragment" of the minimal domain of ZEBRA as defined above is preferably to be understood as a truncated sequence thereof, i.e. an amino acid sequence, which is N-terminally, C-terminally and/or intrasequentially truncated compared to the amino acid sequence of the native sequence. Moreover, such a "fragment" of the minimal domain of ZEBRA has preferably a length of 5 to 50 amino acids in total, preferably of 10 to 45 amino acids in total, more preferably of 15 to 45 amino acids in total.

[0108] Accordingly, the term "sequence variant" as used in the context of the present invention, i.e. throughout the present application, refers to any alteration in a reference sequence. The term "sequence variant" includes nucleotide sequence variants and amino acid sequence variants. Preferably, a reference sequence is any of the sequences listed in the "Table of Sequences and SEQ ID Numbers" (Sequence listing), i.e. SEQ ID NO: 1 to SEQ ID NO: 47. Preferably, a sequence variant shares, in particular over the whole length of the sequence, at least 70%, at least 75%, preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, particularly preferably at least 95%, most preferably at least 99% sequence identity with a reference sequence, whereby sequence identity is calculated as described below. In particular, a sequence variant preserves the specific function of the reference sequence. Sequence identity is calculated as described below. In particular, an amino acid sequence variant has an altered sequence in which one or more of the amino acids in the reference sequence is deleted or substituted, or one or more amino acids are inserted into the sequence of the reference amino acid sequence. As a result of the alterations, the amino acid sequence variant has an amino acid sequence which is at least 70%, at least 75%, preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, particularly preferably at least 95%, most preferably at least 99% identical to the reference sequence. For example, variant sequences which are at least 90% identical have no more than 10 alterations, i.e. any combination of deletions, insertions or substitutions, per 100 amino acids of the reference sequence.

[0109] In the context of the present invention, an amino acid sequence "sharing a sequence identity" of at least, for example, 95% to a query amino acid sequence of the present invention, is intended to mean that the sequence of the subject amino acid sequence is identical to the query sequence except that the subject amino acid sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain an amino acid sequence having a sequence of at least 95% identity to a query amino acid sequence, up to 5% (5 of 100) of the amino acid residues in the subject sequence may be

inserted or substituted with another amino acid or deleted, preferably within the above definitions of variants or fragments. The same, of course, also applies similarly to nucleic acid sequences.

[0110] For (amino acid or nucleic acid) sequences without exact correspondence, a "% identity" of a first sequence may be determined with respect to a second sequence. In general, these two sequences to be compared are aligned to give a maximum correlation between the sequences. This may include inserting "gaps" in either one or both sequences, to enhance the degree of alignment. A % identity may then be determined over the whole length of each of the sequences being compared (so-called global alignment), that is particularly suitable for sequences of the same or similar length, or over shorter, defined lengths (so-called local alignment), that is more suitable for sequences of unequal length.

[0111] Methods for comparing the identity and homology of two or more sequences are well known in the art. The percentage to which two sequences are identical can e.g. be determined using a mathematical algorithm. A preferred example of a mathematical algorithm which can be used is the algorithm of Karlin et al. (1993), PNAS USA, 90:5873-5877. Such an algorithm is integrated in the BLAST family of programs, e.g. BLAST or NBLAST program (see also Altschul et al., 1990, J. Mol. Biol. 215, 403-410 or Altschul et al. (1997), Nucleic Acids Res, 25:3389-3402), accessible through the home page of the NCBI at world wide web site ncbi.nlm.nih.gov) and FASTA (Pearson (1990), Methods Enzymol. 183, 63-98; Pearson and Lipman (1988), Proc. Natl. Acad. Sci. U. S. A 85, 2444-2448.). Sequences which are identical to other sequences to a certain extent can be identified by these programmes. Furthermore, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux et al., 1984, Nucleic Acids Res., 387-395), for example the programs BESTFIT and GAP, may be used to determine the % identity between two polynucleotides and the % identity and the % homology or identity between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of (Smith and Waterman (1981), J. Mol. Biol. 147, 195-197.) and finds the best single region of similarity between two sequences.

[0112] More preferably, the fragments of the cell penetrating peptide or the variants thereof as described above further retain said peptide's ability to present a cargo molecule such as antigens or antigenic epitopes at the surface of a cell, such as an antigen-presenting cell, in the context of MHC class I and/or MHC class II molecules. The ability of a cell penetrating peptide or complex comprising said cell penetrating peptide to present a cargo molecule such as antigens or antigenic epitopes at the surface of a cell in the context of MHC class I and/or MHC class II molecules can be checked by standard methods known to one skilled in the art, including capacity to stimulate proliferation and/or function of MHC-restricted CD4⁺ or CD8⁺ T cells with specificity for these epitopes.

[0113] The preferred cell penetrating peptide, which

1. i) has a length of the amino acid sequence of said peptide of 5 to 50 amino acids in total, preferably of 10 to 45 amino acids in total, more preferably of 15 to 45 amino acids in

total; and/or

2. ii) has an amino acid sequence comprising a fragment of the minimal domain of ZEBRA, said minimal domain extending from residue 170 to residue 220 of the ZEBRA amino acid sequence according to SEQ ID NO: 3, wherein, optionally, 1, 2, 3, 4, or 5 amino acids have been substituted, deleted, and/or added without abrogating said peptide's cell penetrating ability, or a variant of such a fragment

preferably comprises an amino acid sequence having at least one conservatively substituted amino acid compared to the referenced sequence, meaning that a given amino acid residue is replaced by a residue having similar physiochemical characteristics.

[0114] Generally, substitutions for one or more amino acids present in the referenced amino acid sequence should be made conservatively. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity properties, are well known (Kyte and Doolittle, 1982, J. Mol. Biol. 157(1):105- 132). Substitutions of one or more L-amino acids with one or more D-amino acids are to be considered as conservative substitutions in the context of the present invention. Exemplary amino acid substitutions are presented in Table 1 below:

(Table 1)

Original residues	Examples of substitutions
Ala (A)	Val, Leu, Ile, Gly
Arg (R)	His, Lys
Asn (N)	Gln
Asp (D)	Glu
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Pro, Ala
His (H)	Lys, Arg
Ile (I)	Leu, Val, Met, Ala, Phe
Leu (L)	Ile, Val, Met, Ala, Phe
Lys (K)	Arg, His
Met (M)	Leu, Ile, Phe
Phe (F)	Leu, Val, Ile, Tyr, Trp, Met
Pro (P)	Ala, Gly
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr, Phe

Original residues	Examples of substitutions
Tyr (Y)	Trp, Phe
Original residues	Examples of substitutions
Val (V)	Ile, Met, Leu, Phe, Ala

[0115] Particularly preferably, the preferred cell penetrating peptide, which

1. i) has a length of the amino acid sequence of said peptide of 5 to 50 amino acids in total, preferably of 10 to 45 amino acids in total, more preferably of 15 to 45 amino acids in total; and/or
2. ii) has an amino acid sequence comprising a fragment of the minimal domain of ZEBRA, said minimal domain extending from residue 170 to residue 220 of the ZEBRA amino acid sequence according to SEQ ID NO: 3, wherein, optionally, 1, 2, 3, 4, or 5 amino acids have been substituted, deleted, and/or added without abrogating said peptide's cell penetrating ability, or a variant of such a fragment

comprises a Cys (C) substituted into a Ser (S), at the equivalent of position 189 relative to ZEBRA amino acid sequence of SEQ ID NO: 3.

[0116] Thereby, it is preferred that such a preferred cell penetrating peptide has an amino acid sequence comprising a sequence according to the following general formula (I):

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}SX_{13}X_{14}X_{15}X_{16}X_{17}$

with 0, 1, 2, 3, 4, or 5 amino acids which are substituted, deleted, and/or added without abrogating said peptide's cell penetrating ability, wherein

X_1 is K, R, or H, preferably X_1 is K or R;

X_2 is R, K, or H, preferably X_2 is R or K;

X_3 is Y, W, or F, preferably X_3 is Y, W, or F;

X_4 is K, R, or H, preferably X_4 is K or R;

X_5 is N or Q;

X_6 is R, K, or H, preferably X_6 is R or K;

X_7 is V, I, M, L, F, or A, preferably X_7 is V, I, M or L;

X_8 is A, V, L, I, or G, preferably X_8 is A or G;

X_9 is S or T;

X_{10} is R, K, or H, preferably X_{10} is R or K;

X₁₁ is K, R, or H, preferably X₁₁ is K or R;

X₁₃ is R, K, or H, preferably X₁₃ is R or K;

X₁₄ is A, V, L, I, or G, preferably X₁₄ is A or G;

X₁₅ is K, R, or H, preferably X₁₅ is K or R;

X₁₆ is F, L, V, I, Y, W, or M, preferably X₁₆ is F, Y or W; and

X₁₇ is K, R, or H, preferably X₁₇ is K or R.

[0117] Preferably, such a peptide, polypeptide or protein is either (entirely) composed of L-amino acids or (entirely) of D-amino acids, thereby forming "retro-inverso peptide sequences". The term "retro-inverso (peptide) sequences" refers to an isomer of a linear peptide sequence in which the direction of the sequence is reversed and the chirality of each amino acid residue is inverted (see e.g. Jameson et al., Nature, 368,744-746 (1994); Brady et al., Nature, 368,692-693 (1994)).

[0118] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X₁ is K.

[0119] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X₂ is R.

[0120] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X₃ is Y.

[0121] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X₄ is K.

[0122] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X₅ is N.

[0123] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X₆ is R.

[0124] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X₇ is V.

[0125] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X₈ is A.

[0126] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X_9 is S.

[0127] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X_{10} is R.

[0128] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X_{11} is K.

[0129] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X_{13} is R.

[0130] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X_{14} is A.

[0131] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X_{15} is K.

[0132] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X_{16} is F.

[0133] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein X_{17} is K.

[0134] In a particular embodiment, the cell penetrating peptide is as generically defined above by general formula (I), wherein the amino acid at position equivalent to position 12 relative to general formula (I) is a Ser (S).

[0135] It is also particularly preferred, that the preferred cell penetrating peptide, which

1. i) has a length of the amino acid sequence of said peptide of 5 to 50 amino acids in total, preferably of 10 to 45 amino acids in total, more preferably of 15 to 45 amino acids in total; and/or
2. ii) has an amino acid sequence comprising a fragment of the minimal domain of ZEBRA, said minimal domain extending from residue 170 to residue 220 of the ZEBRA amino acid sequence according to SEQ ID NO: 3, wherein, optionally, 1, 2, 3, 4, or 5 amino acids have been substituted, deleted, and/or added without abrogating said peptide's cell penetrating ability, or a variant of such a fragment

comprises or consists of an amino acid sequence selected from the group consisting of amino acid sequences according to SEQ ID NO: 4 - 13, or sequence variants thereof without abrogating said peptide's cell penetrating ability, preferably sequence variants having 0, 1, 2, 3, 4, or 5 amino acids substituted, deleted and/or added without abrogating said peptide's cell

penetrating ability.

CPP1 (Z11):

KRYKNRVASRKCRKFKQLLQHYREVAAKSSSENDRLRLLLKQMC
(SEQ ID NO: 4)

CPP2 (Z12):

KRYKNRVASRKCRKFKQLLQHYREVAAKSSSENDRLRLLLK
(SEQ ID NO: 5)

CPP3 (Z13):

KRYKNRVASRKSRKFKQLLQHYREVAAKSSSENDRLRLLLK
(SEQ ID NO: 6)

CPP4 (Z14):

KRYKNRVASRKSRKFKQLLQHYREVAAK
(SEQ ID NO: 7)

CPP5 (Z15):

KRYKNRVASRKSRKFK
(SEQ ID NO: 8)

CPP6 (Z16):

QHYREVAAKSSSEND
(SEQ ID NO: 9)

CPP7 (Z17):

QLLQHYREVAAK
(SEQ ID NO: 10)

CPP8 (Z18):

REVAAKSS END RLRLLLK
(SEQ ID NO: 11)

CPP9 (Z19):

KRYKNRVA
(SEQ ID NO: 12)

CPP10 (Z20):

VASRKSRKFK
(SEQ ID NO: 13)

[0136] Thereby, a cell penetrating peptide is particularly preferred, which has an amino acid sequence comprising or consisting of an amino acid sequence according to SEQ ID NO: 6 (CPP3/Z13), SEQ ID NO: 7 (CPP4/Z14), SEQ ID NO: 8 (CPP5/Z15), or SEQ ID NO: 11 (CPP8/Z18), or sequence variants thereof without abrogating said peptide's cell penetrating

ability, preferably sequence variants having 0, 1, 2, 3, 4, or 5 amino acids substituted, deleted and/or added without abrogating said peptide's cell penetrating ability. Moreover, a cell penetrating peptide is more preferred, which has an amino acid sequence comprising or consisting of an amino acid sequence according to SEQ ID NO: 6 (CPP3/Z13) or SEQ ID NO: 7 (CPP4/Z14) or sequence variants thereof without abrogating said peptide's cell penetrating ability, preferably sequence variants having 0, 1, 2, 3, 4, or 5 amino acids substituted, deleted and/or added without abrogating said peptide's cell penetrating ability. Moreover, a cell penetrating peptide is most preferred, which has an amino acid sequence comprising or consisting of an amino acid sequence according to SEQ ID NO: 6 (CPP3/Z13) or sequence variants thereof without abrogating said peptide's cell penetrating ability, preferably sequence variants having 0, 1, 2, 3, 4, or 5 amino acids substituted, deleted and/or added without abrogating said peptide's cell penetrating ability.

[0137] In one preferred embodiment, the cell penetrating peptide according to the invention has an amino acid sequence comprising or consisting of SEQ ID NO: 6 (CPP3/Z13).

[0138] In another preferred embodiment, the cell penetrating peptide according to the invention has an amino acid sequence comprising or consisting of SEQ ID NO: 7 (CPP4/Z14).

[0139] In another preferred embodiment, the cell penetrating peptide according to the invention has an amino acid sequence comprising or consisting of SEQ ID NO: 8 (CPP5/Z15).

[0140] In another preferred embodiment, the cell penetrating peptide according to the invention has an amino acid sequence comprising or consisting of SEQ ID NO: 11 (CPP8/Z18).

[0141] It will be understood by one skilled in the art that the primary amino acid sequence of the cell penetrating peptide may further be post-translationally modified, such as by glycosylation or phosphorylation, without departing from the invention.

[0142] In a further embodiment, the cell penetrating peptide optionally further comprises, in addition to its amino acid sequence as described above, any one of, or any combination of:

1. (i) a nuclear localization signal (NLS). Such signals are well known to the skilled person and are described in Nair et al. (2003, *NucleicAcids Res.* 31(1): 397-399)
2. (ii) a targeting peptide, including tumor homing peptides such as those described in Kapoor et al. (2012, *PLoS ONE* 7(4): e35187) and listed in-
<http://crdd.osdd.net/raghava/tumorhope/general.php?>

[0143] The cell penetrating peptide is linked to an antigen or antigenic epitope and facilitates the cellular internalization of said antigen or antigenic epitope.

[0144] The complex comprised by the combination for use according to the present invention may comprise one single cell penetrating peptide or more than one cell penetrating peptides.

Preferably, the complex comprised by the combination for use according to the present invention comprises no more than five cell penetrating peptides, more preferably the complex comprised by the combination for use according to the present invention comprises no more than four cell penetrating peptides, even more preferably the complex comprised by the combination for use according to the present invention comprises no more than three cell penetrating peptides, particularly preferably the complex comprised by the combination for use according to the present invention comprises no more than two cell penetrating peptides and most preferably the complex comprised by the combination for use according to the present invention comprises one single cell penetrating peptide.

Component b) - Antigen / antigenic epitope

[0145] The complex comprised by the combination for use according to the present invention comprises as component b) at least one antigen or antigenic epitope.

[0146] As used herein, an "antigen" is any structural substance which serves as a target for the receptors of an adaptive immune response, in particular as a target for antibodies, T cell receptors, and/or B cell receptors. An "epitope", also known as "antigenic determinant", is the part (or fragment) of an antigen that is recognized by the immune system, in particular by antibodies, T cell receptors, and/or B cell receptors. Thus, one antigen has at least one epitope, i.e. a single antigen has one or more epitopes. In the context of the present invention, the term "epitope" is mainly used to designate T cell epitopes, which are presented on the surface of an antigen-presenting cell, where they are bound to Major Histocompatibility Complex (MHC). T cell epitopes presented by MHC class I molecules are typically, but not exclusively, peptides between 8 and 11 amino acids in length, whereas MHC class II molecules present longer peptides, generally, but not exclusively, between 12 and 25 amino acids in length.

[0147] Preferably, in the complex comprised by the combination for use according to the present invention, the at least one antigen or antigenic epitope is selected from the group consisting of: (i) a peptide, a polypeptide, or a protein, (ii) a polysaccharide, (iii) a lipid, (iv) a lipoprotein or a lipopeptide, (v) a glycolipid, (vi) a nucleic acid, and (vii) a small molecule drug or a toxin. Thus, the at least one antigen or antigenic epitope may be a peptide, a protein, a polysaccharide, a lipid, a combination thereof including lipoproteins and glycolipids, a nucleic acid (e.g. DNA, siRNA, shRNA, antisense oligonucleotides, decoy DNA, plasmid), or a small molecule drug (e.g. cyclosporine A, paclitaxel, doxorubicin, methotrexate, 5-aminolevulinic acid), or any combination thereof in particular if more than one antigen or antigenic epitope is comprised by the complex comprised by the combination for use according to the present invention.

[0148] It is understood that the at least one antigen or antigenic epitope can comprise for example at least one, i.e. one or more, peptides, polypeptides or proteins linked together and/or at least one, i.e. one or more, nucleic acids, e.g. where each one encodes one peptide

or polypeptide. Also the at least one antigen or antigenic epitope can be a combination of a protein, a lipid, and/or a polysaccharide including lipoproteins and glycolipids. Thus, in particular if the complex comprised by the combination for use according to the present invention comprises more than one antigen or antigenic epitope, it can comprise more than one peptide, polypeptide, or protein, more than one polysaccharide, more than one lipid, more than one lipoprotein, more than one glycolipid, more than one nucleic acid, more than one small molecule drug or toxin, or a combination thereof.

[0149] Preferably, the complex comprised by the combination for use according to the invention comprises at least one antigen or antigenic epitope comprising one or more epitope(s) from a cancer/tumor-associated antigen, a cancer/tumor-specific antigen, and/or an antigenic protein from a pathogen, including viral, bacterial, fungal, protozoal and multicellular parasitic antigenic protein.

[0150] More preferably, the at least one antigen or antigenic epitope comprises or consists of (i) at least one pathogen epitope and/or (ii) at least one cancer/tumor epitope, in particular at least one tumor epitope. Most preferably, the at least one antigen or antigenic epitope comprises or consists of at least one cancer/tumor epitope, in particular at least one tumor epitope.

[0151] It is particularly preferred that the complex comprised by the combination for use according to the present invention comprises only such antigen(s) or antigenic epitope(s), which are cancer/tumor-associated antigen(s), cancer/tumor-specific antigen(s) and/or cancer/tumor epitope(s); in particular, which are tumor-associated antigen(s), tumor-specific antigen(s), and/or tumor epitope(s).

[0152] As used herein, "cancer epitope" means an epitope from a cancer-associated antigen or from a cancer-specific antigen. Accordingly, "tumor epitope" means an epitope from a tumor-associated antigen or from a tumor-specific antigen. Such epitopes are typically specific (or associated) for a certain kind of cancer/tumor. For instance, cancer/tumor epitopes include glioma epitopes. In particular, cancer/tumor-associated (also cancer/tumor-related) antigens are antigens, which are expressed by both, cancer/tumor cells and normal cells. Accordingly, those antigens are normally present since birth (or even before). Accordingly, there is a chance that the immune system developed self-tolerance to those antigens. Cancer/tumor-specific antigens, in contrast, are antigens, which are expressed specifically by cancer/tumor cells, but not by normal cells. Cancer/tumor-specific antigens include in particular neoantigens. In general neoantigens are antigens, which were not present before and are, thus, "new" to the immune system. Neoantigens are typically due to somatic mutations. In the context of cancer/tumors, cancer/tumor-specific neoantigens were typically not present before the cancer/tumor developed and cancer/tumor-specific neoantigens are usually encoded by somatic gene mutations in the cancerous cells/tumor cells. Since neoantigens are new to the immune system, the risk of self-tolerance of those antigens is considerably lower as compared to cancer/tumor-associated antigens. However, every cancer's set of tumor-specific mutations appears to be unique. Accordingly, in the context of the present invention it is preferred that

such cancer/tumor-specific antigens, in particular neoantigens, are identified in a subject diagnosed with a cancer by methods known to the skilled person, e.g., cancer genome sequencing. After identification, the respective cancer/tumor-specific neoantigens and/or cancer/tumor-specific neoantigenic epitopes are used in the complex comprised by the combination for use according to the present invention.

[0153] Preferably, a complex comprised by the combination for use according to the present invention comprises one or more cancer/tumor-associated epitopes and/or one or more cancer/tumor-associated antigens (but preferably no cancer/tumor-specific epitopes). It is also preferred that a complex comprised by the combination for use according to the present invention comprises one or more cancer/tumor-specific epitopes and/or one or more cancer/tumor-specific antigens (but preferably no cancer/tumor-associated epitopes). A complex comprised by the combination for use according to the present invention may also preferably comprise both, (i) one or more cancer/tumor-associated epitopes and/or one or more cancer/tumor-associated antigens and (ii) one or more cancer/tumor-specific epitopes and/or one or more cancer/tumor-specific antigens.

[0154] Suitable cancer/tumor epitopes can be retrieved for example from cancer/tumor epitope databases, e.g. from van der Bruggen P, Stroobant V, Vigneron N, Van den Eynde B. Peptide database: T cell-defined tumor antigens. Cancer Immun 2013; URL: <http://www.cancerimmunity.org/peptide/>, wherein human tumor antigens recognized by CD4+ or CD8+ T cells are classified into four major groups on the basis of their expression pattern, or from the database "Tantigen" (TANTIGEN version 1.0, Dec 1, 2009; developed by Bioinformatics Core at Cancer Vaccine Center, Dana-Farber Cancer Institute; URL: <http://cvc.dfci.harvard.edu/tadb/>). Examples of cancer/tumor epitopes include e.g. TRP2-derived epitopes, glycoprotein 100 (gp100) melanoma antigen-derived epitopes, glycoprotein 70 (gp70) antigen-derived epitopes, survivin epitopes, IEa epitopes, IL13 α 2, EphA2 (ephrin type-A receptor 2), immunogenic fragments thereof, and fusions of such antigens and/or fragments. Furthermore, examples of cancer/tumor epitopes include epitopes of neoantigens, such as, for example, a neoantigen from MC-38 tumor cell line as described by Yadav et al. Nature. 2014 Nov 27;515(7528):572-6. As described above, neoantigens are antigens, which are entirely absent from the normal human genome. As compared with nonmutated self-antigens, neoantigens are of relevance to tumor control, as the quality of the T cell pool that is available for these antigens is not affected by central T cell tolerance. In particular, neoantigens may be based on individual tumor genomes. Potential neoantigens may be predicted by methods known to the skilled person, such as cancer genome sequencing or deep-sequencing technologies identifying mutations within the protein-coding part of the (cancer) genome.

[0155] Specific examples of cancer/tumor-associated, in particular tumor-related, or tissue-specific antigens useful in a complex comprised by the combination for use according to the present invention include the following antigens: Prostate: prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), PAP, PSCA (PNAS 95(4) 1735-1740 1998), prostate mucin antigen (PMA) (Beckett and Wright, 1995, Int. J. Cancer 62: 703-710),

Prostate, Her-2neu, SPAS-1; Melanoma: TRP-2, tyrosinase, Melan A/Mart-1, gp100, BAGE, GAGE, GM2 ganglioside; Breast: Her2-neu, kinesin 2, TATA element modulatory factor 1, tumor protein D52, MAGE D, ING2, HIP-55, TGF-1 anti-apoptotic factor, HOM-Mel-40/SSX2, epithelial antigen (LEA 135), DF31MUC1 antigen (Apostolopoulos et al., 1996 Immunol. Cell. Biol. 74: 457-464; Pandey et al., 1995, Cancer Res. 55: 4000-4003); Testis: MAGE-1, HOM-Mel-40/SSX2, NY-ESO-1; Colorectal: EGFR, CEA; Lung: MAGE D, EGFR Ovarian Her-2neu; Bladder: transitional cell carcinoma (TCC) (Jones et al., 1997, Anticancer Res. 17: 685-687), Several cancers: EphA2, EphA4, PCDGF, HAAH, Mesothelin; EPCAM; NY-ESO-1, glycoprotein MUC1 and NIUC10 mucins p5 (especially mutated versions), EGFR; Miscellaneous tumor: cancer-associated serum antigen (CASA) and cancer antigen 125 (CA 125) (Kierkegaard et al., 1995, Gynecol. Oncol. 59: 251-254), the epithelial glycoprotein 40 (EGP40) (Kievit et al., 1997, Int. J. Cancer 71: 237-245), squamous cell carcinoma antigen (SCC) (Lozza et al., 1997 Anticancer Res. 17: 525-529), cathepsin E (Mota et al., 1997, Am. J. Pathol. 150: 1223-1229), tyrosinase in melanoma (Fishman et al., 1997 Cancer 79: 1461-1464), cell nuclear antigen (PCNA) of cerebral cavernomas (Notelet et al., 1997 Surg. Neurol. 47: 364-370), a 35 kD tumor-associated autoantigen in papillary thyroid carcinoma (Lucas et al., 1996 Anticancer Res. 16: 2493-2496), CDC27 (including the mutated form of the protein), antigens triosephosphate isomerase, 707-AP, A60 mycobacterial antigen (Macs et al., 1996, J. Cancer Res. Clin. Oncol. 122: 296-300), Annexin II, AFP, ART-4, BAGE, β -catenin/m, BCL-2, bcr-abl, bcr-abl p190, bcr-abl p210, BRCA-1, BRCA-2, CA 19-9 (Tolliver and O'Brien, 1997, South Med. J. 90: 89-90; Tsuruta et al., 1997 Urol. Int. 58: 20-24), CAMEL, CAP-1, CASP-8, CDC27/m, CDK-4/m, CEA (Huang et al., Exper Rev. Vaccines (2002)1:49-63), CT9, CT10, Cyp-B, Decalin, DAM-6 (MAGE-B2), DAM-10 (MAGE-B1), EphA2 (Zantek et al., Cell Growth Differ. (1999) 10:629-38; Carles-Kinch et al., Cancer Res. (2002) 62:2840-7), EphA4 (Cheng et al., 2002, Cytokine Growth Factor Rev. 13:75-85), tumor associated Thomsen-Friedenreich antigen (Dahlenborg et al., 1997, Int. J. Cancer 70: 63-71), ELF2M, ETV6-AML1, G250, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7B, GAGE-8, GnT-V, gp100 (Zajac et al., 1997, Int. J. Cancer 71: 491-496), HAGE, HER2/neu, HLA-A*0201-R170I, HPV-E7, HSP70-2M, HST-2, hTERT, hTRT, iCE, inhibitors of apoptosis (e.g., survivin), KH-1 adenocarcinoma antigen (Deshpande and Danishefsky, 1997, Nature 387: 164-166), KIAA0205, K-ras, LAGE, LAGE-1, LDLR/FUT, MAGE-1, MAGE-2, MAGE-3, MAGE-6, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A10, MAGE-A12, MAGE-B5, MAGE-B6, MAGE-C2, MAGE-C3, MAGE D, MART-1, MART-1/Melan-A (Kawakami and Rosenberg, 1997, Int. Rev. Immunol. 14: 173-192), MC1R, MDM-2, Myosin/m, MUC1, MUC2, MUM-1, MUM-2, MUM-3, neo-polyA polymerase, NA88-A, NY-ESO-1, NY-ESO-1a (CAG-3), PAGE-4, PAP, Proteinase 3 (Molldrem et al., Blood (1996) 88:2450-7; Molldrem et al., Blood (1997) 90:2529-34), P15, p190, Pm1/RAR α , PRAME, PSA, PSM, PSMA, RAGE, RAS, RCAS1, RU1, RU2, SAGE, SART-1, SART-2, SART-3, SP17, SPAS-1, TEL/AML1, TPI/m, Tyrosinase, TARP, TRP-1 (gp75), TRP-2, TRP-2/INT2, WT-1, and alternatively translated NY-ESO-ORF2 and CAMEL proteins, derived from the NY-ESO-1 and LAGE-1 genes. Numerous other cancer antigens are well known in the art.

[0156] Preferably, the cancer/tumor antigen or the cancer/tumor epitope is a recombinant cancer/tumor antigen or a recombinant cancer/tumor epitope. Such a recombinant

cancer/tumor antigen or a recombinant cancer/tumor epitope may be designed by introducing mutations that change (add, delete or substitute) particular amino acids in the overall amino acid sequence of the native cancer/tumor antigen or the native cancer/tumor epitope. The introduction of mutations does not alter the cancer/tumor antigen or the cancer/tumor epitope so much that it cannot be universally applied across a mammalian subject, and preferably a human or dog subject, but changes it enough that the resulting amino acid sequence breaks tolerance or is considered a foreign antigen in order to generate an immune response. Another manner may be creating a consensus recombinant cancer/tumor antigen or cancer/tumor epitope that has at least 85% and up to 99% amino acid sequence identity to its' corresponding native cancer/tumor antigen or native cancer/tumor epitope; preferably at least 90% and up to 98% sequence identity; more preferably at least 93% and up to 98% sequence identity; or even more preferably at least 95% and up to 98% sequence identity. In some instances the recombinant cancer/tumor antigen or the recombinant cancer/tumor epitope has 95%, 96%, 97%, 98%, or 99% amino acid sequence identity to its' corresponding native cancer/tumor antigen or cancer/tumor epitope. The native cancer/tumor antigen is the antigen normally associated with the particular cancer or cancer tumor. Depending upon the cancer/tumor antigen, the consensus sequence of the cancer/tumor antigen can be across mammalian species or within subtypes of a species or across viral strains or serotypes. Some cancer/tumor antigen do not vary greatly from the wild type amino acid sequence of the cancer/tumor antigen. The aforementioned approaches can be combined so that the final recombinant cancer/tumor antigen or cancer/tumor epitope has a percent similarity to native cancer antigen amino acid sequence as discussed above. Preferably, however, the amino acid sequence of an epitope of a cancer/tumor antigen as described herein is not mutated and, thus, identical to the reference epitope sequence.

[0157] As used herein "pathogen epitope" means an epitope from an antigenic protein, an antigenic polysaccharide, an antigenic lipid, an antigenic lipoprotein or an antigenic glycolipid from a pathogen including viruses, bacteria, fungi, protozoa and multicellular parasites. Antigenic proteins, polysaccharides, lipids, lipoproteins or glycolipids from pathogens include, herewith, proteins, polysaccharides, lipids, lipoproteins and glycolipids, respectively, from pathogens responsible of diseases which can be a target for vaccination including, for instance, Amoebiasis, Anthrax, Buruli Ulcer (*Mycobacterium ulcerans*), Caliciviruses associated diarrhoea, *Campylobacter* diarrhoea, Cervical Cancer (Human papillomavirus), Chlamydia trachomatis associated genital diseases, Cholera, Crimean-Congo haemorrhagic fever, Dengue Fever, Diphtheria, Ebola haemorrhagic fever, Enterotoxigenic *Escherichia coli* (ETEC) diarrhoea, Gastric Cancer (*Helicobacter pylori*), Gonorrhea, Group A *Streptococcus* associated diseases, Group B *Streptococcus* associated diseases, *Haemophilus influenzae* B pneumonia and invasive disease, Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis E diarrhoea, Herpes simplex type 2 genital ulcers, HIV/AIDS, Hookworm Disease, Influenza, Japanese encephalitis, Lassa Fever, Leishmaniasis, Leptospirosis, Liver cancer (Hepatitis B), Liver Cancer (Hepatitis C), Lyme Disease, Malaria, Marburg haemorrhagic fever, Measles, Mumps, Nasopharyngeal cancer (Epstein-Barr virus), *Neisseria meningitidis* Meningitis, Parainfluenza associated pneumonia, Pertussis, Plague, Poliomyelitis, Rabies, Respiratory syncytial virus (RSV) pneumonia, Rift Valley fever, Rotavirus diarrhoea, Rubella, Schistosomiasis, Severe Acute

Respiratory Syndrome (SARS), Shigellosis, Smallpox, Staphylococcus aureus associated diseases, Stomach Cancer (Helicobacter pylori), Streptococcus pneumoniae and invasive disease, Tetanus, Tick-borne encephalitis, Trachoma, Tuberculosis, Tularaemia, Typhoid fever, West-Nile virus associated disease, Yellow fever.

[0158] Preferably, the at least one antigen or antigenic epitope will be presented at the cell surface in an MHC class I and/or MHC class II context and/or in a CD1 context, whereby presentation at the cell surface in an MHC class I and/or MHC class II context is preferred. The phrase "epitope presentation in the MHC class I context" refers in particular to a CD8⁺ epitope lying in the groove of a MHC class I molecule at the surface of a cell. The phrase "epitope presentation in the MHC class II context" refers in particular to a CD4⁺ epitope lying in the groove of a MHC class II molecule at the surface of a cell. The phrase "epitope presentation in the CD1 context" refers in particular to a lipidic epitope lying in the groove of a cluster of differentiation 1 molecule at the surface of a cell.

[0159] Advantageously, the complex comprised by the combination for use according to the invention comprises a cell penetrating peptide and at least one antigen or antigenic epitope, and allows the transport and presentation of said epitopes at the cell surface of antigen presenting cells in an MHC class I and MHC class II context, and is, thus, useful in vaccination and immunotherapy.

[0160] Preferably, the complex comprised by the combination for use according to the present invention comprises at least one antigen or antigenic epitope, which is at least one CD4⁺ epitope and/or at least one CD8⁺ epitope.

[0161] The terms "CD4⁺ epitope" or "CD4⁺-restricted epitope", as used herein, designate an epitope recognized by a CD4⁺ T cell, said epitope in particular consisting of an antigen fragment lying in the groove of a MHC class II molecule. A single CD4⁺ epitope comprised in the complex comprised by the combination for use according to the present invention preferably consists of about 12-25 amino acids. It can also consist of, for example, about 8-25 amino acids or about 6-100 amino acids.

[0162] The terms "CD8⁺ epitope" or "CD8⁺-restricted epitope", as used herein, designate an epitope recognized by a CD8⁺ T cell, said epitope in particular consisting of an antigen fragment lying in the groove of a MHC class I molecule. A single CD8⁺ epitope comprised in the complex comprised by the combination for use according to the present invention preferably consists of about 8-11 amino acids. It can also consist of, for example, about 8-15 amino acids or about 6-100 amino acids.

[0163] Preferably, the at least one antigen can comprise or the at least one antigenic epitope can consist of a CD4⁺ epitope and/or a CD8⁺ epitope corresponding to antigenic

determinant(s) of a cancer/tumor-associated antigen, a cancer/tumor-specific antigen, or an antigenic protein from a pathogen. More preferably, the at least one antigen can comprise or the at least one antigenic epitope can consist of a CD4⁺ epitope and/or a CD8⁺ epitope corresponding to antigenic determinant(s) of a cancer/tumor-associated antigen or a cancer/tumor-specific antigen. Most preferably, the at least one antigen can comprise or the at least one antigenic epitope can consist of a CD4⁺ epitope and/or a CD8⁺ epitope corresponding to antigenic determinant(s) of a tumor-associated antigen or a tumor-specific antigen.

[0164] It is also preferred that the complex comprised by the combination for use according to the present invention comprises at least two antigens or antigenic epitopes, wherein at least one antigen or antigenic epitope comprises or consists a CD4⁺ epitope and at least one antigen or antigenic epitope comprises or consists a CD8⁺ epitope. It is now established that T_h cells (CD4⁺) play a central role in the anti-tumor immune response both in DC licensing and in the recruitment and maintenance of CTLs (CD8⁺) at the tumor site. Therefore, a complex comprised by the combination for use according to the present invention comprising at least two antigens or antigenic epitopes, wherein at least one antigen or antigenic epitope comprises or consists of a CD4⁺ epitope and at least one antigen or antigenic epitope comprises or consists a CD8⁺ epitope, provides an integrated immune response allowing simultaneous priming of CTLs and T_h cells and is thus preferable to immunity against only one CD8⁺ epitope or only one CD4⁺ epitope. For example, the complex comprised by the combination for use according to the present invention may preferably comprise an Ealpha-CD4⁺ epitope and a gp100-CD8⁺ epitope.

[0165] Preferably, the complex comprised by the combination for use according to the present invention comprises at least two antigens or antigenic epitopes, wherein the at least two antigens or antigenic epitopes comprise or consist of at least two, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or more, CD4⁺ epitopes and/or at least two, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or more, CD8⁺ epitopes. Thereby, the at least two antigens or antigenic epitopes are preferably different antigens or antigenic epitopes, more preferably the at least two antigens or antigenic epitopes are different from each other but relating to the same kind of tumor. A multi-antigenic vaccine will (i) avoid outgrowth of antigen-loss variants, (ii) target different tumor cells within a heterogeneous tumor mass and (iii) circumvent patient-to-patient tumor variability. Thus, the complex comprised by the combination for use according to the present invention particularly preferably comprises at least four antigens or antigenic epitopes, in particular with at least two CD8⁺ epitopes and at least two CD4⁺ epitopes. Such a complex comprised by the combination for use according to the present invention induces multi-epitopic CD8 CTLs and CD4 T_h cells to function synergistically to counter tumor cells and promote efficient anti-tumor immunity. T_h cells are also involved in the maintenance of long-lasting cellular immunity that was monitored after vaccination. Such a complex comprised by the combination for use according to the present

invention induces polyclonal, multi-epitopic immune responses and poly-functional CD8⁺ and CD4⁺ T cells, and thus efficacious anti-tumor activity.

[0166] Preferably, the complex comprised by the combination for use according to the present invention comprises at least two antigens or antigenic epitopes, more preferably the complex comprised by the combination for use according to the present invention comprises at least three antigens or antigenic epitopes, even more preferably the complex comprised by the combination for use according to the present invention comprises at least four antigens or antigenic epitopes, particularly preferably the complex comprised by the combination for use according to the present invention comprises at least five antigens or antigenic epitopes and most preferably the complex comprised by the combination for use according to the present invention comprises at least six antigens or antigenic epitopes. The antigens or antigenic epitopes comprised by the complex may be the same or different, preferably the antigens or antigenic epitopes comprised by the complex are different from each other. Preferably, the complex comprises at least one CD4⁺ epitope and at least one CD8⁺ epitope.

[0167] Preferably, the complex comprised by the combination for use according to the present invention comprises more than one CD4⁺ epitope, e.g. two or more CD4⁺ epitopes from the same antigen or from different antigens, and preferably no CD8⁺ epitope. It is also preferred that the complex comprised by the combination for use according to the present invention comprises more than one CD8⁺ epitope, e.g. two or more CD8⁺ epitopes from the same antigen or from different antigens, and preferably no CD4⁺ epitope. Most preferably, however, the complex comprised by the combination for use according to the present invention comprises (i) at least one CD4⁺ epitope, e.g. two or more CD4⁺ epitopes from the same antigen or from different antigens, and (ii) at least one CD8⁺ epitope, e.g. two or more CD8⁺ epitopes from the same antigen or from different antigens.

[0168] For example, the complex comprised by the combination for use according to the present invention may preferably comprise a gp100-CD8⁺ epitope, an Ealpha-CD4⁺ epitope, and a further CD4⁺ epitope and a further CD8⁺ epitope. Even more preferably, the complex comprised by the combination for use according to the present invention may comprise a polypeptide or protein comprising a gp100-CD8⁺ epitope and an Ealpha-CD4⁺ epitope. For example, such a polypeptide or protein comprised by the complex comprised by the combination for use according to the present invention comprises or consists of an amino acid sequence according to SEQ ID NO: 14 or sequence variants thereof as defined above:

ESLKIS QAVHAAHAEI NEAGREVVGV GALKVPRNQD WLGVPFRAKF ASFEAQGALA

NIAVDKANLD VEQLESIINF EKLTEWTGS

SEQ ID NO: 14 (MAD5-cargo comprising OVA-CD4⁺, gp100-CD8⁺, Ealpha-CD4⁺, and OVA-CD8⁺ epitopes)

[0169] For example, the complex comprised by the combination for use according to the present invention may preferably comprise a gp70-CD8⁺ epitope and/or a gp70-CD4⁺ epitope. Even more preferably, the complex comprised by the combination for use according to the present invention may comprise a polypeptide or protein comprising a gp70-CD8⁺ epitope and/or a gp70-CD4⁺ epitope. For example, such a polypeptide or protein comprised by the complex comprised by the combination for use according to the present invention comprises or consists of an amino acid sequence according to SEQ ID NO: 36 or sequence variants thereof as defined above:

VTYHSPSYAYHQFERRAILNRLVQFIKDRI

SEQ ID NO: 36 (Mad8-cargo comprising a gp70-CD8⁺ and a gp70-CD4⁺ epitope)

[0170] For example, the complex comprised by the combination for use according to the present invention may preferably comprise at least one survivin epitope, such as a survivin CD8⁺ epitope and/or a survivin CD4⁺ epitope. More preferably, the complex comprised by the combination for use according to the present invention may comprise a polypeptide or protein comprising a survivin CD8⁺ epitope and/or a survivin CD4⁺ epitope. More preferably, the complex comprised by the combination for use according to the present invention may comprise a polypeptide or protein comprising more than one survivin CD8⁺ epitope and/or more than one survivin CD4⁺ epitope, such as two different survivin CD8⁺ epitopes. For example, such a polypeptide or protein comprised by the complex comprised by the combination for use according to the present invention comprises or consists of an amino acid sequence according to SEQ ID NO: 37 or sequence variants thereof as defined above:

NYRIATFKNWPFLDCAMEELTVSEFLKLDRQR

SEQ ID NO: 37 (Mad11-cargo comprising survivin CD8⁺ epitope 1 and survivin CD8⁺ epitope 2)

[0171] For example, the complex comprised by the combination for use according to the present invention may preferably comprise an epitope from a neoantigen. Even more preferably, the complex comprised by the combination for use according to the present invention may comprise a polypeptide or protein comprising an epitope from a neoantigen, such as the neoantigen from MC-38 tumor cell line identified by Yadav et al. Nature. 2014 Nov 27;515(7528):572-6. For example, such a polypeptide or protein comprised by the complex comprised by the combination for use according to the present invention comprises or consists of an amino acid sequence according to SEQ ID NO: 38 or sequence variants thereof as defined above:

HLELASMTNMELMSSIV

SEQ ID NO: 38 (Mad9-cargo comprising the epitope from a neoantigen as described by Yadav et al. Nature. 2014 Nov 27;515(7528):572-6).

[0172] For example, the complex comprised by the combination for use according to the present invention may preferably comprise more than one, e.g. two or three, epitopes from neoantigens. Even more preferably, the complex comprised by the combination for use

according to the present invention may comprise a polypeptide or protein comprising more than one, e.g. two or three, epitopes from neoantigens, such as the neoantigens from MC-38 tumor cell line identified by Yadav et al. Nature. 2014 Nov 27;515(7528):572-6. For example, such a polypeptide or protein comprised by the complex comprised by the combination for use according to the present invention comprises or consists of an amino acid sequence according to SEQ ID NO: 39 or sequence variants thereof as defined above:

LFRAAQLAN DVVLQIMEH LELASMTNMELMSSIIVVISASIIVFN LLELEG

SEQ ID NO: 39 (Mad12-cargo comprising the epitope from a neoantigen as described by Yadav et al. Nature. 2014 Nov 27;515(7528):572-6).

[0173] Preferably, the at least one antigen or antigenic epitope comprised by the complex comprised by the combination for use according to the present invention is a peptide, polypeptide, or a protein. Examples of antigen or antigenic epitope of peptidic, polypeptidic, or proteic nature useful in the invention, include cancer/tumor antigens or antigenic epitopes thereof, allergy antigens or antigenic epitopes thereof, auto-immune self-antigens or antigenic epitopes thereof, pathogenic antigens or antigenic epitopes thereof, and antigens or antigenic epitopes thereof from viruses, preferably from cytomegalovirus (CMV), orthopox variola virus, orthopox alastrim virus, parapox ovis virus, molluscum contagiosum virus, herpes simplex virus 1, herpes simplex virus 2, herpes B virus, varicella zoster virus, pseudorabies virus, human cytomegaly virus, human herpes virus 6, human herpes virus 7, Epstein-Barr virus, human herpes virus 8, hepatitis B virus, chikungunya virus, O'nyong'nyong virus, rubivirus, hepatitis C virus, GB virus C, West Nile virus, dengue virus, yellow fever virus, louping ill virus, St. Louis encephalitis virus, Japan B encephalitis virus, Powassan virus, FSME virus, SARS, SARS-associated corona virus, human corona virus 229E, human corona virus Oc43, Torovirus, human T cell lymphotropic virus type I, human T cell lymphotropic virus type II, HIV (AIDS), i.e. human immunodeficiency virus type 1 or human immunodeficiency virus type 2, influenza virus, Lassa virus, lymphocytic choriomeningitis virus, Tacaribe virus, Junin virus, Machupo virus, Borna disease virus, Bunyamwera virus, California encephalitis virus, Rift Valley fever virus, sand fly fever virus, Toscana virus, Crimean-Congo haemorrhagic fever virus, Hazara virus, Khasan virus, Hantaan virus, Seoul virus, Prospect Hill virus, Puumala virus, Dobrava Belgrade virus, Tula virus, sin nombre virus, Lake Victoria Marburg virus, Zaire Ebola virus, Sudan Ebola virus, Ivory Coast Ebola virus, influenza virus A, influenza virus B, influenza viruses C, parainfluenza virus, malaria parasite (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium knowlesi*), Marburg virus, measles virus, mumps virus, respiratory syncytial virus, human metapneumovirus, vesicular stomatitis Indiana virus, rabies virus, Mokola virus, Duvenhage virus, European bat lyssavirus 1 + 2, Australian bat lyssavirus, adenoviruses A-F, human papilloma viruses, condyloma virus 6, condyloma virus 11, polyoma viruses, adeno-associated virus 2, rotaviruses, orbiviruses, varicella including varicella zoster, or antigens or antigenic epitopes from leishmania, typanosomes, amibes, bacteria, or may be selected from epitopes or from variants of the above antigens or antigenic epitopes. Preferably, epitopes as well as variants of antigens as defined above exhibit a sequence homology or identity of about 10 %, in particular at least 10 %, about 20 %, in particular at least 20 %, about 30 %, in particular at least 30 %, about 40 %, in particular at least 40 %, about 50 %, in particular at least 50 %, about 60 %, in particular at

least 60 %, about 70 %, in particular at least 70 %, about 80 %, in particular at least 80 %, about 90 % in particular at least 90 %, at least 95 % or at least 98 % with one of the antigens or antigen sequences as shown or described above. In this context, the definition of epitopes and variants similarly applies as defined.

[0174] Examples of antigens or antigenic epitopes in the category of peptide, polypeptide or protein include a combination of multiple glioma epitopes such as those described in Novellino et al. (2005, *Cancer Immunol Immunother*, 54(3):187-207), Vigneron et al. (2013, *Cancer Immun.* 13:15). However, a single complex comprised by the combination for use according to the present invention may also comprise only a subset, i.e. one or more of all of said glioma epitopes. In such a case preferably different complexes comprised by the combination for use according to the present invention comprise different subsets of all of said glioma epitopes, so that for example a vaccine according to the present invention comprising such different complexes comprises all of said glioma epitopes but distributed in the different complexes.

[0175] Moreover, a complex comprised by the combination for use according to the invention may also comprise at least one antigen or antigenic epitope, wherein said antigen or antigenic epitope is a polysaccharide, a lipid, a lipoprotein, and/or a glycolipid, in particular a polysaccharidic, lipidic, lipoproteic, and/or glycolipidic epitope, which can be, for example, pathogen epitopes as defined herewith.

[0176] In particular, the complex comprised by the combination for use according to the invention may comprise at least one antigen or antigenic epitope, wherein said antigen or antigenic epitope is polysaccharidic, lipidic, lipoproteic, and/or glycolipidic, including viral, bacterial, fungal, protozoal and multicellular parasitic antigens or antigenic epitopes.

[0177] Preferably, said epitopes will be presented at the cell surface in an MHC class I and/or MHC class II context.

[0178] Preferably, said lipidic epitopes will be presented at the cell surface in a CD1 (cluster of differentiation 1) context.

[0179] The complex comprised by the combination for use according to the present invention may also comprise at least one antigen or antigenic epitope, wherein said antigen or antigenic epitope is a small molecule drug or toxin.

[0180] Examples of cargo molecules within the category of small molecule drugs or toxins useful in the invention include cyclosporine A, paclitaxel, doxorubicin, methotrexate, 5-aminolevulinic acid, diphtheria toxin, sunitinib and those molecules reviewed in De wit Amer (2010, *Neuro Oncol*, 12(3):304-16).

[0181] The complex comprised by the combination for use according to the present invention comprises at least one antigen or antigenic epitope, preferably the complex comprised by the combination for use according to the present invention comprises more than one antigen or

antigenic epitope, in particular 2, 3, 4, 5, 6, 7, 8, 9, 10 or more antigens or antigenic epitopes, more preferably the complex comprised by the combination for use according to the present invention comprises (at least) two or three antigens or antigenic epitopes, even more preferably the complex comprised by the combination for use according to the present invention comprises (at least) four or five antigens or antigenic epitopes. If more than one antigen or antigenic epitope is comprised by the complex comprised by the combination for use according to the present invention it is understood that said antigen or antigenic epitope is in particular also covalently linked in the complex comprised by the combination for use according to the present invention, e.g. to another antigen or antigenic epitope and/or to a component a), i.e. a cell penetrating peptide, and/or to a component c), i.e. a TLR peptide agonist.

[0182] The various antigens or antigenic epitopes comprised by the complex may be the same or different. Preferably, the various antigens or antigenic epitopes comprised by the complex are different from each other, thus providing a multi-antigenic and/or multi-epitopic complex.

[0183] Moreover, it is preferred that the more than one antigen or antigenic epitope, in particular 2, 3, 4, 5, 6, 7, 8, 9, 10 or more antigens or antigenic epitopes, are positioned consecutively in the complex comprised by the combination for use according to the present invention. This means in particular that all antigens and/or antigenic epitopes comprised by the complex are positioned in a stretch, which is neither interrupted by component a), i.e. a cell penetrating peptide, nor by component c), i.e. a TLR peptide agonist. Rather, component a) and component c) are positioned in the complex for example before or after such a stretch of all antigens and/or antigenic epitopes. However, the antigens and/or antigenic epitopes positioned consecutively in such a way may be linked to each other for example by a spacer or linker as described below, which is neither component a), i.e. a cell penetrating peptide, nor component c), i.e. a TLR peptide agonist.

[0184] Alternatively, however, the various antigens and/or antigenic epitopes may also be positioned in any other way in the complex comprised by the combination for use according to the present invention, for example with component a) and/or component c) positioned in between two or more antigens and/or antigenic epitopes, i.e. with one or more antigens and/or antigenic epitopes positioned between component a) and component c) (or vice versa) and, optionally, one or more antigens and/or antigenic epitopes positioned at the respective other end of component a) and/or component c).

[0185] It is understood that a number of different antigens or antigenic epitopes relating to the same kind of disease, in particular to the same kind of tumor, may be advantageously comprised by a single complex. Alternatively, a number of different antigens or antigenic epitopes relating to the same kind of disease, in particular to the same kind of tumor, may be distributed to subsets of different antigens or antigenic epitopes, in particular subsets complementing each other in the context of a certain kind of disease, e.g. tumor, which are comprised by different complexes, whereby such different complexes comprising different subsets may advantageously be administered simultaneously, e.g. in a single vaccine, to a

subject in need thereof.

[0186] Preferably, the complex according to the present invention comprises at least one tumor epitope of an antigen selected from the group consisting of EpCAM, HER-2/neu, MUC-1, TOMM34, RNF 43, KOC1, VEGFR, β hCG, survivin, CEA, TGF β R2, p53, KRas, OGT, CASP5, COA-1, MAGE, SART, IL13Ralpha2, CMV, EGFRvIII, EphA2, gp100, hTert, TRP-2, YKL-40, brevican, neuroligin 4 and PTPRz1. More preferably, the complex according to the present invention comprises at least one tumor antigen selected from the group consisting of EpCAM, HER-2/neu, MUC-1, TOMM34, RNF 43, KOC1, VEGFR, β hCG, survivin, CEA, TGF β R2, p53, KRas, OGT, CASP5, COA-1, MAGE, SART, IL13Ralpha2, CMV, EGFRvIII, EphA2, gp100, hTert, TRP-2, YKL-40, brevican, neuroligin 4 and PTPRz1 or a fragment thereof, or a sequence variant of a tumor antigen or a sequence variant of a fragment thereof. It is also preferred that the complex according to the present invention comprises at least one tumor epitope of an antigen selected from the glioma antigens disclosed by Reardon, D.A., et al., An update on vaccine therapy and other immunotherapeutic approaches for glioblastoma. Expert Rev Vaccines, 2013. 12(6): p. 597-615.

[0187] As used herein, a "fragment" of an antigen comprises at least 10 consecutive amino acids of the antigen, preferably at least 15 consecutive amino acids of the antigen, more preferably at least 20 consecutive amino acids of the antigen, even more preferably at least 25 consecutive amino acids of the antigen and most preferably at least 30 consecutive amino acids of the antigen. A "sequence variant" is as defined above, namely a sequence variant has an (amino acid) sequence which is at least 70%, at least 75%, preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, particularly preferably at least 95%, most preferably at least 99% identical to the reference sequence. A "functional" sequence variant means in the context of an antigen/antigen fragment/epitope, that the function of the epitope(s), e.g. comprised by the antigen (fragment), is not impaired or abolished. Preferably, however, the amino acid sequence of the epitope(s), e.g. comprised by the cancer/tumor antigen (fragment) as described herein, is not mutated and, thus, identical to the reference epitope sequence.

[0188] As described above, suitable cancer/tumor epitopes of those antigens are known from the literature or can be identified by using cancer/tumor epitope databases, e.g. from van der Bruggen P, Stroobant V, Vigneron N, Van den Eynde B. Peptide database: T cell-defined tumor antigens. Cancer Immun 2013; URL: <http://www.cancerimmunity.org/peptide/>, wherein human tumor antigens recognized by CD4+ or CD8+ T cells are classified into four major groups on the basis of their expression pattern, or from the database "Tantigen" (TANTIGEN version 1.0, Dec 1, 2009; developed by Bioinformatics Core at Cancer Vaccine Center, Dana-Farber Cancer Institute; URL: <http://cvc.dfci.harvard.edu/tadb/>).

Component c) - TLR peptide agonist

[0189] In the complex comprised by the combination for use according to the present

invention, the TLR peptide agonist allows an increased targeting of the vaccine towards dendritic cells along with self-adjuvancy. Physical linkage of a TLR peptide agonist to the CPP and the at least one antigen or antigenic epitope in the complex comprised by the combination for use according to the present invention provides an enhanced immune response by simultaneous stimulation of antigen presenting cells, in particular dendritic cells, that internalize, metabolize and display antigen(s).

[0190] As used in the context of the present invention, a "TLR peptide agonist" is an agonist of a Toll-like receptor (TLR), i.e. it binds to a TLR and activates the TLR, in particular to produce a biological response. Moreover, the TLR peptide agonist is a peptide, a polypeptide or a protein as defined above. Preferably, the TLR peptide agonist comprises from 10 to 150 amino acids, more preferably from 15 to 130 amino acids, even more preferably from 20 to 120 amino acids, particularly preferably from 25 to 110 amino acids, and most preferably from 30 to 100 amino acids.

[0191] Toll like receptors (TLRs) are transmembrane proteins that are characterized by extracellular, transmembrane, and cytosolic domains. The extracellular domains containing leucine-rich repeats (LRRs) with horseshoe-like shapes are involved in recognition of common molecular patterns derived from diverse microbes. Toll like receptors include TLRs1 - 10. Compounds capable of activating TLR receptors and modifications and derivatives thereof are well documented in the art. TLR1 may be activated by bacterial lipoproteins and acetylated forms thereof, TLR2 may in addition be activated by Gram positive bacterial glycolipids, LPS, LP A, LTA, fimbriae, outer membrane proteins, heat shock proteins from bacteria or from the host, and Mycobacterial lipoarabinomannans. TLR3 may be activated by dsRNA, in particular of viral origin, or by the chemical compound poly(LC). TLR4 may be activated by Gram negative LPS, LTA, Heat shock proteins from the host or from bacterial origin, viral coat or envelope proteins, taxol or derivatives thereof, hyaluronan containing oligosaccharides and fibronectins. TLR5 may be activated with bacterial flagellae or flagellin. TLR6 may be activated by mycobacterial lipoproteins and group B streptococcus heat labile soluble factor (GBS- F) or staphylococcus modulins. TLR7 may be activated by imidazoquinolines. TLR9 may be activated by unmethylated CpG DNA or chromatin - IgG complexes.

[0192] Preferably, the TLR peptide agonist comprised by the complex comprised by the combination for use according to the present invention is an agonist of TLR1, 2, 4, 5, 6, and/or 10. TLRs are expressed either on the cell surface (TLR1, 2, 4, 5, 6, and 10) or on membranes of intracellular organelles, such as endosomes (TLR3, 4, 7, 8, and 9). The natural ligands for the endosomal receptors turned out to be nucleic acid-based molecules (except for TLR4). The cell surface-expressed TLR1, 2, 4, 5, 6, and 10 recognize molecular patterns of extracellular microbes (Monie, T. P., Bryant, C. E., et al. 2009: Activating immunity: Lessons from the TLRs and NLRs. Trends Biochem. Sci. 34(11), 553-561). TLRs are expressed on several cell types but virtually all TLRs are expressed on DCs allowing these specialized cells to sense all possible pathogens and danger signals.

[0193] However, TLR2, 4, and 5 are constitutively expressed at the surface of DCs.

Accordingly, the TLR peptide agonist comprised by the complex comprised by the combination for use according to the present invention is more preferably a peptide agonist of TLR2, TLR4 and/or TLR5. Even more preferably, the TLR peptide agonist is a TLR2 peptide agonist and/or a TLR4 peptide agonist. Particularly preferably, the TLR peptide agonist is a TLR4 peptide agonist. Most preferably, the TLR peptide agonist is one TLR peptide agonist, which is both, a TLR2 and a TLR4 agonist. TLR2 can detect a wide variety of ligands derived from bacteria, viruses, parasites, and fungi. The ligand specificity is often determined by the interaction of TLR2 with other TLRs, such as TLR1, 6, or 10, or non-TLR molecules, such as dectin-1, CD14, or CD36. The formation of a heterodimer with TLR1 enables TLR2 to identify triacyl lipoproteins or lipopeptides from (myco)bacterial origin, such as Pam3CSK4 and peptidoglycan (PGA; Gay, N. J., and Gangloff, M. (2007): Structure and function of Toll receptors and their ligands. *Annu. Rev. Biochem.* 76, 141-165; Spohn, R., Buwitt-Beckmann, U., et al. (2004): Synthetic lipopeptide adjuvants and Toll-like receptor 2-Structure-activity relationships. *Vaccine* 22(19), 2494-2499). Heterodimerization of TLR2 and 6 enables the detection of diacyl lipopeptides and zymosan. Lipopolysaccharide (LPS) and its derivatives are ligands for TLR4 and flagellin for TLR5 (Bryant, C. E., Spring, D. R., et al. (2010). The molecular basis of the host response to lipopolysaccharide. *Nat. Rev. Microbiol.* 8(1), 8-14).

[0194] TLR2 interacts with a broad and structurally diverse range of ligands, including molecules expressed by microbes and fungi. Multiple TLR2 agonists have been identified, including natural and synthetic lipopeptides (e.g. *Mycoplasma fermentas* macrophage-activating lipopeptide (MALP-2)), peptidoglycans (PG such as those from *S. aureus*), lipopolysaccharides from various bacterial strains (LPS), polysaccharides (e.g. zymosan), glycosylphosphatidyl-inositol-anchored structures from gram positive bacteria (e.g. lipoteichoic acid (LTA) and lipo-arabinomannan from mycobacteria and lipomannans from *M. tuberculosis*). Certain viral determinants may also trigger via TLR2 (Barbalat R, Lau L, Locksley RM, Barton GM. Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. *Nat Immunol.* 2009; 10(11):1200-7). Bacterial lipopeptides are structural components of cell walls. They consist of an acylated s-glycerylcysteine moiety to which a peptide can be conjugated via the cysteine residue. Examples of TLR2 agonists, which are bacterial lipopeptides, include MALP-2 and its synthetic analogue di-palmitoyl-S-glyceryl cysteine (Pam₂Cys) or tri-palmitoyl-S-glyceryl cysteine (Pam₃Cys).

[0195] A diversity of ligands interact with TLR4, including Monophosphoryl Lipid A from *Salmonella minnesota* R595 (MPLA), lipopolysaccharides (LPS), mannans (*Candida albicans*), glycoinositolphospholipids (*Trypanosoma*), viral envelope proteins (RSV and MMTV) and endogenous antigens including fibrinogen and heat-shock proteins. Such agonists of TLR4 are for example described in Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell.* Feb 24; 2006; 124(4):783-801 or in Kumar H, Kawai T, Akira S. Toll-like receptors and innate immunity. *Biochem Biophys Res Commun.* Oct 30; 2009 388(4):621-5. LPS, which is found in the outer membrane of gram negative bacteria, is the most widely studied of the TLR4 ligands. Suitable LPS-derived TLR4 agonist peptides are described for example in WO 2013/120073 (A1).

[0196] TLR5 is triggered by a region of the flagellin molecule expressed by nearly all motile bacteria. Thus, flagellin, or peptides or proteins derived from flagellin and/or variants or fragments of flagellin are also suitable as TLR peptide agonists comprised by the complex.

[0197] Examples of TLR peptide agonists thus include the TLR2 lipopeptide agonists MALP-2, Pam₂Cys and Pam₃Cys or modifications thereof, different forms of the TLR4 agonist LPS, e.g. N. meningitidis wild-type L3-LPS and mutant penta-acylated LpxL1-LPS, and the TLR5 agonist flagellin.

[0198] However, it is preferred that the TLR peptide agonist comprised by the complex is neither a lipopeptide nor a lipoprotein, neither a glycopeptide nor a glycoprotein, more preferably, the TLR peptide agonist comprised by the complex is a classical peptide, polypeptide or protein as defined herein.

[0199] A preferred TLR2 peptide agonist is annexin II or an immunomodulatory fragment thereof, which is described in detail in WO 2012/048190 A1 and US patent application 13/0331546, in particular a TLR2 peptide agonist comprising an amino acid sequence according to SEQ ID NO: 4 or SEQ ID NO: 7 of WO 2012/048190 A1 or fragments or variants thereof are preferred.

[0200] Thereby, a TLR2 peptide agonist comprising or consisting of an amino acid sequence according to SEQ ID NO: 15 or a sequence variant thereof as described above is particularly preferred as component c), i.e. as the at least one TLR peptide agonist, comprised by the complex.

STVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE

SEQ ID NO: 15 (TLR2 peptide agonist Anaxa)

[0201] A particularly preferred functional sequence variant of the TLR peptide agonist according to SEQ ID NO: 15 is the TLR peptide agonist according to SEQ ID NO: 47:

STVHEILSKLSLEGDHSTPPSAYGSVKPYTNFDAE

SEQ ID NO: 47

[0202] Accordingly, a TLR2 peptide agonist comprising or consisting of an amino acid sequence according to SEQ ID NO: 47 or a sequence variant thereof as described above is particularly preferred as component c), i.e. as the at least one TLR peptide agonist, comprised by the complex.

[0203] Regarding TLR4, TLR peptides agonists are particularly preferred, which in particular correspond to motifs that bind to TLR4, in particular (i) peptides mimicking the natural LPS ligand (RS01: Gln-Glu-Ile-Asn-Ser-Ser- Tyr and RS09: Ala-Pro-Pro-His-Ala-Leu-Ser) and (ii) Fibronectin derived peptides. The cellullarglycoprotein Fibronectin (FN) has multiple isoforms generated from a single gene by alternative splicing of three exons. One of these isoforms is the extra domain A (EDA), which interacts with TLR4.

[0204] Further suitable TLR peptide agonists comprise a fibronectin EDA domain or a fragment or variant thereof. Such suitable fibronectin EDA domains or a fragments or variants thereof are disclosed in EP 1 913 954 B1, EP 2 476 440 A1, US 2009/0220532 A1, and WO 2011/101332 A1. Thereby, a TLR4 peptide agonist comprising or consisting of an amino acid sequence according to SEQ ID NO: 40 or a sequence variant thereof as described above is particularly preferred as component c), i.e. as the at least one TLR peptide agonist, comprised by the complex comprised by the combination for use according to the present invention.

NIDRPKGLAFTDVIDVDSIKIAWESPQGQVSRVRYTYSSPEDGIRELFPAPDGEDDTAELQGLRP

GSEYTVSVVALHDDMESQPLIGIQST

SEQ ID NO: 40 (TLR4 peptide agonist EDA)

[0205] In addition, high-mobility group box 1 protein (HMGB1) and peptide fragments thereof are assumed to be TLR4 agonists. Such HMGB1-derived peptides are for example disclosed in US 2011/0236406 A1.

[0206] Moreover, also the TLR agonist according to SEQ ID NO: 15 and the TLR agonist according to SEQ ID NO: 47 may act as TLR4 agonist. Accordingly, a TLR4 peptide agonist comprising or consisting of an amino acid sequence according to SEQ ID NO: 15 or 47 or a functional sequence variant thereof is particularly preferred as component c), i.e. as the at least one TLR peptide agonist, comprised by the complex.

[0207] The complex comprised by the combination for use according to the present invention comprises at least one TLR peptide agonist, preferably the complex comprised by the combination for use according to the present invention comprises more than one TLR peptide agonist, in particular 2, 3, 4, 5, 6, 7, 8, 9, 10 or more TLR peptide agonists, more preferably the complex comprised by the combination for use according to the present invention comprises (at least) two or three TLR peptide agonists, even more preferably the complex comprised by the combination for use according to the present invention comprises (at least) four or five TLR peptide agonists. If more than one TLR peptide agonist is comprised by the complex it is understood that said TLR peptide agonist is in particular also covalently linked in the complex comprised by the combination for use according to the present invention, e.g. to another TLR peptide agonist and/or to a component a), i.e. a cell penetrating peptide, and/or to a component b), i.e. an antigen or antigenic epitope.

[0208] In a particularly preferred embodiment, the complex comprised by the combination for use according to the present invention comprises one single TLR peptide agonist. In particular, in this particularly preferred embodiment, the complex comprised by the combination for use according to the present invention comprises one single TLR peptide agonist and no further component having TLR agonist properties except the one single TLR peptide agonist as described.

[0209] The various TLR peptide agonists comprised by the complex may be the same or different. Preferably, the various TLR peptide agonists comprised by the complex are different

from each other.

[0210] Moreover, it is preferred that the more than one antigen or antigenic epitope, in particular 2, 3, 4, 5, 6, 7, 8, 9, 10 antigens or antigenic epitopes, or more TLR peptide agonists, in particular 2, 3, 4, 5, 6, 7, 8, 9, 10 TLR agonists, are positioned consecutively in the complex comprised by the combination for use according to the present invention. This means in particular that all TLR peptide agonists comprised by the complex are positioned in a stretch, which is neither interrupted by component a), i.e. a cell penetrating peptide, nor by component b), i.e. at least one antigen or antigenic epitope. Rather, component a) and component b) are positioned in the complex for example before or after such a stretch of all TLR peptide agonists. However, the TLR peptide agonists positioned consecutively in such a way may be linked to each other for example by a spacer or linker as described below, which is neither component a), i.e. a cell penetrating peptide, nor component b), i.e. at least one antigen or antigenic epitope.

[0211] Alternatively, however, the various TLR peptide agonists may also be positioned in any other way in the complex comprised by the combination for use according to the present invention, for example with component a) and/or component b) positioned in between two or more TLR peptide agonists, i.e. with one or more TLR peptide agonist positioned between component a) and component b) (or vice versa) and, optionally, one or more TLR peptide agonists positioned at the respective other end of component a) and/or component b).

[0212] It is understood that a number of different TLR peptide agonists activating the same or different TLR receptors may be advantageously comprised by a single complex. Alternatively, a number of different TLR peptide agonists activating the same or different TLR receptors may be distributed to subsets of different TLR peptide agonists activating the same or different TLR receptors, which are comprised by different complexes, whereby such different complexes comprising different subsets may advantageously be administered simultaneously, e.g. in a single vaccine, to a subject in need thereof.

Linkage of components a), b), and c) in the complex

[0213] In the complex comprised by the combination for use according to the present invention, components a), b) and c) are covalently linked, i.e. the linkage between two out of the three components a), b), and c) of the complex is a covalent linkage. Preferably, two out of the three components a), b), and c) of the complex are covalently linked to each other (i.e. the "first" and the "second" component), and the third component out of the three components a), b), and c) is covalently linked either to the first component out of the three components a), b), and c) or to the second component out of the three components a), b), and c). Thereby, preferably a linear molecule is formed. However, it is also conceivable that each of the three components a), b), and c) is covalently linked to both of the other components out of the three components a), b), and c).

[0214] A "covalent linkage" (also covalent bond), as used in the context of the present invention, refers to a chemical bond that involves the sharing of electron pairs between atoms. A "covalent linkage" (also covalent bond) in particular involves a stable balance of attractive and repulsive forces between atoms when they share electrons. For many molecules, the sharing of electrons allows each atom to attain the equivalent of a full outer shell, corresponding to a stable electronic configuration. Covalent bonding includes many kinds of interactions, including for example σ -bonding, π -bonding, metal-to-metal bonding, agostic interactions, and three-center two-electron bonds. Accordingly, the complex comprised by the combination for use according to the present invention, may also be referred to as "compound", in particular it may be referred to as "molecule".

[0215] Preferably, in the complex comprised by the combination for use according to the present invention, components a), b), and c) are covalently linked by chemical coupling in any suitable manner known in the art, such as cross-linking methods. However, attention is drawn to the fact that many known chemical cross-linking methods are non-specific, i.e., they do not direct the point of coupling to any particular site on the components a), b), and c). Thus, the use of non-specific cross-linking agents may attack functional sites or sterically block active sites, rendering the fused components of the complex biologically inactive. It is referred to the knowledge of the skilled artisan to block potentially reactive groups by using appropriate protecting groups. Alternatively, the use of the powerful and versatile oxime and hydrazone ligation techniques, which are chemo-selective entities that can be applied for the cross-linking of components a), b), and c) may be employed. This linking technology is described e.g. by Rose et al. (1994), JACS 116, 30.

[0216] Coupling specificity can be increased by direct chemical coupling to a functional group found only once or a few times in components a), b), and/or c), which functional group is to be cross-linked to the another of the components a), b), and c). As an example, the cystein thiol group may be used, if just one cystein residue is present in a certain component a), b), or c) of complex comprised by the combination for use according to the present invention. Also, for example, if a certain component a), b), or c) contains no lysine residues, a cross-linking reagent specific for primary amines will be selective for the amino terminus of the respective component. Alternatively, cross-linking may also be carried out via the side chain of a glutamic acid residue placed at the N-terminus of the peptide such that a amide bond can be generated through its side-chain. Therefore, it may be advantageous to link a glutamic acid residue to the N-terminus of a certain component a), b), or c). However, if a cysteine residue is to be introduced into a certain component a), b), or c), introduction at or near its N- or C-terminus is preferred. Conventional methods are available for such amino acid sequence alterations based on modifications of certain component a), b), or c) by either adding one or more additional amino acids, e.g. inter alia an cystein residue, to the translocation sequence or by substituting at least one residue of the translocation sequence(s) being comprised in the respective component. In case a cystein side chain is used for coupling purposes, a certain component a), b), or c) has preferably one cystein residue. Any second cystein residue should preferably be avoided and can, optionally, be replaced when they occur in the respective component comprised by the complex. When a cysteine residue is replaced in the original sequence of a

certain component a), b), or c), it is typically desirable to minimize resulting changes in the peptide folding of the respective component. Changes in folding are minimized when the replacement is chemically and sterically similar to cysteine. Therefore, serine is preferred as a replacement for cysteine.

[0217] Coupling of two out of the three components a), b), and c) can be accomplished via a coupling or conjugating agent including standard peptide synthesis coupling reagents such as HOBt, HBTU, DICl, TBTU. There are several intermolecular cross-linking agents which can be utilized, see for example, Means and Feeney, *Chemical Modification of Proteins*, Holden-Day, 1974, pp. 39-43. Among these reagents are, for example, N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) or N,N'-(1,3-phenylene)bismaleimide; N,N'-ethylene-bis-(iodoacetamide) or other such reagent having 6 to 11 carbon methylene bridges; and 1,5-difluoro-2,4-dinitrobenzene. Other cross-linking agents useful for this purpose include: p,p'-difluoro-m,m'-dinitrodiphenylsulfone; dimethyl adipimidate; phenol-1,4-disulfonylchloride; hexamethylenediisocyanate or diisothiocyanate, or azophenyl-p-diisocyanate; glutaraldehyde and disdiazobenzidine. Cross-linking agents may be homobifunctional, i.e., having two functional groups that undergo the same reaction. A preferred homobifunctional cross-linking agent is bismaleimido-hexane (BMH). BMH contains two maleimide functional groups, which react specifically with sulfhydryl-containing compounds under mild conditions (pH 6.5-7.7). The two maleimide groups are connected by a hydrocarbon chain. Therefore, BMH is useful for irreversible cross-linking of proteins (or polypeptides) that contain cysteine residues. Cross-linking agents may also be heterobifunctional. Heterobifunctional cross-linking agents have two different functional groups, for example an amine-reactive group and a thiol-reactive group, that will cross-link two proteins having free amines and thiols, respectively. Examples of heterobifunctional cross-linking agents are Succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), and succinimide 4-(p-maleimidophenyl)butyrate (SMPB), an extended chain analog of MBS. The succinimidyl group of these cross-linkers reacts with a primary amine, and the thiol-reactive maleimide forms a covalent bond with the thiol of a cysteine residue. Because cross-linking agents often have low solubility in water, a hydrophilic moiety, such as a sulfonate group, may be added to the cross-linking agent to improve its water solubility. Sulfo-MBS and sulfo-SMCC are examples of cross-linking agents modified for water solubility. Many cross-linking agents yield a conjugate that is essentially non-cleavable under cellular conditions. Therefore, some cross-linking agents contain a covalent bond, such as a disulfide, that is cleavable under cellular conditions. For example, Traut's reagent, dithiobis (succinimidylpropionate) (DSP), and N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) are well-known cleavable cross-linkers. The use of a cleavable cross-linking agent permits the cell penetrating peptide, the at least one antigen or antigenic epitope and the at least one TLR peptide agonist comprised by the complex to separate from each other after delivery into the target cell. For this purpose, direct disulfide linkage may also be useful. Chemical cross-linking may also include the use of spacer arms. Spacer arms provide intramolecular flexibility or adjust intramolecular distances between conjugated moieties and thereby may help preserve biological activity. A spacer arm may be in the form of a protein (or polypeptide) moiety that includes spacer amino acids, e.g. proline. Alternatively, a spacer arm may be part of the cross-linking agent, such as in "long-chain

SPDP" (Pierce Chem. Co., Rockford, Ill., cat. No. 21651 H). Numerous cross-linking agents, including the ones discussed above, are commercially available. Detailed instructions for their use are readily available from the commercial suppliers. More detailed information on protein cross-linking and conjugate preparation, which is useful in the context of linkage of components a), b), and c) comprised by the complex can be retrieved from: Wong, Chemistry of Protein Conjugation and Cross-Linking, CRC Press (1991).

[0218] Cross-linking agents for peptide or protein crosslinking include for example (i) amine-to-amine crosslinkers, e.g. homobifunctional amine-specific protein crosslinking reagents based on NHS-ester and imidoester reactive groups for selective conjugation of primary amines; available in short, long, cleavable, irreversible, membrane permeable, and cell surface varieties; (ii) sulfhydryl-to-carbohydrate crosslinkers, e.g. crosslinking reagents based on maleimide and hydrazide reactive groups for conjugation and formation of covalent crosslinks; (iii) sulfhydryl-to-sulfhydryl crosslinkers, e.g. homobifunctional sulfhydryl-specific crosslinking reagents based on maleimide or pyridyldithiol reactive groups for selective covalent conjugation of protein and peptide thiols (reduced cysteines) to form stable thioether bonds; (iv) photoreactive crosslinkers, e.g. aryl azide, diazirine, and other photo-reactive (light-activated) chemical heterobifunctional crosslinking reagents to conjugate proteins, nucleic acids and other molecular structures involved in receptor-ligand interaction complexes via two-step activation; (v) amine-to-sulfhydryl crosslinkers, e.g. heterobifunctional protein crosslinking reagents for conjugation between primary amine (lysine) and sulfhydryl (cysteine) groups of proteins and other molecules; available with different lengths and types of spacer arms; and (vi) amine-to-amine crosslinkers, e.g. carboxyl-to-amine crosslinkers, e.g. Carbodiimide crosslinking reagents, DCC and EDC (EDAC), for conjugating carboxyl groups (glutamate, aspartate, C-termini) to primary amines (lysine, N-termini) and also N-hydroxysuccinimide (NHS) for stable activation of carboxylates for amine-conjugation.

[0219] Examples of crosslinkers in general, which can be used in the complex comprised by the combination for use according to the present invention, include *N*-(α -Maleimidoacetoxy)-succinimide ester, *N*-5-Azido-2-nitrobenzyloxy-succinimide, 1,4-*Bis*-Maleimidobutane, 1,4-*Bis*-Maleimimidyl-2,3-dihydroxy-butane, *Bis*-Maleimidohexane, *Bis*-Maleimidoethane, *N*-(β -Maleimidopropionic acid)hydrazide*TFA, *N*-(β -Maleimidopropoxy)succinimide ester, 1,8-*Bis*-Maleimidodiethylene-glycol, 1,11-*Bis*-Maleimidotriethyleneglycol, *Bis* (sulfosuccinimidyl)suberate, *Bis* (sulfosuccinimidyl)glutarate-d0, *Bis* (sulfosuccinimidyl)2,2,4,4-glutarate-d4, *Bis* (sulfosuccinimidyl)suberate-d0, *Bis* (sulfosuccinimidyl)2,2,7,7-suberate-d4, *Bis* (NHS)PEG5, *Bis* (NHS)PEG9, *Bis* (2-[succinimidoxycarbonyloxy]ethyl)sulfone, *N,N*-Dicyclohexylcarbodiimide, 1-5-Difluoro-2,4-dinitrobenzene, Dimethyl adipimidate*2HCl, Dimethyl pimelimidate*2HCl, Dimethyl suberimidate*2HCl, Disuccinimidyl glutarate, Dithiobis(succinimidylpropionate) (Lomant's Reagent), Disuccinimidyl suberate, Disuccinimidyl tartarate, Dimethyl 3,3'-dithiobispropionimidate*2HCl, Dithiobis-maleimidoethane, 3,3'-Dithiobis (sulfosuccinimidylpropionate), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, Ethylene glycol *bis* (succinimidylsuccinate), *N*- ϵ -Maleimidocaproic acid, *N*-(ϵ -Maleimidocaproic acid)hydrazide, *N*-(ϵ -Maleimidocaproyloxy)succinimide ester, *N*-(γ -Maleimidobutyryloxy)succinimide ester, *N*-(κ -Maleimidoundecanoic acid)hydrazide, NHS-LC-

Diazirine, Succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxy-(6-amidocaproate, Succinimidyl 6-(3'-[2-pyridyldithio] propionamido)hexanoate, L-Photo-Leucine, L-Photo-Methionine, *m*-Maleimidobenzoyl-*N*-hydroxysuccinimide ester, 4-(4-*N*-Maleimidophenyl)-butyric acid hydrazide*HCl, 2-[N2-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-N6-(6-biotinamidocaproyl)-L-lysiny]ethylmethanethiosulfate, 2-{N2-[N6-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-N6-(6-biotinamidocaproyl)-L-lysiny]}ethylmethanethiosulfate, *N*-Hydroxysuccinimide, *N*-hydroxysuccinimide ester ethane azide, *N*-hydroxysuccinimide ester tetraoxapentadecane azide, *N*-hydroxysuccinimide ester dodecaoxanonatriacontane azide, NHS-Phosphine, 3-(2-Pyridyldithio)propionylhydrazide, 2-pyridyldithiol-tetraoxatetradecane-*N*-hydroxysuccinimide, 2-pyridyldithiol-tetraoxaoctatriacontane-*N*-hydroxysuccinimide, *N*-(*p*-Maleimidophenyl)isocyanate, Succinimidyl 3-(bromoacetamido)propionate, NHS-Diazirine, NHS-SS-Diazirine, *N*-succinimidyl iodoacetate, *N*-Succinimidyl(4-iodoacetyl)aminobenzoate, Succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate, NHS-PEG2-Maleimide, NHS-PEG4-Maleimide, NHS-PEG6-Maleimide, NHS-PEG8-Maleimide, NHS-PEG12-Maleimide, NHS-PEG24-Maleimide, Succinimidyl 4-(*p*-maleimido-phenyl)butyrate, Succinimidyl-6-(β -maleimidopropionamido)hexanoate, 4-Succinimidyloxycarbonyl-methyl- α -(2-pyridyldithio)toluene, Succinimidyl-(4-psoralen-8-yloxy)butyrate, *N*-Succinimidyl 3-(2-pyridyldithio)propionate, Ethylene glycol bis (sulfo-succinimidyl succinate), *N*-(ϵ -Maleimidocaproxyloxy)sulfosuccinimide ester, *N*-(γ -Maleimidobutyloxy)sulfosuccinimide ester, *N*-(κ -Maleimidoundecanoyloxy)sulfosuccinimide ester, Sulfo-NHS-LC-Diazirine, Sulfosuccinimidyl 6-(3'-[2-pyridyldithio]propionamido)hexanoate, *m*-Maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester, *N*-Hydroxysuccinimide, Sulfo-NHS-Phosphine, Sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino)hexanoate, Sulfo-NHS-(2-6-[Biotinamido]-2-(*p*-azidobezamido), Sulfo-NHS-Diazirine, Sulfo-NHS-SS-Diazirine, Sulfosuccinimidyl(4-iodoacetyl)aminobenzoate, Sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate, Sulfosuccinimidyl 4-(*p*-maleimidophenyl)butyrate, *Tris*-(2-Maleimidoethyl)amine (Trifunctional), and *Tris*-(succinimidyl aminotricetate) (Trifunctional).

[0220] The linkage between two out of the three components a), b), and c) of the complex comprised by the combination for use according to the present invention may be directly or indirectly, i.e. two components directly adjoin or they may be linked by an additional component of the complex, e.g. a spacer or a linker.

[0221] A direct linkage may be realized preferably by an amide bridge, if the components to be linked have reactive amino or carboxy groups. More specifically, if the components to be linked are peptides, polypeptides or proteins, a peptide bond is preferred. Such a peptide bond may be formed using a chemical synthesis involving both components (an N-terminal end of one component and the C-terminal end of the other component) to be linked, or may be formed directly via a protein synthesis of the entire peptide sequence of both components, wherein both (protein or peptide) components are preferably synthesized in one step. Such protein synthesis methods include e.g., liquid phase peptide synthesis methods or solid peptide synthesis methods, e.g. solid peptide synthesis methods according to Merrifield, *t*-Boc solid-phase peptide synthesis, Fmoc solid-phase peptide synthesis, BOP (Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate) based solid-phase peptide synthesis.

Alternatively, ester or ether linkages are preferred.

[0222] Moreover, in particular if the components to be linked are peptides, polypeptides or proteins, a linkage may occur via the side chains, e.g. by a disulfide bridge. Further components of other chemical nature, e.g. the at least one antigen or antigenic epitope if it is not of peptidic nature, may be likewise attached to the components of peptidic nature, e.g. the cell penetrating peptide, the at least one TLR peptide agonist, and the at least one antigen or antigenic epitope if it is of peptidic nature. The linkage via a side chain will preferably be based on side chain amino, thiol or hydroxyl groups, e.g. via an amide or ester or ether linkage. A linkage of a peptidic main chain with a peptidic side chain of another component may also be via an isopeptide bond. An isopeptide bond is an amide bond that is not present on the main chain of a protein. The bond forms between the carboxyl terminus of one peptide or protein and the amino group of a lysine residue on another (target) peptide or protein.

[0223] The complex comprised by the combination for use according to the present invention may optionally comprise a spacer or linker, which are non-immunologic moieties, which are preferably cleavable, and which link component a) and b) and/or component a) and c), and/or component b) and c), and/or link consecutive antigens or antigenic epitopes, and/or link consecutive TLR peptide agonists, and/or link consecutive cell penetrating peptides, and/or which can be placed at the C-terminal part of components b) and/or c). A linker or spacer may preferably provide further functionalities in addition to linking of the components, and preferably being cleavable, more preferably naturally cleavable inside the target cell, e.g. by enzymatic cleavage. However, such further functionalities do in particular not include any immunological functionalities. Examples of further functionalities, in particular regarding linkers in fusion proteins, can be found in Chen X. et al., 2013: Fusion Protein Linkers: Property, Design and Functionality. Adv Drug Deliv Rev. 65(10): 1357 - 1369, wherein for example also in vivo cleavable linkers are disclosed. Moreover, Chen X. et al., 2013: Fusion Protein Linkers: Property, Design and Functionality. Adv Drug Deliv Rev. 65(10): 1357- 1369 also discloses various linkers, e.g. flexible linkers and rigid linkers, and linker designing tools and databases, which can be useful in the complex comprised by the combination for use according to the present invention or to design a linker to be used in the complex.

[0224] Said spacer may be peptidic or non-peptidic, preferably the spacer is peptidic. Preferably, a peptidic spacer consists of about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, more preferably of about 1, 2, 3, 4, or 5 amino acids. The amino acid sequence of the peptidic spacer may be identical to that of the N-terminal or C-terminal flanking region of any of the components a), b), or c). Alternatively a peptidic spacer can consist of non-natural amino acid sequences such as an amino acid sequence resulting from conservative amino acid substitutions of said natural flanking regions or sequences of known cleavage sites for proteases such as an enterokinase target site (amino acid sequence: DDDK, SEQ ID NO: 16), factor Xa target site (amino acid sequence: IEDGR, SEQ ID NO: 17), thrombin target site (amino acid sequence: LVPRGS, SEQ ID NO: 18), protease TEV target site (amino acid sequence: ENLYFQG, SEQ ID NO: 19), PreScission protease target site (amino acid sequence: LEVLFQGP, SEQ ID NO: 20), polycationic amino acids, e.g. poly K, furin target site (amino acid

sequence RX(R/K)R, SEQ ID NO: 21). In a particular embodiment, the peptidic spacer does not contain any Cys (C) residues. In a preferred embodiment the linker sequence contains at least 20%, more preferably at least 40% and even more preferably at least 50% Gly or β -alanine residues, e.g. GlyGlyGlyGlyGly (SEQ ID NO: 22), GlyGlyGlyGly (SEQ ID NO: 23), GlyGlyGly, CysGlyGly or GlyGlyCys. Appropriate linker sequences can be easily selected and prepared by a person skilled in the art. They may be composed of D and/or L amino acids. Further examples of a peptidic spacer include the amino acid sequences EQLE (SEQ ID NO: 24) or TEWT (SEQ ID NO: 25) or any conservative substitutions thereof.

[0225] A non-peptidic spacer can include or may be an ester, a thioester, and a di-sulfide.

[0226] In particular, the complex comprised by the combination for use according to the invention may comprise a spacer or linker, in particular a peptidic spacer, placed between component a) and b) and/or between component a) and c), and/or between component b) and c),. This peptidic spacer can be chosen by one skilled in the art so that it may be cut by the cell machinery once the complex comprising the cell penetrating peptide and the cargo molecule has been internalized.

[0227] When the complex comprises several antigens or antigenic epitopes or when the complex comprises several TLR peptide agonists, it will be clear for one skilled in the art that each of the antigens or antigenic epitopes and/or each of the TLR peptide agonists comprised in the complex can be either directly linked to each other or linked via spacers or linkers such as, e.g., a peptidic spacer consisting of a few amino acids. Alternatively, when the complex comprised by the combination for use according to the present invention comprises several antigens or antigenic epitopes or when the complex comprises several TLR peptide agonists, it is also possible that some antigens or antigenic epitopes and/or some TLR peptide agonists comprised by the complex are directly linked to each other and some other antigens or antigenic epitopes and/or some other TLR peptide agonists are linked via spacers or linkers such as a peptidic spacer consisting of a few amino acids.

[0228] For example, two successive antigens or antigenic epitopes or two successive TLR peptide agonists comprised in the complex are linked to each other by spacers consisting of the natural flanking regions of said antigens or antigenic epitopes or of said TLR peptide agonists, respectively. For example, the spacer used to link a first antigen/antigenic epitope or a first TLR peptide agonist to a second antigen/antigenic epitope or to a second TLR peptide agonist, respectively, may consist of up to about 8 amino acids corresponding to up to about 4 amino acids of the N-terminal or C-terminal flanking region of the first antigen/antigenic epitope or the first TLR peptide agonist, followed by up to about 4 amino acids of the N-terminal or C-terminal flanking region of the second antigen/antigenic epitope or the second TLR peptide agonist. In an illustration of the complex comprised by the combination for use according to the present invention, the spacer used to link a first antigen/antigenic epitope or a first TLR peptide agonist ("antigen/epitope/TLR peptide agonist 1") to a second epitope ("antigen/epitope/TLR peptide agonist 2") consists of about 8 amino acids corresponding to any possible combination ranging from: 0 flanking amino acid of antigen/epitope/TLR peptide agonist 1 and 8 flanking

amino acids of antigen/epitope/TLR peptide agonist 2, to 8 flanking amino acids of antigen/epitope/TLR peptide agonist 1 and 0 flanking amino acid of antigen/epitope/TLR peptide agonist 2, i.e. including 1 flanking amino acid of antigen/epitope/TLR peptide agonist 1 and 7 flanking amino acids of antigen/epitope/TLR peptide agonist 2, 2 flanking amino acid of antigen/epitope/TLR peptide agonist 1 and 6 flanking amino acids of antigen/epitope/TLR peptide agonist 2, 3 flanking amino acid of antigen/epitope/TLR peptide agonist 1 and 5 flanking amino acids of antigen/epitope/TLR peptide agonist 2, 4 flanking amino acid of antigen/epitope/TLR peptide agonist 1 and 4 flanking amino acids of antigen/epitope/TLR peptide agonist 2, 5 flanking amino acid of antigen/epitope/TLR peptide agonist 1 and 3 flanking amino acids of antigen/epitope/TLR peptide agonist 2, 6 flanking amino acid of antigen/epitope/TLR peptide agonist 1 and 2 flanking amino acids of antigen/epitope/TLR peptide agonist 2, 7 flanking amino acid of antigen/epitope/TLR peptide agonist 1 and 1 flanking amino acid of antigen/epitope/TLR peptide agonist 2, 8 flanking amino acid of antigen/epitope/TLR peptide agonist 1 and 0 flanking amino acids of antigen/epitope/TLR peptide agonist 2. It will be understood that the total of 8 amino acids constituting a spacer linking two consecutive antigen/epitope/TLR peptide agonist is not an absolute value and the spacer could also be composed of a total of, for instance, 3 amino acids, 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, 9 amino acids or 10 amino acids. Similarly, equivalent combinations as mentioned above are also an illustration of the complex in the situation where a spacer has less or more than 8 amino acids.

[0229] In another particular illustration of the complex comprised by the combination for use according to the present invention, the spacer used to link a first antigen/antigenic epitope or a first TLR peptide agonist ("antigen/epitope/TLR peptide agonist 1") to a second antigen/antigenic epitope or to a second TLR peptide agonist, respectively, ("antigen/epitope/TLR peptide agonist 2") consists of e.g. 1, 2, 3, 4, or 5 amino acids. More particularly, said spacer's amino acid sequence can correspond to the 4 amino acids of the N-terminal or C-terminal flanking region of antigen/epitope/TLR peptide agonist 1 or antigen/epitope/TLR peptide agonist 2. A spacer as described above may also be placed at the C-terminal part of the last antigen/epitope/TLR peptide agonist comprised in the complex.

[0230] The techniques for linking two of the three components a), b), and c) are well documented in the literature and can depend on the nature of the at least one antigen or antigenic epitope. For instance, linkages between two of the three components a), b), and c) can be achieved via cleavable disulphide linkages through total stepwise solid-phase synthesis or solution-phase or solid-phase fragment coupling, stable amide, thiazolidine, oxime and hydrazine linkage, disulphide linkage, stable thiomaleimide linkage, peptide bond (including peptide bonds between amino acids of a fusion protein), or electrostatic or hydrophobic interactions.

[0231] Preferably, the at least one antigen or antigenic epitope comprised by the complex as well as any optional spacer or linker comprised by the complex are of peptidic nature. More preferably, all components of the complex comprised by the combination for use according to the present invention, e.g. the cell penetrating peptide, the at least one antigen or antigenic epitope, which is a peptide, polypeptide or protein, the at least one TLR peptide agonist and

any optional peptidic linker or spacer are linked in the complex comprised by the combination for use according to the present invention by a peptide bond. Most preferably, the complex comprised by the combination for use according to the present invention is thus a peptide, polypeptide or protein, such as a fusion protein, e.g. a recombinant fusion protein.

[0232] In this context, a complex comprising or consisting of an amino acid sequence according to SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, or SEQ ID NO: 45; or a complex comprising or consisting of an amino acid sequence sharing at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with any of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, or SEQ ID NO: 45 is preferred. A complex comprising or consisting of an amino acid sequence according to SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43 or SEQ ID NO: 45 or a complex comprising or consisting of an amino acid sequence sharing at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with any of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43 or SEQ ID NO: 45 is more preferred. A complex comprising or consisting of an amino acid sequence according to SEQ ID NO: 28, SEQ ID NO: 31, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43 or SEQ ID NO: 45 or a complex comprising or consisting of an amino acid sequence sharing at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with SEQ ID NO: 28, SEQ ID NO: 31, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43 or SEQ ID NO: 45 is even more preferred. A complex comprising or consisting of an amino acid sequence according to SEQ ID NO: 28, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43 or SEQ ID NO: 45 or a complex comprising or consisting of an amino acid sequence sharing at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with SEQ ID NO: 28, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43 or SEQ ID NO: 45 is particularly preferred.

SEQ ID NO: 26:

MHHHHHHNID RPKGLAFTDV DVDSIKIAWE SPQGQVSRYSR VTYSSPEDGI
RELFPAPDGEDDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQSTKRY KNRVASRKS
AKFKQLLQHY REVAAKSSE NDRLRLLLKE SLKISQAVHA AHAEINEAGR EVVGVGALKV
PRNQDWLGVP RFAKFASFEA QGALANIAVD KANLDVEQLE SIINFELTE WTGS

SEQ ID NO: 27:

MHHHHHHSTV HEILCKLSLE GDHSTPPSAY GSVKPYTNFD AEKRYKNRVA SRKSRAKFKQ
LLQHYREVAA AKSENDRLR LLLKESLKIS QAVHAAHAEI NEAGREVGV GALKVPRNQD
WLGVPRAKFAK ASFEAQGALA NIAVDKANLD VEQLESIIINF EKLTEWTGS

SEQ ID NO: 28:

MHHHHHHHKRYKNRVA SRKSRAKFKQ LLQHYREVAA AKSENDRLR LLLKESLKIS
QAVHAAHAEI NEAGREVGV GALKVPRNQD WLGVPRAKFAK ASFEAQGALA

QAVHAAHAEI EINEAGREVGV GALKVPRNQD WLGVPKFAE ASFEAQGALA
 NIAVDKANLD VEQLESIINF EKLTEWTGSS TVHEILCKLS LEGDHSTPPS AYGSVKPYTN FDAE

SEQ ID NO: 29:

MHHHHHHKRY KNRVASRKS AKFKQLLQHY REVAAAKESL KISQAVHAAH AEINEAGREV
 VGVGALKVPR NQDWLGVPF AKFASFEAQG ALANIAVDKA NLDVEQLESI INFEKLTEWT
 GSSTVHEILC KLSLEGDHST PPSAYGSVKP YTNFDAE

SEQ ID NO: 30:

MHHHHHHREV AAAKSENDRLRLLLKESLK ISQAVHAAHA EINEAGREVV GVGALKVPRN
 QDWLGVPFRA KASFEAQGA LANIAVDKAN LDVEQLESII NFEKLTEWTG SSTVHEILCK
 LSLEGDHSTP PSAYGSVKPY TNFDAE

SEQ ID NO: 31:

MHHHHHHNID RPKGLAFTDV DVDSIKIAWE SPQGQVSRYP VTYSSPEDGI RELFPAPDGE
 DDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQTSTKRY KNRVASRKS AKFKQLLQHY
 REVAAAKESL KISQAVHAAH AEINEAGREV VGVGALKVPR NQDWLGVPF AKFASFEAQG
 ALANIAVDKA NLDVEQLESI INFEKLTEWT GS

SEQ ID NO: 32:

MHHHHHHNID RPKGLAFTDV DVDSIKIAWE SPQGQVSRYP VTYSSPEDGI RELFPAPDGE
 DDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQTSTREV AAAXSENDRLRLLLKESLK
 ISQAVHAAHA EINEAGREVV GVGALKVPRN QDWLGVPFRA KASFEAQGA LANIAVDKAN
 LDVEQLESII NFEKLTEWTG S

SEQ ID NO: 41:

KRYKNRVASRKSRAKFKQLLQHYREVAAAKSENDRLRLLLKVITYHSPSYAYHQFERRAILNRLV
 QFIKDRISVVQALVLTSTVHEILCKLSLEGDHSTPPSAYGSVKPYTN FDAE

SEQ ID NO: 42:

KRYKNRVASRKSRAKFKQLLQHYREVAAAKSENDRLRLLLKNYRIATFKNWPFLDCAMEELT
 VSEFLKLDQRSTVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE

SEQ ID NO: 43:

KRYKNRVASRKSRAKFKQLLQHYREVAAAKSENDRLRLLLKHLELASMTNMELMSSIVSTVHEI
 LCKLSLEGDHSTPPSAYGSVKPYTNFDAE

SEQ ID NO: 44:

RKKRRQRRRRVKRISQAVHAAHAEINEAGRRVKRKVPRNQDWLRVKRASFEAQGALANIAVD
 KARVKRSIINFELRVKRSTVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE

SEQ ID NO: 45:

KRYKNRVASRKSRAKFKQLLQHYREVAAAKSENDRLRLLLKLFRAAQLANDVVLQIMEHLELA
 SMTNMELMSSIVVISASIIVNLELEGSTVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE

Arrangement of components a), b), and c) in the complex

[0233] The components a), b), and c) may be arranged in the complex comprised by the combination for use according to the present invention in any way.

[0234] In particular if more than one cell penetrating peptide and/or more than one antigen or antigenic epitope and/or more than one TLR peptide agonist are comprised by the complex, the more than one cell penetrating peptide may be positioned in a non-consecutive manner, i.e. at least one antigen or antigenic epitope (component b)) and/or at least one TLR peptide agonist (component c)) may interrupt a stretch of consecutively positioned cell penetrating peptides and/or the cell penetrating peptides may be positioned with component b) and/or with component c) in an alternating manner. Similarly, the more than one antigen or antigenic epitope may be positioned in a non-consecutive manner, i.e. at least one cell penetrating peptide (component a)) and/or at least one TLR peptide agonist (component c)) may interrupt a stretch of consecutively positioned antigens or antigenic epitopes and/or the antigens or antigenic epitopes may be positioned with component a) and/or with component c) in an alternating manner. Similarly, the more than one TLR peptide agonist may be positioned in a non-consecutive manner, i.e. at least one cell penetrating peptide (component a)) and/or at least one antigen or antigenic epitope (component b)) may interrupt a stretch of consecutively positioned TLR peptide agonists and/or the TLR peptide agonists may be positioned with component a) and/or with component b) in an alternating manner.

[0235] However, it is preferred that the more than one cell penetrating peptide is positioned in the complex comprised by the combination for use according to the present invention in a consecutive manner and/or the more than one antigen or antigenic epitope is positioned in the complex comprised by the combination for use according to the present invention in a consecutive manner and/or the more than one TLR peptide agonist is positioned in the complex comprised by the combination for use according to the present invention in a consecutive manner. This means in particular that all single units of a certain component, i.e. all cell penetrating peptides, all antigens or antigenic epitopes or all TLR peptide agonists, which are comprised by the complex are positioned in a stretch, which is not interrupted by any of the other two components. Rather, the other two components are positioned in the complex for example before or after such a stretch of all single units of said certain component. However, the single units of said certain component positioned consecutively in such a way may be linked to each other for example by a spacer or linker as described herein, which is not of the other two components.

[0236] It is particularly preferred that each of the components a), b), and c) is positioned in a consecutive manner.

[0237] Structurally each component a), b), and c) typically comprises a single main chain and at least one side chain. The term "main chain" (also "backbone chain"), as used in the context of the present invention, refers to the main continuous chain of covalently bond atoms in a molecule. For example, in peptides, polypeptides and proteins, the main chain (backbone)

typically comprises alpha-carbon atoms and nitrogen atoms of the constituent amino acids linked by the peptide bond. The backbone does not include the side chains. The term "side chain" (also "pendant chain"), as used in the context of the present invention, refers to a chemical group that is attached to a core part of the molecule called "main chain" or backbone. For example, in peptides, polypeptides and proteins, the side chains typically represent the (main) parts of the constituent amino acids, which are attached to the alpha-carbon atoms of the backbone.

[0238] In the complex comprised by the combination for use according to the present invention, the components a), b), and c) may be covalently linked via a linker or spacer as described herein or they may be directly covalently linked. Independently of whether a spacer or linker is used for covalent linkage or not, there are in principle four options of how two of the three components are linked to each other in the complex comprised by the combination for use according to the present invention, namely:

1. (i) via main-chain/main-chain linkage,
2. (ii) via main-chain/side-chain linkage,
3. (iii) via side-chain/main-chain linkage or
4. (iv) via side-chain/side chain linkage.

[0239] Preferably, all three components a), b), and c) are linked via main-chain/main-chain linkage, thus resulting in particular in a main chain of the complex, which comprises the main chain of one or more cell penetrating peptide(s), the main chain of one or more antigen(s) or antigenic epitope(s), and the main chain of one or more TLR peptide agonist(s). In other words, the main chain of one or more cell penetrating peptide(s), the main chain of one or more antigen(s) or antigenic epitope(s), and the main chain of one or more TLR peptide agonist(s) constitute the main chain of the complex, optionally together with further components, for example linker(s) and spacer(s). Accordingly, the following arrangements of the components a), b), and c) are preferred, in particular if the at least one antigen or antigenic epitope is a peptide, polypeptide or protein, whereby said preferred arrangements are shown below in N-terminus -> C-terminus direction of the main chain of the complex and wherein all three components a), b), and c) are linked via main-chain/main-chain linkage and may be optionally linked by a linker, a spacer or another additional component:

(α) component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope) - component c) (at least one TLR peptide agonist);

(β) component c) (at least one TLR peptide agonist) - component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope);

(γ) component a) (cell penetrating peptide) - component c) (at least one TLR peptide agonist) - component b) (at least one antigen or antigenic epitope);

(δ) component c) (at least one TLR peptide agonist) - component b) (at least one antigen or

antigenic epitope) - component a) (cell penetrating peptide);

(ε) component b) (at least one antigen or antigenic epitope) - component a) (cell penetrating peptide) - component c) (at least one TLR peptide agonist); or

(ζ) component b) (at least one antigen or antigenic epitope) - component c) (at least one TLR peptide agonist) - component a) (cell penetrating peptide).

[0240] In particular if all three components a), b), and c) are linked via main-chain/main-chain linkage, it is preferred that the at least one antigen or antigenic epitope is positioned C-terminally of the cell penetrating peptide, whereby the cell penetrating peptide and the at least one antigen or antigenic epitope are optionally linked by a further component, e.g. a linker, a spacer, or by the at least one TLR peptide agonist. Accordingly, this corresponds to the arrangements (α), (β), and (γ) from the arrangements shown above, i.e. from the above arrangements arrangements (α), (β), and (γ) are more preferred.

[0241] Even more preferably, the at least one antigen or antigenic epitope is positioned C-terminally of the cell penetrating peptide, whereby the cell penetrating peptide and the at least one antigen or antigenic epitope are optionally linked by a further component, e.g. a linker, a spacer, but not by the at least one TLR peptide agonist. Accordingly, this corresponds to the arrangements (α) and (β) from the arrangements shown above, i.e. from the above arrangements arrangements (α) and (β) are even more preferred. Particularly preferably, the complex comprised by the combination for use according to the present invention is a recombinant polypeptide or a recombinant protein and the components a) to c) are positioned in N-terminus -> C-terminus direction of the main chain of said complex in the order:

(α)	component a) - component b) - component c); or
(β)	component c) - component a) - component b),

wherein the components may be linked by a further component, in particular by a linker or a spacer.

[0242] Particularly preferred is arrangement (α), wherein the at least one TLR agonist comprises or consists of at least one TLR2 agonist, for example:

(α1)	component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope) - one or more TLR2 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR2 peptide agonist(s);
(α2)	component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope) - one or more TLR2 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR2 peptide agonist(s), one or more TLR4 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR4 peptide
	agonist(s) and one or more TLR5 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR5 peptide agonist(s);
(α3)	component a) (cell penetrating peptide) - component b) (at least one antigen

	or antigenic epitope) - one or more TLR2 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR2 peptide agonist(s) and one or more TLR4 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR4 peptide agonist(s); or
(α4)	component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope) - one or more TLR2 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR2 peptide agonist(s) and one or more TLR5 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR5 peptide agonist(s).

[0243] Alternatively, in such an arrangement comprising a TLR2 peptide agonist, additional TLR peptide agonists may also be arranged at other positions in the complex, for example:

(α5)	one or more TLR4 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR4 peptide agonist(s) - component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope) - one or more TLR2 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR2 peptide agonist(s);
(α6)	one or more TLR5 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR5 peptide agonist(s) - component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope) - one or more TLR2 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR2 peptide agonist(s); or
(α7)	one or more TLR4 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR4 peptide agonist(s) and one or more TLR5 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR5 peptide agonist(s) - component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope) - one or more TLR2 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR2 peptide agonist(s).

[0244] Particularly preferred is arrangement (β), wherein the at least one TLR agonist comprises or consists of at least one TLR4 agonist, for example:

(β1)	one or more TLR4 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR4 peptide agonist(s) - component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope);
(β2)	one or more TLR4 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR4 peptide agonist(s), one or more TLR2 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR2 peptide agonist(s) and one or more TLR5 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR5 peptide agonist(s) - component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope);
(β3)	one or more TLR4 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR4 peptide agonist(s) and one or more TLR2 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR2 peptide agonist(s) - component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope); or
(β4)	one or more TLR4 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR4 peptide agonist(s) and one or more TLR5 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR5 peptide agonist(s) - component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope).

[0245] Alternatively, in such an arrangement comprising a TLR4 peptide agonist, additional TLR peptide agonists may also be arranged at other positions in the complex, for example:

(β5)	one or more TLR4 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR4 peptide agonist(s) - component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope) - one or more TLR2 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR2 peptide agonist(s);
(β6)	one or more TLR4 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR4 peptide agonist(s) - component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope) - one or more TLR5 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR5 peptide agonist(s); or
(β7)	one or more TLR4 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR4 peptide agonist(s) - component a) (cell penetrating peptide) - component b) (at least one antigen or antigenic epitope) - one or more TLR2 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR2 peptide agonist(s) and one or more TLR5 peptide agonist, e.g. 1, 2, 3, 4, or 5 TLR5 peptide agonist(s).

[0246] Alternatively, only two of the three components a), b), and c) are linked via main-chain/main-chain linkage in the complex comprised by the combination for use according to the present invention.

[0247] For example components a) and b) are linked via main-chain/main-chain linkage, resulting thus in the following arrangements of the components a) and b) in the complex, shown in N-terminus -> C-terminus direction of the main chain of the complex, whereby the components a) and b) may be optionally linked by a further component, e.g. a linker or a spacer:

1. (1) cell penetrating peptide (a) - antigen/antigenic epitope (b); or
2. (2) antigen/antigenic epitope (b) - cell penetrating peptide (a).

[0248] In such a case, component c), i.e. the at least one TLR peptide agonist, may then be arranged via main-chain/side-chain linkage, via side-chain/main-chain linkage or via side-chain/side chain linkage to either the cell penetrating peptide (a) or to the antigen/antigenic epitope (b) or, if present, to an additional component like a spacer or linker, which may be, for example, positioned between the cell penetrating peptide (a) and the antigen/antigenic epitope (b). This includes the following arrangements:

1. (i) component c) may be linked - optionally via a spacer or a linker - via main-chain/side-chain linkage to component a), i.e. the main chain of the at least one TLR peptide agonist is covalently linked - optionally via a spacer or a linker - to the side chain of the cell penetrating peptide;
2. (ii) component c) may be linked - optionally via a spacer or a linker - via side-chain/main-chain linkage to component a), i.e. the side chain of the at least one TLR peptide agonist is covalently linked - optionally via a spacer or a linker - to the main chain of the cell

penetrating peptide;

3. (iii) component c) may be linked - optionally via a spacer or a linker- via side-chain/side-chain linkage to component a), i.e. the side chain of the at least one TLR peptide agonist is covalently linked - optionally via a spacer or a linker - to the side chain of the cell penetrating peptide;
4. (iv) component c) may be linked - optionally via a spacer or a linker - via main-chain/side-chain linkage to component b), i.e. the main chain of the at least one TLR peptide agonist is covalently linked - optionally via a spacer or a linker - to the side chain of the at least one antigen or antigenic epitope;
5. (v) component c) may be linked - optionally via a spacer or a linker - via side-chain/main-chain linkage to component b), i.e. the side chain of the at least one TLR peptide agonist is covalently linked - optionally via a spacer or a linker - to the main chain of the at least one antigen or antigenic epitope;
6. (vi) component c) may be linked - optionally via a spacer or a linker- via side-chain/side-chain linkage to component b), i.e. the side chain of the at least one TLR peptide agonist is covalently linked - optionally via a spacer or a linker - to the side chain of the at least one antigen or antigenic epitope;
7. (vii) component c) may be linked - optionally via a spacer or a linker - via main-chain/side-chain linkage to a linker or a spacer positioned between component a) and component b), i.e. the main chain of the at least one TLR peptide agonist is covalently linked - optionally via a spacer or a linker - to the side chain of a linker or a spacer positioned between component a) and component b);
8. (viii) component c) may be linked - optionally via a spacer or a linker - via side-chain/main-chain linkage to a linker or a spacer positioned between component a) and component b), i.e. the side chain of the at least one TLR peptide agonist is covalently linked - optionally via a spacer or a linker - to the main chain of a linker or a spacer positioned between component a) and component b); or
9. (ix) component c) may be linked - optionally via a spacer or a linker- via side-chain/side-chain linkage to a linker or a spacer positioned between component a) and component b), i.e. the side chain of the at least one TLR peptide agonist is covalently linked - optionally via a spacer or a linker - to the side chain of a linker or a spacer positioned between component a) and component b).

[0249] For example components b) and c) are linked via main-chain/main-chain linkage, resulting thus in the following arrangements of the components b) and c) in the complex, shown in N-terminus -> C-terminus direction of the main chain of the complex, whereby the components b) and c) may be optionally linked by a further component, e.g. a linker or a spacer:

(3) antigen/antigenic epitope (b) - TLR peptide agonist (c); or

(4) TLR peptide agonist (c) - antigen/antigenic epitope (b).

[0250] In such a case, component a), i.e. the cell penetrating peptide, may then be arranged via main-chain/side-chain linkage, via side-chain/main-chain linkage or via side-chain/side chain linkage to either the antigen/antigenic epitope (b) or to the TLR peptide agonist (c) or, if present, to an additional component like a spacer or linker, which may be, for example, positioned between the antigen/antigenic epitope (b) and the TLR peptide agonist (c). This includes the following arrangements:

(x) component a) may be linked - optionally via a spacer or a linker - via main-chain/side-chain linkage to component b), i.e. the main chain of the cell penetrating peptide is covalently linked - optionally via a spacer or a linker - to the side chain of the at least one antigen or antigenic epitope;

(xi) component a) may be linked - optionally via a spacer or a linker - via side-chain/main-chain linkage to component b), i.e. the side chain of the cell penetrating peptide is covalently linked - optionally via a spacer or a linker - to the main chain of the at least one antigen or antigenic epitope;

(xii) component a) may be linked - optionally via a spacer or a linker - via side-chain/side-chain linkage to component b), i.e. the side chain of the cell penetrating peptide is covalently linked - optionally via a spacer or a linker - to the side chain of the at least one antigen or antigenic epitope;

(xiii) component a) may be linked - optionally via a spacer or a linker - via main-chain/side-chain linkage to component c), i.e. the main chain of the cell penetrating peptide is covalently linked - optionally via a spacer or a linker - to the side chain of the at least one TLR peptide agonist;

(xiv) component a) may be linked - optionally via a spacer or a linker - via side-chain/main-chain linkage to component c), i.e. the side chain the cell penetrating peptide is covalently linked - optionally via a spacer or a linker - to the main chain of the at least one TLR peptide agonist;

(xv) component a) may be linked - optionally via a spacer or a linker - via side-chain/side-chain linkage to component c), i.e. the side chain of the cell penetrating peptide is covalently linked - optionally via a spacer or a linker - to the side chain of the at least one TLR peptide agonist;

(xvi) component a) may be linked - optionally via a spacer or a linker - via main-chain/side-chain linkage to a linker or a spacer positioned between component b) and component c), i.e. the main chain of the cell penetrating peptide is covalently linked - optionally via a spacer or a linker - to the side chain of a linker or a spacer positioned between component b) and component c);

(xvii) component a) may be linked - optionally via a spacer or a linker - via side-chain/main-chain linkage to a linker or a spacer positioned between component b) and component c), i.e. the side chain of the cell penetrating peptide is covalently linked - optionally via a spacer or a

linker - to the main chain of a linker or a spacer positioned between component b) and component c); or

(xviii) component a) may be linked - optionally via a spacer or a linker - via side-chain/side-chain linkage to a linker or a spacer positioned between component b) and component c), i.e. the side chain of the cell penetrating peptide is covalently linked - optionally via a spacer or a linker - to the side chain of a linker or a spacer positioned between component b) and component c).

[0251] For example components a) and c) are linked via main-chain/main-chain linkage, resulting thus in the following arrangements of the components a) and b) in the complex, shown in N-terminus -> C-terminus direction of the main chain of the complex, whereby the components a) and c) may be optionally linked by a further component, e.g. a linker or a spacer:

(5) cell penetrating peptide (a) - TLR peptide agonist (c); or

(6) TLR peptide agonist (c) - cell penetrating peptide (a).

[0252] In such a case, component b), i.e. the at least one antigen or antigenic epitope, may then be arranged via main-chain/side-chain linkage, via side-chain/main-chain linkage or via side-chain/side chain linkage to either the cell penetrating peptide (a) or to the TLR peptide agonist (c) or, if present, to an additional component like a spacer or linker, which may be, for example, positioned between the cell penetrating peptide (a) and the TLR peptide agonist (c). This includes the following arrangements:

(xix) component b) may be linked - optionally via a spacer or a linker - via main-chain/side-chain linkage to component a), i.e. the main chain of the at least one antigen or antigenic epitope is covalently linked - optionally via a spacer or a linker - to the side chain of the cell penetrating peptide;

(xx) component b) may be linked - optionally via a spacer or a linker - via side-chain/main-chain linkage to component a), i.e. the side chain of the at least one antigen or antigenic epitope is covalently linked - optionally via a spacer or a linker - to the main chain of the cell penetrating peptide;

(xxi) component b) may be linked - optionally via a spacer or a linker - via side-chain/side-chain linkage to component a), i.e. the side chain of the at least one antigen or antigenic epitope is covalently linked - optionally via a spacer or a linker - to the side chain of the cell penetrating peptide;

(xxii) component b) may be linked - optionally via a spacer or a linker - via main-chain/side-chain linkage to component c), i.e. the main chain of the at least one antigen or antigenic

epitope is covalently linked - optionally via a spacer or a linker - to the side chain of the at least one TLR peptide agonist;

(xxiii) component b) may be linked - optionally via a spacer or a linker - via side-chain/main-chain linkage to component c), i.e. the side chain of the at least one antigen or antigenic epitope is covalently linked - optionally via a spacer or a linker - to the main chain of the at least one TLR peptide agonist;

(xxiv) component b) may be linked - optionally via a spacer or a linker - via side-chain/side-chain linkage to component c), i.e. the side chain of the at least one antigen or antigenic epitope is covalently linked - optionally via a spacer or a linker - to the side chain of the at least one TLR peptide agonist;

(xxv) component b) may be linked - optionally via a spacer or a linker - via main-chain/side-chain linkage to a linker or a spacer positioned between component a) and component c), i.e. the main chain of the at least one antigen or antigenic epitope is covalently linked - optionally via a spacer or a linker - to the side chain of a linker or a spacer positioned between component a) and component c);

(xxvi) component b) may be linked - optionally via a spacer or a linker - via side-chain/main-chain linkage to a linker or a spacer positioned between component a) and component c), i.e. the side chain of the at least one antigen or antigenic epitope is covalently linked - optionally via a spacer or a linker - to the main chain of a linker or a spacer positioned between component a) and component c); or

(xxvii) component b) may be linked - optionally via a spacer or a linker - via side-chain/side-chain linkage to a linker or a spacer positioned between component a) and component c), i.e. the side chain of the at least one antigen or antigenic epitope is covalently linked - optionally via a spacer or a linker - to the side chain of a linker or a spacer positioned between component a) and component c).

[0253] Alternatively, it is also conceivable that in the complex comprised by the combination for use according to the present invention all three of the components a), b), and c) are arranged via main-chain/side-chain linkage, via side-chain/main-chain linkage or via side-chain/side chain linkage, optionally linked by an additional component, e.g. a spacer or a linker.

Preferred combinations of a preferred immune checkpoint modulator and a preferred complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope

[0254] As described above, a preferred combination for use according to the present invention comprises a preferred immune checkpoint modulator as described herein. Moreover, a preferred combination for use according to the present invention comprises a preferred

complex as described herein comprising a (preferred) cell penetrating peptide, at least one (preferred) antigen or antigenic epitope and at least one (preferred) TLR peptide agonist.

[0255] A more preferred combination for use according to the present invention comprises (i) a preferred immune checkpoint modulator as described herein and (ii) a preferred complex comprising as described herein comprising a (preferred) cell penetrating peptide, at least one (preferred) antigen or antigenic epitope and at least one (preferred) TLR peptide agonist. In the following preferred embodiments of a preferred combination for use according to the present invention are described.

[0256] In a preferred combination for use according to the present invention the complex is a recombinant polypeptide or a recombinant protein wherein

1. a) the cell penetrating peptide has an amino acid sequence comprising or consisting of an amino acid sequence according to SEQ ID NO: 6 (CPP3/Z13), SEQ ID NO: 7 (CPP4/Z14), SEQ ID NO: 8 (CPP5/Z15), or SEQ ID NO: 11 (CPP8/Z18), or sequence variants thereof as described herein without abrogating said peptide's cell penetrating ability;
2. b) the at least one antigen or antigenic epitope is a peptide, polypeptide or protein and, preferably, comprises or consists of at least one cancer epitope or tumor epitope; and
3. c) the TLR peptide agonist is a TLR2 peptide agonist and/or a TLR4 peptide agonist.

[0257] It is also preferred in the combination for use according to the present invention that the immune checkpoint modulator is a modulator of CD40, CTLA-4, PD-L1, PD-L2, PD-1 and/or IDO, preferably the immune checkpoint modulator is an inhibitor of CTLA-4, PD-L1, PD-L2, PD-1 and/or IDO or an activator of CD40, more preferably the immune checkpoint modulator is an inhibitor of CTLA-4, PD-L1, PD-1 and/or IDO and even more preferably the immune checkpoint modulator is an inhibitor of CTLA-4 and/or PD-1.

[0258] It is also preferred in the combination for use according to the present invention that the immune checkpoint modulator is a modulator of CD40, LAG3, CTLA-4, PD-L1, PD-L2, PD-1 and/or IDO, preferably the immune checkpoint modulator is an inhibitor of LAG3, CTLA-4, PD-L1, PD-L2, PD-1 and/or IDO or an activator of CD40, more preferably the immune checkpoint modulator is an inhibitor of LAG3, CTLA-4, PD-L1, PD-1 and/or IDO and even more preferably the immune checkpoint modulator is an inhibitor of LAG3, CTLA-4 and/or PD-1.

[0259] For example, in a preferred combination for use according to the present invention

- the complex is a recombinant polypeptide or a recombinant protein wherein
 1. a) the cell penetrating peptide has an amino acid sequence comprising or consisting of an amino acid sequence according to SEQ ID NO: 6 (CPP3/Z13), SEQ ID NO: 7 (CPP4/Z14), SEQ ID NO: 8 (CPP5/Z15), or SEQ ID NO: 11 (CPP8/Z18), or sequence variants thereof as described herein without abrogating

said peptide's cell penetrating ability;

2. b) the at least one antigen or antigenic epitope is a peptide, polypeptide or protein and, preferably, comprises or consists of at least one cancer epitope or tumor epitope; and
 3. c) the TLR peptide agonist is a TLR2 peptide agonist and/or a TLR4 peptide agonist; and
- the immune checkpoint modulator is a modulator of the CD40 pathway, of the CTLA-4 pathway and/or of the PD-1 pathway, in particular the immune checkpoint modulator is an activator of CD40 or an inhibitor of CD40, CTLA-4, PD-L1, PD-L2 and/or PD-1, more preferably the immune checkpoint modulator is an inhibitor of CTLA-4, PD-L1, PD-L2 and/or PD-1, and even more preferably the immune checkpoint modulator is an inhibitor of CTLA-4 and/or PD-1.

[0260] For example, in a preferred combination for use according to the present invention

- the complex is a recombinant polypeptide or a recombinant protein wherein
 1. a) the cell penetrating peptide has an amino acid sequence comprising or consisting of an amino acid sequence according to SEQ ID NO: 6 (CPP3/Z13), SEQ ID NO: 7 (CPP4/Z14), SEQ ID NO: 8 (CPP5/Z15), or SEQ ID NO: 11 (CPP8/Z18), or sequence variants thereof as described herein without abrogating said peptide's cell penetrating ability;
 2. b) the at least one antigen or antigenic epitope is a peptide, polypeptide or protein and, preferably, comprises or consists of at least one cancer epitope or tumor epitope; and
 3. c) the TLR peptide agonist is a TLR2 peptide agonist and/or a TLR4 peptide agonist; and

the immune checkpoint modulator is a modulator of the CD40 pathway, of the CTLA-4 pathway, of the LAG3 pathway, and/or of the PD-1 pathway, in particular the immune checkpoint modulator is an activator of CD40 or an inhibitor of CD40, LAG3, CTLA-4, PD-L1, PD-L2 and/or PD-1, more preferably the immune checkpoint modulator is an inhibitor of LAG3, CTLA-4, PD-L1, PD-L2 and/or PD-1, and even more preferably the immune checkpoint modulator is an inhibitor of LAG3, CTLA-4 and/or PD-1.

[0261] In a preferred combination for use as described herein, in particular in such a preferred combination for use as described above, it is preferred if more than one immune checkpoint modulator, e.g. checkpoint inhibitor, is used, in particular if at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 distinct immune checkpoint modulators, e.g. checkpoint inhibitors, are used, preferably 2, 3, 4 or 5 distinct immune checkpoint modulators, e.g. checkpoint inhibitors, are used, more preferably 2, 3 or 4 distinct immune checkpoint modulators, e.g. checkpoint inhibitors, are used, even more preferably 2 or 3 distinct immune checkpoint modulators, e.g. checkpoint inhibitors, are used and most preferably 2 distinct immune checkpoint modulators, e.g. checkpoint inhibitors, are used.

[0262] Thereby it is preferred that at least (i) an inhibitor of the CTLA-4 pathway, in particular an inhibitor of CTLA-4 and (ii) an inhibitor of the PD-1 pathway, in particular an inhibitor of PD-1, PD-L1 or PD-L2 are used; preferably at least (i) an inhibitor of CTLA-4 and (ii) an inhibitor of PD-1 are used.

Use in medicine

[0263] The combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein is for use in medicine.

[0264] Such a combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein is able to initiate or enhance the efficacy of checkpoint modulators. Moreover, such a combination as described herein can simultaneously (i) stimulate multi-epitopic cytotoxic T cell-mediated immunity, (ii) induce T_h cells and (iii) promote immunological memory, thereby generating potent, long lasting (anti-tumor) immunity. Immunological memory is essential to protect against relapse, in particular against tumor relapse. Taken together, the present invention thus provides to a combination as described herein which is able to initiate, enable, enhance or improve an immune response, in particular which enables, enhances or improves the subject's or tumor response to checkpoint modulators.

[0265] The combination for use according to the present invention can be useful in a variety of diseases. Preferably the combination as described herein is for use (for the preparation of a medicament) for the prevention, treatment or stabilization of a disease or disorder, such as those which can be treated by immunotherapy, including cancers, infectious diseases, autoimmunity disorders, hematological diseases and transplant rejections. Accordingly, a combination as described herein for use in the prevention, treatment or stabilization of a disease or disorder, such as those which can be treated by immunotherapy, including cancers, infectious diseases, autoimmunity disorders, hematological diseases and transplant rejections is preferred.

[0266] In the context of the present invention, it is particularly preferred that the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein is used in the prevention and/or treatment of cancer.

[0267] The term "disease" as used in the context of the present invention is intended to be generally synonymous, and is used interchangeably with, the terms "disorder" and "condition" (as in medical condition), in that all reflect an abnormal condition of the human or animal body or of one of its parts that impairs normal functioning, is typically manifested by distinguishing

signs and symptoms, and causes the human or animal to have a reduced duration or quality of life.

[0268] Preferred diseases to be treated and/or prevented by use of the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein include cancer, hematological disorders, infectious diseases, autoimmunity disorders and transplant rejections. Thereby, treatment and/or prevention of cancer and infectious diseases is preferred and treatment and/or prevention of cancer is more preferred. For cancer, a malignant neoplasm of the brain or a malignant neoplasm of lymphoid, hematopoietic and related tissue are preferred and glioblastoma is more preferred.

[0269] Preferably, the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein may be used for (the preparation of a medicament for) the prophylaxis, treatment and/or amelioration of cancer or tumor diseases, including diseases caused by defective apoptosis, preferably selected from acoustic neurinoma, anal carcinoma, astrocytoma, basalioma, Behcet's syndrome, bladder cancer, blastomas, bone cancer, brain metastases, brain tumors, brain cancer (glioblastomas), breast cancer (mamma carcinoma), Burkitt's lymphoma, carcinoids, cervical cancer, colon carcinoma, colorectal cancer, corpus carcinoma, craniopharyngeomas, CUP syndrome, endometrial carcinoma, gall bladder cancer, genital tumors, including cancers of the genitourinary tract, glioblastoma, gliomas, head/neck tumors, hepatomas, histocytic lymphoma, Hodgkin's syndromes or lymphomas and non-Hodgkin's lymphomas, hypophysis tumor, intestinal cancer, including tumors of the small intestine, and gastrointestinal tumors, Kaposi's sarcoma, kidney cancer, kidney carcinomas, laryngeal cancer or larynx cancer, leukemia, including acute myeloid leukaemia (AML), erythroleukemia, acute lymphoid leukaemia (ALL), chronic myeloid leukaemia (CML), and chronic lymphocytic leukaemia (CLL), lid tumor, liver cancer, liver metastases, lung carcinomas (= lung cancer = bronchial carcinoma), small cell lung carcinomas and non-small cell lung carcinomas, and lung adenocarcinoma, lymphomas, lymphatic cancer, malignant melanomas, mammary carcinomas (= breast cancer), medulloblastomas, melanomas, meningiomas, Mycosis fungoides, neoplastic diseases neurinoma, oesophageal cancer, oesophageal carcinoma (= oesophageal cancer), oligodendroglioma, ovarian cancer (= ovarian carcinoma), ovarian carcinoma, pancreatic carcinoma (= pancreatic cancer), penile cancer, penis cancer, pharyngeal cancer, pituitary tumour, plasmocytoma, prostate cancer (= prostate tumors), rectal carcinoma, rectal tumors, renal cancer, renal carcinomas, retinoblastoma, sarcomas, Schneeberger's disease, skin cancer, e.g. melanoma or non-melanoma skin cancer, including basal cell and squamous cell carcinomas as well as psoriasis, pemphigus vulgaris, soft tissue tumours, spinalioma, stomach cancer, testicular cancer, throat cancer, thymoma, thyroid carcinoma, tongue cancer, urethral cancer, uterine cancer, vaginal cancer, various virus-induced tumors such as, for example, papilloma virus-induced carcinomas (e.g. cervical carcinoma = cervical cancer), adenocarcinomas, herpes virus-induced tumors (e.g. Burkitt's lymphoma, EBV-induced B-cell lymphoma, cervix carcinoma), heptatitis B-induced tumors (hepatocell carcinomas), HTLV-1-

and HTLV-2-induced lymphomas, vulval cancer, wart conditions or involvement. In the present context, the terms "therapy" and "therapeutic" preferably mean to have at least some minimal physiological effect upon being administered to a living body. For example, a physiological effect upon administering a "therapeutic" anti-tumor compound may be the inhibition of tumor growth, or decrease in tumor size, or prevention reoccurrence of the tumor. Preferably, in the treatment of cancer or neoplastic disease, a compound which inhibits the growth of a tumor or decreased the size of the tumor or prevents the reoccurrence of the tumor would be considered therapeutically effective. The term "anti-tumor drug" therefore preferably means any therapeutic agent having therapeutic effect against a tumor, neoplastic disease or cancer.

[0270] Further preferred examples of cancers to be treated with the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein include brain cancer, prostate cancer, breast cancer, ovarian cancer, esophageal cancer, lung cancer, liver cancer, kidney cancer, melanoma, gut carcinoma, lung carcinoma, head and neck squamous cell carcinoma, chronic myeloid leukemia, colorectal carcinoma, gastric carcinoma, endometrial carcinoma, myeloid leukemia, lung squamous cell carcinoma, acute lymphoblastic leukemia, acute myelogenous leukemia, bladder tumor, promyelocytic leukemia, non-small cell lung carcinoma, and sarcoma. Most preferably, the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein are used to treat colorectal cancer.

[0271] The cancer may be a solid tumor, blood cancer, or lymphatic cancer. The cancer may be benign or metastatic.

[0272] Preferably, the cancer to be prevented and/or treated is a glioma, more preferably highly invasive glioblastoma multiforme (GBM). Gliomas are the most frequent form of primary brain tumors in adults, with glioblastoma multiforme (GBM) having the poorest prognosis. This tumor is notorious for its highly invasive and aggressive behavior. In particular, the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein may be used in conjunction with existing modalities for glioma, more specifically highly invasive GBM. T lymphocytes can actively seek out neoplastic cells in the brain, and have the potential to safely eliminate specific tumor cells without damaging the surrounding healthy tissues.

[0273] Moreover, the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein may be used for (the preparation of a medicament for) the prophylaxis, treatment and/or amelioration of infectious diseases, preferably viral, retroviral, bacterial or protozoological infectious diseases. Such infectious diseases are typically selected from AIDS, anthrax, Japanese encephalitis, bacterial infectious diseases such as miscarriage (prostate inflammation), anthrax, appendicitis,

borreliosis, botulism, *Camphylobacter*, *Chlamydia trachomatis* (inflammation of the urethra, conjunctivitis), cholera, diphtheria, donovanosis, epiglottitis, typhus fever, gas gangrene, gonorrhoea, rabbit fever, *Helicobacter pylori*, whooping cough, climatic bubo, osteomyelitis, Legionnaire's disease, chicken-pox, condyloma acuminata, cytomegalic virus (CMV), dengue fever, early summer meningoencephalitis (ESME), Ebola virus, colds, fifth disease, foot-and-mouth disease, herpes simplex type I, herpes simplex type II, herpes zoster, HSV, infectious diseases caused by parasites, protozoa or fungi, such as amoebiasis, bilharziosis, Chagas disease, *Echinococcus*, fish tapeworm, fish poisoning (*Ciguatera*), fox tapeworm, athlete's foot, canine tapeworm, candidosis, yeast fungus spots, scabies, cutaneous Leishmaniasis, lambliasis (giardiasis), lice, onchocercosis (river blindness), fungal diseases, bovine tapeworm, schistosomiasis, porcine tapeworm, toxoplasmosis, trichomoniasis, trypanosomiasis (sleeping sickness), visceral Leishmaniasis, nappy/diaper dermatitis or miniature tapeworm, infectious erythema, influenza, Kaposi's sarcoma, Lassa fever, Leishmaniasis, leprosy, listeriosis, Lyme borreliosis, malaria, Marburg virus infection, measles, meningitis, including bacterial meningitis, molluscum contagiosum, mononucleosis, mumps, *Mycoplasma hominis*, neonatal sepsis (Chorioamnionitis), noma, Norwalk virus infection, otitis media, paratyphus, Pfeiffer's glandular fever, plague, pneumonia, polio (poliomyelitis, childhood lameness), pseudo-croup, rabies, Reiter's syndrome, Rocky Mountain spotted fever, *Salmonella paratyphus*, *Salmonella typhus*, SARS, scarlet fever, shingles, hepatitis, smallpox, soft chancre, syphilis, tetanus, three-day fever, tripper, tsutsugamushi disease, tuberculosis, typhus, vaginitis (colpitis), viral diseases caused by cytomegalovirus (CMV), orthopox variola virus, orthopox alastrim virus, parapox ovis virus, molluscum contagiosum virus, herpes simplex virus 1, herpes simplex virus 2, herpes B virus, varicella zoster virus, pseudorabies virus, human cytomegaly virus, human herpes virus 6, human herpes virus 7, Epstein-Barr virus, human herpes virus 8, hepatitis B virus, chikungunya virus, O'nyong'nyong virus, rubivirus, hepatitis C virus, GB virus C, West Nile virus, dengue virus, yellow fever virus, louping ill virus, St. Louis encephalitis virus, Japan B encephalitis virus, Powassan virus, FSME virus, SARS, SARS-associated corona virus, human corona virus 229E, human corona virus Oc43, Torovirus, human T cell lymphotropic virus type I, human T cell lymphotropic virus type II, HIV (AIDS), i.e. human immunodeficiency virus type 1 or human immunodeficiency virus type 2, influenza virus, Lassa virus, lymphocytic choriomeningitis virus, Tacaribe virus, Junin virus, Machupo virus, Borna disease virus, Bunyamwera virus, California encephalitis virus, Rift Valley fever virus, sand fly fever virus, Toscana virus, Crimean-Congo haemorrhagic fever virus, Hazara virus, Khasan virus, Hantaan virus, Seoul virus, Prospect Hill virus, Puumala virus, Dobrava Belgrade virus, Tula virus, sin nombre virus, Lake Victoria Marburg virus, Zaire Ebola virus, Sudan Ebola virus, Ivory Coast Ebola virus, influenza virus A, influenza virus B, influenza viruses C, parainfluenza virus, measles virus, mumps virus, respiratory syncytial virus, human metapneumovirus, vesicular stomatitis Indiana virus, rabies virus, Mokola virus, Duvenhage virus, European bat lyssavirus 1 + 2, Australian bat lyssavirus, adenoviruses A-F, human papilloma viruses, condyloma virus 6, condyloma virus 11, polyoma viruses, adeno-associated virus 2, rotaviruses, or orbiviruses, Varicella including Varicella zoster, and malaria parasite (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium knowlesi*), viral infectious diseases such as AIDS, infectious diseases caused by *Condyloma acuminata*, hollow warts, Dengue fever, three-day fever, Ebola virus, cold, early summer meningoencephalitis (FSME),

flu, shingles, hepatitis, herpes simplex type I, herpes simplex type II, Herpes zoster, influenza, Japanese encephalitis, Lassa fever, Marburg virus, warts, West Nile fever, yellow fever.

[0274] Further preferred examples of infectious diseases include diseases caused by viruses, bacteria, fungi, protozoa and multicellular parasites. They include, for instance, Amoebiasis, Anthrax, Buruli Ulcer (*Mycobacterium ulcerans*), Caliciviruses associated diarrhoea, *Campylobacter* diarrhoea, Cervical Cancer (Human papillomavirus), *Chlamydia trachomatis* associated genital diseases, Cholera, Crimean-Congo haemorrhagic fever, Dengue Fever, Diphtheria, Ebola haemorrhagic fever, Enterotoxigenic *Escherichia coli* (ETEC) diarrhoea, Gastric Cancer (*Helicobacter pylori*), Gonorrhea, Group A *Streptococcus* associated diseases, Group B *Streptococcus* associated diseases, *Haemophilus influenzae* B pneumonia and invasive disease, Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis E diarrhoea, Herpes simplex type 2 genital ulcers, HIV/AIDS, Hookworm Disease, Influenza, Japanese encephalitis, Lassa Fever, Leishmaniasis, Leptospirosis, Liver cancer (Hepatitis B), Liver Cancer (Hepatitis C), Lyme Disease, Malaria, Marburg haemorrhagic fever, Measles, Mumps, Nasopharyngeal cancer (Epstein-Barr virus), *Neisseria meningitidis* Meningitis, Parainfluenza associated pneumonia, Pertussis, Plague, Poliomyelitis, Rabies, Respiratory syncytial virus (RSV) pneumonia, Rift Valley fever, Rotavirus diarrhoea, Rubella, Schistosomiasis, Severe Acute Respiratory Syndrome (SARS), Shigellosis, Smallpox, *Staphylococcus aureus* associated diseases, Stomach Cancer (*Helicobacter pylori*), *Streptococcus pneumoniae* and invasive disease, Tetanus, Tick-borne encephalitis, Trachoma, Tuberculosis, Tularaemia, Typhoid fever, West-Nile virus associated disease, Yellow fever.

[0275] Moreover, the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein may be used for (the preparation of a medicament for) the prophylaxis, treatment and/or amelioration of autoimmune disorders, for example autoimmune diseases of the CNS, auto-inflammatory diseases, Celiac disease; Sjogren's syndrome, systemic lupus erythematosus. Typically, autoimmune diseases arise from an abnormal immune response of the body against substances and tissues normally present in the body (autoimmunity). This may be restricted to certain organs (e.g. in autoimmune thyroiditis) or may involve a particular tissue in different places (e.g. Goodpasture's disease which may affect the basement membrane in both the lung and the kidney). Autoimmune diseases may be classified by corresponding type of hypersensitivity: type I (i.e. urticaria induced by autologous serum), type II, type III, or type IV.

[0276] Examples of autoimmune diseases include Blau syndrome, Bullous pemphigoid, Cancer, Castleman's disease, Celiac disease, Chagas disease, Chronic inflammatory demyelinating polyneuropathy, Chronic recurrent multifocal osteomyelitis, chronic obstructive pulmonary disease, Churg-Strauss syndrome, Cicatricial pemphigoid, Cogan syndrome, Cold agglutinin disease, Complement component 2 deficiency, Contact dermatitis, Cranial arteritis, CREST syndrome, Crohn's disease, Cushing's Syndrome, Dercum's disease, Dermatitis herpetiformis, Dermatomyositis, Diabetes mellitus type 1, Diffuse cutaneous systemic sclerosis, Dressler's syndrome, lupus, Discoid lupus erythematosus, Eczema, Acute disseminated

encephalomyelitis (ADEM), Addison's disease, Agammaglobulinemia, Amyotrophic lateral sclerosis (Also Lou Gehrig's disease; Motor Neuron Disease), Ankylosing Spondylitis, Antiphospholipid syndrome, Antisynthetase syndrome, Atopic dermatitis, Autoimmune aplastic anemia, Autoimmune cardiomyopathy, Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune inner ear disease, Autoimmune lymphoproliferative syndrome, Autoimmune peripheral neuropathy, Autoimmune pancreatitis, Autoimmune polyendocrine syndrome, Autoimmune progesterone dermatitis, Autoimmune thrombocytopenic purpura, Autoimmune urticarial, Autoimmune uveitis, Balo disease/Balo concentric sclerosis, Behcet's disease, Berger's disease, Bickerstaff's encephalitis, Endometriosis, Enthesitis-related arthritis, Eosinophilic gastroenteritis, Epidermolysis bullosa acquisita, Erythroblastosis fetalis, Evan's syndrome, Fibrodysplasia ossificans, Fibrosing alveolitis (or Idiopathic pulmonary fibrosis), Gastritis, Glomerulonephritis, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome, Hashimoto's encephalopathy, Hashimoto's thyroiditis, Gestational Pemphigoid, Hidradenitis suppurativa, Hypogammaglobulinemia, Idiopathic thrombocytopenic purpura (Autoimmune thrombocytopenic purpura), IgA nephropathy, Occular cicatricial pemphigoid, Inclusion body myositis, Rheumatoid arthritis, Chronic inflammatory Rheumatic fever, demyelinating polyneuropathy, Sarcoidosis, Palindromic rheumatism, Interstitial cystitis, Juvenile idiopathic Schizophrenia, PANDAS (pediatric arthritis aka Juvenile autoimmune rheumatoid arthritis), Schmidt syndrome, neuropsychiatric Kawasaki's disease another form of APS, Schnitzler syndrome, Paraneoplastic cerebellar myasthenic syndrome, Leukocytoclastic Serum Sickness, Lichen planus, Sjögren's syndrome, Lichen sclerosus, Parsonage-Turner, Linear IgA disease, Still's disease, Pemphigus vulgaris, Lupoid hepatitis, Autoimmune hepatitis, Stiff person syndrome, Pernicious anaemia, Subacute bacterial endocarditis (SBE), POEMS syndrome, Lupus erythematosus, Sweet's syndrome, Sympathetic ophthalmia, Meniere's disease, Systemic lupus, Primary biliary cirrhosis, Miller-Fisher syndrome, Takayasu's arteritis, cholangitis, Progressive inflammatory neuropathy, Mucha-Habermann disease, Psoriasis, Psoriatic arthritis, Pyoderma gangrenosum, Multiple sclerosis, Pure red cell aplasia, Rasmussen's encephalitis, Myasthenia gravis, Transverse myelitis, Raynaud phenomenon, Microscopic colitis, Ulcerative colitis, Myositis, idiopathic inflammatory bowel disease (IBD), Neuromyelitis optica, Devic's disease, and Neuromyotonia.

[0277] Moreover, the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein may be used for (the preparation of a medicament for) the prophylaxis, treatment and/or amelioration of hematological disorders, which are typically disorders which primarily affect the blood. Thereby, hematological malignancies are preferred.

[0278] Examples of hematological diseases include myeloid disorders, including Hemoglobinopathies (congenital abnormality of the hemoglobin molecule or of the rate of hemoglobin synthesis), e.g. Sickle-cell disease, Thalassemia, Methemoglobinemia; Anemias (lack of red blood cells or hemoglobin), e.g. Iron deficiency anemia, Megaloblastic anemia including Vitamin B₁₂ deficiency, Pernicious anemia, and Folate deficiency, Hemolytic anemias (destruction of red blood cells) including Genetic disorders of RBC membrane such as

Hereditary spherocytosis, Hereditary elliptocytosis, and Congenital dyserythropoietic anemia, Genetic disorders of RBC metabolism such as Glucose-6-phosphate dehydrogenase deficiency (G6PD), and Pyruvate kinase deficiency, Immune mediated hemolytic anemia (direct Coombs test is positive) such as Autoimmune hemolytic anemia including Warm antibody autoimmune hemolytic anemia (such as Idiopathic, Systemic lupus erythematosus (SLE), and Evans' syndrome (antiplatelet antibodies and hemolytic antibodies)) and Cold antibody autoimmune hemolytic anemia (such as Idiopathic cold hemagglutinin syndrome, Infectious mononucleosis, and Paroxysmal cold hemoglobinuria), Alloimmune hemolytic anemia including Hemolytic disease of the newborn (HDN) (such as Rh disease (Rh D), ABO hemolytic disease of the newborn, Anti-Kell hemolytic disease of the newborn, Rhesus c hemolytic disease of the newborn, Rhesus E hemolytic disease of the newborn, and other blood group incompatibility (RhC, Rhe, Kid, Duffy, MN, P and others)), Drug induced immune mediated hemolytic anemia including Penicillin (high dose) and Methyldopa, Hemoglobinopathies (i.e. where there is an unstable or crystalline hemoglobin), Paroxysmal nocturnal hemoglobinuria (rare acquired clonal disorder of red blood cell surface proteins), Direct physical damage to RBCs including Microangiopathic hemolytic anemia and Secondary to artificial heart valve(s), Aplastic anemia such as Fanconi anemia, Diamond-Blackfan anemia (inherited pure red cell aplasia), and Acquired pure red cell aplasia; Decreased numbers of cells, e.g. Myelodysplastic syndrome, Myelofibrosis, Neutropenia (decrease in the number of neutrophils), Agranulocytosis, Glanzmann's thrombasthenia, and Thrombocytopenia (decrease in the number of platelets) including Idiopathic thrombocytopenic purpura (ITP), Thrombotic thrombocytopenic purpura (TTP), and Heparin-induced thrombocytopenia (HIT); Myeloproliferative disorders (Increased numbers of cells), e.g. Polycythemia vera (increase in the number of cells in general), Erythrocytosis (increase in the number of red blood cells), Leukocytosis (increase in the number of white blood cells), Thrombocytosis (increase in the number of platelets), and Myeloproliferative disorder; Coagulopathies (disorders of bleeding and coagulation), e.g. Thrombocytosis, Recurrent thrombosis, Disseminated intravascular coagulation, Disorders of clotting proteins including Hemophilia such as Hemophilia A, Hemophilia B (also known as Christmas disease), and Hemophilia C, Von Willebrand disease, Disseminated intravascular coagulation, Protein S deficiency, and Antiphospholipid syndrome, and Disorders of platelets including Thrombocytopenia, Glanzmann's thrombasthenia, and Wiskott-Aldrich syndrome. Moreover, examples of hematological diseases also include hematological malignancies, e.g. lymphomas including Hodgkin's disease and Non-Hodgkin's lymphoma such as Burkitt's lymphoma, Anaplastic large cell lymphoma, Splenic marginal zone lymphoma, Hepatosplenic T-cell lymphoma, and Angioimmunoblastic T-cell lymphoma (AILT), Myelomas such as Multiple myeloma, Waldenström macroglobulinemia, and Plasmacytoma, Leukemias such as Acute lymphocytic leukemia (ALL), Chronic lymphocytic leukemia (CLL), Acute myelogenous leukemia (AML), Chronic Idiopathic Myelofibrosis (MF), Chronic myelogenous leukemia (CML), T-cell prolymphocytic leukemia (T-PLL), B-cell prolymphocytic leukemia (B-PLL), Chronic neutrophilic leukemia (CNL), Hairy cell leukemia (HCL), T-cell large granular lymphocyte leukemia (T-LGL), and Aggressive NK-cell leukemia. Moreover, examples of hematological diseases also include miscellaneous haematological diseases including Hemochromatosis, Asplenia, Hypersplenism such as Gaucher's disease, Monoclonal gammopathy of undetermined significance, Hemophagocytic lymphohistiocytosis, and Tempel's syndrome.

Moreover, examples of hematological diseases also include hematological changes secondary to non-hematological disorders including Anemia of chronic disease, Infectious mononucleosis, AIDS, Malaria, and Leishmaniasis.

[0279] Moreover, the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein may be used for (the preparation of a medicament for) the prophylaxis, treatment and/or amelioration of transplant rejection, including e.g. graft-versus-host reaction. Transplant rejection includes hyperacute rejection, acute rejection and chronic rejection of a transplant. Examples of transplant rejection include skin, kidney, heart, lung, pancreas, liver, blood cell, bone marrow, cornea, accidental severed limb, in particular fingers, hand, foot, face, nose, bone, cardiac valve, blood vessel or intestine transplant rejection reaction.

[0280] In general, "combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein" means that the therapy with the immune checkpoint modulator as described herein is combined with the therapy with the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein. In other words, even if one component (the checkpoint modulator or the complex) is not administered, e.g., at the same day as the other component (the other of checkpoint modulator or complex), their treatment schedules are typically intertwined. This means that "a combination" in the context of the present invention does in particular not include the start of a therapy with one component (the checkpoint modulator or the complex) after the therapy with the other component (the other of checkpoint modulator or complex) is finished. Thereby, a "finished" therapy means in particular that the active component does not exert its effects anymore - i.e. a "therapy" may in particular be finished several minutes, hours or days after the last administration of the active component, depending on how long the active component exerts its effects. In more general, an "intertwined" treatment schedule of the checkpoint modulator and the complex - and, thus, a combination of the checkpoint modulator and the complex - means that

1. (i) not every administration of the checkpoint modulator (and therefore the complete checkpoint modulator therapy) is completed for more than one week (preferably for more than 3 days, more preferably for more than 2 days, even more preferably for more than a day) before the first administration of the complex (and therefore the complete therapy with the complex) starts; or
2. (ii) not every administration of the complex (and therefore the complete therapy with the complex) is completed for more than one week (preferably for more than 3 days, more preferably for more than 2 days, even more preferably for more than a day) before the first administration of the checkpoint modulator (and therefore the complete checkpoint modulator therapy) starts.

[0281] For example, in the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein for use according to the present invention, one component (the checkpoint modulator or the complex) may be administered once a week and the other component (the other of checkpoint modulator or complex) may be administered once a month. To achieve in this example "a combination" in the sense of the present invention the monthly administered component is to be administered at least once in the same week, in which also the weekly administered other component is administered.

[0282] As outlined above, the administration of the immune checkpoint modulator and/or of the complex comprised by the combination for use according to the present invention may require multiple successive administrations, e.g. multiple injections. Thus, the administration may be repeated at least two times, for example once as primary immunization injections and, later, as booster injections.

[0283] In particular, the immune checkpoint modulator and/or the complex comprised by the combination for use according to the present invention may be administered repeatedly or continuously. The immune checkpoint modulator and/or the complex comprised by the combination for use according to the present invention may be administered repeatedly or continuously for a period of at least 1, 2, 3, or 4 weeks; 2, 3, 4, 5, 6, 8, 10, or 12 months; or 2, 3, 4, or 5 years. For example, the immune checkpoint modulator comprised by the combination for use according to the present invention may be administered twice per day, once per day, every two days, every three days, once per week, every two weeks, every three weeks, once per month or every two months. For example, the complex comprised by the combination for use according to the present invention may be administered twice per day, once per day, every two days, every three days, once per week, every two weeks, every three weeks, once per month or every two months. Preferably, the complex comprised by the combination for use according to the present invention may be administered repeatedly, for example once per week or (once) every two weeks.

[0284] In the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein for use according to the present invention, the immune checkpoint modulator and the complex are preferably administered at about the same time.

[0285] "At about the same time", as used herein, means in particular simultaneous administration or that directly after administration of the immune checkpoint modulator the complex is administered or directly after administration of the complex the immune checkpoint modulator is administered. The skilled person understands that "directly after" includes the time necessary to prepare the second administration - in particular the time necessary for exposing and disinfecting the location for the second administration as well as appropriate preparation of the "administration device" (e.g., syringe, pump). Simultaneous administration

also includes if the periods of administration of the checkpoint modulator and of the complex overlap or if, for example, one component (checkpoint modulator or complex) is administered over a longer period of time, such as 30 min, 1 h, 2 h or even more, e.g. by infusion, and the other component (checkpoint modulator or complex) is administered at some time during such a long period. Administration of the immune checkpoint modulator and of the complex at about the same time is in particular preferred if different routes of administration and/or different administration sites are used.

[0286] It is also preferred in the combination of the immune checkpoint modulator as described herein and of the complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope and at least one TLR peptide agonist as described herein for use according to the present invention that the immune checkpoint modulator and the complex are administered consecutively. For example, the immune checkpoint modulator is preferably administered before the complex. It is also preferred that the immune checkpoint modulator is administered after the complex.

[0287] In consecutive administration, the time between administration of the first component (the checkpoint modulator or the complex) and administration of the second component (the other of the checkpoint modulator and the complex) is preferably no more than one week, more preferably no more than 3 days, even more preferably no more than 2 days and most preferably no more than 24 h. It is particularly preferred that the checkpoint modulator and the complex are administered at the same day with the time between administration of the first component (the checkpoint modulator or the complex) and administration of the second component (the other of the checkpoint modulator and the complex) being preferably no more than 6 hours, more preferably no more than 3 hours, even more preferably no more than 2 hours and most preferably no more than 1 h.

[0288] Preferably, the immune checkpoint modulator comprised by the combination for use according to the present invention and the complex comprised by the combination for use according to the present invention are administered in a therapeutically effective amount. A "therapeutically effective amount", as used herein, is the amount which is sufficient for the alleviation of the symptoms of the disease or condition being treated and/or for prophylaxis of the symptoms of the disease or condition being prevented. In other words, a "therapeutically effective amount" means an amount of the complex and/or of the checkpoint modulator that is sufficient to significantly induce a positive modification of a disease or disorder, i.e. an amount of the complex and/or of the checkpoint modulator, that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought. The term also includes the amount of the complex and/or of the immune checkpoint modulator sufficient to reduce the progression of the disease, notably to reduce or inhibit the tumor growth or infection and thereby elicit the response being sought, in particular such response could be an immune response directed against the antigens or antigenic epitopes comprised in by the complex (i.e. an "inhibition effective amount"). At the same time, however, a "therapeutically effective amount" is preferably small enough to avoid serious side-effects, that is to say to permit a sensible relationship between advantage and risk. The determination of these limits typically

lies within the scope of sensible medical judgment. A "therapeutically effective amount" of the complex and/or of the checkpoint modulator, will furthermore vary in connection with the particular condition to be treated and also with the age and physical condition of the patient to be treated, the body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, the activity of the specific components (checkpoint modulator and complex), the severity of the condition, the duration of the treatment, the nature of the accompanying therapy, of the particular pharmaceutically acceptable carrier used, and similar factors, within the knowledge and experience of the accompanying doctor.

[0289] The dosage administered, as single or multiple doses, to an individual will thus vary depending upon a variety of factors, including pharmacokinetic properties, subject conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired.

[0290] Preferably, for cancer treatment, the therapeutically effective dose of the complex comprised by the combination for use according to the present invention is from about 0.001 mg to 10 mg, preferably from about 0.01 mg to 5 mg, more preferably from about 0.1 mg to 2 mg per injection or from about 0.01 nmol to 1 mmol per injection, in particular from 1 nmol to 1 mmol per injection, preferably from 1 μ mol to 1 mmol per injection. It is also preferred if the therapeutically effective dose of the complex comprised by the combination for use according to the present invention is (per kg body weight), in particular for cancer treatment, from about 0.01 mg/kg to 100 mg/kg, preferably from about 0.05 mg/kg to 50 mg/kg, more preferably from about 0.1 mg/kg to 25 mg/kg, even more preferably from about 0.5 mg/kg to 10 mg/kg and most preferably from about 1 mg/kg to 5 mg/kg.

[0291] Preferably, the therapeutically effective dose of the immune checkpoint modulator comprised by the combination for use according to the present invention is (per kg body weight), in particular for cancer treatment, from about 0.01 mg/kg to 100 mg/kg, preferably from about 0.05 mg/kg to 50 mg/kg, more preferably from about 0.1 mg/kg to 25 mg/kg, even more preferably from about 0.5 mg/kg to 15 mg/kg and most preferably from about 1 mg/kg to 10 mg/kg.

[0292] The complex comprised by the combination for use according to the present invention and the immune checkpoint modulator comprised by the combination for use according to the present invention can be administered by various routes of administration, for example, systemically or locally. Routes for systemic administration in general include, for example, transdermal, oral and parenteral routes, which include subcutaneous, intravenous, intramuscular, intraarterial, intradermal and intraperitoneal routes and/or intranasal administration routes. Routes for local administration in general include, for example, topical administration routes, but also administration directly at the site of affliction, such as intratumoral administration.

[0293] Preferably, the complex comprised by the combination for use according to the present invention and the immune checkpoint modulator comprised by the combination for use

according to the present invention are administered by a parenteral route of administration. More preferably, the complex comprised by the combination for use according to the present invention and the immune checkpoint modulator comprised by the combination for use according to the present invention are administered via intravenous, intratumoral, intradermal, subcutaneous, intramuscular, intranasal, or intranodal route. Even more preferably, the complex comprised by the combination for use according to the present invention and the immune checkpoint modulator comprised by the combination for use according to the present invention are administered intravenously and/or subcutaneously. It is also more preferred that the complex comprised by the combination for use according to the present invention and the immune checkpoint modulator comprised by the combination for use according to the present invention are administered intradermally and/or subcutaneously

[0294] Preferably, the complex comprised by the combination for use according to the present invention and the immune checkpoint modulator comprised by the combination for use according to the present invention are administered via the same route of administration, preferably via the same parenteral route of administration, more preferably intravenously or subcutaneously.

[0295] However, it is more preferred that the complex comprised by the combination for use according to the present invention and the immune checkpoint modulator comprised by the combination for use according to the present invention are administered via distinct routes of administration, preferably via distinct parenteral routes of administration, more preferably the immune checkpoint modulator comprised by the combination for use according to the present invention is administered intravenously and the complex comprised by the combination for use according to the present invention is administered via intratumoral, intradermal, subcutaneous, intramuscular, intranasal, or intranodal route, preferably the complex comprised by the combination for use according to the present invention is administered subcutaneously. Even more preferably the immune checkpoint modulator comprised by the combination for use according to the present invention is administered intravenously and/or the complex comprised by the combination for use according to the present invention is administered subcutaneously or intradermally.

[0296] The complex comprised by the combination for use according to the present invention and the immune checkpoint modulator comprised by the combination for use according to the present invention may be provided in the same or in distinct compositions.

[0297] Preferably, the complex comprised by the combination for use according to the present invention and the immune checkpoint modulator comprised by the combination for use according to the present invention are provided in distinct compositions. Thereby, different other components, e.g. different vehicles, can be used for the complex and for the checkpoint modulator. Moreover, the complex and the immune checkpoint modulator can be administered via different routes of administration and the doses (in particular the relation of the doses) can be adjusted according to the actual need.

[0298] However, it is also preferred that the immune checkpoint modulator and the complex are provided in the same composition. Such a composition comprising both, the immune checkpoint modulator and the complex is described in more detail below ("composition according to the present invention").

[0299] No matter whether a composition comprises only the immune checkpoint modulator (and not the complex), only the complex (and not the checkpoint modulator) or both, such a composition may be a pharmaceutical composition and/or a vaccine composition.

[0300] In particular, such a composition, which comprises only the immune checkpoint modulator (and not the complex), only the complex (and not the checkpoint modulator) or both, is preferably a (pharmaceutical) composition which optionally comprises a pharmaceutically acceptable carrier and/or vehicle, or any excipient, buffer, stabilizer or other materials well known to those skilled in the art.

[0301] As a further ingredient, the (pharmaceutical) composition may in particular comprise a pharmaceutically acceptable carrier and/or vehicle. In the context of the present invention, a pharmaceutically acceptable carrier typically includes the liquid or non-liquid basis of the (pharmaceutical) composition. If the (pharmaceutical) composition is provided in liquid form, the carrier will typically be pyrogen-free water; isotonic saline or buffered (aqueous) solutions, e.g. phosphate or citrate buffered solutions. Particularly for injection of the (pharmaceutical) composition, water or preferably a buffer, more preferably an aqueous buffer, may be used, containing a sodium salt, preferably at least 30 mM of a sodium salt, a calcium salt, preferably at least 0.05 mM of a calcium salt, and optionally a potassium salt, preferably at least 1 mM of a potassium salt. According to a preferred embodiment, the sodium, calcium and, optionally, potassium salts may occur in the form of their halogenides, e.g. chlorides, iodides, or bromides, in the form of their hydroxides, carbonates, hydrogen carbonates, or sulfates. Examples of sodium salts include e.g. NaCl, NaI, NaBr, Na₂CO₃, NaHCO₃, Na₂SO₄, examples of the optional potassium salts include e.g. KCl, KI, KBr, K₂CO₃, KHCO₃, K₂SO₄, and examples of calcium salts include e.g. CaCl₂, CaI₂, CaBr₂, CaCO₃, CaSO₄, Ca(OH)₂. Furthermore, organic anions of the aforementioned cations may be contained in the buffer. According to a more preferred embodiment, the buffer suitable for injection purposes as defined above, may contain salts selected from sodium chloride (NaCl), calcium chloride (CaCl₂) and optionally potassium chloride (KCl), wherein further anions may be present additional to the chlorides. CaCl₂ can also be replaced by another salt like KCl. Typically, the salts in the injection buffer are present in a concentration of at least 30 mM sodium chloride (NaCl), at least 1 mM potassium chloride (KCl) and at least 0.05 mM calcium chloride (CaCl₂). The injection buffer may be hypertonic, isotonic or hypotonic with reference to the specific reference medium, i.e. the buffer may have a higher, identical or lower salt content with reference to the specific reference medium, wherein preferably such concentrations of the afore mentioned salts may be used, which do not lead to damage of cells due to osmosis or other concentration effects. Reference media are e.g. liquids occurring in "*in vivo*" methods, such as blood, lymph, cytosolic liquids, or other body liquids, or e.g. liquids, which may be used as reference media in "*in vitro*"

methods, such as common buffers or liquids. Such common buffers or liquids are known to a skilled person. Saline (0.9% NaCl) and Ringer-Lactate solution are particularly preferred as a liquid basis.

[0302] Preferably, the (pharmaceutical) composition, which comprises the immune checkpoint modulator and/or the complex as described herein, further comprises arginine, such as L-arginine.

[0303] However, one or more compatible solid or liquid fillers or diluents or encapsulating compounds may be used as well for the (pharmaceutical) composition, which are suitable for administration to a subject to be treated. The term "compatible" as used herein means that these constituents of the (pharmaceutical) composition are capable of being mixed with the complex according to the present invention as defined above in such a manner that no interaction occurs which would substantially reduce the pharmaceutical effectiveness of the (pharmaceutical) composition under typical use conditions. Pharmaceutically acceptable carriers, fillers and diluents must, of course, have sufficiently high purity and sufficiently low toxicity to make them suitable for administration to a subject to be treated. Some examples of compounds which can be used as pharmaceutically acceptable carriers, fillers or constituents thereof are sugars, such as, for example, lactose, glucose and sucrose; starches, such as, for example, corn starch or potato starch; cellulose and its derivatives, such as, for example, sodium carboxymethylcellulose, ethylcellulose, cellulose acetate; powdered tragacanth; malt; gelatin; tallow; solid glidants, such as, for example, stearic acid, magnesium stearate; calcium sulfate; vegetable oils, such as, for example, groundnut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil from theobroma; polyols, such as, for example, polypropylene glycol, glycerol, sorbitol, mannitol and polyethylene glycol; alginic acid.

[0304] As described above, the (pharmaceutical) composition may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, and sublingual injection or infusion techniques.

[0305] Preferably, the (pharmaceutical) composition may be administered by parenteral injection, more preferably by subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, and sublingual injection or via infusion techniques. Sterile injectable forms of the (pharmaceutical) compositions may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are

conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation of the (pharmaceutical) composition.

[0306] For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will preferably be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be included, as required. Whether it is a polypeptide, peptide, or nucleic acid molecule, other pharmaceutically useful compound according to the present invention that is to be given to an individual, administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated.

[0307] The (pharmaceutical) composition as described herein may also be administered orally in any orally acceptable dosage form including capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient, i.e. the inventive transporter cargo conjugate molecule as defined above, is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

[0308] The (pharmaceutical) composition may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, e.g. including diseases of the skin or of any other accessible epithelial tissue. Suitable topical formulations are readily prepared for each of these areas or organs. For topical applications, the (pharmaceutical) composition may be formulated in a suitable ointment, containing the inventive immunostimulatory composition, particularly its components as defined above, suspended or dissolved in one or more carriers. Carriers for topical administration include mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the (pharmaceutical) composition can be formulated in a suitable lotion or cream. In the context of the present

invention, suitable carriers include mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[0309] In this context, prescription of treatment, e.g. decisions on dosage when using the above (pharmaceutical) composition is typically within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in REMINGTON'S PHARMACEUTICAL SCIENCES, 16th edition, Osol, A. (ed), 1980.

[0310] Accordingly, the (pharmaceutical) composition typically comprises a therapeutically effective amount of the components of the (pharmaceutical) composition, in particular of the complex and/or of the checkpoint modulator. The (pharmaceutical) composition may be used for human and also for veterinary medical purposes, preferably for human medical purposes, as a (pharmaceutical) composition in general or as a vaccine.

[0311] (Pharmaceutical) compositions, in particular vaccine compositions, or formulations according to the invention may be administered as a pharmaceutical formulation which can contain the complex as described herein and/or the checkpoint modulator as described herein in any form described herein.

[0312] The terms "pharmaceutical formulation" and "pharmaceutical composition" as used in the context of the present invention refer in particular to preparations which are in such a form as to permit biological activity of the active ingredient(s) to be unequivocally effective and which contain no additional component which would be toxic to subjects to which the said formulation would be administered.

[0313] In the context of the present invention, an "efficacy" of a treatment can be measured based on changes in the course of a disease in response to a use according to the present invention. For example, the efficacy of a treatment of cancer can be measured by a reduction of tumor volume, and/or an increase of progression free survival time, and/or a decreased risk of relapse post-resection for primary cancer. More specifically for cancer treated by immunotherapy, assessment of efficacy can be by the spectrum of clinical patterns of antitumor response for immunotherapeutic agents through novel immune-related response criteria (irRC), which are adapted from Response Evaluation Criteria in Solid Tumors (RECIST) and World Health Organization (WHO) criteria (J. Natl. Cancer Inst. 2010, 102(18): 1388-1397). The efficacy of prevention of infectious disease is ultimately assessed by epidemiological studies in human populations, which often correlates with titres of neutralizing antibodies in sera, and induction of multifunctional pathogen specific T cell responses. Preclinical assessment can include resistance to infection after challenge with infectious pathogen. Treatment of an infectious disease can be measured by inhibition of the pathogen's growth or elimination of the pathogen (and, thus, absence of detection of the pathogen), correlating with pathogen specific antibodies and/or T cell immune responses.

[0314] (Pharmaceutical) compositions, in particular vaccine compositions, or formulations according to the invention may also be administered as a pharmaceutical formulation which can contain antigen presenting cells loaded with the complex as described herein in any form described herein.

[0315] The vaccine and/or the composition according to the present invention may also be formulated as (pharmaceutical) compositions and unit dosages thereof, in particular together with a conventionally employed adjuvant, immunomodulatory material, carrier, diluent or excipient as described above and below, and in such form may be employed as solids, such as tablets or filled capsules, or liquids such as solutions, suspensions, emulsions, elixirs, or capsules filled with the same, all for oral use, or in the form of sterile injectable solutions for parenteral (including subcutaneous and intradermal) use by injection or continuous infusion.

[0316] In the context of the present invention, in particular in the context of a (pharmaceutical) composition and vaccines according to the present invention, injectable compositions are typically based upon injectable sterile saline or phosphate-buffered saline or other injectable carriers known in the art. Such (pharmaceutical) compositions and unit dosage forms thereof may comprise ingredients in conventional proportions, with or without additional active compounds or principles, and such unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed.

[0317] Examples of suitable adjuvants and/or immunomodulatory materials in the context of the present invention include MPL[®] (Corixa), aluminum-based minerals including aluminum compounds (generically called Alum), ASO1-4, MF59, CalciumPhosphate, Liposomes, Iscom, polyinosinic:polycytidylic acid (polyIC), including its stabilized form poly-ICLC (Hiltonol), CpG oligodeoxynucleotides, Granulocyte-macrophage colony-stimulating factor (GM-CSF), lipopolysaccharide (LPS), Montanide, polylactide co-glycolide (PLG), Flagellin, Soap Bark tree saponins (QS21), amino alkyl glucosamide compounds (e.g. RC529), two component antibacterial peptides with synthetic oligodeoxynucleotides (e.g. IC31), Imiquimod, Resiquimod, Immunostimulatory sequences (ISS), monophosphoryl lipid A (MPLA), and Fibroblast-stimulating lipopeptide (FSL1).

[0318] Compositions, in particular pharmaceutical compositions and vaccines, according to the present invention may be liquid formulations including aqueous or oily suspensions, solutions, emulsions, syrups, and elixirs. The compositions may also be formulated as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain additives including suspending agents, emulsifying agents, nonaqueous vehicles and preservatives. Suspending agents include sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminum stearate gel, and hydrogenated edible fats. Emulsifying agents include lecithin, sorbitan monooleate, and acacia. Preservatives include methyl or propyl p-hydroxybenzoate and sorbic acid. Dispersing or wetting agents include poly(ethylene glycol), glycerol, bovine serum albumin, Tween[®], Span[®].

[0319] Compositions, in particular pharmaceutical compositions and vaccines, according to the present invention may also be formulated as a depot preparation, which may be administered by implantation or by intramuscular injection.

[0320] Compositions, in particular pharmaceutical compositions and vaccines, according to the present invention may also be solid compositions, which may be in the form of tablets or lozenges formulated in a conventional manner. For example, tablets and capsules for oral administration may contain conventional excipients including binding agents, fillers, lubricants, disintegrants and wetting agents. Binding agents include syrup, accacia, gelatin, sorbitol, tragacanth, mucilage of starch and polyvinylpyrrolidone. Fillers include lactose, sugar, microcrystalline cellulose, maizestarch, calcium phosphate, and sorbitol. Lubricants include magnesium stearate, stearic acid, talc, polyethylene glycol, and silica. Disintegrants include potato starch and sodium starch glycollate. Wetting agents include sodium lauryl sulfate. Tablets may be coated according to methods well known in the art.

[0321] Compositions, in particular pharmaceutical compositions and vaccines, according to the present invention may also be administered in sustained release forms or from sustained release drug delivery systems.

[0322] Moreover, the compositions, in particular pharmaceutical compositions and vaccines, according to the present invention may be adapted for delivery by repeated administration.

[0323] Further materials as well as formulation processing techniques, which are useful in the context of compositions, in particular pharmaceutical compositions and vaccines, according to the present invention or in the context of their preparation are set out in "Part 5 of Remington's "The Science and Practice of Pharmacy", 22nd Edition, 2012, University of the Sciences in Philadelphia, Lippincott Williams & Wilkins".

Kit according to the present invention

[0324] In a further aspect, the present invention also provides a kit, in particular a kit of parts, comprising

1. (i) an immune checkpoint modulator and
2. (ii) a complex comprising:
 1. a) a cell penetrating peptide;
 2. b) at least one antigen or antigenic epitope; and
 3. c) at least one TLR peptide agonist,

wherein the components a) - c) comprised by the complex are covalently linked.

[0325] In particular, such a kit according to the present invention comprises (i) the immune checkpoint modulator as described above (in the context of the combination for use according

to the present invention) and (ii) the complex as described above (in the context of the combination for use according to the present invention). In other words, preferred embodiments of the immune checkpoint modulator as described above (in the context of the combination for use according to the present invention) are also preferred in the kit according to the present invention. Accordingly, preferred embodiments of the complex as described above (in the context of the combination for use according to the present invention) are also preferred in the kit according to the present invention.

[0326] The various components of the kit may be packaged in one or more containers. The above components may be provided in a lyophilized or dry form or dissolved in a suitable buffer. For example, the kit may comprise a (pharmaceutical) composition comprising the immune checkpoint modulator as described above and a (pharmaceutical) composition comprising the complex as described above, e.g. with each composition in a separate container. The kit may also comprise a (pharmaceutical) composition comprising both, the immune checkpoint modulator and the complex, as described above.

[0327] The kit may also comprise additional reagents including, for instance, preservatives, growth media, and/or buffers for storage and/or reconstitution of the above-referenced components, washing solutions.

[0328] In addition, the kit-of-parts according to the present invention may optionally contain instructions of use. Preferably, the kit further comprises a package insert or label with directions to treat a disease as described herein, for example cancer, by using a combination of the immune checkpoint modulator and the complex.

[0329] Such a kit may preferably be for use in medicine as described herein, in particular for use in the prevention and/or treatment of cancer as described herein.

[0330] Moreover, the present invention also provides a vaccination kit for treating, preventing and/or stabilizing a disease, e.g. a cancer or an infectious disease, comprising the pharmaceutical composition comprising the immune checkpoint modulator as described herein and the pharmaceutical composition, in particular the vaccine, comprising the complex as described herein (wherein the pharmaceutical compositions may be the same or different) and instructions for use of said pharmaceutical composition or of said vaccine.

Composition according to the present invention

[0331] In a further aspect, the present invention also provides a composition comprising

1. (i) an immune checkpoint modulator and
2. (ii) a complex comprising:
 1. a) a cell penetrating peptide;
 2. b) at least one antigen or antigenic epitope; and

3. c) at least one TLR peptide agonist,

wherein the components a) - c) comprised by the complex are covalently linked.

[0332] In particular, such a composition according to the present invention comprises (i) the immune checkpoint modulator as described above (in the context of the combination for use according to the present invention) and (ii) the complex as described above (in the context of the combination for use according to the present invention). In other words, preferred embodiments of the immune checkpoint modulator as described above (in the context of the combination for use according to the present invention) are also preferred in the composition according to the present invention. Accordingly, preferred embodiments of the complex as described above (in the context of the combination for use according to the present invention) are also preferred in the composition according to the present invention.

[0333] Moreover, a composition comprising the immune checkpoint modulator as described above and the complex as described above and preferred embodiments of such a composition are described above (in the context of the combination for use according to the present invention). It is understood that the same description, in particular the same preferred embodiments as described above for the composition apply accordingly to the composition as described here.

[0334] Accordingly, the composition preferably comprises a pharmaceutically acceptable carrier. Preferred examples of such a pharmaceutically acceptable carrier are as described above.

[0335] It is also preferred that the composition comprises at least two different complexes. Moreover, a preferred composition comprises at least two different immune checkpoint modulators.

[0336] Such a composition may preferably be for use in medicine as described herein, in particular for use in the prevention and/or treatment of cancer as described herein.

[0337] Moreover, it is preferred that the composition is a vaccine.

[0338] As used in the context of the present invention, the term "vaccine" refers to a biological preparation that provides innate and/or adaptive immunity, typically to a particular disease, preferably cancer. Thus, a vaccine supports in particular an innate and/or an adaptive immune response of the immune system of a subject to be treated. For example, the antigen or antigenic epitope of the complex as described herein typically leads to or supports an adaptive immune response in the patient to be treated, and the TLR peptide agonist of the complex as described herein may lead to or support an innate immune response.

[0339] The vaccine may also comprise a pharmaceutically acceptable carrier, adjuvant, and/or vehicle as defined below for the inventive pharmaceutical composition. In the specific context

of the vaccine, the choice of a pharmaceutically acceptable carrier is determined in principle by the manner in which the inventive vaccine is administered. The inventive vaccine can be administered, for example, systemically or locally as described above. More preferably, vaccines may be administered by an intravenous, intradermal, subcutaneous, or intramuscular route. The vaccine is therefore preferably formulated in liquid (or sometimes in solid) form. The suitable amount of the vaccine to be administered can be determined by routine experiments with animal models. Such models include rabbit, sheep, mouse, rat, dog and non-human primate models. Preferred unit dose forms for injection include sterile solutions of water, physiological saline or mixtures thereof. The pH of such solutions should be adjusted to about 7.4. Suitable carriers for injection include hydrogels, devices for controlled or delayed release, polylactic acid and collagen matrices. Suitable pharmaceutically acceptable carriers for topical application include those which are suitable for use in lotions, creams, gels. If the inventive vaccine is to be administered orally, tablets and capsules are the preferred unit dose form. The pharmaceutically acceptable carriers for the preparation of unit dose forms which can be used for oral administration are well known in the prior art. The choice thereof will depend on secondary considerations such as taste, costs and storability, which are not critical for the purposes of the present invention, and can be made without difficulty by a person skilled in the art.

[0340] The vaccine can additionally contain one or more auxiliary substances in order to further increase its immunogenicity. A synergistic action of the inventive complex as defined above and of an auxiliary substance, which may be optionally contained in the inventive vaccine as described above, is preferably achieved thereby. Depending on the various types of auxiliary substances, various mechanisms can come into consideration in this respect. For example, compounds that permit the maturation of dendritic cells (DCs), for example lipopolysaccharides or TNF-alpha, form a first class of suitable auxiliary substances. In general, it is possible to use as auxiliary substance any agent that influences the immune system in the manner of a "danger signal" (LPS, GP96) or cytokines, such as GM-CSF, which allow an immune response produced by the immune-stimulating adjuvant according to the invention to be enhanced and/or influenced in a targeted manner. Particularly preferred auxiliary substances are cytokines, such as monokines, lymphokines, interleukins or chemokines, that further promote the innate immune response, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IFN-alpha, IFN-beta, IFN-gamma, GM-CSF, G-CSF, M-CSF, LT-beta or TNF-alpha, growth factors, such as hGH.

[0341] Further additives which may be included in the vaccine are emulsifiers, such as, for example, Tween®; wetting agents, such as, for example, sodium lauryl sulfate; colouring agents; taste-imparting agents, pharmaceutical carriers; tablet-forming agents; stabilizers; antioxidants; preservatives.

[0342] The inventive vaccine can also additionally contain any further compound, which is known to be immune-stimulating due to its binding affinity (as ligands) to human Toll-like

receptors TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, or due to its binding affinity (as ligands) to murine Toll-like receptors TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12 or TLR13.

[0343] Another class of compounds, which may be added to the vaccine in this context, may be CpG nucleic acids, in particular CpG-RNA or CpG-DNA. A CpG-RNA or CpG-DNA can be a single-stranded CpG-DNA (ss CpG-DNA), a double-stranded CpG-DNA (dsDNA), a single-stranded CpG-RNA (ss CpG-RNA) or a double-stranded CpG-RNA (ds CpG-RNA). The CpG nucleic acid is preferably in the form of CpG-RNA, more preferably in the form of single-stranded CpG-RNA (ss CpG-RNA). The CpG nucleic acid preferably contains at least one or more (mitogenic) cytosine/guanine dinucleotide sequence(s) (CpG motif(s)). According to a first preferred alternative, at least one CpG motif contained in these sequences, in particular the C (cytosine) and the G (guanine) of the CpG motif, is unmethylated. All further cytosines or guanines optionally contained in these sequences can be either methylated or unmethylated. According to a further preferred alternative, however, the C (cytosine) and the G (guanine) of the CpG motif can also be present in methylated form.

[0344] Preferably, the (pharmaceutical) composition, in particular the vaccine, as described above is for use in the prevention and/or treatment of diseases or disorders including for example cancers, hematological disorders, infectious diseases, autoimmunity disorders and transplant rejections, whereby cancer is preferred.

[0345] It is also preferred that the (pharmaceutical) composition, in particular the vaccine, is for use in treating a subject, preferably a mammalian subject, and most preferably a human subject, who is suffering from a disease or disorder, in particular from a disorder that can be treated by immunotherapy such as cancers, infectious diseases, autoimmunity disorders and transplant rejections.

Nucleic acid molecule

[0346] In another aspect, the invention also provides a nucleic acid molecule comprising a polynucleotide encoding the immune checkpoint modulator according to the present invention as described above, wherein the immune checkpoint modulator is a (poly)peptide or a protein, and/or encoding the complex according to the present invention as described above, wherein the complex is a (poly)peptide or a protein, in particular a fusion (poly)peptide or a fusion protein. Most preferably, the nucleic acid (molecule) encodes at least an exemplified complex as described herein or a functional sequence variant thereof. Accordingly, the nucleic acid (molecule) most preferably comprises a polynucleotide encoding an amino acid sequence according to any of SEQ ID NOs: 26 - 32, or 41 - 45 or an amino acid sequence sharing at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with any of SEQ ID NOs: 26 - 32, or 41 - 45.

[0347] Examples of nucleic acid molecules and/or polynucleotides include, e.g., a recombinant

polynucleotide, a vector, an oligonucleotide, an RNA molecule such as an rRNA, an mRNA, an miRNA, an siRNA, or a tRNA, or a DNA molecule such as a cDNA.

[0348] The term "vector" refers to a nucleic acid molecule, preferably to a recombinant nucleic acid molecule, i.e. a nucleic acid molecule which does not occur in nature. A vector in the context of the present invention is suitable for incorporating or harboring a desired nucleic acid sequence. Such vectors may be storage vectors, expression vectors, cloning vectors, transfer vectors. A storage vector is a vector which allows the convenient storage of a nucleic acid molecule. Thus, the vector may comprise a sequence corresponding, e.g., to a desired complex according to the present invention as described above and/or a sequence corresponding, e.g., to a desired immune checkpoint modulator according to the present invention as described above. An expression vector may be used for production of expression products such as RNA, e.g. mRNA, or peptides, polypeptides or proteins. For example, an expression vector may comprise sequences needed for transcription of a sequence stretch of the vector, such as a promoter sequence. A cloning vector is typically a vector that contains a cloning site, which may be used to incorporate nucleic acid sequences into the vector. A cloning vector may be, e.g., a plasmid vector or a bacteriophage vector. A transfer vector may be a vector which is suitable for transferring nucleic acid molecules into cells or organisms, for example, viral vectors. A vector in the context of the present invention may be, e.g., an RNA vector or a DNA vector. Preferably, a vector is a DNA molecule. For example, a vector in the sense of the present application comprises a cloning site, a selection marker, such as an antibiotic resistance factor, and a sequence suitable for multiplication of the vector, such as an origin of replication. Preferably, a vector in the context of the present application is a plasmid vector

[0349] In particular, the present invention provides a kit comprising

1. (i) a nucleic acid molecule comprising a polynucleotide encoding an immune checkpoint modulator, wherein the immune checkpoint modulator is a (poly)peptide or a protein; and
2. (ii) a nucleic acid molecule comprising a polynucleotide encoding a complex, the complex comprising:
 1. a) a cell penetrating peptide;
 2. b) at least one antigen or antigenic epitope; and
 3. c) at least one TLR peptide agonist,

wherein the complex is a (poly)peptide or a protein, in particular a fusion (poly)peptide or a fusion protein.

[0350] In such a kit according to the present invention preferred immune checkpoint modulator are those as described herein and preferred complexes are those as described herein.

[0351] Moreover, a kit preferably also comprises a package insert or label, for example with directions to express the immune checkpoint modulator and the complex and/or to treat cancer by using a combination of the immune checkpoint modulator and the complex.

[0352] Such a kit is preferably for use in medicine, for example for use in the prevention and/or treatment of cancer.

[0353] As described above, the nucleic acid molecule(s) in the kit are preferably selected from the group consisting of a vector as described above, such as an expression vector; an RNA molecule such as an rRNA, an mRNA, an miRNA, an siRNA, or a tRNA; or a DNA molecule, such as a cDNA.

[0354] In the kit according to the present invention the immune checkpoint modulator and the complex may be encoded by the same nucleic acid molecule or, preferably, by distinct nucleic acid molecules.

[0355] Accordingly, the present invention also provides a nucleic acid molecule comprising

1. (i) a polynucleotide encoding an immune checkpoint modulator, wherein the immune checkpoint modulator is a (poly)peptide or a protein; and
2. (ii) a polynucleotide encoding a complex, the complex comprising:
 1. a) a cell penetrating peptide;
 2. b) at least one antigen or antigenic epitope; and
 3. c) at least one TLR peptide agonist,

wherein the complex is a (poly)peptide or a protein, in particular a fusion (poly)peptide or a fusion protein.

[0356] In such a nucleic acid molecule according to the present invention preferred immune checkpoint modulator are those as described herein and preferred complexes are those as described herein. Moreover, the nucleic acid molecule is preferably selected from the group consisting of a vector as described above, such as an expression vector; an RNA molecule such as an rRNA, an mRNA, an miRNA, an siRNA, or a tRNA; or a DNA molecule, such as a cDNA. For example, the nucleic acid molecule may be an expression vector or an RNA molecule, e.g. an mRNA molecule, encoding the complex and the immune checkpoint inhibitor in a bicistronic manner.

[0357] Furthermore, the present invention also provides a composition comprising

1. (i) a nucleic acid molecule comprising a polynucleotide encoding an immune checkpoint modulator, wherein the immune checkpoint modulator is a (poly)peptide or a protein; and
2. (ii) a nucleic acid molecule comprising a polynucleotide encoding a complex, the complex comprising:
 1. a) a cell penetrating peptide;
 2. b) at least one antigen or antigenic epitope; and
 3. c) at least one TLR peptide agonist,

wherein the complex is a (poly)peptide or a protein, in particular a fusion (poly)peptide or a fusion protein.

[0358] In such a composition according to the present invention preferred immune checkpoint modulator are those as described herein and preferred complexes are those as described herein. Moreover, the nucleic acid molecule(s) are preferably selected from the group consisting of a vector as described above, such as an expression vector; an RNA molecule such as an rRNA, an mRNA, an miRNA, an siRNA, or a tRNA; or a DNA molecule, such as a cDNA.

[0359] The composition may also comprise further components, for example those described above for a (pharmaceutical) composition according to the present invention comprising the complex and/or the checkpoint inhibitor as described herein.

Method and combination therapy

[0360] In a further aspect, the present description provides a method for treating a subject, preferably a mammalian subject, and most preferably a human subject, who is suffering from a disease or disorder, in particular from a disorder that can be treated by immunotherapy such as cancers, infectious diseases, autoimmunity disorders and transplant rejections, comprising administering to the subject

1. (i) an immune checkpoint modulator and
2. (ii) a complex comprising:
 1. a) a cell penetrating peptide;
 2. b) at least one antigen or antigenic epitope; and
 3. c) at least one TLR peptide agonist,

wherein the components a) - c) comprised by the complex are covalently linked.

[0361] In particular, the present description provides a method for treating cancer or initiating, enhancing or prolonging an anti-tumor-response in a subject in need thereof comprising administering to the subject

1. (i) an immune checkpoint modulator and
2. (ii) a complex comprising:
 1. a) a cell penetrating peptide;
 2. b) at least one antigen or antigenic epitope; and
 3. c) at least one TLR peptide agonist,

wherein the components a) - c) comprised by the complex are covalently linked.

[0362] In more general, the present invention can be applied to any subject suffering from any disease or disorder, depending on the specificity in particular of the at least one antigen or

antigenic epitope comprised by the complex comprised by the combination for use according to the present invention. In particular, the therapeutic effect of the complex may be to elicit an immune response directed against said antigens or antigenic epitopes, in particular a response that is dependent on CD4⁺ helper T cells and/or CD8⁺ cytotoxic T cells and/or that is restricted by MHC class I molecules and/or MHC class II molecules and the therapeutic effect of the checkpoint modulator may be to enable or to enhance such an effect of the complex.

[0363] Preferably, subjects according to the invention are subjects suffering from a cancer, for instance from a cancer of the brain, colon, head or neck, or from a cervical cancer. More preferably, subjects according to the invention are subjects suffering from a brain cancer including glioma.

[0364] It is also preferred that subjects according to the invention have been subjected to a surgical removal of a tumor.

[0365] Alternatively, subjects according to the invention may preferably be subjects suffering from an infectious disease.

[0366] In a further aspect, the present description also provides a combination therapy for preventing and/or treating cancer, wherein the combination therapy comprises administration of

1. (i) an immune checkpoint modulator and
2. (ii) a complex comprising:
 1. a) a cell penetrating peptide;
 2. b) at least one antigen or antigenic epitope; and
 3. c) at least one TLR peptide agonist,

wherein the components a) - c) comprised by the complex are covalently linked.

[0367] Preferred embodiments of such a combination therapy are preferred embodiments of the complex as described above, embodiments of the checkpoint modulator as described above, and/or - in more general - preferred embodiments of the combination for use according to the present invention. The kit according to the present invention and the (pharmaceutical) composition, in particular the vaccine, according to the present invention may be used in the combination therapy. Subjects to be treated with such a combination therapy are the same as described for the methods as described herein and diseases to be prevented and/or treated are the same as described herein, in the context of the combination for use according to the present invention.

BRIEF DESCRIPTION OF THE FIGURES

[0368] In the following a brief description of the appended figures will be given. The figures are

intended to illustrate the present invention in more detail. However, they are not intended to limit the subject matter of the invention in any way.

Figure 1

shows for Example 1 the tumor growth (A) and the survival rate (B) of 7 mice per group (mean \pm SEM). C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank. Mice of the groups "Z13Mad5Anaxa + Isotype" and "Z13Mad5Anaxa + anti-PD1" were vaccinated twice (d5 and d13) by subcutaneous injection of 2 nmol of Z13Mad5Anaxa in the right flank. 200 μ g of anti-PD1 antibody were administered i.p. on each of days 5, 9 and 13 to mice of groups "anti-PD1" and "Z13Mad5Anaxa + anti-PD1". For control, 200 μ g of isotype 2A3 were administered i.p. on each of days 5, 9 and 13 to mice of groups "isotype" and "Z13Mad5Anaxa + isotype". Tumor size was measured with a caliper. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, ****, $p < 0.0001$.

Figure 2

shows for Example 2 tumor growth of 8 mice per group (mean \pm SEM). C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank. Mice of the groups "Z13Mad5Anaxa + Isotype" and "Z13Mad5Anaxa + anti-PD1" were vaccinated twice (d5 and d13) by subcutaneous injection of 2 nmol of Z13Mad5Anaxa in the right flank. 200 μ g of anti-PD1 antibody were administered i.p. on each of days 5, 9 and 13 to mice of groups "anti-PD1" and "Z13Mad5Anaxa + anti-PD1". For control, 200 μ g of isotype 2A3 were administered i.p. on each of days 5, 9 and 13 to mice of groups "isotype" and "Z13Mad5Anaxa + isotype". Tumor size was measured with a caliper.

Figure 3

shows for Example 2 the percentage of multimer-positive cells (in % of CD8 T cells) at the tumor site (Tumor-infiltrating cells, TILs) for the different experimental groups (A) and a correlation of multimer-positive cells (in % of CD8 T cells) at the tumor site (TILs) with tumor size (B). **, $p < 0.01$; ***, $p < 0.001$.

Figure 4

shows for Example 2 the percentage of granulocytic MDSCs (in % of CD45+CD11b+ cells) for the different experimental groups in spleen (A) and TILs (B). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 5

shows for Example 2 the correlation of granulocytic MDSCs (in % of CD45+CD11b+ cells) with tumor size for the different experimental groups in spleen (A) and TILs (B).

Figure 6

shows for Example 3 the tumor growth of mice treated with anti-PD1 and Z13Mad5Anaxa in distinct treatment protocols. (A) Mice treated with anti-PD1 and Z13Mad5Anaxa according to the protocol of Example 1. (B) Tumor growth of mice treated with anti-PD1 only after vaccination with Z13Mad5Anaxa was finished. ****, $p < 0.0001$.

Figure 7

shows for Example 4 the percentage of SIINFEKL-specific CD8 T cells in blood (A) and the percentage of SIINFEKL-specific CD8 T cells in BILs (B). Fig. 7C shows a summary of the percentage of SIINFEKL-specific CD8 T cells in blood and BILs. C57BL/6 mice

were implanted intracranially with 5×10^5 GI261-Quad tumor cells at day 0. After tumor implantation, mice of the groups "Z13Mad5Anaxa + Isotype" and "Z13Mad5Anaxa + anti-PD1" were vaccinated at days 7 and 21 by subcutaneous injection of 2 nmol of Z13Mad5Anaxa in the right flank. 200 μ g of anti-PD1 antibody were administered i.p. on each of days 7, 10, 14, 17 and 21 to mice of groups "anti-PD1" and "Z13Mad5Anaxa + anti-PD1". For control, 200 μ g of isotype mAB 2A3 were administered i.p. on each of days 7, 10, 14, 17 and 21 to mice of groups "isotype" and "Z13Mad5Anaxa + isotype". SIINFEKL-specific CD8 T cells were quantified in blood and in brain infiltrating leukocytes (BILs) at day 28 by multimer staining (5-8 mice per group). *, $p < 0.05$.

Figure 8

shows for Example 4 the percentage of IFN- γ producing cells (% of CD8 T cells) in BILs (A) and a summary of IFN- γ and/or TNF α producing cells (% of CD8 T cells) in BILs (B) after stimulation with SIINFEKL peptide for 6h. *, $p < 0.05$; **, $p < 0.01$.

Figure 9

shows for Example 5 the survival rate of mice. C57BL/6 mice were implanted intracranially with 5×10^5 GI261-Quad tumor cells at day 0. After tumor implantation, mice of the groups "Z13Mad5Anaxa + Isotype" and "Z13Mad5Anaxa + anti-PD1" were vaccinated at days 7, 21 and 35 by subcutaneous injection of 2 nmol of Z13Mad5Anaxa in the right flank. 200 μ g of anti-PD1 antibody were administered i.p. on each of days 7, 10, 14, 17 and 21 to mice of the group "Z13Mad5Anaxa + anti-PD1". For control, 200 μ g of isotype mAB 2A3 were administered i.p. on each of days 7, 10, 14, 17 and 21 to mice of groups "isotype" and "Z13Mad5Anaxa + isotype". At days 7 and 21, when both, Z13Mad5Anaxa and antibody, were administered, the antibody was administered i.p. just after s.c. administration of Z13Mad5Anaxa. Mice were weighed daily and euthanized when weight loss was more than 15%. *, $p < 0.05$; **, $p < 0.01$.

Figure 10

shows for Example 6 the number of metastases. C57BL/6 mice were implanted i.v. with 1×10^5 B16-OVA melanoma tumor cells at day 0. After tumor implantation, mice of the group "Z13Mad5Anaxa + anti-PD1" ("Vaccine + anti-PD1") were vaccinated at days 0 and 10 by subcutaneous injection of 0.5 nmol of Z13Mad5Anaxa in the right flank. 200 μ g of anti-PD1 antibody were administered i.p. on each of days 0, 3 and 7 to mice of the groups "Z13Mad5Anaxa + anti-PD1" ("Vaccine + anti-PD1") and "anti-PD1". For control, 200 μ g of isotype mAB 2A3 were administered i.p. on each of days 0, 3 and 7 to mice of the group "isotype". Mice were euthanized at day 17 and lung recovered. Number of metastasis foci was counted for each lung. **, $p < 0.01$ (Unpaired T test).

Figure 11

shows for Example 7 the tumor growth in the EG7 tumor model. C57BL/6 mice (5-6 mice per group) were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank (day 0). After tumor implantation, mice of the group "Z13Mad5Anaxa + aCTLA4 ip" were vaccinated at days 5 and 13 subcutaneously with 2 nmol of Z13Mad5Anaxa in the right flank. 100 μ g of anti-CTLA4 antibody were administered i.p. on each of days 5 and 13 to mice of groups "aCTLA4 ip" and "Z13Mad5Anaxa + aCTLA4 ip". In group "Z13Mad5Anaxa + aCTLA4 ip" the antibody was administered i.p. just after s.c.

administration of Z13Mad5Anaxa. For control, 100 µg of isotype mAB MPC-11 were administered i.p. on each of days 5 and 13 to mice of the group "control". Tumor size was measured with a caliper. (A) Summary of all experimental groups. (B) Tumor growth in each mouse of the control group. (C) Tumor growth in each mouse of the anti-CTLA4 alone group. (D) Tumor growth in each mouse of the Z13Mad5Anaxa + aCTLA4 group. **, $p < 0.01$; ***, $p < 0.001$.

Figure 12

shows for Example 8 the effect of the route of administration on tumor growth in the EG7 tumor model. C57BL/6 mice (5-6 mice per group) were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank (day 0). After tumor implantation, mice of the groups "Z13Mad5Anaxa + aCTLA4 ip" and "Z13Mad5Anaxa + aCTLA4 sc" were vaccinated at days 5 and 13 subcutaneously with 2 nmol of Z13Mad5Anaxa in the right flank. 100 µg of anti-CTLA4 antibody were administered i.p. on each of days 5 and 13 to mice of the group "Z13Mad5Anaxa + aCTLA4 ip" just after s.c. administration of Z13Mad5Anaxa. In group "Z13Mad5Anaxa + aCTLA4 sc" on each of days 5 and 13 100 µg of anti-CTLA4 antibody were administered s.c. at the same site as Z13Mad5Anaxa 1 h after injection of Z13Mad5Anaxa. For control, 100 µg of isotype mAB MPC-11 were administered i.p. on each of days 5 and 13 to mice of the group "control". Tumor size was measured with a caliper. (A) Summary of all experimental groups. (B) Tumor growth in each mouse of the control group. (C) Tumor growth in each mouse of the Z13Mad5Anaxa + aCTLA4 sc group. (D) Tumor growth in each mouse of the Z13Mad5Anaxa + aCTLA4 ip group. **, $p < 0.01$.

Figure 13

shows for Example 9 the effect of a combination of a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist and a CTLA4 inhibitor in the CT26 tumor model. BALB/c mice (5 to 6 mice per group) were implanted s.c. with 2×10^5 CT26 tumor cells in the left flank (day 0). After tumor implantation, mice of the group "Z13Mad8Anaxa + aCTLA4" were vaccinated at days 3 and 9 subcutaneously with 2 nmol of Z13Mad8Anaxa in the right flank and 100 µg of anti-CTLA4 antibody were administered i.p. on each of days 3, 6 and 9 (on days 3 and 9 just after s.c. administration of Z13Mad8Anaxa). In the control group, 100 µg of isotype mAB MPC-11 were administered i.p. on each of days 3, 6 and 9 to mice of the group "control". Tumor size was measured with a caliper.

Figure 14

shows for Example 10 the effect of a combination of a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist, a PD1 inhibitor and a CTLA4 inhibitor. BALB/c mice (5 to 6 mice per group) were implanted s.c. with 2×10^5 CT26 tumor cells in the left flank (day 0). After tumor implantation, mice of the groups "Z13Mad8Anaxa + aCTLA4" and "Z13Mad8Anaxa + aCTLA4/aPD1" were vaccinated at days 3 and 9 by subcutaneous injection of 2 nmol of Z13Mad8Anaxa in the right flank. 200 µg of anti-PD1 antibody were administered i.p. on each of days 3, 6 and 9 to mice of groups "aCTLA4/aPD1" and "Z13Mad8Anaxa + aCTLA4/aPD1". 100 µg of anti-CTLA4 antibody were administered i.p. on each of days 3, 6 and 9 to groups "aCTLA4/aPD1",

"Z13Mad8Anaxa + aCTLA4" and "Z13Mad8Anaxa + aCTLA4/aPD1". At days 3 and 9, when Z13Mad8Anaxa and the antibodies were administered, the antibodies were administered i.p. just after s.c. administration of Z13Mad8Anaxa. In the control group, 100 µg of isotype mAB MPC-11 and 200 µg of isotype 2A3 were administered i.p. on each of days 3, 6 and 9 to mice of the group "control". Tumor size was measured with a caliper. (A) Summary of all experimental groups. (B) Tumor growth in each mouse of the control group. (C) Tumor growth in each mouse of the Z13Mad8Anaxa + aCTLA4 group. (D) Tumor growth in each mouse of the aCTLA4/aPD1 group. (E) Tumor growth in each mouse of the Z13Mad8Anaxa + aCTLA4/aPD1 group.

Figure 15

shows for Example 11 the effect of a combination of a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist, a CD40 agonist. C57BL/6 mice (4 mice per group) were vaccinated at days 0, 14, 28 and 42 by subcutaneous injection of 2 nmol of Z13Mad5Anaxa in the right flank. 100 µg of anti-CD40 antibody were administered s.c. at days 0 and days 0 and 14 to mice of groups "Z13Mad5Anaxa + aCD40 at vac1" and "Z13Mad5Anaxa + aCD40 at vac1 and vac2" respectively. (A) The percentage of multimer-positive cells (in % of CD8 T cells) for the different experimental groups. (B) The percentage of KLRG1 positive cells among multimer-positive cells. All groups are significantly different compared to naïve control group. *, $p < 0.05$.

Figure 16

shows for Example 11 (A) the amount of IFN-γ producing SIINFEKL-specific CD8 T cells (Elispot assay) and (B) the results of an intracellular staining of splenocytes for IFN-γ, CD107 and TNFα for SIINFEKL-specific CD8 T cells.

Figure 17

shows for Example 11 the FACS plots of the intracellular staining of splenocytes for IFN-γ, CD107 and TNFα.

Figure 18

shows for Example 12 the tumor growth of 7 mice per group (mean ± SEM); *, $p < 0.05$ EDAZ13Mad5 versus control group (2-way Anova test). C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and vaccinated twice (d5 and d13) by subcutaneous injection of 10nmol of EDAZ13Mad5, EDAMad5, Mad5 or Mad5 and MPLA (equimolar to EDA) s.c. in the right flank. Tumor size was measured with a caliper.

Figure 19

shows for Example 12 individual tumor growth curves (7 individual mice per group). C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and vaccinated twice (d5 and d13) by subcutaneous injection of 10nmol of EDAZ13Mad5, EDAMad5, Mad5 or Mad5 and MPLA (equimolar to EDA) s.c. in the right flank. Tumor size was measured with a caliper.

Figure 20

shows for Example 12 (A) the survival curve of 7 mice per group; *, $p < 0.05$ EDAZ13Mad5 versus control group (Log-rank test) and (B) the tumor-free progression curve of 7 mice per group; *, $p < 0.05$ EDAZ13Mad5 versus control group (Log-rank test).

Figure 21

shows for Example 13 the effect of complexes having different CPPs on the immune response. C57BL/6 mice were vaccinated five times (Wk0, Wk2, Wk4, Wk6 and Wk8) s.c. with either 2nmol (A) or 0.5 nmol (B) of Z13Mad5Anaxa, Z14Mad5Anaxa or Z18Mad5Anaxa. Mice were bled 7 days after the 2nd, 3rd, 4th and 5th vaccination and multimer staining was performed (one experiment with 4 mice per group). *, p<0.05 between vaccinated versus naïve mice at each time point except after Vac2 for Z18Mad5Anaxa-vaccinated mice.

Figure 22

shows for Example 14 the effect of complexes having different CPPs on CD8 T cells in spleen (A), draining lymph nodes (B) and bone marrow (C). C57BL/6 mice were vaccinated five times (Wk0, Wk2, Wk4, Wk6 and Wk8) s.c. with 2 nmol of Z13Mad5Anaxa or Z14Mad5Anaxa. Nine days after the 5th vaccination, mice were euthanized, organs recovered and multimer staining was performed.

Figure 23

shows for Example 14 the effect of complexes having different CPPs on T cells in spleen (CD8 T cell response (A) and CD4 T cell response (B)). C57BL/6 mice were vaccinated five times (Wk0, Wk2, Wk4, Wk6 and Wk8) s.c. with 2 nmol of Z13Mad5Anaxa or Z14Mad5Anaxa. (A) nine days after the 5th vaccination, Elispot assay was performed on spleen cells stimulated with SIINFEKL OVACD8 peptide. (B) nine days after the 5th vaccination, Elispot assay was performed on spleen cells stimulated with OVACD4 peptide.

Figure 24

shows for Example 14 the effect of complexes having different CPPs on CD8 T cell effector function. C57BL/6 mice were vaccinated five times (Wk0, Wk2, Wk4, Wk6 and Wk8) s.c. with 2 nmol of Z13Mad5Anaxa or Z14Mad5Anaxa. Nine days after the 5th vaccination, intracellular staining was performed on spleen cells stimulated with SIINFEKL OVACD8 peptide.

Figure 25

shows for Example 15 the effect of complexes having different CPPs on tumor growth (A) and survival rates (B). C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and vaccinated twice (d5 and d13) by s.c. injection of 0.5 nmol of Z13Mad5Anaxa or Z14Mad5Anaxa in the right flank. (A) Tumor growth of 7 mice per group (mean \pm SEM); *, p<0.05; ****, p<0.0001 (2-way Anova test at day 28). (B) Survival curve of 7 mice per group. Median survival is indicated on the graph (m.s.). *, p<0.05; **, p<0.01; ***, p<0.001 (Log-rank test).

Figure 26

shows for Example 16 the effect of complexes having different CPPs on the immune response. C57BL/6 mice were vaccinated three times (Wk0, Wk2 and Wk4) s.c. with 2 nmol (A) or 0.5 nmol (B) of EDAZ13Mad5, EDAZ14Mad5 or EDAZ18Mad5. Mice were bled 7 days after the 3rd vaccination and multimer staining was performed (one experiment with 4 mice per group). *, p<0.05

Figure 27

shows for Example 17 the effect of EDAZ14Mad5 on tumor growth (A) and survival rates (B). C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and vaccinated twice (d5 and d13) by s.c. injection of 2nmoles of EDAZ14Mad5 in the right flank. Left panel: Tumor growth of 7 mice per group (mean \pm SEM); **, $p < 0.01$ (2-way Anova test at day 27). Right panel: Survival curve of 7 mice per group. Median survival is indicated on the graph (m.s.)

Figure 28

shows for Example 18 the tumor growth of 7 mice per group (mean \pm SEM). C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and vaccinated twice (d5 and d13) by subcutaneous injection of 10 nmol of either AnaxZ13Mad5, Z13Mad5Anaxa or co-injection of Z13Mad5 + Pam3CSK4 (equimolar to Anaxa) in the right flank. Tumor size was measured with a caliper. *, $p < 0.05$; ***, $p < 0.001$, ****, $p < 0.0001$.

Figure 29

shows for Example 18 the individual tumor growth curves (7 individual mice per group). C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and vaccinated twice (d5 and d13) by subcutaneous injection of 10 nmol of either AnaxZ13Mad5, Z13Mad5Anaxa or co-injection of Z13Mad5 + Pam3CSK4 (equimolar to Anaxa) s.c. in the right flank. Tumor size was measured with a caliper.

Figure 30

shows for Example 18 the survival curve of 7 mice per group. C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and vaccinated twice (d5 and d13) by subcutaneous injection of 10 nmol of either AnaxZ13Mad5, Z13Mad5Anaxa or co-injection of Z13Mad5 + Pam3CSK4 (equimolar to Anaxa) in the right flank. Tumor size was measured with a caliper. *, $p < 0.05$, **, $p < 0.01$, ****, $p < 0.0001$ (Log-rank test).

Figure 31

shows for Example 19 the tumor growth of 7 mice per group (mean \pm SEM). C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and vaccinated twice (d5 and d13) by subcutaneous injection of 2nmoles of Hp91Z13Mad5, EDAZ13Mad5, Z13Mad5Anaxa, Z13Mad5EDA or Z13Mad5 and MPLA (equimolar to EDA) in the right flank. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$ (2-way Anova test at day 23).

Figure 32

shows for Example 19 the individual tumor growth curves (7 individual mice per group). C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and vaccinated twice (d5 and d13) by subcutaneous injection of 2nmoles of Hp91Z13Mad5, EDAZ13Mad5, Z13Mad5Anaxa, Z13Mad5EDA or Z13Mad5 and MPLA (equimolar to EDA) s.c. in the right flank.

Figure 33

shows for Example 19 the survival curves of all 7 mice per group. Median survival is indicated on the graph (m.s.). *, $p < 0.05$; **, $p < 0.01$ (Log-rank test).

Figure 34

shows for Example 20 the quantification of SIINFEKL-specific CD8 T cells in a Quad-

GI261 glioblastoma model. Briefly, C57BL/6 mice were implanted i.c. with 5×10^5 GI261-Quad tumor cells and vaccinated twice (d7 and 21) by s.c. injection of 2 nmol of Z13Mad5Anaxa or 2 nmol of Z13Mad5 and 2 nmol of Anaxa. SIINFEKL-specific CD8 T cells were quantified in blood and in BILs at d28 by multimer staining (7-16 mice per group).

Figure 35

shows for Example 20 the cytokine secretion. Briefly, C57BL/6 mice were implanted i.c. with 5×10^5 GI261-Quad tumor cells and vaccinated twice (d7 and 21) by s.c. injection of 2 nmol of Z13Mad5Anaxa or 2 nmol of Z13Mad5 and 2 nmol of Anaxa. BILs were isolated and cultured during 6h with matured BMDCs loaded or not with SIINFEKL peptide in presence of BrefeldinA before intracellular staining for cytokines. % of CD8 T cells secreting cytokine (7-16 mice per group).

Figure 36

shows for Example 21 the quantification of SIINFEKL-specific CD8 T cells in naïve mice. Briefly, C57BL/6 mice were vaccinated once (day0) by s.c. injection of 2 nmol of Z13Mad5Anaxa (group "Z13Mad5Anaxa") or 2 nmol of Z13Mad5 and 2 nmol of Anaxa (group "Z13Mad5+Anaxa"). SIINFEKL-specific CD8 T cells were quantified in blood at d7 by multimer staining (4-8 mice per group).

Figure 37

shows for Example 22 the tumor growth (A) and the survival rate (B) of 7 mice per group (mean \pm SEM). C57BL/6 mice were implanted s.c. with 2×10^5 MC-38 tumor cells in the left flank. Mice of the groups "Z13Mad12Anaxa" and "Z13Mad12Anaxa + anti-PD1" were vaccinated 3 times (d3, d10 and d17) by subcutaneous injection of 2 nmol of Z13Mad12Anaxa at the tail base. 200 μ g of anti-PD1 antibody were administered i.p. on each of days 6, 10, 13, 17, 20, 24, 27 and 31 to mice of groups "anti-PD1" and "Z13Mad12Anaxa + anti-PD1". Tumor size was measured with a caliper. The number of tumor-free mice of each group is indicated for each tumor growth curve. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 38

shows for Example 22 individual tumor growth curves of 7 mice per group. C57BL/6 mice were implanted s.c. with 2×10^5 MC-38 tumor cells in the left flank. Mice of the groups "Z13Mad12Anaxa" and "Z13Mad12Anaxa + anti-PD1" were vaccinated 3 times (d3, d10 and d17) by subcutaneous injection of 2 nmol of Z13Mad12Anaxa at the tail base. 200 μ g of anti-PD1 antibody were administered i.p. on each of days 6, 10, 13, 17, 20, 24, 27 and 31 to mice of groups "anti-PD1" and "Z13Mad12Anaxa + anti-PD1". Tumor size was measured with a caliper.

Figure 39

shows for Example 23 the tumor growth (A) and the survival rate (B) of 13 to 14 mice per group (mean \pm SEM). C57BL/6 mice were implanted s.c. with 2×10^5 MC-38 tumor cells on the back. Mice of the groups "Z13Mad12Anaxa" and "Z13Mad12Anaxa + anti-PD1" were vaccinated 3 times (d3, d10 and d17) by subcutaneous injection of 2 nmol of Z13Mad12Anaxa at the tail base. 200 μ g of anti-PD1 antibody were administered i.p. on each of days 6, 10, 13, 17, 20, 24 and 27 to mice of groups "anti-PD1" and

"Z13Mad12Anaxa + anti-PD1". Tumor size was measured with a caliper. The number of tumor-free mice of each group is indicated for each tumor growth curve. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Figure 40

shows for Example 23 individual tumor growth curves of 13 to 14 mice per group. C57BU6 mice were implanted s.c. with 2×10^5 MC-38 tumor cells on the back. Mice of the groups "Z13Mad12Anaxa" and "Z13Mad12Anaxa + anti-PD1" were vaccinated 3 times (d3, d10 and d17) by subcutaneous injection of 2 nmol of Z13Mad12Anaxa at the tail base. 200 μ g of anti-PD1 antibody were administered i.p. on each of days 6, 10, 13, 17, 20, 24 and 27 to mice of groups "anti-PD1" and "Z13Mad12Anaxa + anti-PD1". Tumor size was measured with a caliper.

EXAMPLES

[0369] In the following, particular examples illustrating various embodiments and aspects of the invention are presented. However, the present invention shall not be limited in scope by the specific embodiments described herein. The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. The present invention, however, is not limited in scope by the exemplified embodiments, which are intended as illustrations of single aspects of the invention only, and methods which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention in addition to those described herein will become readily apparent to those skilled in the art from the foregoing description, accompanying figures and the examples below.

Example 1: Effects of combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist on tumor growth and survival rate

[0370] In order to assess the effects of combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist in treating cancer, the E.G7 tumor model was used. E.G7 is an OVA transfectant derived from the EL4 Thymoma cell line.

[0371] To this end, "Z13Mad5Anaxa" was provided, which is a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist as described herein. Specifically, "Z13Mad5Anaxa" is a fusion protein comprising the cell-penetrating peptide "Z13", the antigenic cargo "MADS" comprising OVA-CD4⁺, gp100-CD8⁺, Ealpha-CD4⁺, and OVA-CD8⁺ epitopes, and the TLR peptide agonist "Anaxa". In the following, the amino acid

sequence of Z13Mad5Anaxa is shown with the cell-penetrating peptide "Z13" shown underlined and the TLR peptide agonist "Anaxa" shown in italics:

MHHHHHHKRYKNRVA SRKSRKAFKQ LLQHYREVAA AKSSENDRLR LLLKESLKIS

QAVHAAHAEI NEAGREVVGV GALKVPRNQD WLCVPRFAKF ASFEAQGALA
NIAVDKANLD VEQLESIINF EKLTEWTGSS *TVHEILCKLS LEGDHSTPPS* *AYGSVKPYTN* FDAE

[SEQ ID NO: 28]

[0372] C57BL/6 mice (seven mice per group) were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank (day 0). After tumor implantation, mice of the groups "Z13Mad5Anaxa + Isotype" and "Z13Mad5Anaxa + anti-PD1" were vaccinated at days 5 and 13 subcutaneously with 2 nmol of Z13Mad5Anaxa in the right flank. 200 µg of anti-PD1 antibody RMP1-14 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 5, 9 and 13 to mice of groups "anti-PD1" and "Z13Mad5Anaxa + anti-PD1". For control, 200 µg of isotype mAB 2A3 were administered i.p. on each of days 5, 9 and 13 to mice of groups "isotype" and "Z13Mad5Anaxa + isotype". At days 5 and 13, when both, Z13Mad5Anaxa and antibody, were administered, the antibody was administered i.p. just after s.c. administration of Z13Mad5Anaxa. Tumor size was measured with a caliper.

[0373] As shown in Figure 1, treatment with the PD1 inhibitor alone or with Z13Mad5Anaxa alone resulted in significantly reduced tumor volume (Fig. 1A) and increased survival (Fig. 1B) as compared to the control group ("isotype"). However, the combination of both, the PD1 inhibitor and Z13Mad5Anaxa, resulted in the most pronounced improvement, namely in strongly decreased tumor volume and strongly increased survival rates. These data show that a combination of both, anti-PD1 therapy and Z13Mad5Anaxa vaccination, is more efficient than anti-PD1 therapy alone or Z13Mad5Anaxa vaccination alone. These results thus indicate a synergistic effect of anti-PD1 therapy and Z13Mad5Anaxa vaccination.

Example 2: Effects of combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist on homing of T cells to the E.G7 tumor site and on MDSCs

[0374] In order to assess the effects of combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist on homing of T cells to the tumor site and on MDSCs (myeloid-derived suppressor cells), the previous experiment (Example 1) was repeated with 8 mice per group and on day 22, mice were euthanized and FACS staining was performed to monitor the OVA-specific immune response and tumor microenvironment in the spleen and TILs (tumor-infiltrating lymphocytes).

[0375] Figure 2 shows the tumor growth in the experimental groups (same experimental method as in Example 1). Again, treatment with the PD1 inhibitor alone or with Z13Mad5Anaxa alone resulted in significantly reduced tumor volume (Fig. 2) as compared to the control group

("isotype"). However, the combination of both, the PD1 inhibitor and Z13Mad5Anaxa, resulted in the most pronounced improvement, namely in strongly decreased tumor volume and strongly increased survival rates. Accordingly, these data are in conformity with the results of Example 1 (cf. Fig. 1A). Thus, the data confirm that a combination of both, anti-PD1 therapy and Z13Mad5Anaxa vaccination, is more efficient than anti-PD1 therapy alone or Z13Mad5Anaxa vaccination alone and indicate a synergistic effect of anti-PD1 therapy and Z13Mad5Anaxa vaccination.

[0376] Figure 3 shows the percentage of multimer-positive cells (in % of CD8 T cells) at the tumor site (TILs) for the different experimental groups (Fig. 3A) and a correlation of multimer-positive cells (in % of CD8 T cells) among TILs with tumor size (Fig. 3B). These data show that antigen-specific T cells accumulate at the tumor site in vaccinated mice. The lowest percentage of multimer-positive cells was found in control mice and in mice, which were treated with anti-PD1 only ("anti-PD1" group). In mice vaccinated with Z13Mad5Anaxa, the percentage of multimer-positive cells was significantly increased with the strongest and most pronounced increase observed in mice treated with both, Z13Mad5Anaxa and anti-PD1. In addition, a significant inverse correlation was observed between the percentage of multimer-positive cells (in % of CD8 T cells) and tumor size, i.e. the more the percentage of multimer-positive cells (in % of CD8 T cells), the smaller the tumor.

[0377] Figure 4 shows the percentage of granulocytic MDSCs (in % of CD45+CD11b+ cells) for the different experimental groups in spleen (Fig. 4A) and TILs (Fig. 4B). The highest percentage of granulocytic MDSCs (in % of CD45+CD11b+ cells) was observed for the control group ("Isotype") in the periphery (spleen) and at the tumor site (TILs). Significantly lower percentages of granulocytic MDSCs (in % of CD45+CD11b+ cells) were observed all other experimental groups with the strongest and most pronounced decrease in granulocytic MDSCs observed in mice treated with both, Z13Mad5Anaxa and anti-PD1. Figure 5 shows a significant correlation between the percentage of granulocytic MDSCs (in % of CD45+CD11b+ cells) and tumor size for the different experimental groups in spleen (Fig. 5A) and TILs (Fig. 5B).

[0378] Taken together, the present T cell data and MDSC data strongly support the synergistic effect of a combination of anti-PD1 therapy and Z13Mad5Anaxa vaccination observed for tumor growth and survival rate.

Example 3: Effect of the treatment schedule of a combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist on tumor growth

[0379] In order to assess the effects of the treatment schedule of a combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist in treating cancer, again the E.G7 tumor model was used.

[0380] In a first experimental group "Z13Mad5Anaxa + anti-PD1" the PD1 inhibitor and

Z13Mad5Anaxa were administered as described in Example 1. Briefly, C57BL/6 mice (six-seven mice per group) were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank (day 0). After tumor implantation, mice were vaccinated at days 5 and 13 subcutaneously with 2 nmol of Z13Mad5Anaxa in the right flank. 200 µg of anti-PD1 antibody RMP1-14 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 5, 9 and 13. In the respective control group, 200 µg of isotype mAB 2A3 were administered i.p. on each of days 5, 9 and 13. At days 5 and 13, when both, Z13Mad5Anaxa and antibody, were administered, the antibody was administered i.p. just after s.c. administration of Z13Mad5Anaxa.

[0381] In a second experimental group "Z13Mad5Anaxa + anti-PD1" the PD1 inhibitor and Z13Mad5Anaxa were administered as follows: C57BL/6 mice (six-seven mice per group) were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank (day 0). After tumor implantation, mice were vaccinated at days 5 and 13 subcutaneously with 2 nmol of Z13Mad5Anaxa in the right flank - as in the first experimental group. However, in contrast to the first experimental group, in the second experimental group 200 µg of anti-PD1 antibody RMP1-14 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 17 and 20. In other words, the anti-PD1 treatment started only after the end of the Z13Mad5Anaxa treatment. In the respective control group, 200 µg of isotype mAB 2A3 were administered i.p. on each of days 17 and 20.

[0382] Results are shown in Figure 6 with Fig. 6A showing the tumor growth in the first experimental group (protocol according to Example 1) and the respective control group and Fig. 6B showing the tumor growth in the second experimental group (anti-PD1 treatment only started after Z13Mad5Anaxa treatment was finished) and the respective control group. As can be retrieved from Figure 6, the latter treatment protocol, wherein anti-PD1 treatment only started after Z13Mad5Anaxa treatment was finished, resulted in a slight improvement (slightly decreased tumor growth, cf. Fig. 6B), whereas the "true combination" of anti-PD1 and Z13Mad5Anaxa (protocol as in Example 1, cf. Fig. 6A) resulted in a considerably stronger improvement, i.e. in a strongly pronounced decrease in tumor growth.

[0383] These data further support the synergistic effect of a combination of anti-PD1 therapy and Z13Mad5Anaxa vaccination observed in the previous Examples, since the effect was much smaller when anti-PD1 therapy was only started after Z13Mad5Anaxa vaccination was finished.

Example 4: Effects of combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist on T cell homing at the tumor site in a glioblastoma model

[0384] To investigate the effects of the combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist in a different tumor model (other than the E.G7 model used in Examples 1 - 3), a murine

glioblastoma model was used. T cell homing at the tumor site was thus analyzed in GL261-Quad tumor-bearing mice vaccinated twice (week 1 and week 3 after tumor implantation) with Z13Mad5Anaxa vaccine and treated or not with anti-PD1. At week 4, blood and the brain infiltrating leukocytes (BILs) were analyzed.

[0385] In particular, C57BL/6 mice were implanted intracranially with 5×10^5 GL261-Quad tumor cells at day 0. After tumor implantation, mice of the groups "Z13Mad5Anaxa + Isotype" and "Z13Mad5Anaxa + anti-PD1" were vaccinated at days 7 and 21 by subcutaneous injection of 2 nmol of Z13Mad5Anaxa in the right flank. 200 μ g of anti-PD1 antibody RMP1-14 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 7, 10, 14, 17 and 21. to mice of groups "anti-PD1" and "Z13Mad5Anaxa + anti-PD1". For control, 200 μ g of isotype mAB 2A3 were administered i.p. on each of days 7, 10, 14, 17 and 21 to mice of groups "isotype" and "Z13Mad5Anaxa + isotype". At days 7 and 21, when both, Z13Mad5Anaxa and antibody, were administered, the antibody was administered i.p. just after s.c. administration of Z13Mad5Anaxa. SIINFEKL-specific CD8 T cells were quantified in blood and in brain infiltrating leukocytes (BILs) at day 28 by multimer staining (5-8 mice per group).

[0386] Results are shown in Figure 7 with the percentage of SIINFEKL-specific CD8 T cells in blood shown in Fig. 7A and the percentage of SIINFEKL-specific CD8 T cells in BILs shown in Fig. 7B. Fig. 7C shows a summary of the percentage of SIINFEKL-specific CD8 T cells in blood and BILs. As previously observed, low frequency of SIINFEKL-specific CD8 T cells was quantified in the blood. However, a higher percentage of SIINFEKL-specific CD8 T cells was observed in the blood of Z13Mad5Anaxa-vaccinated mice treated or not with anti-PD1. In all groups, there was a sensibly stronger accumulation of SIINFEKL-specific CD8 T cells in the BILs than in blood. Importantly, the anti-PD1 therapy was able to increase the frequency of specific CD8 T cells to 42% without vaccination and even to 61 % when combined with Z13Mad5Anaxa vaccination suggesting again a synergistic effect of the vaccination and the anti-PD1 therapy.

[0387] Moreover, the percentage of cytokine-producing cells was also assessed. To this end, intracellular cytokines were stained after restimulation with the SIINFEKL peptide for 6 hours in the presence of Brefeldin A (GolgiPlug, BD Biosciences) with mAb to IFN- γ (XMG1.2), tumor necrosis factor (MP6-XT22) and corresponding isotype controls (BD Biosciences).

[0388] Results are shown in Figure 8 with the percentage of IFN- γ producing cells (% of CD8 T cells) shown in Figure 8A and a summary of IFN- γ and/or TNF α producing cells (% of CD8 T cells) shown in Figure 8B. Those data show that vaccination with Z13Mad5Anaxa alone and treatment with anti-PD1 alone result in an increased percentage of cytokine, in particular IFN- γ , producing cells. However, the strongest increase in percentage of cytokine, in particular IFN- γ , producing cells was clearly observed in animals treated with a combination of Z13Mad5Anaxa and anti-PD1 .

[0389] Taken together, the strongest SIINFEKL-specific CD8 T cell immune responses in the brains of tumor bearing mice with potent effector function were clearly observed in animals

treated with a combination of Z13Mad5Anaxa and anti-PD1. These findings also indicate a synergistic effect of Z13Mad5Anaxa and anti-PD1 in the glioblastoma model.

Example 5: Effects of combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist on survival in a glioblastoma model

[0390] Next, the survival rate of mice treated with a combination of anti-PD1 and Z13Mad5Anaxa was assessed in the glioblastoma model in an independent experiment.

[0391] To this end, C57BL/6 mice were implanted intracranially with 5×10^5 GI261-Quad tumor cells at day 0. After tumor implantation, mice of the groups "Z13Mad5Anaxa + Isotype" and "Z13Mad5Anaxa + anti-PD1" were vaccinated at days 7, 21 and 35 by subcutaneous injection of 2 nmol of Z13Mad5Anaxa in the right flank. 200 µg of anti-PD1 antibody RMP1-14 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 7, 10, 14, 17 and 21 to mice of the group "Z13Mad5Anaxa + anti-PD1". For control, 200 µg of isotype mAB 2A3 were administered i.p. on each of days 7, 10, 14, 17 and 21 to mice of groups "isotype" and "Z13Mad5Anaxa + isotype". At days 7 and 21, when both, Z13Mad5Anaxa and antibody, were administered, the antibody was administered i.p. just after s.c. administration of Z13Mad5Anaxa. Mice were weighed daily and euthanized when weight loss was more than 15%.

[0392] Results are shown in Figure 9. These results show that Z13Mad5Anaxa therapeutic vaccination alone is more efficacious than the control group with a 10-days difference in the median survival. Again, the effects, i.e. the increase in the survival rate, are strongest for the combination of anti-PD1 and Z13Mad5Anaxa. In particular, combined anti-PD1/Z13Mad5Anaxa therapy is able to control glioblastoma growth.

[0393] Taken together, combined anti-PD1 therapy increased the effect of the vaccination with Z13Mad5Anaxa. As shown in Example 4, a combination of Z13Mad5Anaxa and anti-PD1 was able to promote the strongest secretion of cytokine by antigen-specific CD8 T cells in the brain.

Example 6: Effects of combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist on the number of metastases in a lung metastasis model

[0394] In this experiment the combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist was investigated again in a different tumor model (other than the E.G7 model used in Examples 1 - 3 and the glioblastoma model used in Examples 4 and 5).

[0395] To this end, C57BL/6 mice were implanted i.v. with 1×10^5 B16-OVA melanoma tumor cells at day 0. After tumor implantation, mice of the group "Z13Mad5Anaxa + anti-PD1" ("Vaccine + anti-PD1") were vaccinated at days 0 and 10 by subcutaneous injection of 0.5 nmol of Z13Mad5Anaxa in the right flank. 200 µg of anti-PD1 antibody RMP1-14 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 0, 3 and 7 to mice of the groups "Z13Mad5Anaxa + anti-PD1" ("Vaccine + anti-PD1") and "anti-PD1". For control, 200 µg of isotype mAB 2A3 were administered i.p. on each of days 0, 3 and 7 to mice of the group "isotype". At day 0, when both, Z13Mad5Anaxa and antibody, were administered, the antibody was administered i.p. just after s.c. administration of Z13Mad5Anaxa. Mice were euthanized at day 17 and lung recovered. Number of metastasis foci was counted for each lung. **, $p < 0.01$ (Unpaired T test).

[0396] Results are shown in Figure 10. Only the combined treatment with anti-PD1 and Z13Mad5Anaxa, but not the treatment with anti-PD1 alone, resulted in a significant decrease in the number of metastases. Accordingly, the combined treatment with anti-PD1 and Z13Mad5Anaxa was highly efficient in inhibiting the growth of melanoma metastasis in the lung.

Example 7: Effects of combination of a CTLA4 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist on tumor growth in the EG7 model

[0397] To assess the effects of a combination of a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist with a different checkpoint inhibitor, an inhibitor of CTLA4 was used in the present experiment.

[0398] C57BL/6 mice (5-6 mice per group) were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank (day 0). After tumor implantation, mice of the group "Z13Mad5Anaxa + aCTLA4 ip" were vaccinated at days 5 and 13 subcutaneously with 2 nmol of Z13Mad5Anaxa in the right flank. 100 µg of anti-CTLA4 antibody 9D9 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 5 and 13 to mice of groups "aCTLA4 ip" and "Z13Mad5Anaxa + aCTLA4 ip". In group "Z13Mad5Anaxa + aCTLA4 ip" the antibody was administered i.p. just after s.c. administration of Z13Mad5Anaxa. For control, 100 µg of isotype mAB MPC-11 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 5 and 13 to mice of the group "control". Tumor size was measured with a caliper.

[0399] As shown in Figure 11, treatment with the CTLA4 inhibitor alone did not result in any effect on tumor growth as compared to the control group. However, the combination of both, the CTLA4 inhibitor and Z13Mad5Anaxa, resulted in significantly decreased tumor volume. These data show that a combination of both, anti-CTLA4 therapy and Z13Mad5Anaxa vaccination efficient in decreasing tumor growth whereas anti-CTLA4 therapy alone did not result in any effect.

Example 8: Effects of the route of administration of the immune checkpoint modulator on tumor growth

[0400] To assess the effects of the route of administration of the immune checkpoint modulator in a combination therapy of a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist with a checkpoint modulator, the EG7 tumor model was used..

[0401] C57BL/6 mice (5-6 mice per group) were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank (day 0). After tumor implantation, mice of the groups "Z13Mad5Anaxa + aCTLA4 ip" and "Z13Mad5Anaxa + aCTLA4 sc" were vaccinated at days 5 and 13 subcutaneously with 2 nmol of Z13Mad5Anaxa in the right flank. 100 µg of anti-CTLA4 antibody were administered i.p. on each of days 5 and 13 to mice of the group "Z13Mad5Anaxa + aCTLA4 ip" just after s.c. administration of Z13Mad5Anaxa. In group "Z13Mad5Anaxa + aCTLA4 sc" on each of days 5 and 13 100 µg of anti-CTLA4 antibody were administered s.c. at the same site as Z13Mad5Anaxa 1 h after injection of Z13Mad5Anaxa. For control, 100 µg of isotype mAB MPC-11 were administered i.p. on each of days 5 and 13 to mice of the group "control". Tumor size was measured with a caliper.

[0402] As shown in Figure 12, the most pronounced effect of the combination therapy of a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist with a checkpoint modulator in decreasing tumor growth was achieved when the checkpoint modulator was administered intraperitoneally. Subcutaneous administration of the checkpoint modulator also resulted in decreased tumor growth, however, the effect was less pronounced than after i.p. administration.

Example 9: Effects of combination of a CTLA4 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist on tumor growth in a CT26 colon carcinoma model

[0403] In this experiment the combination of a CTLA4 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist was investigated in a different tumor model (other than the E.G7 model used in Examples 7 and 8).

[0404] To this end, the murine CT26 colon carcinoma model was used and another complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist was designed ("Z13Mad8Anaxa"). Z13Mad8Anaxa differs from Z13Mad5Anaxa (described in Example 1) in the antigenic cargoes. In particular, "Z13Mad8Anaxa" is a fusion protein comprising the cell-penetrating peptide "Z13", the antigenic cargo "MAD8" comprising CD8 and CD4 epitopes of glycoprotein 70, and the TLR peptide agonist "Anaxa". In the following, the amino acid sequence of Z13Mad8Anaxa is shown with the cell-penetrating peptide "Z13" shown underlined and the TLR peptide agonist "Anaxa" shown in italics:

KRYKNRVASR KSRAKFKQLL QHYREVAATAK SSENDRLRLLLK VTYHSPSYVY HQFERRAILN
 RLVQFIKDRI SVVQALVLT *TVHEILCKLS LEGDHSTPPS AYGSVKPYTN FDAE*
 (SEQ ID NO: 33)

[0405] BALB/c mice (seven mice per group) were implanted s.c. with 2×10^5 CT26 tumor cells in the left flank (day 0). After tumor implantation, mice of the group "Z13Mad8Anaxa + aCTLA4" were vaccinated at days 3 and 9 subcutaneously with 2 nmol of Z13Mad8Anaxa in the right flank and 100 µg of anti-CTLA4 antibody 9D9 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 3, 6 and 9 (on days 3 and 9 just after s.c. administration of Z13Mad8Anaxa). In the control group, 100 µg of isotype mAB MPC-11 were administered i.p. on each of days 3, 6 and 9 to mice of the group "control". Tumor size was measured with a caliper.

[0406] Results are shown in Figure 13. These data show that a combination of an inhibitor of CTLA4 with complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist is able to control tumor growth also in the CT26 colon cancer tumor model.

Example 10: Effects of combination of a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist with two different immune checkpoint modulators on tumor growth

[0407] In this experiment the effects of a combination of a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist with two different immune checkpoint modulators, namely an inhibitor of PD1 and an inhibitor of CTLA4 was assessed. The aim of this experiment was to determine whether a combination including - in addition to the complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist - two different checkpoint modulators were even more efficacious than a combination with a single checkpoint modulator.

[0408] Care must be taken in the selection of an appropriate tumor model to avoid "floor effects" and "ceiling effects", since the effect, e.g. decrease in tumor volume, of combination with one checkpoint modulator should still be able to be improved, e.g. by an even further decrease in tumor volume. Therefore, the CT26 colon carcinoma model was chosen.

[0409] BALB/c mice (5 to 6 mice per group) were implanted s.c. with 2×10^5 CT26 tumor cells in the left flank (day 0). After tumor implantation, mice of the groups "Z13Mad8Anaxa + aCTLA4" and "Z13Mad8Anaxa + aCTLA4/aPD1" were vaccinated at days 3 and 9 by subcutaneous injection of 2 nmol of Z13Mad8Anaxa in the right flank. 200 µg of anti-PD1 antibody RMP1-14 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 3, 6 and 9 to mice of groups "aCTLA4/aPD1" and "Z13Mad8Anaxa + aCTLA4/aPD1". 100 µg of anti-CTLA4 antibody [name of Ab, provider?] were administered i.p. on each of days 3, 6 and 9 to groups

"aCTLA4/aPD1", "Z13Mad8Anaxa + aCTLA4" and "Z13Mad8Anaxa + aCTLA4/aPD1". At days 3 and 9, when Z13Mad8Anaxa and the antibodies were administered, the antibodies were administered i.p. just after s.c. administration of Z13Mad8Anaxa. In the control group, 100 µg of isotype mAb MPC-11 were administered i.p. on each of days 3, 6 and 9 to mice of the group "control". Tumor size was measured with a caliper.

[0410] Results are shown in Figure 14. As shown in Example 9, treatment with a combination of Z13Mad8Anaxa with a CTLA4 inhibitor (but no further checkpoint modulator) resulted in a decrease in tumor growth. Similarly, treatment with a combination of checkpoint modulators (anti-CTLA4 and anti-PD1), but without Z13Mad8Anaxa, also resulted in a decrease in tumor growth. However, the combination of all three, the two distinct checkpoint modulators and Z13Mad8Anaxa, resulted in the most pronounced improvement, namely in strongly decreased tumor volume. Thus, the data show that a combination of all three, anti-PD1 therapy, anti-CTLA4 therapy and Z13Mad5Anaxa vaccination, is more efficacious than a combination of only two components. These results indicate a synergistic effect of anti-PD1 therapy, anti-CTLA4 therapy and Z13Mad5Anaxa vaccination.

Example 11: Immunogenicity of combination of a CD40 agonist and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist

[0411] To assess the potential of a combination of a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist with again a different checkpoint modulator, an agonistic antibody targeting CD40 was used in the present experiment.

[0412] To this end, naïve C57BL/6 mice (4 mice per group) were vaccinated at days 0, 14, 28 and 42 (weeks 0, 2, 4 and 6) subcutaneously with 2 nmol of Z13Mad5Anaxa in the right flank. 100 µg of anti-CD40 antibody FGK45 (BioXcell, West Lebanon, NH, USA) were administered s.c. at day 0 to mice in the group "Z13Mad5Anaxa + aCD40 at vac1" and at days 0 and 14 to mice in the group "Z13Mad5Anaxa + aCD40 at vac1 and vac2" (same days as first and second vaccination). The anti-CD40 antibody was administered s.c. at the same site as Z13Mad5Anaxa 1 h after injection of Z13Mad5Anaxa. Mice were bled on days 21, 35 and 49 (weeks 3, 5 and 7) and the spleen was assessed on week 7. Multimer staining was performed on blood cells. Moreover, the percentage of cytokine-producing cells was also assessed in the spleen. To this end, intracellular cytokines were stained after restimulation with the SIINFEKL peptide for 6 hours in the presence of Brefeldin A (GolgiPlug, BD Biosciences) with mAb to IFN-γ (XMG1.2), tumor necrosis factor (MP6-XT22), CD107a (1 D4B) and corresponding isotype controls (BD Biosciences).

[0413] Figure 15 shows the percentage of multimer-positive cells (in % of CD8 T cells) for the different experimental groups (Fig. 15A) and the percentage of KLRG1 positive cells among multimer-positive cells (Fig. 15B). All groups are significantly different compared to naïve control group, confirming the immunogenicity of Z13Mad5Anaxa. However, the groups treated additionally with an anti-CD40 antibody showed even more multimer-positive cells and KLRG1

positive cells, which was most pronounced in the group receiving two treatments with anti-CD40. These results indicate a synergistic effect of a combination of a CD40 agonist and Z13Mad5Anaxa on immunogenicity.

[0414] These data are supported by the results shown in Figures 16 and 17 relating to the effector function of the OVA-specific CD8 T cells, which show the strongest increase in cytokine producing cells, when vaccination with Z13Mad5Anaxa was combined with treatment with the agonistic anti-CD40 antibody.

Example 12: Efficacy of a complex having a different TLR agonist on tumor growth in a benchmark EG.7-OVA tumor model

[0415] The goal of this study was to investigate the effect of a complex as described herein having a different TLR agonist, namely "EDA" instead of "Z13", on tumor growth and survival. In the present study, the complex is a fusion protein, comprising the cell-penetrating peptide "Z13", a protein "MAD5", which consists of different CD8⁺ and CD4⁺ epitopes from various antigens, and the TLR4 peptide agonist "EDA". Accordingly, a fused protein with the EDA peptide at the N-terminal position and different control conjugated proteins without Z13 or EDA or both were designed.

[0416] Namely, the following constructs were designed, whereby in the amino acid sequence the cell-penetrating peptide "Z13" is shown underlined and the TLR peptide agonist "EDA" is shown in italics:

EDAZ13Mad5

[0417] Sequence:

MHHHHHHH*NID* RPKGLAFTDV DVDSIKIAWE SPQGQVSRYP VTYSSPEDGI
RELFPAPDGEDDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQSTKRY KNRVASRKSR
AKFKQLLQHY *REVAAAKSSE* NDRLRLLLKE SLKISQAVHA AHAEINEAGR EVVGVGALKV
PRNQDWLGVP RFAKFASFEA QGALANIAVD KANLDVEQLE SIINFEKLTE WTGS

[SEQ ID NO: 26]

Molecular weight: 25'057 Da

[0418] Characteristics:

- Mad5 cargo contains OVACD4, gp100CD8, EalphaCD4 and OVACD8 epitopes
- Contains EDA TLR agonist (Lasarte, J J., et al., The extra domain A from fibronectin targets antigens to TLR4-expressing cells and induces cytotoxic T cell responses in vivo. J Immunol, 2007. 178(2): p. 748-56)
- Storage buffer: 50 mM Tris-HCl, , 150 mM NaCl, 10% Glycerol, 2 mM DTT, 1 M L-

Arginine, pH 8

- Endotoxin level: < 0.01 EU/ug

Mad5

[0419] Sequence:

MHHHHHHE SLKISQAVHA AHAEINEAGR EVVGVGALKV PRNQDWLGVP RFAKFASFEA
QGALANIAVD KANLDVEQLE SIINFEKLTE WTGS

[SEQ ID NO: 14]

Molecular weight: 10'154.6 Da

[0420] Characteristics:

- Mad5 cargo contains OVACD4, gp100CD8, EalphaCD4 and OVACD8 epitopes
- Storage buffer: 50 mM Tris-HCl, , 150 mM NaCl, 10% Glycerol, 2 mM DTT, 0.5 M L-Arginine, pH 8
- Endotoxin level: 0.069EU/mg

EDAMad5

[0421] Sequence

MHHHHHHH*NID RPKGLAFTDV DVDSIKIAWE SPQGQVSRYSR VTYSSPEDGI*
RELFAPDGEDDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQSTE SLKISQAVHA
AHAEINEAGR EVVGVGALKV PRNQDWLGVP RFAKFASFEA QGALANIAVD KANLDVEQLE
SIINFEKLTE WTGS

[SEQ ID NO: 34]

Molecular weight: 20'017 Da

[0422] Characteristics:

- Mad5 cargo contains OVACD4, gp100CD8, EalphaCD4 and OVACD8 epitopes
- Contains EDA TLR agonist
- Storage buffer: 50 mM Tris-HCl, , 150 mM NaCl, 10% Glycerol, 2 mM DTT, 0.5 M L-Arginine, pH 8
- Endotoxin level: 1.8EU/mg

[0423] To evaluate the effect of EDA construct proteins on tumor growth control, the s.c. model

of EG.7-OVA thymoma cells was chosen. C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank. After tumor implantation, mice were vaccinated at day 5 and 13 with 10 nmol of one of the following constructs (cf. Examples 1 and 2 for construct description): EDZ13Mad5, EDAMad5, Mad5, or Mad5 and MPLA (equimolar to EDA) s.c. in the right flank. Tumor size was measured with a caliper.

[0424] Fig. 18 shows the tumor growth of 7 mice per group (mean \pm SEM); *, $p < 0.05$ EDZ13Mad5 versus control group (2-way Anova test). Fig. 19 shows individual tumor growth curves (7 individual mice per group). Fig. 20 A shows the survival curve of 7 mice per group; *, $p < 0.05$ EDZ13Mad5 versus control group (Log-rank test). Fig. 20 B shows the tumor-free progression curve of 7 mice per group; *, $p < 0.05$ EDZ13Mad5 versus control group (Log-rank test).

[0425] The results show that in a therapeutic setting, EDZ13Mad5 was the only protein vaccine to significantly control the tumor growth compared to the control group with a significant better tumor free progression curve and survival curve.

[0426] The results therefore suggest that the construct protein EDZ13Mad5 is a highly potent vaccine for controlling the tumor growth in a therapeutic setting.

Example 13: Comparison of the kinetic of immune responses with complexes having different cell penetrating peptides

[0427] To investigate the effect of different CPPs in the complex comprised by the combination for use according to the present invention the fusion protein Z13Mad5Anaxa as described above was used.

[0428] In addition, further fusion proteins were designed, which comprise CPPs other than Z13 - namely Z14 (SEQ ID NO: 7) or Z18 (SEQ ID NO: 11). Those fusion proteins also comprise the protein "MAD5", which consists of different CD8⁺ and CD4⁺ epitopes from various antigens, and the TLR2 peptide agonist "Anaxa". Accordingly, the following constructs were additionally designed:

Z14Mad5Anaxa

[0429] Sequence:

MHHHHHHKRY KNRVASRKSR AKFKQLLQHY REVAAAKESL KISQAVHAAH AEINEAGREV
VGVGALKVPR NQDWLGVPRF AKFASFEAQG ALANIAVDKA NLDVEQLESI INFEKLTWET
GSSTVHEILC KLSLEGDHST PPSAYGSVKP YTNFDEA
(SEQ ID NO: 29)

Z18Mad5Anaxa**[0430] Sequence:**

MHHHHHHHREV AAAKSSENDR LRLLLKESLK ISQAVHAAHA EINEAGREVV GVGALKVPRN
 QDWLGVPRFA KFASFEAQGA LANIAVDKAN LDVEQLESII NFEKLTEWTG SSTVHEILCK
 LSLEGDHSTP PSAYGSVKPY TNFDAE
 (SEQ ID NO: 30)

[0431] C57BL/6 mice were assigned to eight different groups (4 mice per group): three groups receiving 2 nmol of either Z13Mad5Anaxa, Z14Mad5Anaxa or Z18Mad5Anaxa and a respective control and three groups receiving 0.5 nmol of Z13Mad5Anaxa, Z14Mad5Anaxa or Z18Mad5Anaxa and a respective control. The mice were vaccinated five times (Week0, Week2, Week4, Week6 and Week8) s.c.. Mice were bled 7 days after the 2nd, 3rd, 4th and 5th vaccination and multimer staining was performed (one experiment with 4 mice per group).

[0432] The results are shown in Figure 21. All groups vaccinated with Z13Mad5Anaxa, Z14Mad5Anaxa or Z18Mad5Anaxa showed an increased percentage of multimer-positive cells compared to the control group (except for the second vaccination of Z18Mad5Anaxa). These results indicate that complexes according to the present invention having different cell penetrating peptides are able to elicit an immune response at different doses.

Example 14: Comparison of T cell immune responses with complexes having different cell penetrating peptides

[0433] To investigate the CD8 T cell immune responses in more detail, C57BL/6 mice were assigned to three different groups (3 - 4 mice per group): naïve, Z13Mad5Anaxa or Z14Mad5Anaxa. C57BL/6 mice of the Z13Mad5Anaxa group and of the Z14Mad5Anaxa group were vaccinated five times (Week0, Week2, Week4, Week6 and Week8) s.c. with 2 nmol of either Z13Mad5Anaxa or Z14Mad5Anaxa as described above. Nine days after the 5th vaccination, mice were euthanized, organs recovered and multimer staining was performed to identify the percentage of SIINFELK-specific CD8 T cells in the spleen, bone marrow and draining lymph nodes (inguinal and axillary).

[0434] The results are shown in Figure 22. Mice vaccinated with Z13Mad5Anaxa or with Z14Mad5Anaxa showed a similar increase in multimer-positive cells, in particular in the spleen and bone marrow as well as a slight increase in draining lymph nodes.

[0435] To further investigate the CD8 T cell effector function after vaccination with complexes with different CPPs, in the same groups of mice as described above Elispot assay was

performed on spleen cells stimulated with SIINFEKL OVACD8 peptide (SEQ ID NO: 35) nine days after the 5th vaccination in order to quantify IFN- γ producing cells.

[0436] The results are shown in Figure 23A. Mice vaccinated with Z13Mad5Anaxa showed a significant increase in IFN- γ producing cells compared to naïve mice. Mice vaccinated with Z14Mad5Anaxa showed also an increase in IFN- γ producing cells compared to naïve mice, however, the increase was not significant, which may be due to the low number of mice (3 mice in Z14Mad5Anaxa group).

[0437] To investigate the CD4 T cell responses after vaccination with complexes with different CPPs, in the same groups of mice as described above Elispot assay was performed on spleen cells stimulated with OVACD4 peptide (SEQ ID NO: 36) nine days after the 5th vaccination in order to quantify IFN- γ producing cells.

[0438] The results are shown in Figure 23B. Mice vaccinated with Z13Mad5Anaxa showed a highly significant increase in IFN- γ producing cells compared to naïve mice. Mice vaccinated with Z14Mad5Anaxa showed also an increase in IFN- γ producing cells compared to naïve mice, however, the increase was not significant, which may be due to the low number of mice (3 mice in Z14Mad5Anaxa group).

[0439] In addition, in the above described groups of mice, intracellular staining was performed on spleen cells stimulated with SIINFEKL OVACD8 peptide (SEQ ID NO: 35) to identify CD107a⁺IFN- γ ⁺TNF- α ⁺ cells. Results are shown in Figure 24. Mice vaccinated with Z13Mad5Anaxa or with Z14Mad5Anaxa showed a similar increase in CD107a⁺IFN- γ ⁺TNF- α ⁺ cells.

Example 15: Comparison of the effect of complexes having different cell penetrating peptides on tumor growth and survival in the EG.7-OVA s.c. model

[0440] To investigate the effects of complexes having different cell penetrating peptides on tumor growth and survival the EG.7-OVA s.c. model was used. On d0 C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and assigned to three different groups (naïve, Z13Mad5Anaxa and Z14Mad5Anaxa). Mice were vaccinated twice at d5 and d13 after tumor implantation by s.c. injection of either 0.5 nmol of Z13Mad5Anaxa or Z14Mad5Anaxa in the right flank.

[0441] Results are shown in Figure 25. Vaccination with Z13Mad5Anaxa or with Z14Mad5Anaxa resulted in significantly decreased tumor volumes compared to control mice (Figure 25 A) as well as to significantly increased survival rates compared to control mice (Figure 25 B). Those results indicate that both complexes, Z13Mad5Anaxa and Z14Mad5Anaxa, are able to significantly decrease tumor growth and to significantly prolong survival.

Example 16: Comparison of the immune responses after vaccination with complexes having different cell penetrating peptides

[0442] In this experiment the effect of different CPPs in the complex comprised by the combination for use according to the present invention was investigated by using a complex with the TLR agonist "EDA". Therefore, the fusion protein EDAZ13Mad5 as described above was used.

[0443] In addition, further fusion proteins were designed, which comprise CPPs other than Z13 - namely Z14 (SEQ ID NO: 7) or Z18 (SEQ ID NO: 11). Those fusion proteins also comprise the protein "MAD5", which consists of different CD8⁺ and CD4⁺ epitopes from various antigens, and the TLR4 peptide agonist "EDA". Accordingly, the following constructs were additionally designed:

EDAZ14Mad5

[0444] Sequence:

MHHHHHHNID RPKGLAFTDV DVDSIKIAWE SPQGQVSRYP VTYSSPEDGI RELFPAPDGE
DDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQSTKRY KNRVASRKSR AKFKQLLQHY
REVAAAKESL KISQAVHAAH AEINEAGREV VGVGALKVPR NQDWLGVPRF AKFASFEAQG
ALANIAVDKA NLDVEQLESI INFEKLTWT GS
(SEQ ID NO: 31)

EDAZ18Mad5

[0445] Sequence:

MHHHHHHNID RPKGLAFTDV DVDSIKIAWE SPQGQVSRYP VTYSSPEDGI RELFPAPDGE
DDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQSTREV AAKSSENR LRLLLKESLK
ISQAVHAAHA EINEAGREVV GVGALKVPRN QDWLGVPRFA KFASFEAQGA LANIAVDKAN
LDVEQLESII NFEKLTWTG S
(SEQ ID NO: 32)

[0446] C57BL/6 mice were assigned to eight different groups (4 mice per group): three groups receiving 2 nmol of either EDAZ13Mad5, EDAZ14Mad5 or EDAZ18Mad5 and a respective control and three groups receiving 0.5 nmol of either EDAZ13Mad5, EDAZ14Mad5 or EDAZ18Mad5 and a respective control group. The mice were vaccinated three times (Week0,

Week2 and Week4) s.c.. Mice were bled 7 days after the 2nd and 3rd vaccination and multimer staining was performed (one experiment with 4 mice per group).

[0447] The results are shown in Figure 26. All groups vaccinated with EDAZ13Mad5, EDAZ14Mad5 or EDAZ1 8Mad5 showed an increased percentage of multimer-positive cells compared to the control group. These results indicate that complexes according to the present invention having different cell penetrating peptides are able to elicit an immune response at different doses.

Example 17: Effect of EDAZ14Mad5 on tumor growth and survival in the EG.7-OVA s.c. model

[0448] To investigate the effect of EDAZ14Mad5 on tumor growth and survival the EG.7-OVA s.c. model was used (cf. Example 12 and Figures 18 - 20 for the effect of EDAZ13Mad5 in the same model).

[0449] On d0 C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and assigned to two different groups (naïve and EDAZ14Mad5). Mice were vaccinated twice at d5 and d13 after tumor implantation by s.c. injection of 0.5 nmol of EDAZ14Mad5 in the right flank.

[0450] Results are shown in Figure 27. Similarly to EDAZ13Mad5 (cf. Example 12, Figures 18 - 20) vaccination with EDAZ14Mad5 resulted in significantly decreased tumor volumes compared to control mice (Figure 27 A) as well as to significantly increased survival rates compared to control mice (Figure 27 B). Those results indicate that EDAZ14Mad5 is able to significantly decrease tumor growth and to significantly prolong survival - similarly to EDAZ13Mad5 (cf. Example 12, Figures 18 - 20).

Example 18: Superior efficacy of Z13Mad5Anaxa fusion construct compared to Z13Mad5 and a TLR agonist administered separately

[0451] To evaluate the effect of the conjugated TLR agonist "Anaxa" in Z13Mad5Anaxa (SEQ ID NO: 28) on tumor growth control, a benchmark tumor model was used, namely the s.c. implantation of EG.7-OVA thymoma cells. For comparison the construct "AnaxaZ13Mad5" (SEQ ID NO: 27; with N-terminal TLR agonist "Anaxa" and C-terminal antigenic cargo "Mad5") and the construct "Z13Mad5" (SEQ ID NO: 46; without TLR agonist), the latter being administered in combination with a separate TLR agonist (Pam3CSK4) were used.

[0452] C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank. After tumor implantation, the three groups of 7 mice each were vaccinated s.c. in the right flank at day 5 and 13 by subcutaneous injection of 10 nmol of either AnaxZ13Mad5 (group 1),

Z13Mad5Anaxa (group 2) or Z13Mad5 and Pam3CSK4 (equimolar to Anaxa; group 3). Tumor size was measured with a caliper. Results are shown in Fig. 28 - 30.

[0453] In a therapeutic schedule, Z13Mad5Anaxa and AnaxaZ13Mad5 are better protein vaccines for controlling tumor growth compared to the control group, i.e. co-injection of Z13Mad5 and Pam3CSK4, with Z13Mad5Anaxa and AnaxaZ13Mad5 showing a significant better survival curve. In particular, Z13Mad5Anaxa and AnaxaZ13Mad5 demonstrate significantly higher efficacy than Z13Mad5 administered separately with Pam3CSK4. The results therefore suggest that the construct proteins Z13Mad5Anaxa and AnaxaZ13Mad5 are promising conjugate-vaccines for controlling the tumor growth in a therapeutic setting.

Example 19: Therapeutic effect on tumor growth - comparison of constructs with different TLR agonists

[0454] The goal of this study was to compare the efficacy of the different construct protein vaccines conjugated to different TLR agonist, namely EDAZ13Mad5 (SEQ ID NO: 26) and Z13Mad5Anaxa (SEQ ID NO: 28), on tumor growth control. To this end, C57BL/6 mice were implanted s.c. with 3×10^5 EG.7-OVA thymoma cells in the left flank as described previously. Mice (7 individual mice per group) were vaccinated s.c. in the right flank at day 5 and 13 with 2 nmol of either EDAZ13Mad5, Z13Mad5Anaxa or co-injection of Z13Mad5+MPLA (equimolar to EDA).

[0455] Results are shown in Figures 31, 32 and 33. In this experimental setting, Z13Mad5Anaxa (SEQ ID NO: 28), EDAZ13Mad5 (SEQ ID NO: 26), and Z13Mad5(SEQ ID NO: 46)+MPLA were similarly able to significantly control tumor growth. Moreover, these data indicate that Z13Mad5Anaxa is the best construct to significantly control tumor growth and EDAZ13Mad5 was slightly better than Z13Mad5+MPLA in this experimental setting.

Example 20: Superior efficacy of Z13Mad5Anaxa fusion construct compared to Z13Mad5 and Anaxa in a glioblastoma model

[0456] To investigate the efficacy of a complex according to the present invention the glioblastoma model was chosen. Namely, Z13Mad5Anaxa (SEQ ID NO: 28) was administered to one group of mice, whereas Z13Mad5 (SEQ ID NO: 46) and Anaxa (SEQ ID NO: 15) were administered (both together) to another group of mice.

[0457] T cell homing at the tumor site was analyzed in GL261-Quad tumor-bearing mice (7-16 mice per group) vaccinated twice, namely at day 7 and at day 21 after tumor implantation (day 0), with 2 nmol Z13Mad5Anaxa vaccine. A group vaccinated with both, Z13Mad5 and Anaxa (equimolar to Z13Mad5Anaxa), was used as control. Briefly, C57BL/6 mice were implanted i.c. (intracranially) with 5×10^5 GL261-Quad tumor cells and vaccinated twice (at d7 and d21

following implantation) by s.c. injection of 2 nmol of Z13Mad5Anaxa (group 1) or 2 nmol of Z13Mad5 and 2 nmol of Anaxa (group 2). At day 28, the blood and the brain infiltrating leukocytes (BILs) were analyzed, whereby SIINFEKL-specific CD8 T cells were quantified in blood and in BILs at d28 by multimer staining (7 - 16 mice per group).

[0458] Results are shown in Figure 34. A significantly higher percentage of SIINFEKL-specific CD8 T cells was observed in the blood of Z13Mad5Anaxa-vaccinated mice as compared to mice vaccinated with both, Z13Mad5 and Anaxa (Fig. 34A). Similarly, a stronger accumulation of SIINFEKL-specific CD8 T cells was observed in the BILs of Z13Mad5Anaxa-vaccinated mice as compared to mice vaccinated with Z13Mad5 and Anaxa separately (Fig. 34B, $p = 0.0539$).

[0459] Next, cytokine secretion was assessed. To this end, C57BL/6 mice were implanted i.c. with 5×10^5 GI261-Quad tumor cells and vaccinated twice (d7 and 21) by s.c. injection of 2 nmol of Z13Mad5Anaxa or 2 nmol of Z13Mad5 and 2 nmol of Anaxa. BILs were isolated and cultured during 6h with matured BMDCs loaded or not with SIINFEKL peptide (SEQ ID NO: 35) in presence of BrefeldinA before intracellular staining for cytokines.

[0460] Results are shown in Figure 35. In general, a high level of cytokine secretion was observed for brain-infiltrating CD8 T cells from mice vaccinated with Z13Mad5Anaxa. In particular, a significantly higher secretion of total IFN- γ and of IFN- γ and TNF- α together was observed for brain-infiltrating CD8 T cells from mice vaccinated with Z13Mad5Anaxa as compared to mice vaccinated with Z13Mad5 and Anaxa separately.

[0461] Taken together, these results demonstrate that Z13Mad5Anaxa vaccine (as compared to Z13Mad5 and Anaxa administered separately) was able to elicit a stronger SIINFEKL specific CD8 T cell immune response in the brain of tumor-bearing mice with potent effector function.

[0462] The results obtained are indicating that Z13Mad5Anaxa is efficacious for eliciting high brain infiltrating SIINFEKL-specific CD8 immune response. Z13Mad5Anaxa is able to promote the secretion of cytokine by antigen-specific CD8 T cells in the brain.

Example 21: Superior efficacy of Z13Mad5Anaxa fusion construct compared to Z13Mad5 and Anaxa in naïve mice

[0463] Next, the efficacy of a complex according to the present invention was investigated in naïve mice. Namely, Z13Mad5Anaxa (SEQ ID NO: 28) was administered to one group of mice, whereas Z13Mad5 (SEQ ID NO: 46) and Anaxa (SEQ ID NO: 15) were administered (both together) to another group of mice.

[0464] C57BL/6 mice of the Z13Mad5Anaxa group and of the Z13Mad5 + Anaxa group were vaccinated once (Week0) by s.c. injection of 2 nmol of Z13Mad5Anaxa (group 1) or 2 nmol of

Z13Mad5 and 2 nmol of Anaxa (group 2). At day 14, the blood was analyzed, whereby SIINFEKL-specific CD8 T cells were quantified in blood by multimer staining (4 - 8 mice per group).

[0465] Results are shown in Figure 36. A significantly higher percentage of SIINFEKL-specific CD8 T cells was observed in the blood of Z13Mad5Anaxa-vaccinated mice as compared to mice vaccinated with Z13Mad5 and Anaxa separately (Fig. 36).

[0466] Taken together, these results demonstrate that Z13Mad5Anaxa vaccine (as compared to Z13Mad5 and Anaxa administered separately) was able to elicit a stronger SIINFEKL specific CD8 T cell immune response in the periphery.

Example 22: Effects of combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist on tumor growth and survival rate in a colon carcinoma model

[0467] In order to assess the effects of combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist in treating colorectal cancer, the MC-38 tumor model was used. MC-38 is a colon carcinoma cell line.

[0468] To this end, "Z13Mad12Anaxa" was provided, which is a complex comprising a cell penetrating peptide "Z13", the antigenic cargo "MAD12" comprising three neoantigens as identified by Yadav et al. Nature. 2014 Nov 27;515(7528):572-6 from MC-38 tumor cell line, and the TLR peptide agonist "Anaxa". In the following, the amino acid sequence of Z13Mad12Anaxa is shown:

KRYKNRVASRKSRKFKQLLQHYREVAAAKSSENDRLRLLLKLFRAAQLANDVVLQIMEHLELA
SMTNMELMSSIVVISASIIVFNLLELEGSTVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE

(SEQ ID NO: 45)

[0469] C57BL/6 mice (seven mice per group, female, 7 week old) were implanted s.c. with 2×10^5 MC-38 tumor cells in the left flank (day 0). After tumor implantation, mice of the groups "Z13Mad12Anaxa" and "Z13Mad12Anaxa + anti-PD1" were vaccinated at days 3, day10 and 17 subcutaneously with 2 nmol of Z13Mad12Anaxa at the tail base. 200 µg of anti-PD1 antibody RMP1-14 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 6, 10, 13, 17, 20, 24, 27 and 31 to mice of groups "anti-PD1" and "Z13Mad12Anaxa + anti-PD1". At days 10 and 17, when both, Z13Mad12Anaxa and anti-PD1 antibody, were administered to group "Z13Mad12Anaxa + anti-PD1", the antibody was administered i.p. just after s.c. administration of Z13Mad12Anaxa. Tumor size was measured with a caliper.

[0470] As shown in Figures 37 and 38, treatment with the PD1 inhibitor alone or with Z13Mad12Anaxa alone resulted in significantly reduced tumor volume (Fig. 37A) and

increased survival (Fig. 37B), as compared to the control group. However, the combination of both, the PD1 inhibitor and Z13Mad12Anaxa, resulted in the most pronounced improvement, namely in strongly decreased tumor volume and strongly increased survival rates. These data show that a combination of both, anti-PD1 therapy and Z13Mad12Anaxa vaccination, is more efficient than anti-PD1 therapy alone or Z13Mad12Anaxa vaccination alone. Moreover, in the control group all mice showed tumors (0/7 tumor free mice), whereas in Z13Mad12Anaxa group 2/7 mice were tumor free and in the anti-PD1 group 3/7 mice were tumor free. Interestingly, in the "Z13Mad12Anaxa + anti-PD1" only one mouse showed tumors, i.e. 6/7 mice were tumor free. This is more than the sum of tumor-free mice in the Z13Mad12Anaxa group (2/7) and in the anti-PD1 group (3/7). Taken together, these results indicate a strong synergistic effect of a combination of anti-PD1 therapy and Z13Mad12Anaxa vaccination.

Example 23: Effects of combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist on tumor growth and survival rate in colon carcinoma model

[0471] In Example 23, further animals were subdued to the experimental test of Example 22 in order to enlarge the group sizes of the different experimental groups of Example 22. Therefore, the experimental results "include" the results of the animals of Example 22 and those of additional animals.

[0472] Briefly, the effects of combination of a PD1 inhibitor and a complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist in treating cancer, the MC-38 tumor model was used. MC-38 is a colon carcinoma cell line.

[0473] "Z13Mad12Anaxa" was provided, which is a complex comprising a cell penetrating peptide "Z13", the antigenic cargo "MAD12" comprising three neoantigens as identified by Yadav et al. Nature. 2014 Nov 27;515(7528):572-6 from MC-38 tumor cell line, and the TLR peptide agonist "Anaxa". In the following, the amino acid sequence of Z13Mad12Anaxa is shown:

KRYKNRVASRKSRKFKQLLQHYREVAAAQSSNDRLRLLLKLFRAAQLANDVVLQIMEHLELA

SMTNMELMSSIVVISASIVFNLLELEGSTVHEILCKLSLEGDHSTPPSAYGSKPYTNFDEA

(SEQ ID NO: 45)

[0474] C57BL/6 mice (thirteen to fourteen mice per group, female, 7 week old) were implanted s.c. with 2×10^5 MC-38 tumor cells in the left flank (day 0). After tumor implantation, mice of the groups "Z13Mad12Anaxa" and "Z13Mad12Anaxa + anti-PD1" were vaccinated at days 3, 10 and 17 subcutaneously with 2 nmol of Z13Mad12Anaxa at the tail base. 200 µg of anti-PD1 antibody RMP1-14 (BioXcell, West Lebanon, NH, USA) were administered i.p. on each of days 6, 10, 13, 17, 20, 24 and 27 to mice of groups "anti-PD1" and "Z13Mad12Anaxa + anti-PD1". At days 10 and 17, when both, Z13Mad12Anaxa and antibody, were administered, the antibody was administered i.p. just after s.c. administration of Z13Mad12Anaxa. Tumor size

was measured with a caliper.

[0475] As shown in Figures 39 and 40, treatment with the PD1 inhibitor alone or with Z13Mad12Anaxa alone resulted in significantly reduced tumor volume (Fig. 39A) and increased survival (Fig. 39B), as compared to the control group. However, the combination of both, the PD1 inhibitor and Z13Mad12Anaxa, resulted in the most pronounced improvement, namely in strongly decreased tumor volume and strongly increased survival rates. Of note, in the "Z13Mad12Anaxa + aPD1" group only three mice developed tumors (whereas 10 mice remained tumor-free), whereas in the "aPD1" group and in the "Z13Mad12Anaxa" group eight and ten mice, respectively, developed tumors. In the control group all mice developed tumors.

[0476] These data show that a combination of both, anti-PD1 therapy and Z13Mad12Anaxa vaccination, is more efficient than anti-PD1 therapy alone or Z13Mad12Anaxa vaccination alone. These results thus indicate a synergistic effect of anti-PD1 therapy and Z13Mad12Anaxa vaccination.

TABLE OF SEQUENCES AND SEQ ID NUMBERS (SEQUENCE LISTING):

SEQ ID NO	Sequence	Remarks
SEQ ID NO: 1	RQIKIYFQNRRMKWKK	CPP: Penetratin
SEQ ID NO: 2	YGRKKRRQRRR	CPP: TAT minimal
SEQ ID NO: 3	MMDPNSTSEDEVKFTDPYQVPFVQAFDQATRV YQDLGGPSQAPLPCVLWPVLPEPLPQGQLTAY HVSTAPTGSWFSAPQPAPENAYQAYAAPQLFP VSDITQNQQTNQAGGEAPQPGDNSTVQTAA AVVFACPGANQGQQLADIGVPQPAPVAAPAR RTRKPQQPESLEECDSELEIKRYKNRVASRKCRK FKQLLQHYREVAAAKSSENDRLRLLKQMCPSL DVDSIIPRTPDVLHEDLLNF	ZEBRA amino acid sequence (natural sequence from Epstein - Barr virus (EBV)) (YP_401673)
SEQ ID NO: 4	KRYKNRVASRKCRKFKQLLQHYREVAAAKSSE NDRLRLLKQMC	CPP1 (Z11)
SEQ ID NO: 5	KRYKNRVASRKCRKFKQLLQHYREVAAAKSSE NDRLRLLK	CPP2 (Z12)
SEQ ID NO: 6	KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSE NDRLRLLK	CPP3 (Z13)

SEQ ID NO	Sequence	Remarks
SEQ ID NO: 7	KRYKNRVASRKSRKFKQLLQHYREVAAAK	CPP4 (Z14)
SEQ ID NO: 8	KRYKNRVASRKSRKFK	CPP5 (Z15)
SEQ ID NO: 9	QHYREVAAAKSSND	CPP6 (Z16)
SEQ ID NO: 10	QLLQHYREVAAAK	CPP7 (Z17)
SEQ ID NO: 11	REVAAAKSSNDRLRLLK	CPP8 (Z18)
SEQ ID NO: 12	KRYKNRVA	CPP9 (Z19)
SEQ ID NO: 13	VASRKSRKFK	CPP10 (Z20)
SEQ ID NO: 14	ESLKISQAVHAAHAEINEAGREVVGAL KVPRNQDWLGVPFAKFAFSAQGALA NIAVDKANLDVEQLESIINFEKLTWTGS	MAD5 cargo
SEQ ID NO: 15	STVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE	TLR2 peptide agonist Anaxa
SEQ ID NO: 16	DDDK	enterokinase target
SEQ ID NO: 17	IEDGR	factor Xa target site

SEQ ID NO	Sequence	Remarks
SEQ ID NO: 18	LVPRGS	thrombin target site
SEQ ID NO: 19	ENLYFQG	protease TEV target
SEQ ID NO: 20	LEVLFQGP	PreScission protease target
SEQ ID NO: 21	RX(R/K)R	furin target site
SEQ ID NO: 22	GGGGG	peptidic linker
SEQ ID NO: 23	GGGG	peptidic linker
SEQ ID NO: 24	EQLE	peptidic linker
SEQ ID NO: 25	TEWT	peptidic linker
SEQ ID NO: 26	MHHHHHHNIDRPKGLAFTDVDVDSIKIA WESPQQQVSRVRYTVSSPEDGIRELFPAP DGEDDTAELQGLRPGSEYTVSVVALHDD MESQPLIGIQSTKRYKNRVASRKSRAKFKQ LLQHYREVAAAKSSENDRLRLLKESLKISQ AVHAAHAEINEAGREVVGVGALKVPRN QDWLGVPRAFASFEEAQQGALANIAVDK ANLDVEQLESIINFELTEWGTGS	EDAZ13Mad5
SEQ ID NO:	MHHHHHHSTVHEILCKLSLEGDHSTPPSA YGSVKPYTNFDAEKRYKNRVASRKSRAKF	AnaxaZ13Mad5

SEQ ID NO	Sequence	Remarks
27	KQLLQHYREVAAAKSENDRLRLLLKESLKI SQAVHAAHAEINEAGREVVGVGALKVPR NQDWLGVPRFAKFASFEAQGALANIAVD KANLDVEQLESIINFEKLTEWTGS	
SEQ ID NO: 28	MHHHHHHKRYKNRVASRKSRKFKQLL QHYREVAAAKSENDRLRLLLKESLKISQA VHAAHAEINEAGREVVGVGALKVPRNQD WLGVPFAKFASFEAQGALANIAVDKANL DVEQLESIINFEKLTEWTGSSTVHEILCKLSL EGDHSTPPSAYGSVKPYTNFDAE	Z13Mad5Anaxa
SEQ ID NO: 29	MHHHHHHKRYKNRVASRKSRKFKQLL QHYREVAAAKESLKISQAVHAAHAEINE AGREVVGVGALKVPRNQDWLGVPRFA KFASFEAQGALANIAVDKANLDVEQLESI INFEKLTEWTGSSTVHEILCKLSLEGDHST PPSAYGSVKPYTNFDAE	Z14Mad5Anaxa
SEQ ID NO: 30	MHHHHHHHREVAAAKSENDRLRLLLKES LKISQAVHAAHAEINEAGREVVGVGALKV PRNQDWLGVPRFAKFASFEAQGALANIA VDKANLDVEQLESIINFEKLTEWTGSSTVH EILCKLSLEGDHSTPPSAYGSVKPYTNFDA E	Z18Mad5Anaxa
SEQ ID NO: 31	MHHHHHHNIDRPKGLAFTDVDVDSIKIA WESPQQQVSRYRVTYSSPEDGIRELFPAP DGEDDTAELQGLRPGSEYTVSVVALHDD MESQPLIGIQSTKRYKNRVASRKSRKFKQ LLQHYREVAAAKESLKISQAVHAAHAEIN EAGREVVGVGALKVPRNQDWLGVPRFA KFASFEAQGALANIAVDKANLDVEQLESII NFEKLTEWTGS	EDAZ14Mad5
SEQ ID NO: 32	MHHHHHHNIDRPKGLAFTDVDVDSIKIA WESPQQQVSRYRVTYSSPEDGIRELFPAP DGEDDTAELQGLRPGSEYTVSVVALHDD MESQPLIGIQSTREVAAAKSENDRLRLLL KESLKISQAVHAAHAEINEAGREVVGVA LKVPRNQDWLGVPRFAKFASFEAQGALA NIAVDKANLDVEQLESIINFEKLTEWTGS	EDAZ18Mad5
SEQ		Z13Mad8Anaxa

SEQ ID NO	Sequence	Remarks
ID NO: 33	KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSEN DRLRLLLKVITYHSPSYVYHQFERRAILNRLVQFIK DRISVVQALVLTSTVHEILCKLSLEGDHSTPPSAY GSVKPYTN FDAE	
SEQ ID NO: 34	MHHHHHHNIDRPKGLAFTDVDVDSIKIAWESP QGQVSRYRVITYSSPEDGIRELFPAPDGEDDTAEL QGLRPGSEYTVSVVALHDDMESQPLIGIQSTESL KISQAVHAAHAEINEAGREVVGVGALKVPRNQ DWLGVPRFAKFAFEAQGALANIAVDKANLDVE QLE SIINFEKLTE WTGS	EDAMad5
SEQ ID NO: 35	SIINFEKL	SIINFEKL OVACD8 peptide
SEQ ID NO: 36	VTYHSPSYAYHQFERRAILNRLVQFIKDRI	Mad8
SEQ ID NO: 37	NYRIATFKNWPFLEDCAMEELTVSEFLKLDQR	Mad11
SEQ ID NO: 38	HLELASMTNMELMSSIV	Mad9
SEQ ID NO: 39	LFRAAQLANDVVLQIMEHLELASMTNMELMSSI VVISASIIVFNLLELEG	Mad 12
SEQ ID NO: 40	NIDRPKGLAFTDVDVDSIKIAWESPQGQVSRYR VTYSSPEDGIRELFPAPDGEDDTAELQGLRPGSEY TVSVVALHDDMESQPLIGIQST	EDA
SEQ ID NO: 41	KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSEN DRLRLLLKVITYHSPSYAYHQFERRAILNRLVQFIK DRISVVQALVLTSTVHEILCKLSLEGDHSTPPSAY GSVKPYTN FDAE	Z13Mad8Anaxa
SEQ ID	KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSEN	Z13Mad11Anaxa

SEQ ID NO	Sequence	Remarks
NO: 42	DRLRLLKKNYRIATFKNWPFLDCAMEELTVSEFL KLDQRSTVHEILCKLSLEGDHSTPPSAYGSVKPY TNFDAE	
SEQ ID NO: 43	KRYKNRVASRKSRKFKQLLQHYREVAAKSSSEN DRLRLLKHLELASMTNMELMSSIVSTVHEILCKLS LEGDHSTPPSAYGSVKPYTNFDAE	Z13Mad9Anaxa
SEQ ID NO: 44	RKKRRQRRRRVKRISQAVHAAHAEINEAGRVRK RKVPRNQDWLRVKRASFEAQGALANIAVDKAR VKRSIINFELRVKRSTVHEILCKLSLEGDHSTPPSA YGSVKPYTNFDAE	TatFMad5Anaxa
SEQ ID NO: 45	KRYKNRVASRKSRKFKQLLQHYREVAAKSSSEN DRLRLLKLFRAAQLANDVVLQIMEHLELASMTN MELMSSIVVISASIIVFNLELEGSTVHEILCKLSLEG DHSTPPSAYGSVKPYTNFDAE	Z13Mad12Anaxa
SEQ ID NO: 46	MHHHHHHKRYKNRVASRKSRKFKQLLQHYRE VAAKSSSENDRRLLLKESLKISQAVHAAHAEINE AGREVGVGALKVPRNQDWLGVPFAKFAFE AQGALANIAVDKANLDVEQLESINFEKLTEWTG S	Z13Mad5
SEQ ID NO: 47	STVHEILSKLSLEGDHSTPPSAYGSVKPYTNFDAE	TLR peptide agonist: "Anaxa" sequence variant

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P A T E N T K R A V

1. Kombination af

(i) en immuncheckpoint-modulator og

(ii) et kompleks omfattende af:

5 a) et cellepenetrerende peptid;

b) mindst ét antigen eller antigen epitop; og

c) mindst én TLR-peptidagonist,

hvor komponenterne a) - c), som er omfattet af komplekset, er forbundet af kovalente bindinger, til anvendelse i medicin.

10

2. Kombination til anvendelse ifølge krav 1, hvor komplekset er et rekombinant polypeptid eller et rekombinant protein.

3. Kombination til anvendelse ifølge krav 1, hvor det cellepenetrerende peptid,

15 som er omfattet af komplekset;

(i) har en længde af aminosyresekvensen af nævnte peptid på 5 til 50 aminosyrer i alt, fortrinsvist 10 til 45 aminosyrer i alt, mere fortrinsvist 15 til 45 aminosyrer i alt; og/eller

(ii) har en aminosyresekvens som omfatter et fragment af det minimale domæne af
20 ZEBRA, nævnte minimale domæne strækker sig fra aminosyre 170 til aminosyre 220 af ZEBRA-aminosyresekvensen ifølge SEQ ID NO: 3, hvor eventuelt 1, 2, 3, 4 eller 5 aminosyrer er blevet substitueret, slettet og/eller tilføjet uden at ophæve nævnte peptids cellepenetrerende evne, eller en variant deraf.

25 4. Kombination ifølge krav 3, hvor det cellepenetrerende peptid, som er omfattet af komplekset, har en aminosyresekvens som omfatter eller består af en aminosyresekvens ifølge SEQ ID NO: 6 (CPP3/Z13), SEQ ID NO: 7 (CPP4/Z14), SEQ ID NO: 8 (CPP5/Z15) eller SEQ ID NO: 11 (CPP8/Z18), eller sekvensvarianter deraf uden at ophæve nævnte peptids cellepenetrerende evne, fortrinsvist har det cellepenetrerende peptid, som er omfattet af
30 komplekset, en aminosyresekvens som omfatter eller består af en aminosyresekvens ifølge SEQ ID NO: 6 (CPP3/Z13) eller sekvensvarianter deraf uden at ophæve nævnte peptids

cellepenetrerende evne.

5. Kombination til anvendelse ifølge et hvilket som helst af kravene 1 til 4, hvor det mindst éne antigen eller antigen epitop, som er omfattet af komplekset, omfatter eller
5 består af mindst én patogent epitop og/eller mindst en tumor epitop, fortrinsvist omfatter eller består det mindst éne antigen eller antigen epitop af mindst en cancer epitop.

6. Kombination til anvendelse ifølge et hvilket som helst af kravene 1 til 5, hvor komplekset omfatter mere end ét antigen eller antigen epitop, især 2, 3, 4, 5, 6, 7, 8, 9,
10 10 eller flere antigener eller antigene epitoper, fortrinsvist hvor de flere antigener eller antigene epitoper, især 2, 3, 4, 5, 6, 7, 8, 9, 10 eller flere antigener eller antigene epitoper, er placeret fortløbende i komplekset.

7. Kombination til anvendelse ifølge et hvilket som helst af kravene 1 til 6, hvor
15 mindst én TLR-peptidagonist, som er omfattet af komplekset, er en TLR2-, TLR4- og/eller TLR5-peptidagonist, fortrinsvist en TLR2-peptidagonist og/eller en TLR4-peptidagonist, mere fortrinsvist mindst én TLR-peptidagonist, som er omfattet af komplekset, omfatter eller består af en aminosyresekvens ifølge SEQ ID NO: 15 eller 47; eller en sekvensvariant deraf.

20

8. Kombination til anvendelse ifølge et hvilket som helst af kravene 1 til 7, hvor immuncheckpoint-modulatoren er en aktivator eller en inhibitor af én eller flere immunkontrolpunktsmolekyler valgt fra CD27, CD28, CD40, CD122, CD137, OX40, GITR, ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA, CEACAM1, GARP, PS,
25 CSF1R, CD94/NKG2A, TDO, TNFR og/eller FasR/DcR3; eller en aktivator eller en inhibitor af én eller flere ligander dertil.

9. Kombination til anvendelse ifølge krav 8, hvor immuncheckpoint-modulatoren er;
- en inhibitor af et inhibitionscheckpointsmolekyle, fortrinsvist en inhibitor af A2AR,
30 B7-H3, B7-H4, BTLA, CD40, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA, CEACAM1, GARP, PS, CSF1R, CD94/NKG2A, TDO, TNFR og/eller DcR3 eller en ligand deraf; eller

- en aktivator af et stimulerende eller co-stimulerende checkpointsmolekyle, fortrinsvist en aktivator af CD27, CD28, CD40, CD122, CD137, OX40, GITR og/eller ICOS eller en ligand deraf.

5 10. Kombination til anvendelse ifølge krav 8 eller 9, hvor immuncheckpoint-modulatoren er en modulator af CD40, CTLA-4, PD-L1, PD-L2, PD-1 og/eller IDO, fortrinsvist er immuncheckpoint-modulatoren en inhibitor af CTLA-4, PD-L1, PD-L2, PD-1 og/eller IDO eller en aktivator af CD40, mere fortrinsvist immuncheckpoint-modulatoren en inhibitor af CTLA-4, PD-L1, PD-1 og/eller IDO og endnu mere fortrinsvist er immuncheckpoint-modulatoren en inhibitor af CTLA-4 og/eller PD-1.

15 11. Kombination til anvendelse ifølge et hvilket som helst af kravene 8 til 10, hvor der anvendes mere end én immuncheckpoint-modulator, især mindst 2, 3, 4, 5, 6, 7, 8, 9 eller 10 forskellige immuncheckpoint-modulatorer, fortrinsvist 2, 3, 4 eller 5 forskellige immuncheckpoint-modulatorer, mere fortrinsvist 2, 3 eller 4 forskellige immuncheckpoint-modulatorer, endnu mere fortrinsvist 2 eller 3 forskellige immuncheckpoint-modulatorer, og mest foretrukket 2 forskellige immuncheckpoint-modulatorer, såsom mindst (i) en inhibitor af CTLA-4 og (ii) en inhibitor af PD-1, PD-L1 eller PD-L2.

20 12. Kombination til anvendelse ifølge et hvilket som helst af kravene 8 til 11, hvor komplekset er et rekombinant polypeptid eller et rekombinant protein hvor

a) det cellepenetrerende peptid har en aminosyresekvens, der omfatter eller består af en aminosyresekvens ifølge SEQ ID NO: 6 (CPP3/Z13), SEQ ID NO: 7 (CPP4/Z14), SEQ ID NO: 8 (CPP5/Z15) eller SEQ ID NO: 11 (CPP8/Z18), eller sekvensvarianter deraf uden at
25 ophæve nævnte peptids cellepenetrerende evne;

b) mindst ét antigen eller antigen epitop er en peptid, polypeptid eller protein, og fortrinsvist omfatter eller består af mindst én cancer epitop; og

c) TLR-peptidagonisten er en TLR2-peptidagonist og/eller en TLR4-peptidagonist.

30 13. Kombination til anvendelse ifølge et hvilket som helst af kravene 1 til 12, hvor kombinationen anvendes til forebyggelse og/eller behandling af kræft.

14. Sæt som omfatter

(i) en immuncheckpoint-modulator og

(ii) en kompleks omfattende af:

5 a) en cellepenetrerende peptid;

b) mindst én antigen eller antigen epitop; og

c) mindst én TLR-peptidagonist,

hvor komponenterne a) - c), som er omfattet af komplekset, er forbundet af kovalente bindinger; fortrinsvist hvor immuncheckpoint-modulatoren og komplekset er som
10 defineret i et hvilket som helst af kravene 2 til 12.

15 15. Kit ifølge krav 14, hvor sættet yderligere omfatter en indlægningseddelse eller etiket med anvisninger til behandling af kræft ved brug af en kombination af immuncheckpoint-modulatoren og komplekset.

16. Sæt ifølge krav 14 eller 15 til anvendelse i medicin; fortrinsvist til anvendelse i forebyggelse og/eller behandling af kræft.

17. Sammensætning omfattende

20 (i) en immuncheckpoint-modulator og

(ii) en kompleks omfattende af:

a) en cellepenetrerende peptid;

b) mindst én antigen eller antigen epitop; og

c) mindst en TLR-peptidagonist,

25 hvor komponenterne a) - c), som er omfattet af komplekset, er forbundet af kovalente bindinger; fortrinsvist hvor immuncheckpoint-modulatoren og komplekset er som defineret i et hvilket som helst af kravene 2 til 12.

18. Sæt som omfatter

30 (i) et nukleinsyremolekyle bestående af et polynukleotid, som koder for en immuncheckpoint-modulator, hvor immuncheckpoint-modulatoren er et (poly)peptid eller et

protein; og

(ii) et nukleinsyremolekyle bestående af en polynukleotidkodning, der koder for et kompleks, hvor komplekset består af:

a) et cellepenetrerende peptid;

5 b) mindst ét antigen eller antigen epitop; og

c) mindst én TLR-peptidagonist, hvor komplekset er et (poly)peptid eller et protein, især et fusions-(poly)peptid eller et fusionsprotein; fortrinsvis hvor immuncheckpoint-modulatoren og komplekset er som defineret i et hvilket som helst af kravene 2 til 12.

10 19. Sæt ifølge krav 18 til anvendelse i medicin, fortrinsvis til anvendelse i forebyggelse og/eller behandling af kræft.

20. Sæt ifølge krav 18 eller sæt til anvendelse ifølge krav 19, hvor nukleinsyremolekylet er valgt fra gruppen bestående af en vektor, såsom en ekspressionsvektor; et RNA-molekyle, såsom et rRNA, et mRNA, et miRNA, et siRNA eller et tRNA; eller et DNA-molekyle, såsom et cDNA.

15

21. Nukleinsyremolekyle som omfatter

(i) et polynukleotid som koder for en immuncheckpoint-modulator, hvor immuncheckpoint-modulatoren er et (poly)peptid eller et protein; og

20

(ii) et polynukleotid som koder for et kompleks, hvor komplekset omfatter:

a) et cellepenetrerende peptid;

b) mindst ét antigen eller antigen epitop; og

c) mindst én TLR-peptidagonist,

25 hvor komplekset er et (poly)peptid eller et protein, især et fusion- (poly)peptid eller et fusionsprotein; fortrinsvis hvor immuncheckpoint-modulatoren og komplekset er som defineret i et hvilket som helst af kravene 2 til 12.

22. Sammensætning som omfatter

30 (i) et nukleinsyremolekyle som omfatter et polynukleotid som koder for en immuncheckpoint-modulator, hvor immuncheckpoint-modulatoren er et (poly)peptid eller et

protein; og

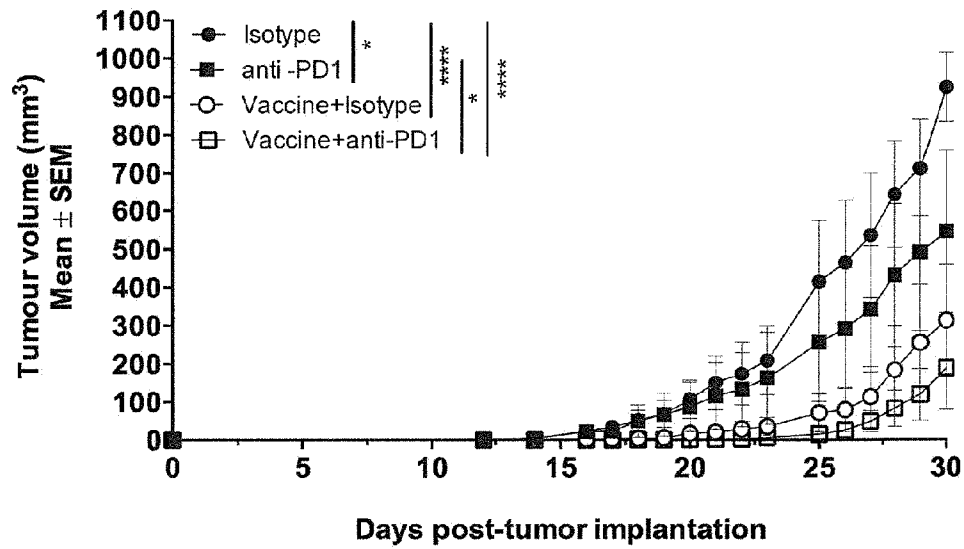
(ii) et nukleinsyremolekyle som omfatter et polynukleotid som koder for et kompleks, hvor komplekset omfatter:

- a) et cellepenetrerende peptid;
- 5 b) mindst ét antigen eller antigen epitop; og
- c) mindst en TLR-peptidagonist,

hvor komplekset er et (poly)peptid eller et protein, især et fusions-(poly)peptid eller et fusionsprotein; fortrinsvist hvor immunccheckpoint-modulatoren og komplekset er som defineret i et hvilket som helst af kravene 2 til 12.

DRAWINGS

A



B

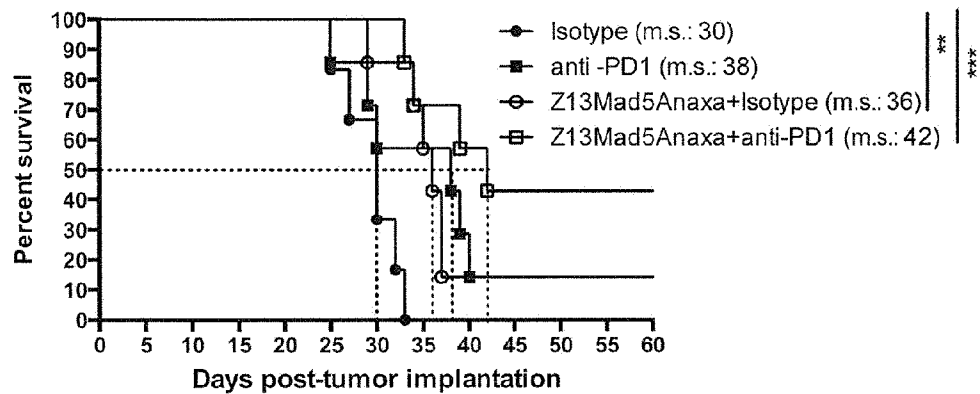


Fig. 1

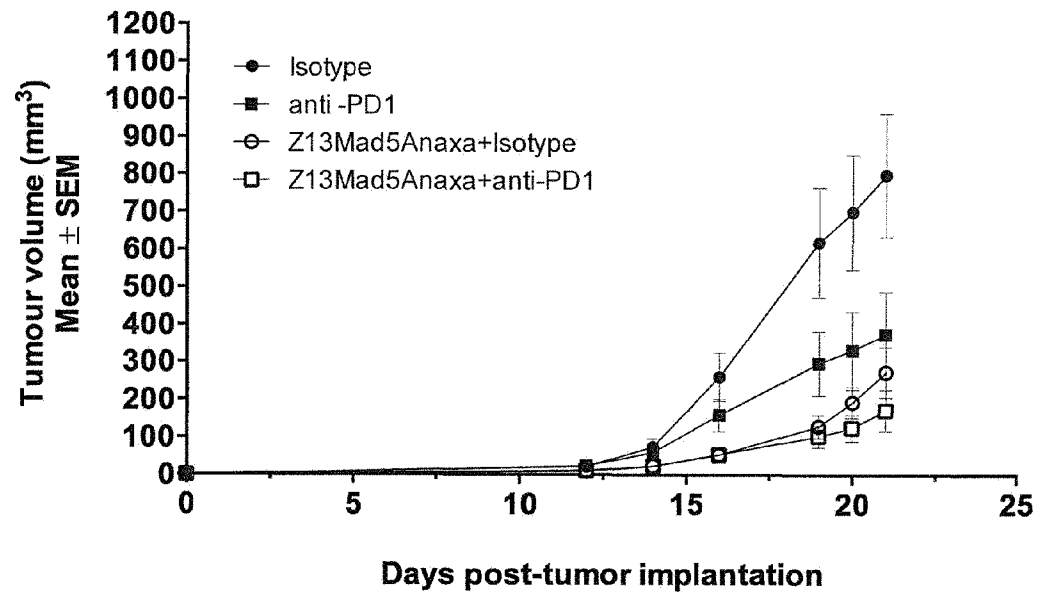
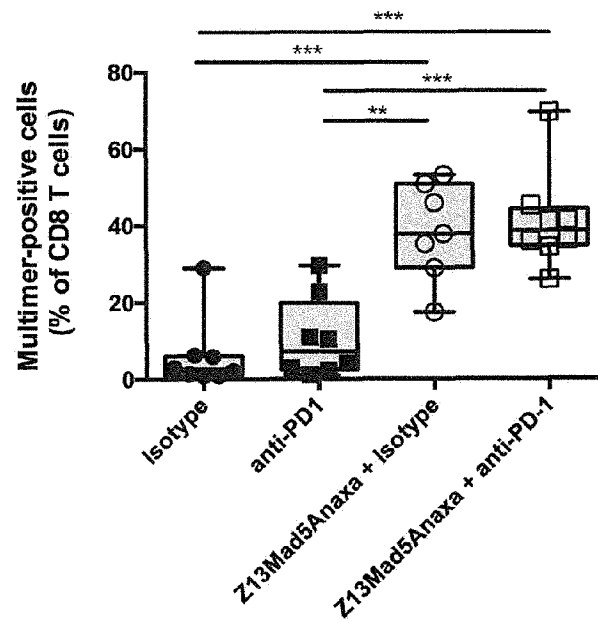


Fig. 2

A



B

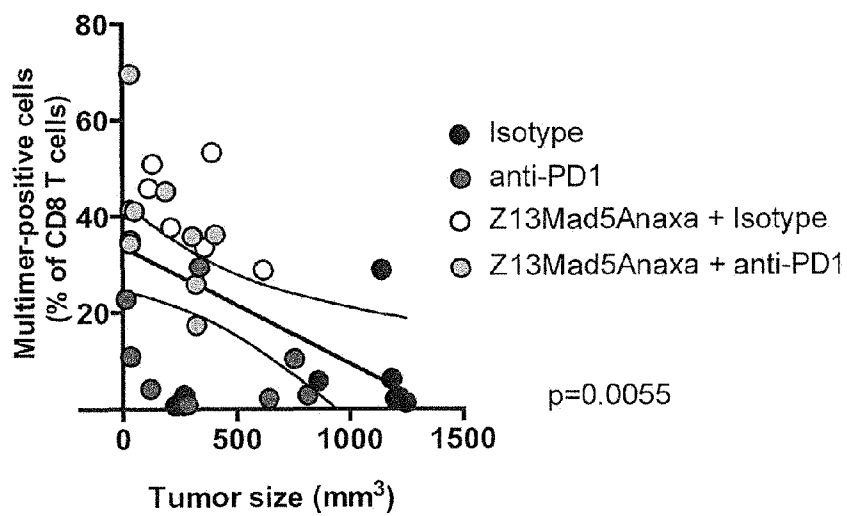
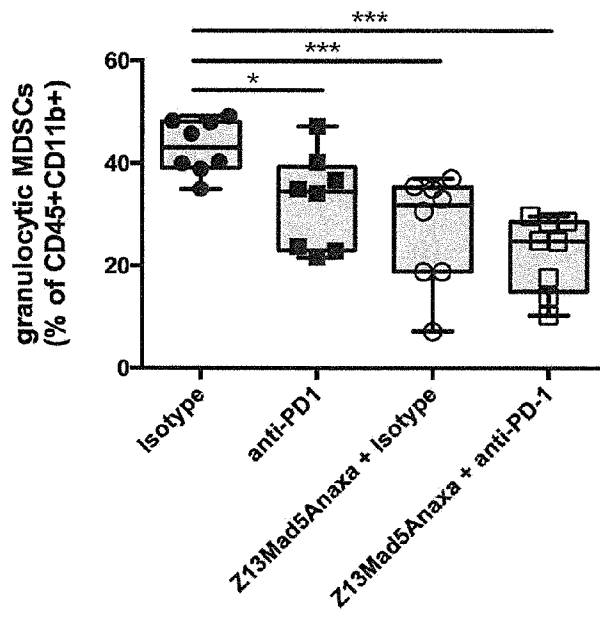


Fig. 3

A



B

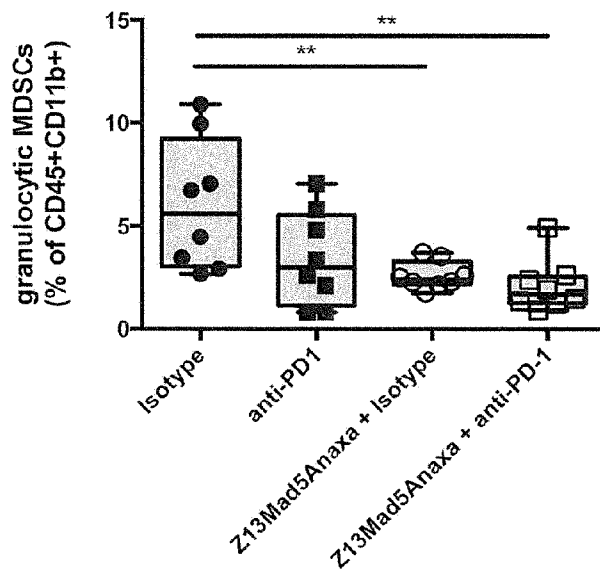
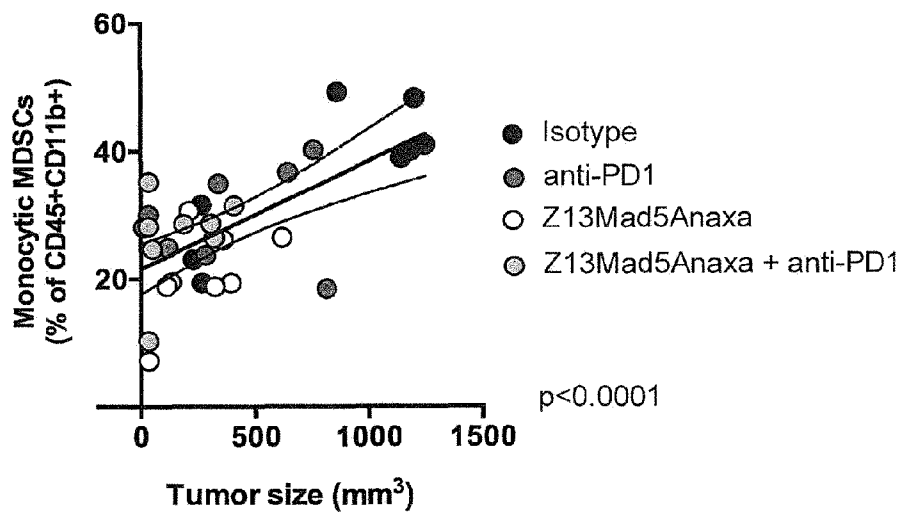


Fig. 4

A



B

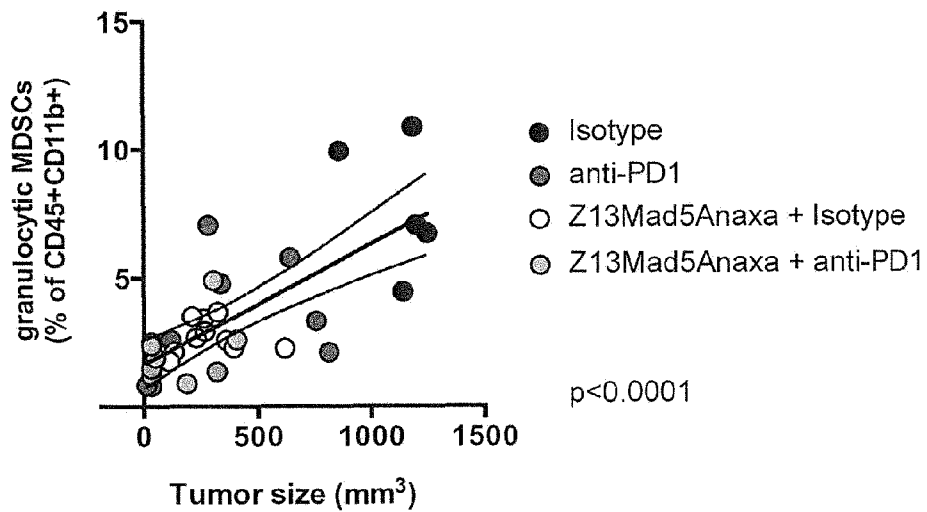
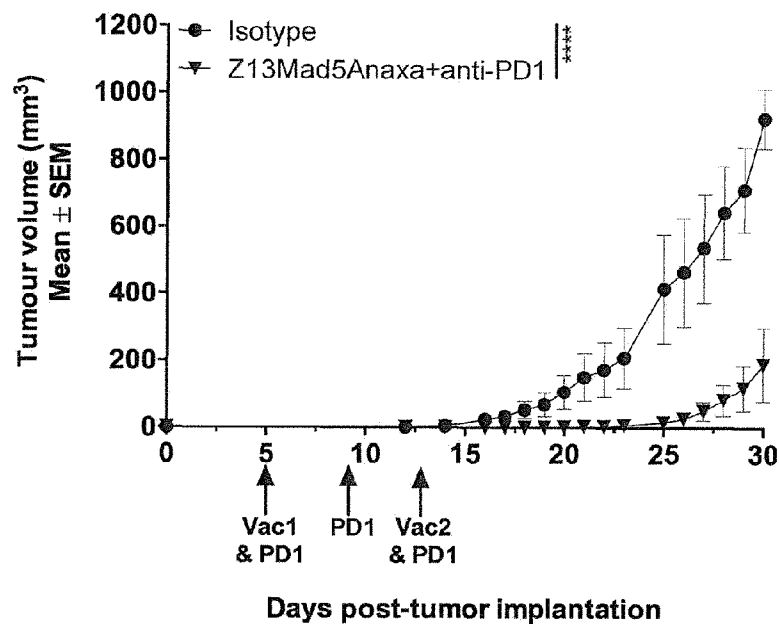


Fig. 5

A



B

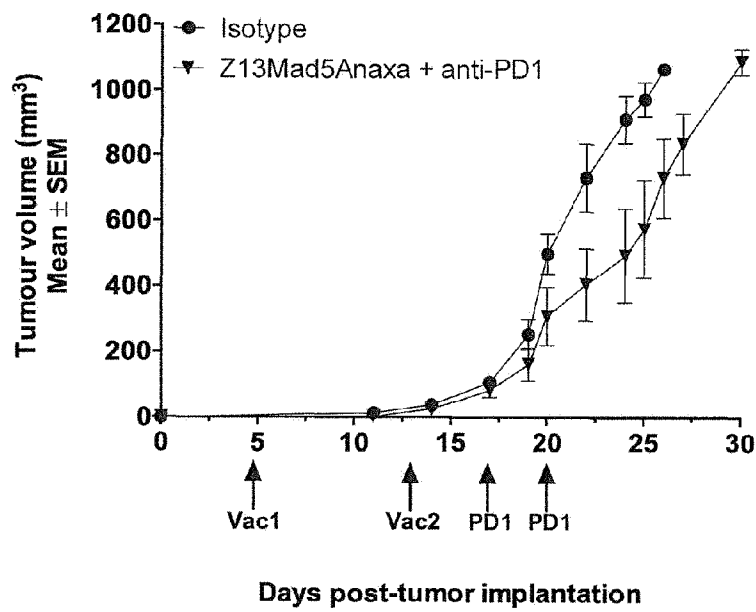


Fig. 6

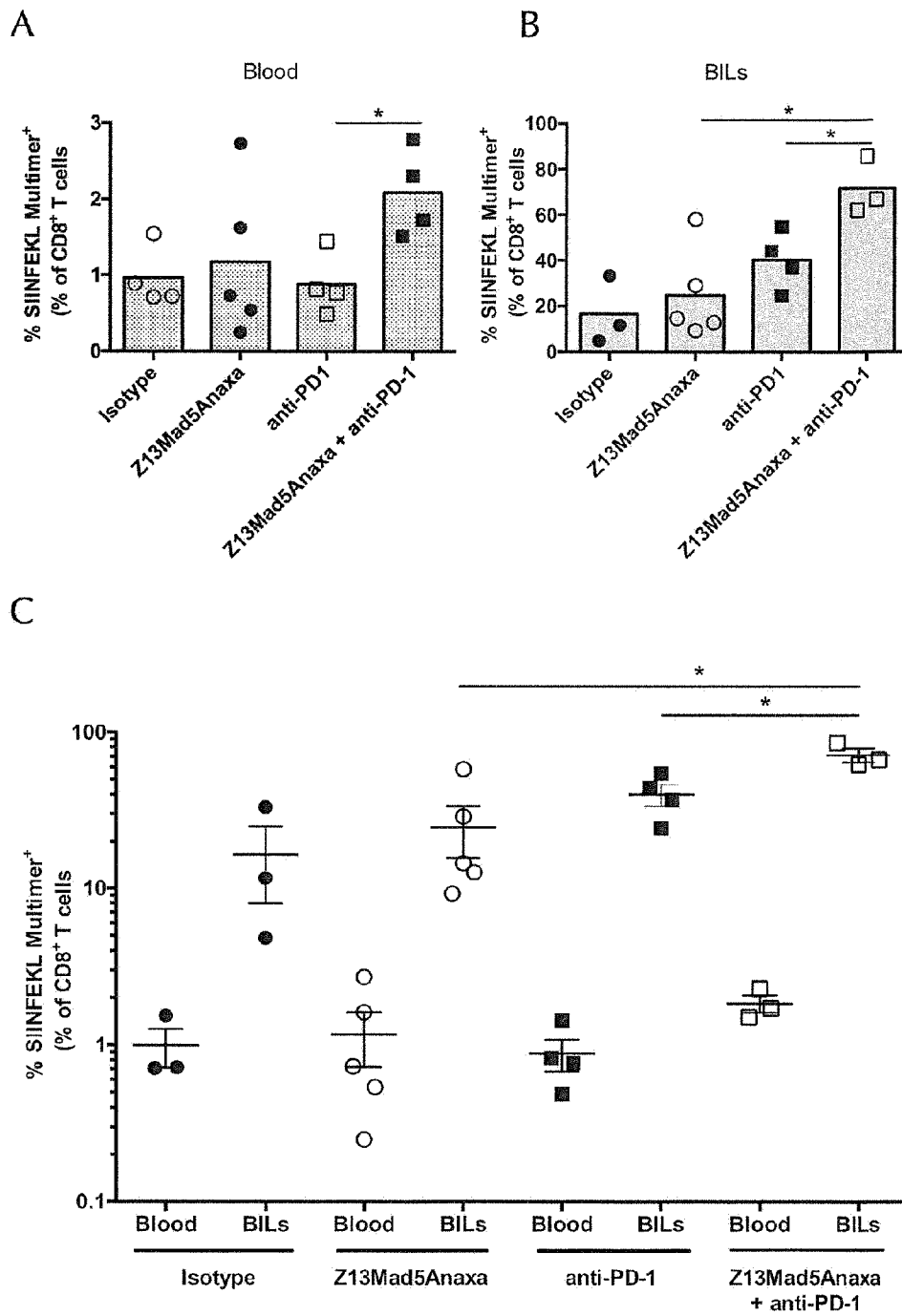
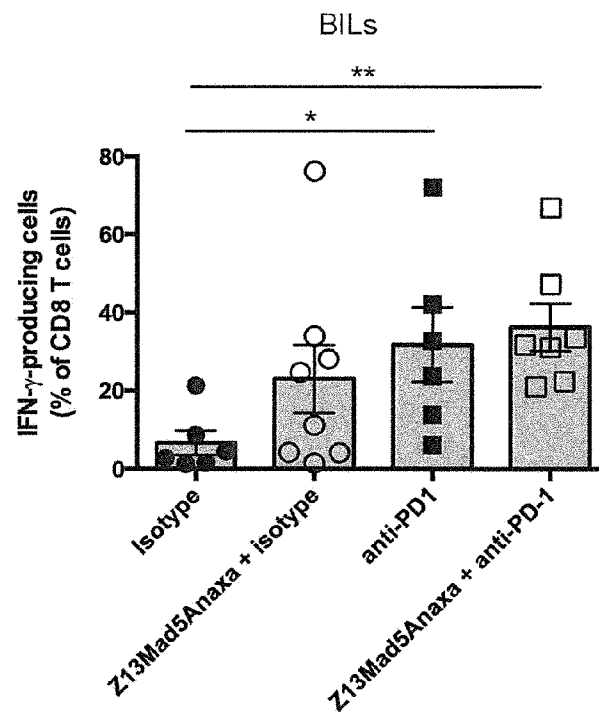


Fig. 7

A



B

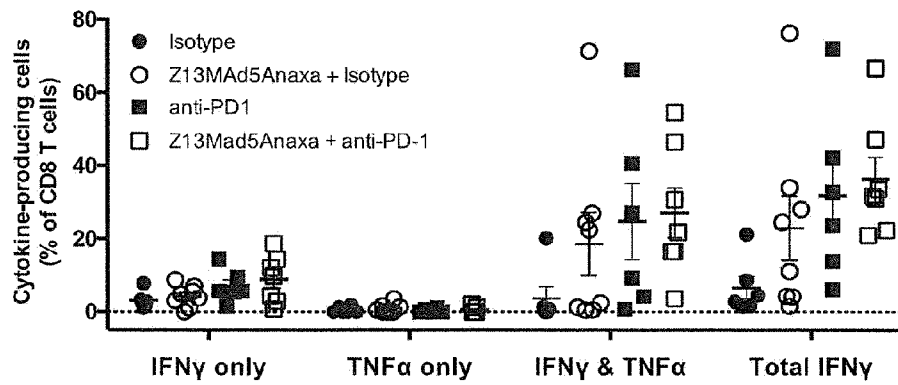


Fig. 8

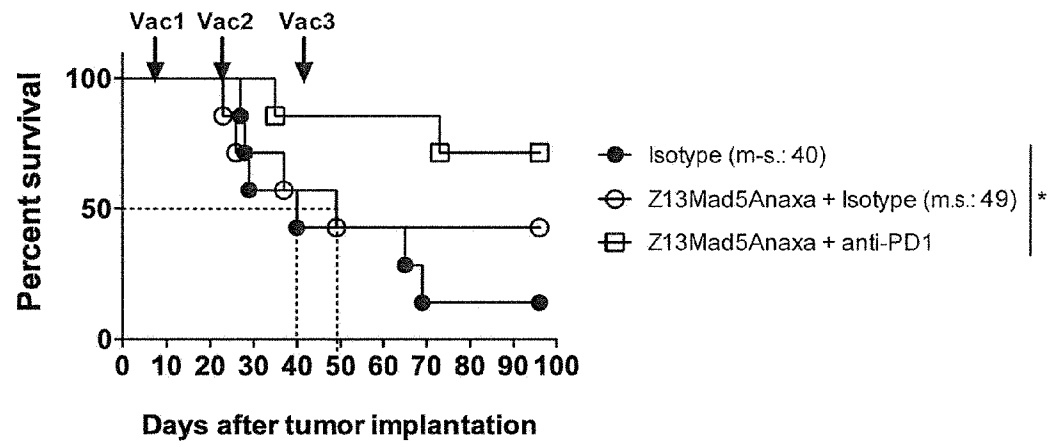


Fig. 9

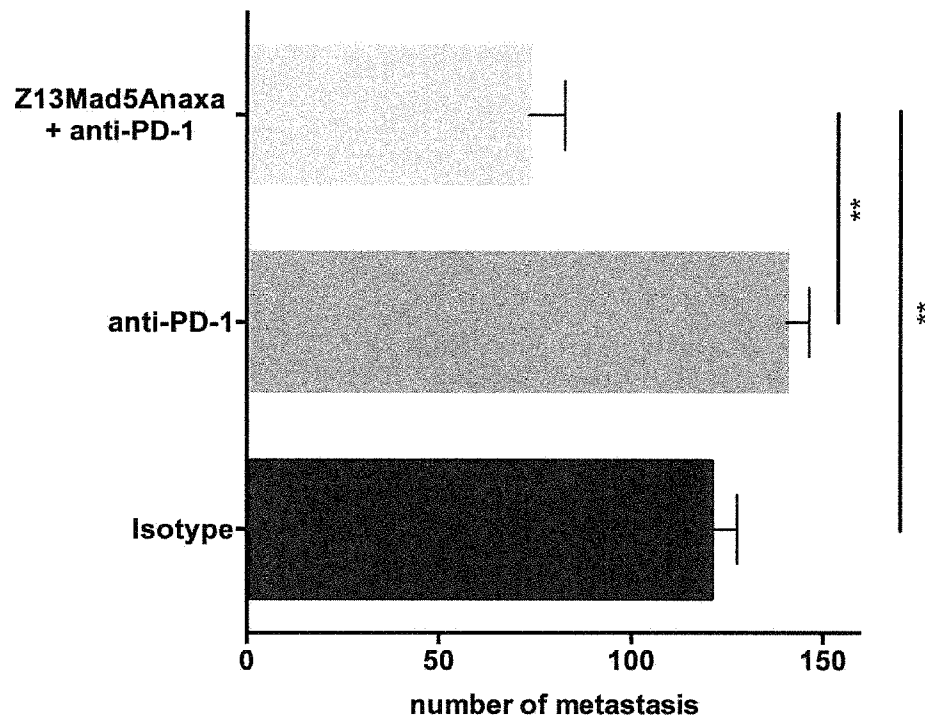


Fig. 10

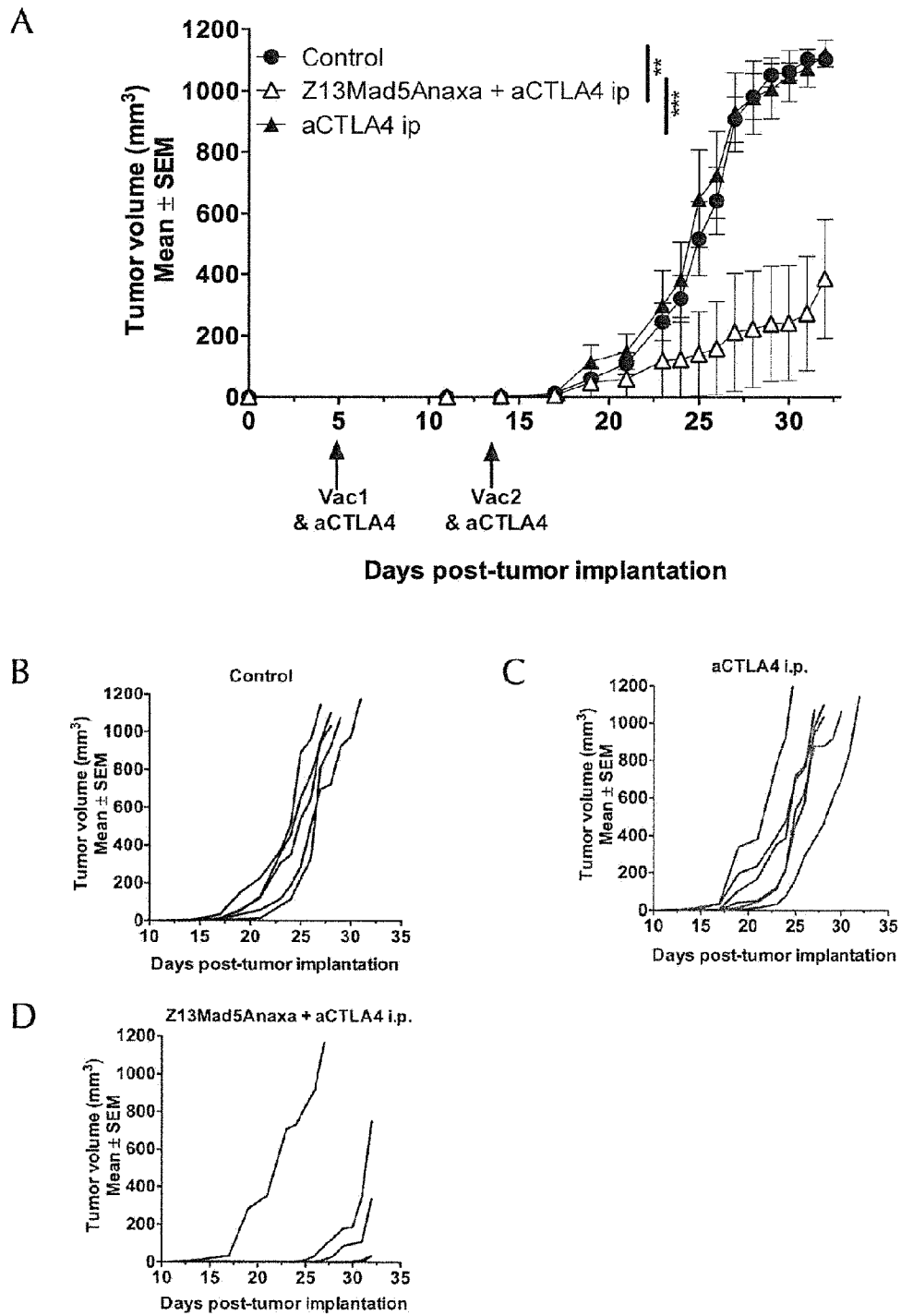


Fig. 11

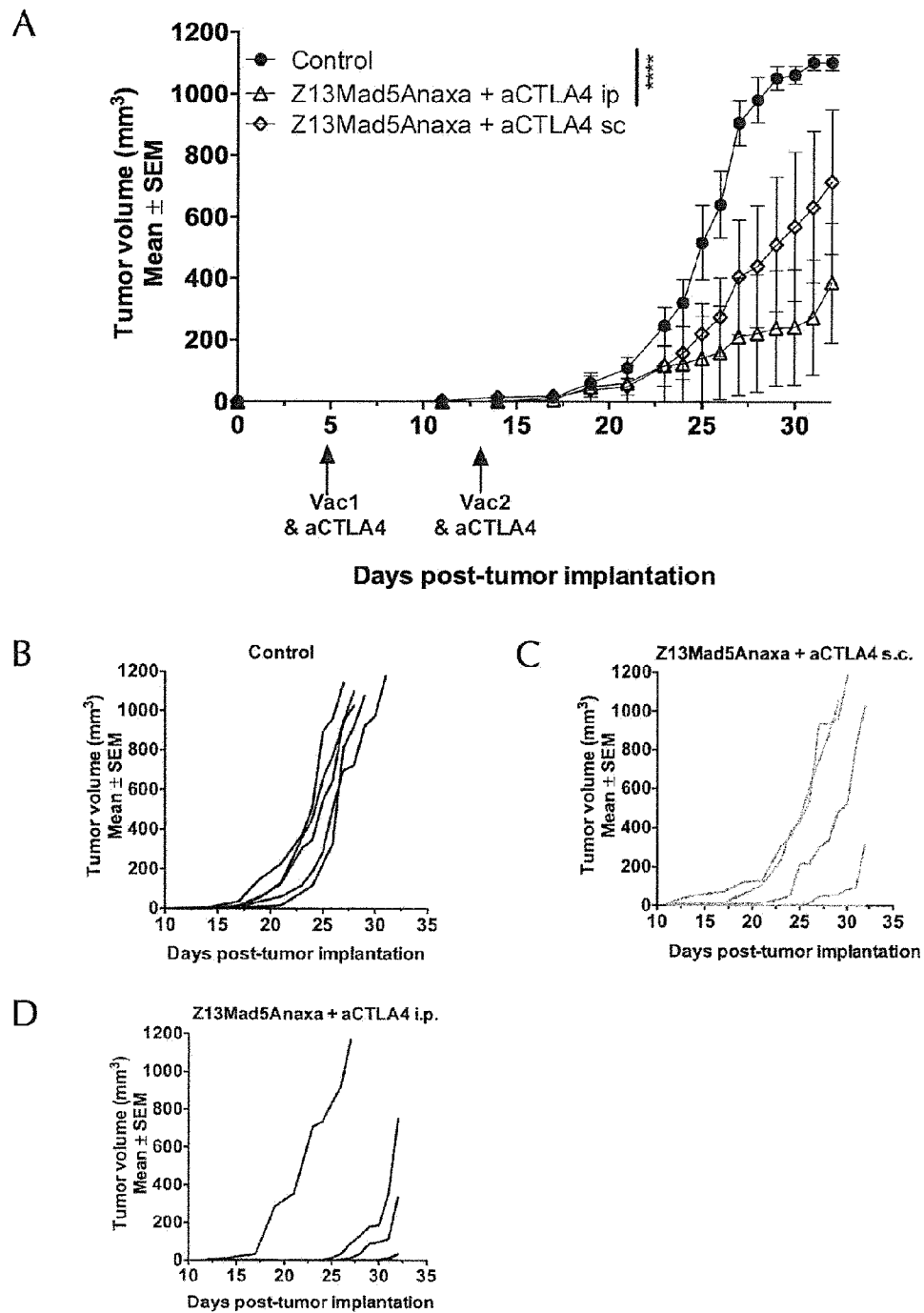
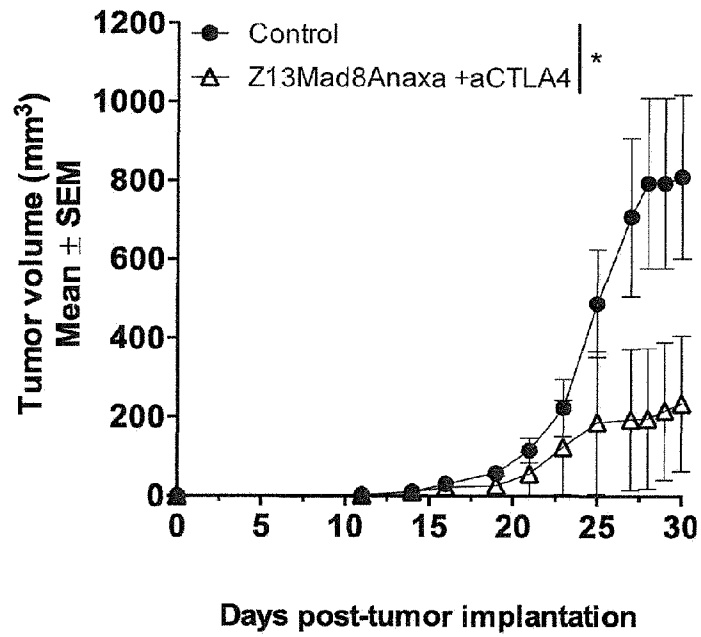
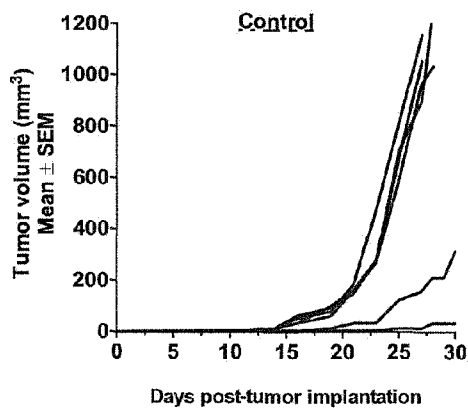


Fig. 12

A



B



C

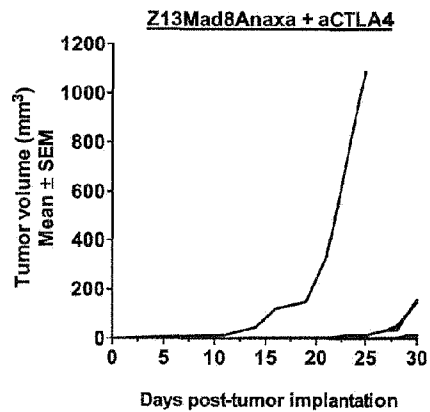
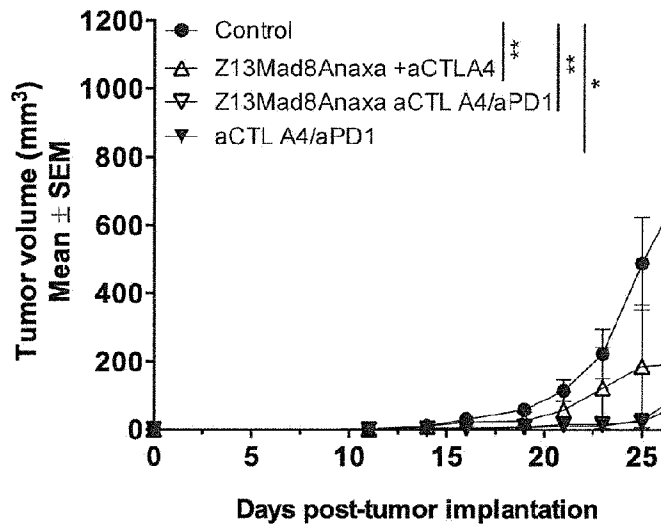
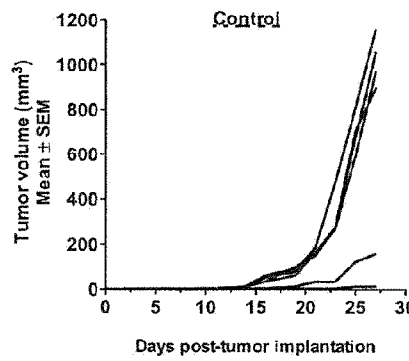


Fig. 13

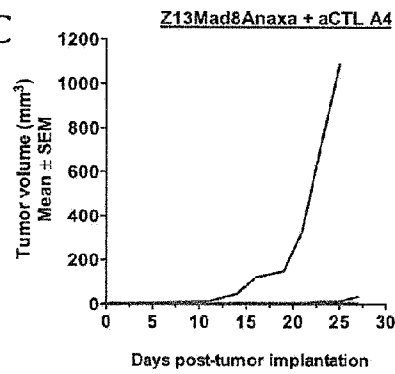
A



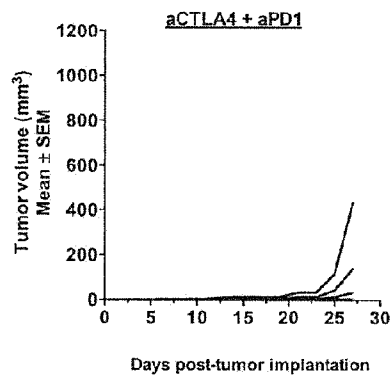
B



C



D



E

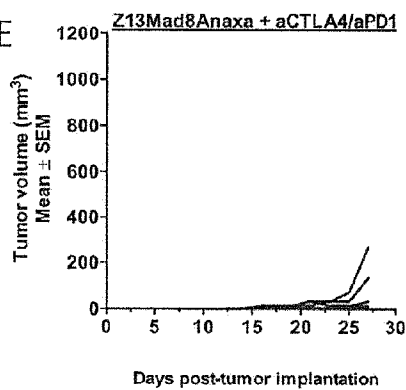
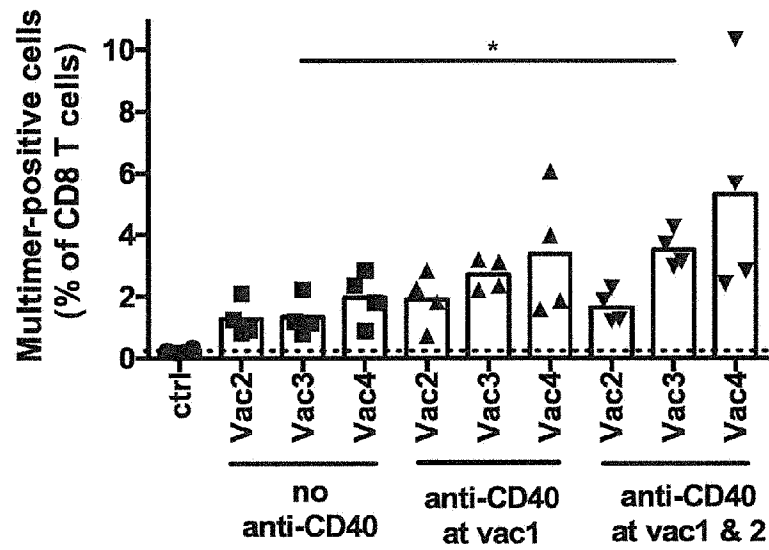


Fig. 14

A



B

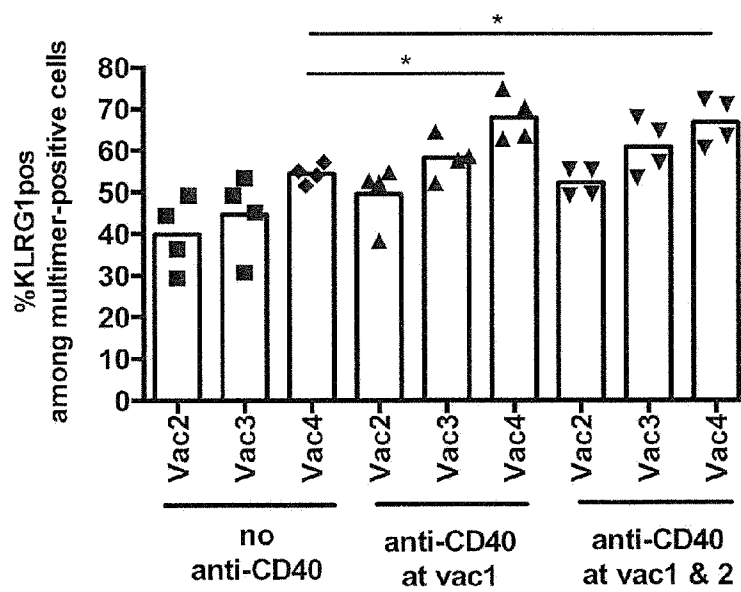
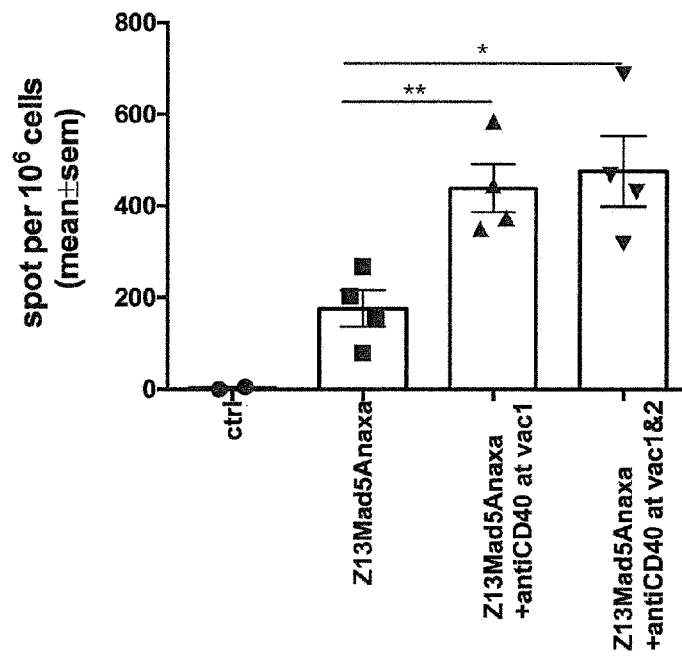


Fig. 15

A



B

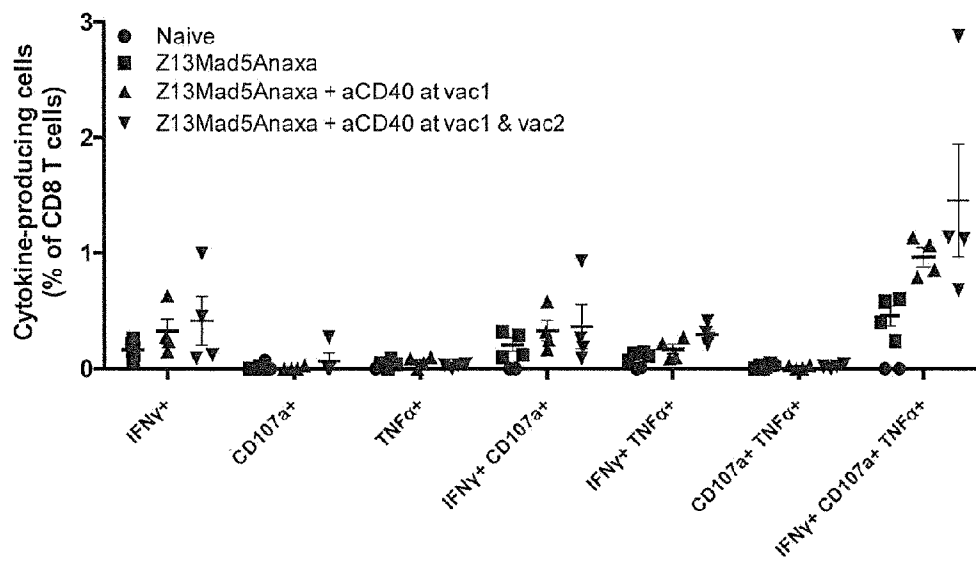


Fig. 16

Z13Mad5Anaxa

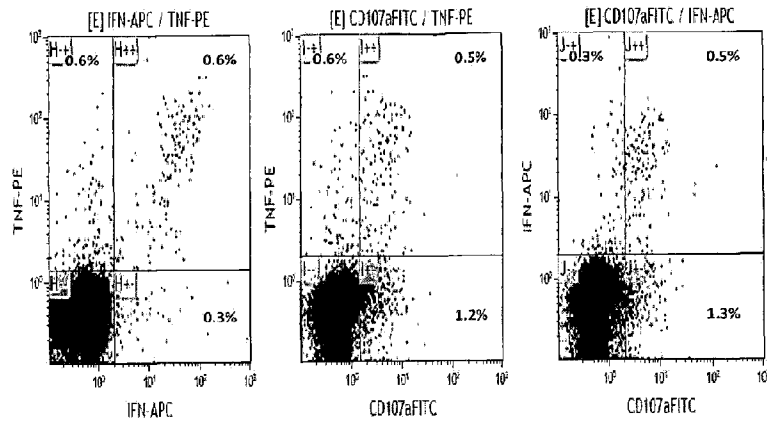
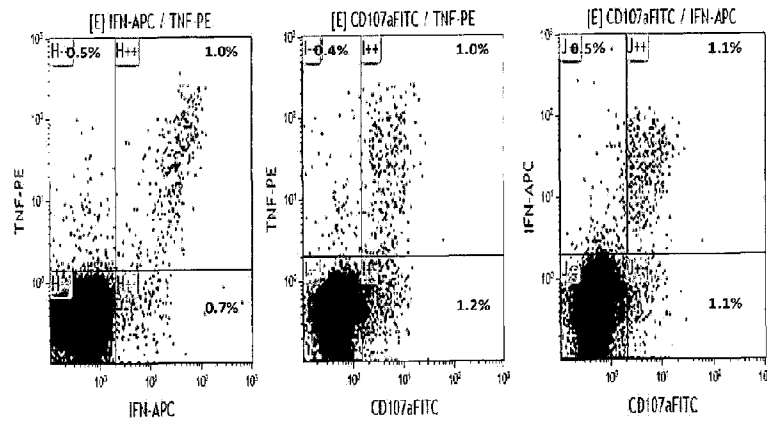
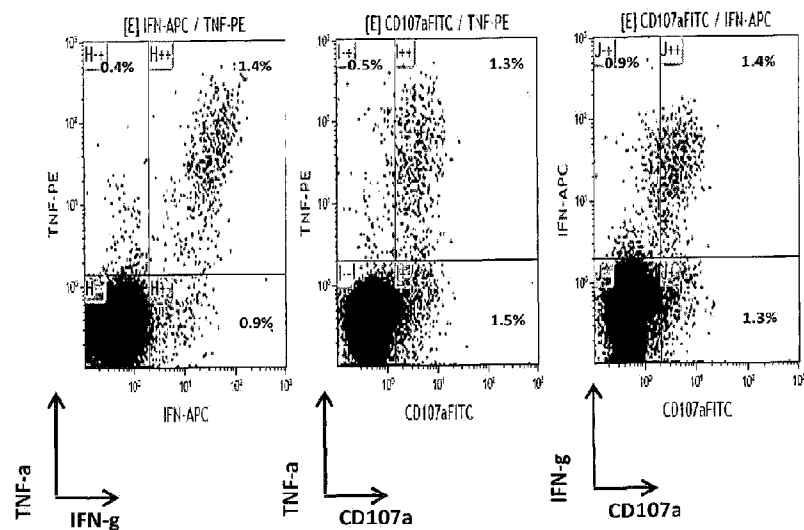
Z13Mad5Anaxa
+ antiCD40 at vac1Z13Mad5Anaxa
+ antiCD40 at vac1 &
vac2

Fig. 17

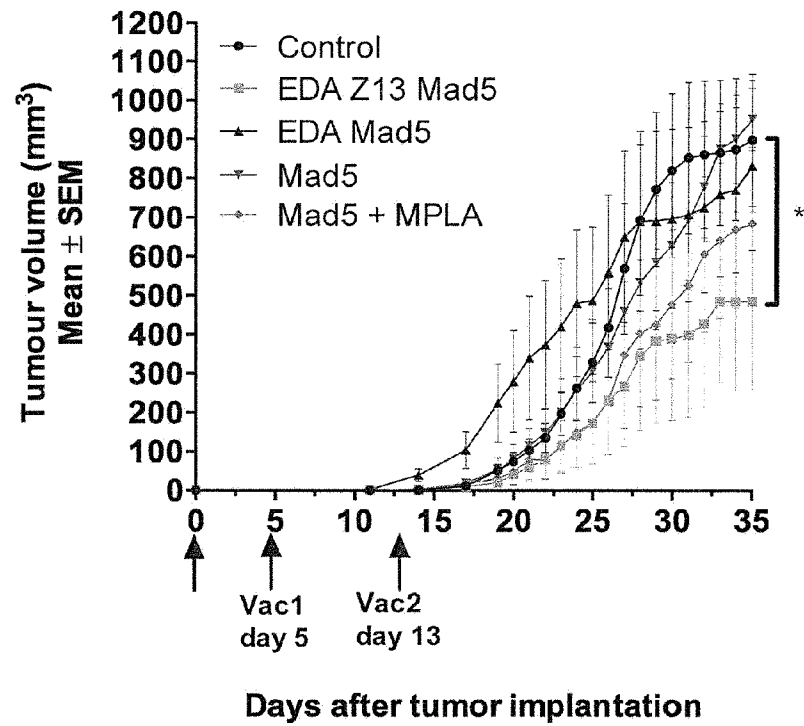


Fig. 18

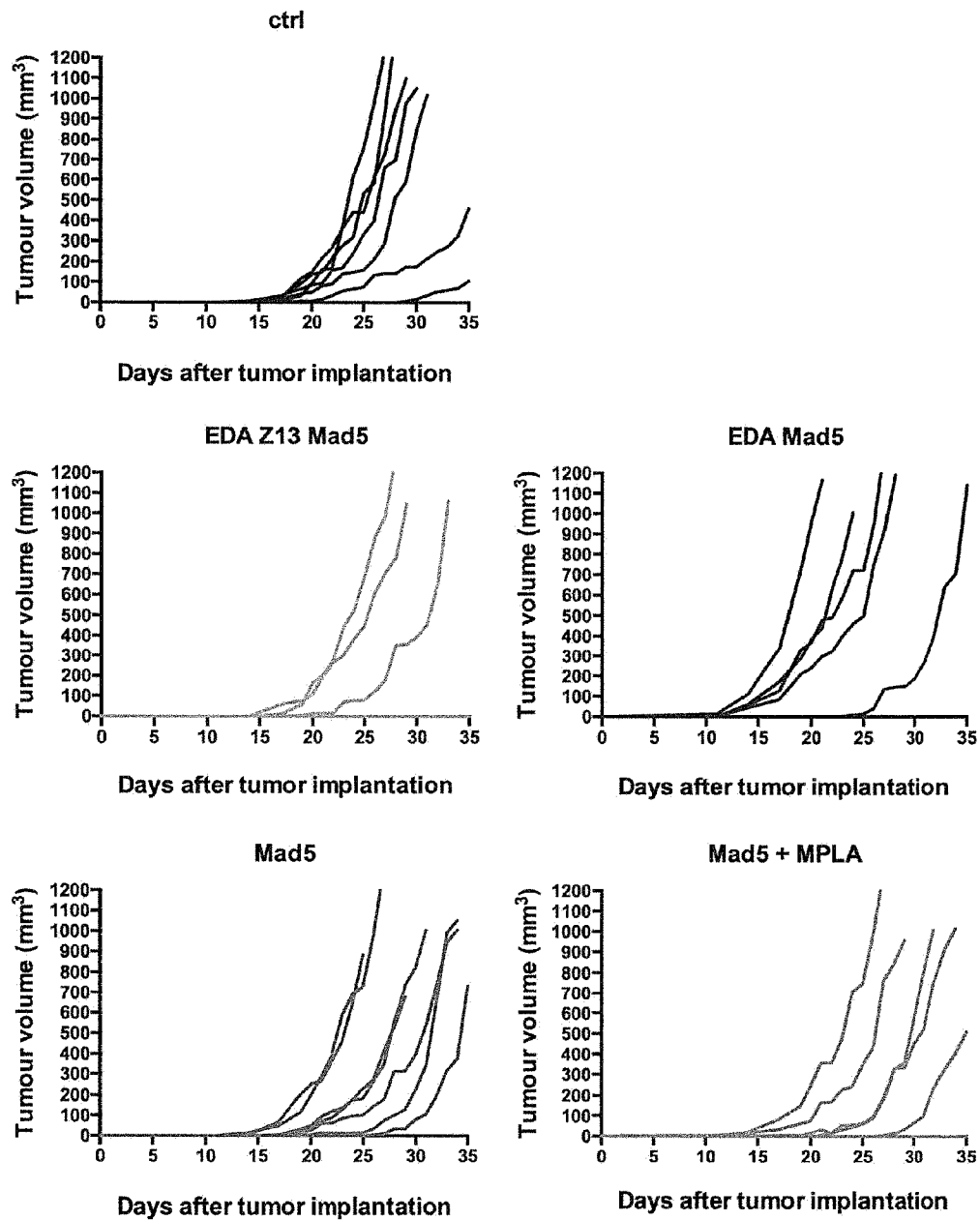
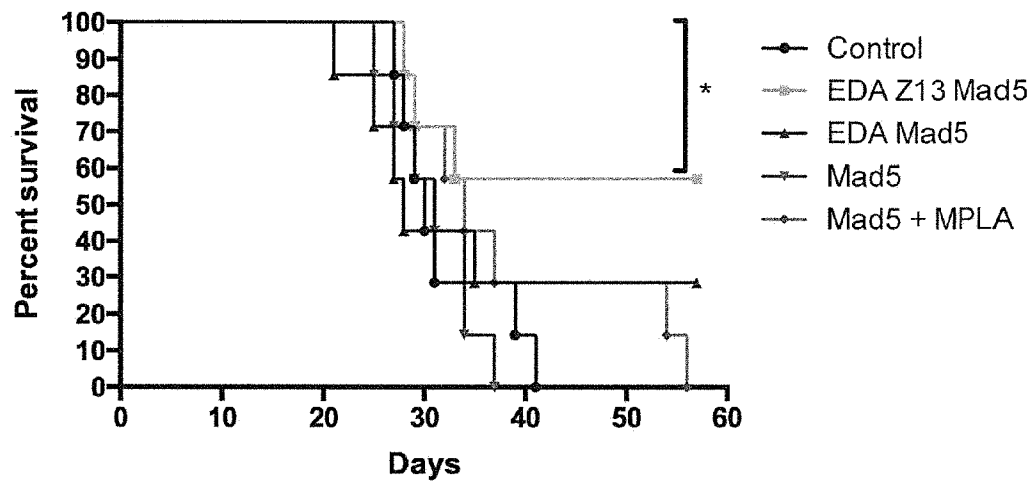


Fig. 19

A



B

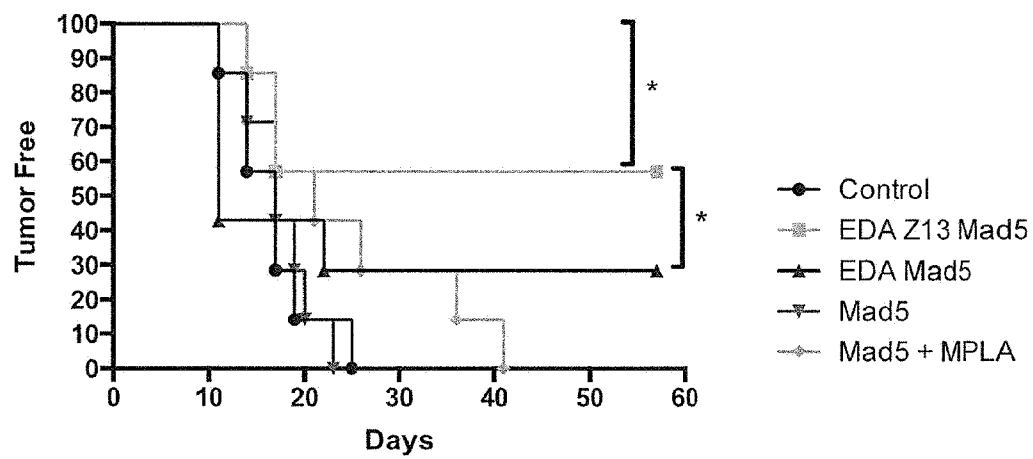
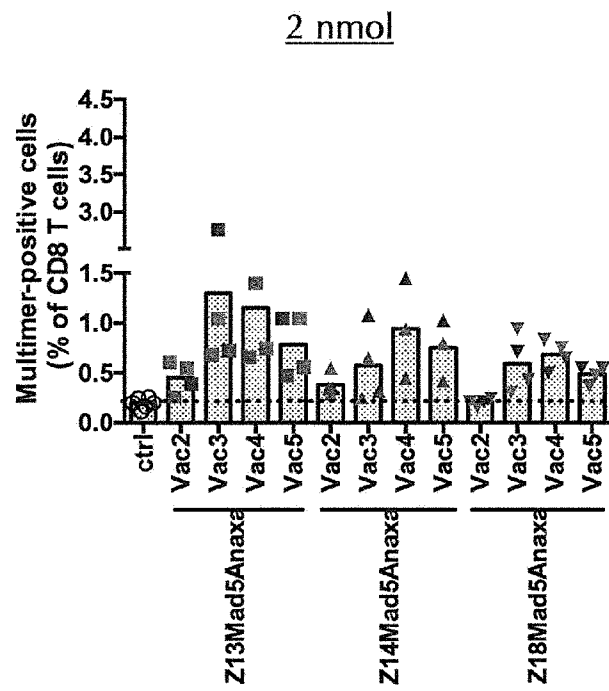


Fig. 20

A



B

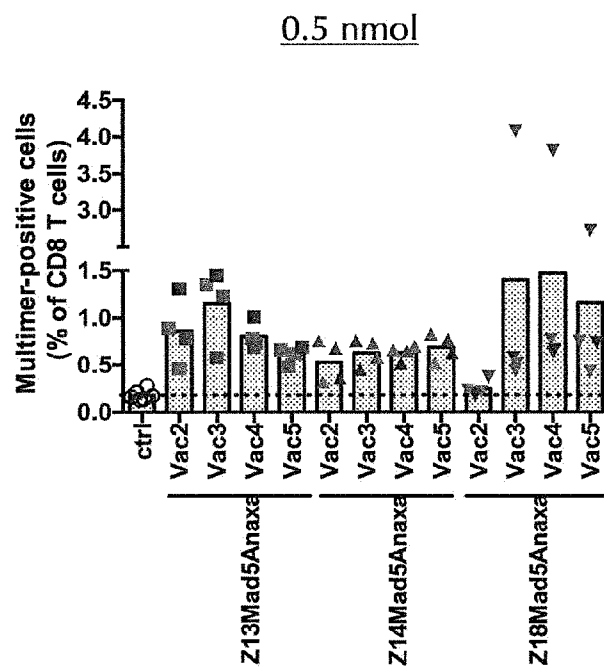


Fig. 21

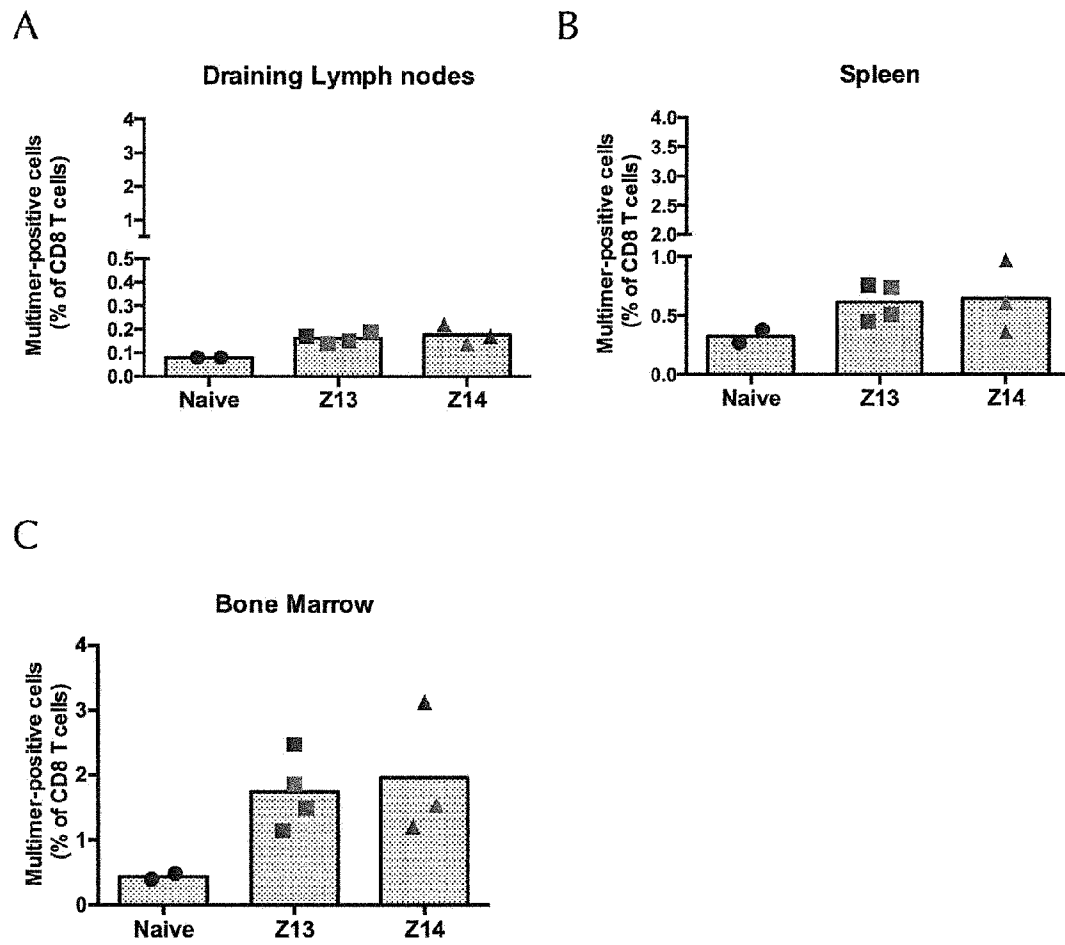
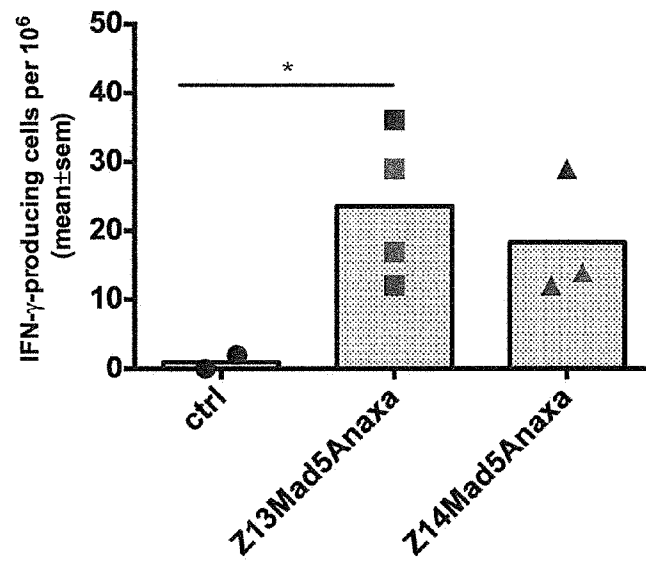


Fig. 22

A



B

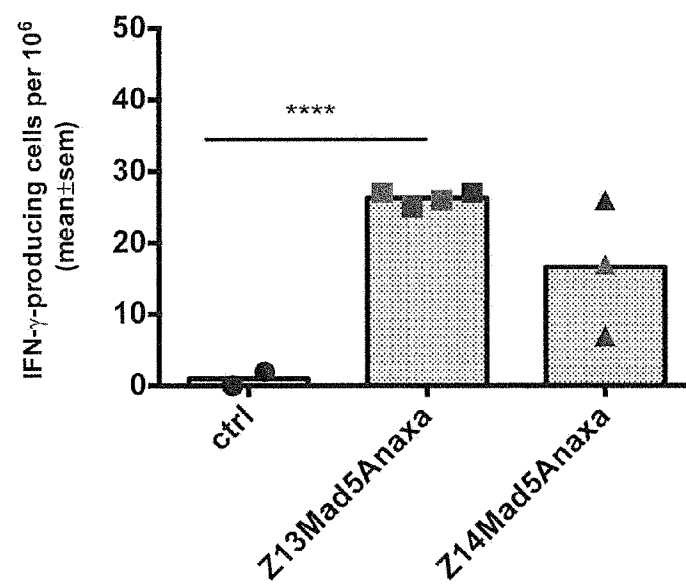


Fig. 23

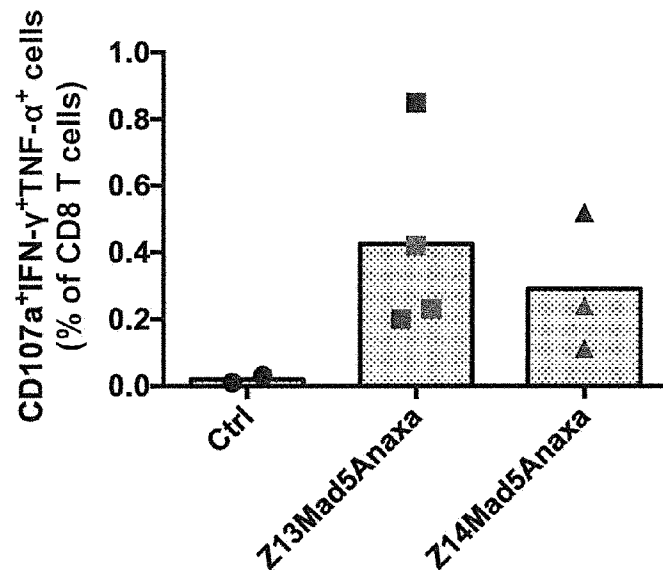
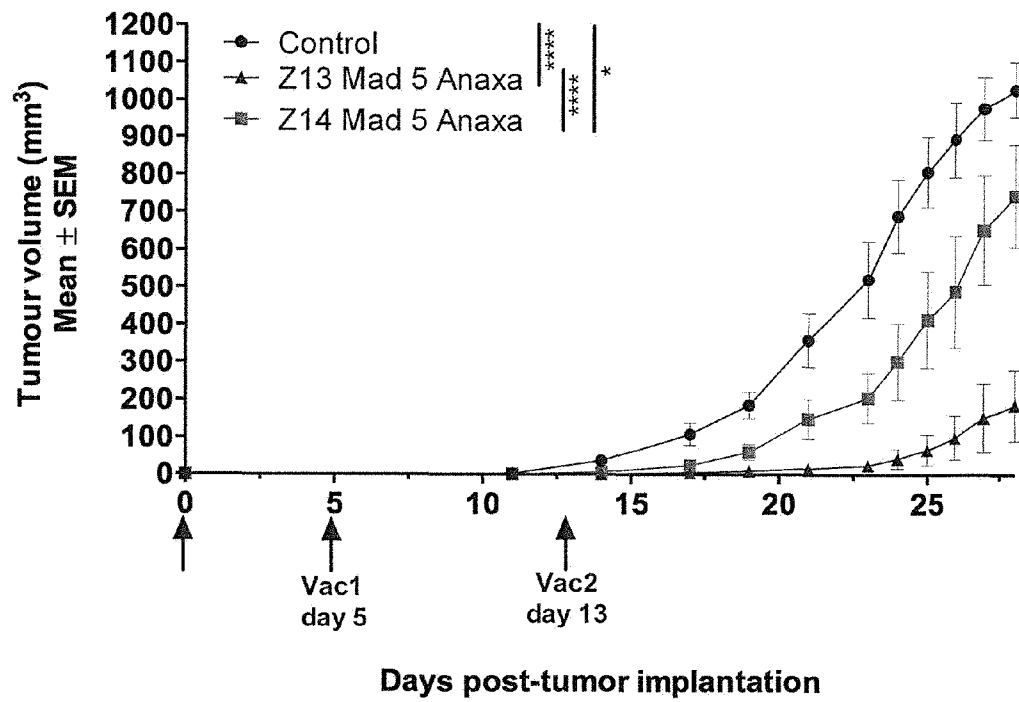


Fig. 24

A



B

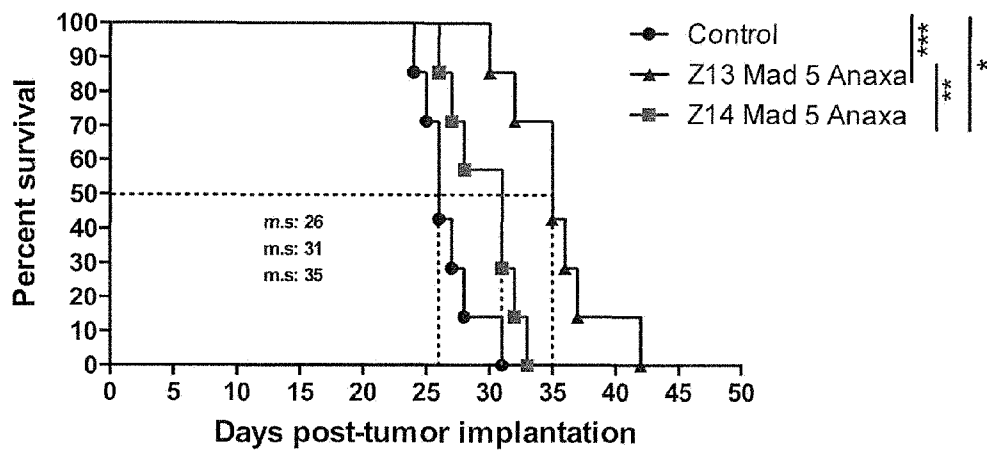
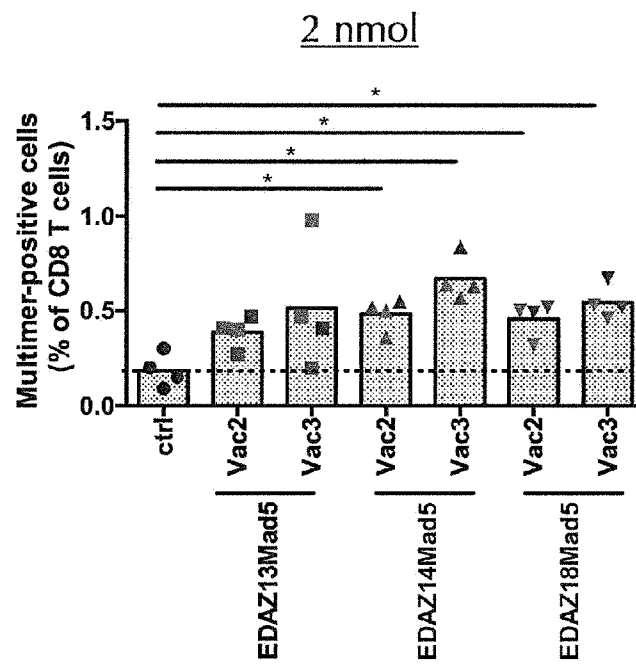


Fig. 25

A



B

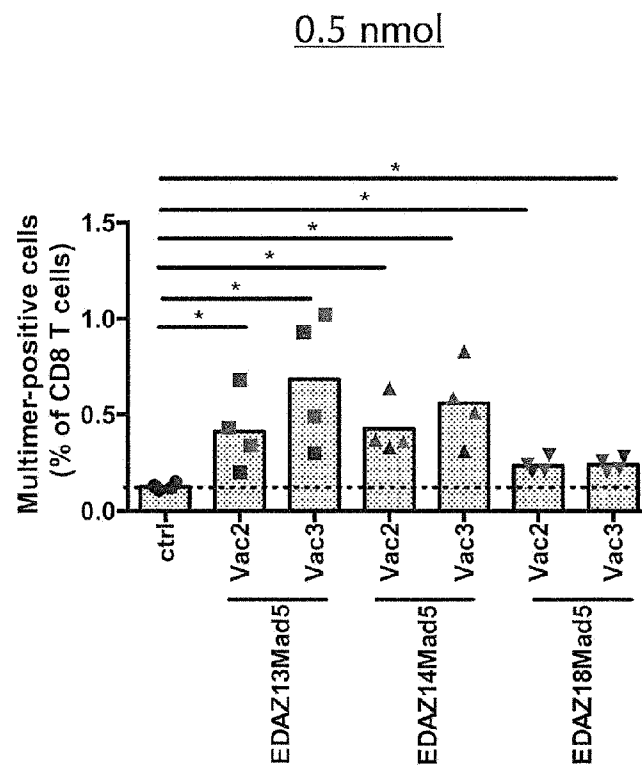
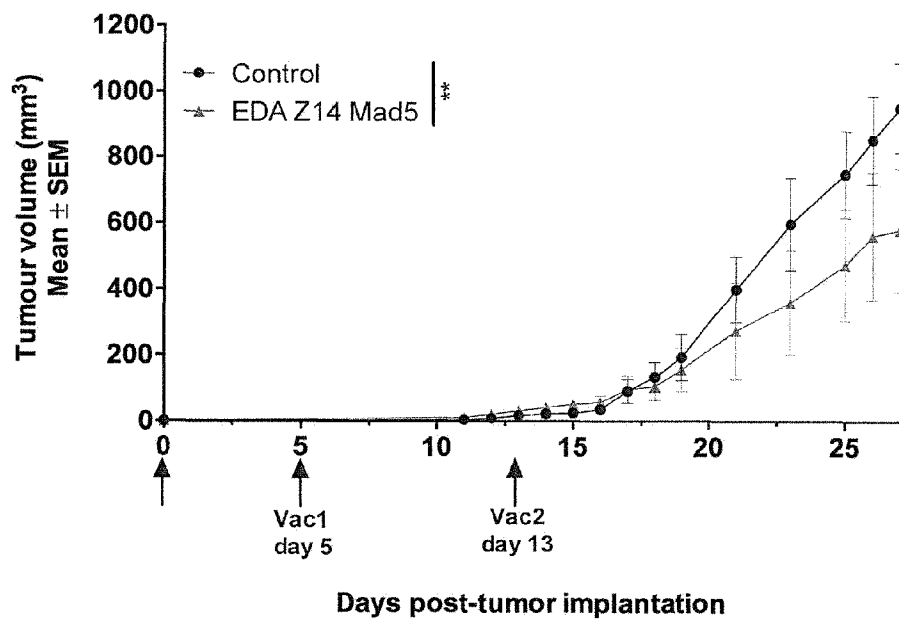


Fig. 26

A



B

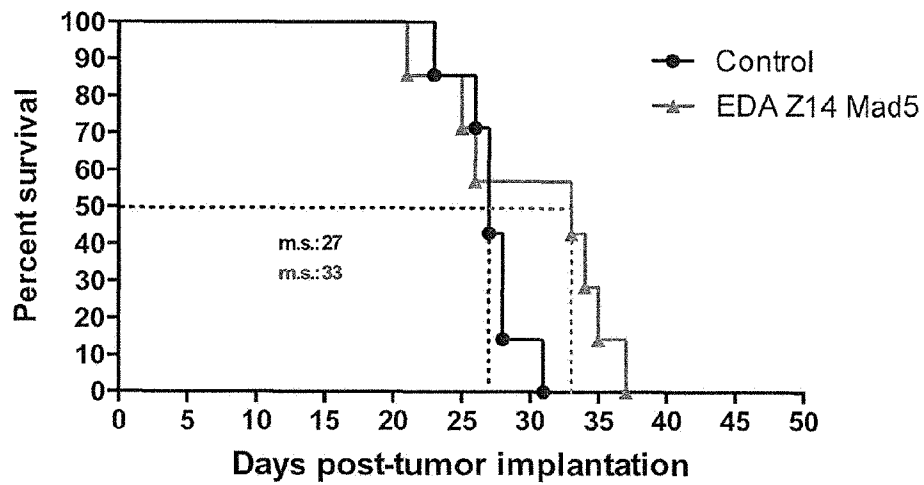


Fig. 27

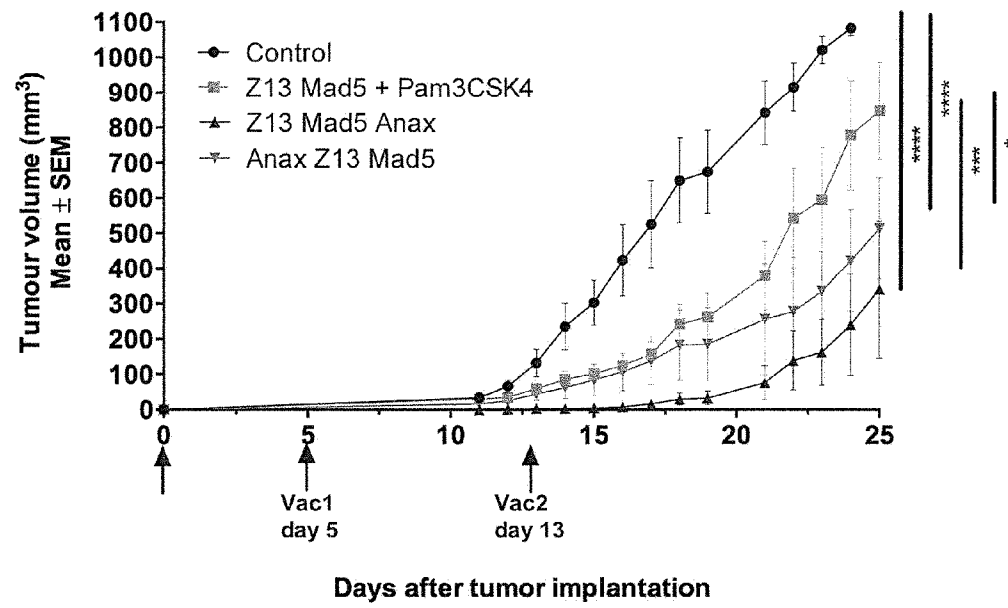


Fig. 28

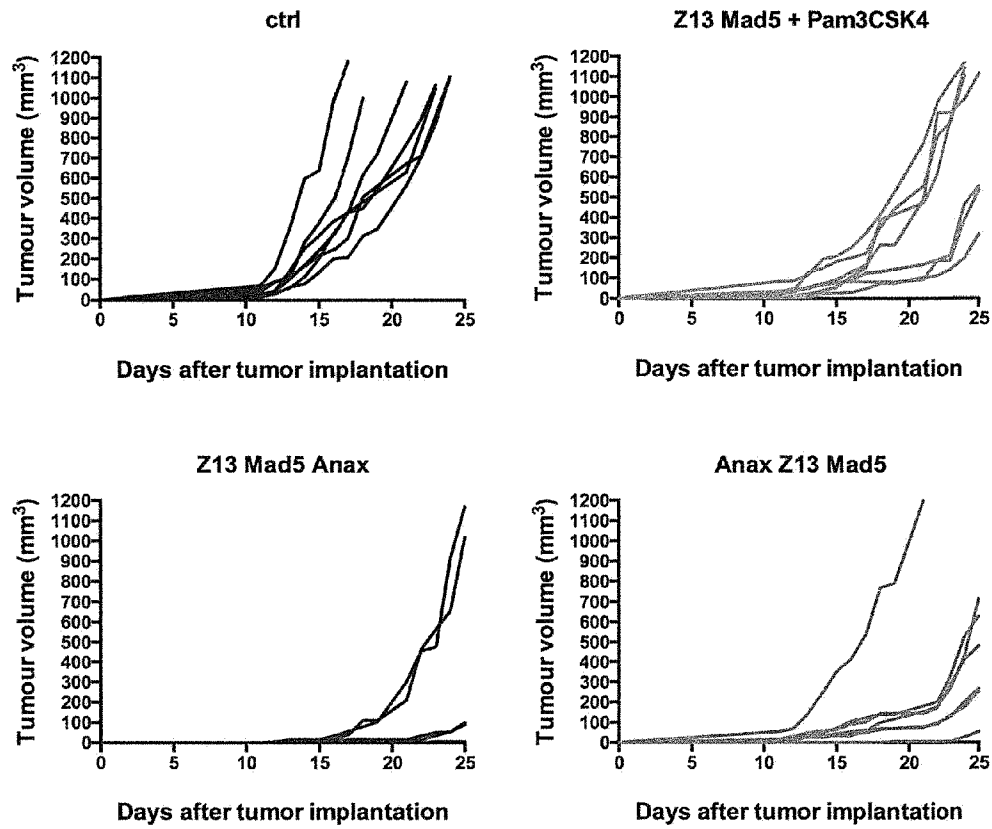


Fig. 29

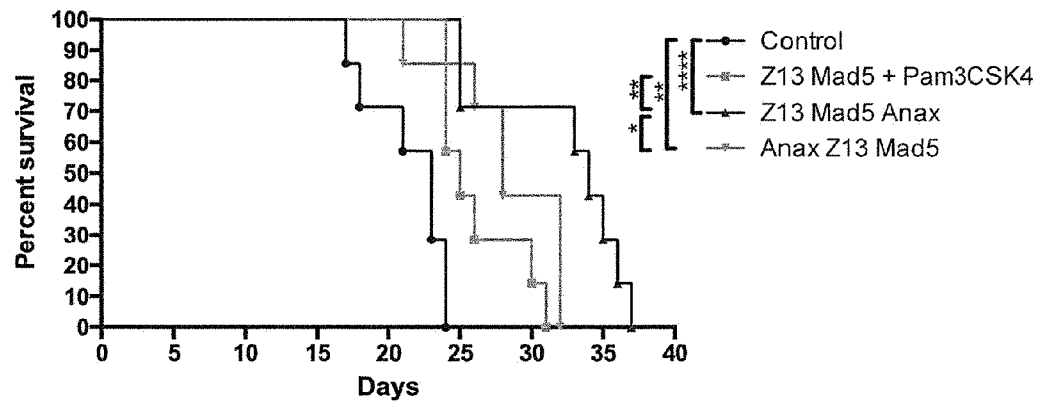


Fig. 30

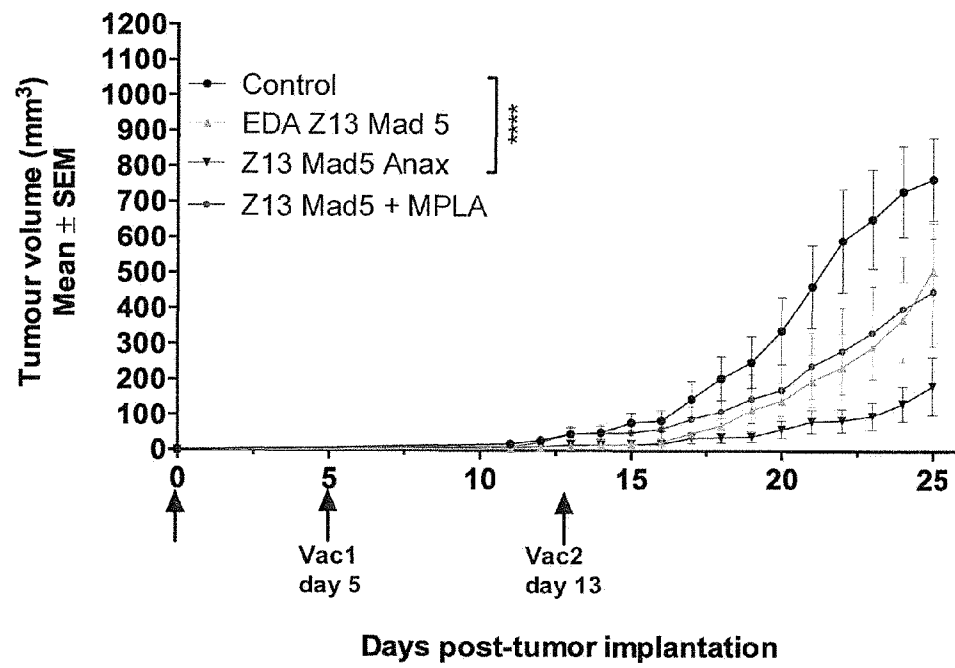


Fig. 31

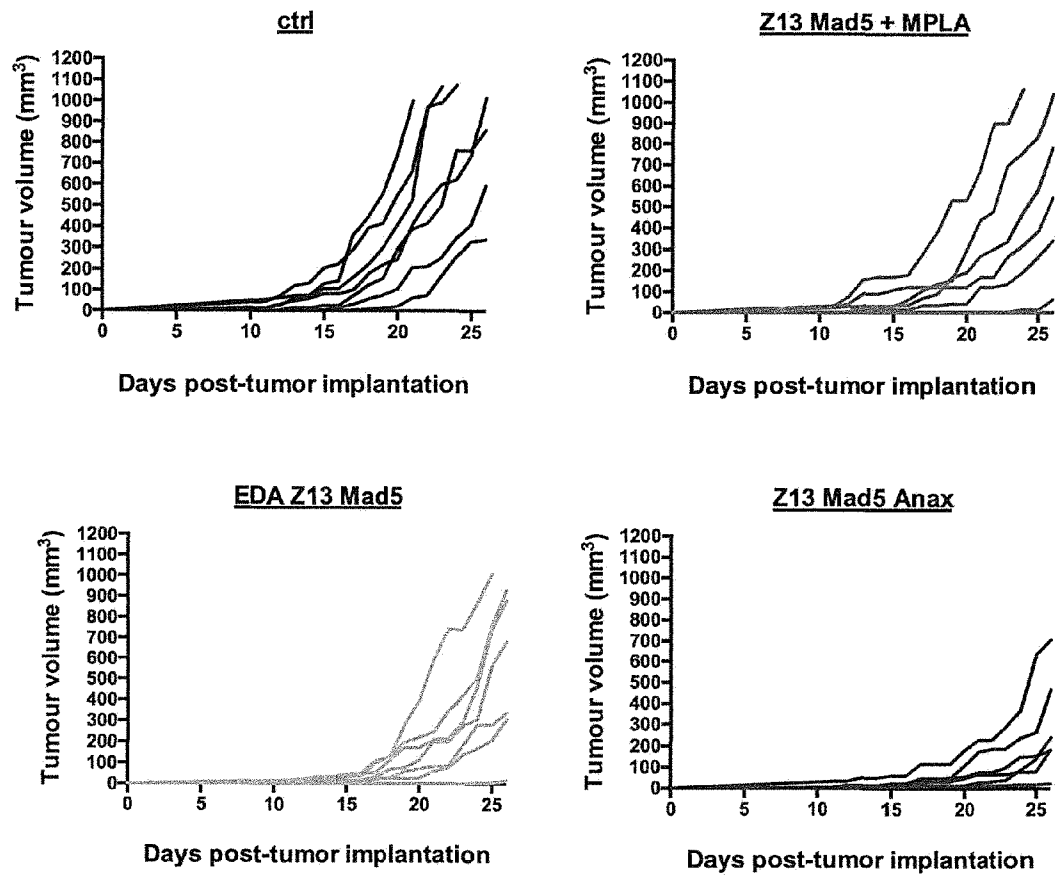


Fig. 32

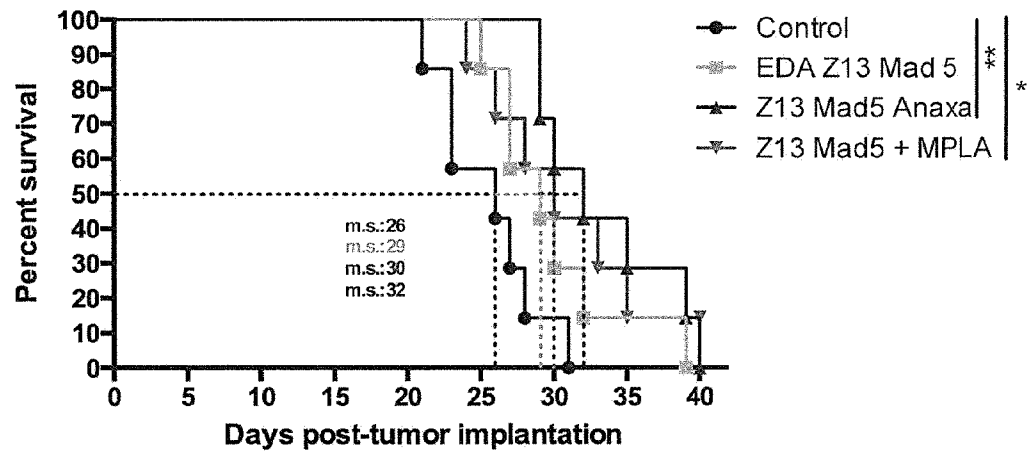
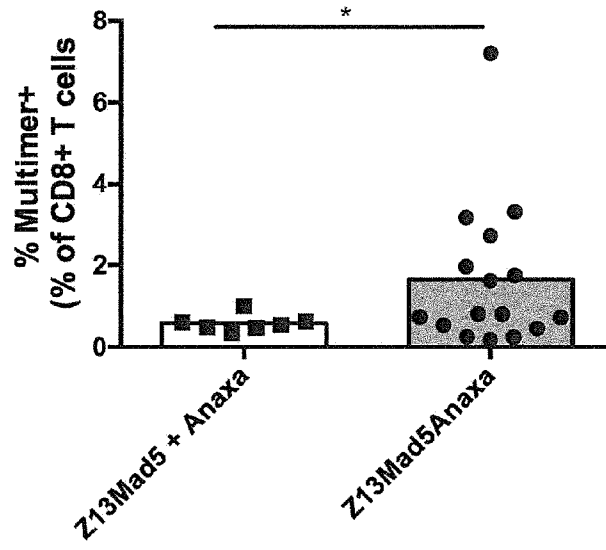


Fig. 33

A



B

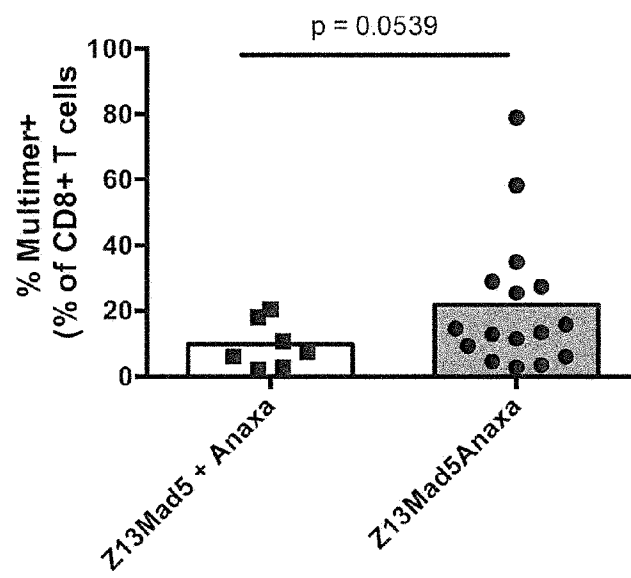


Fig. 34

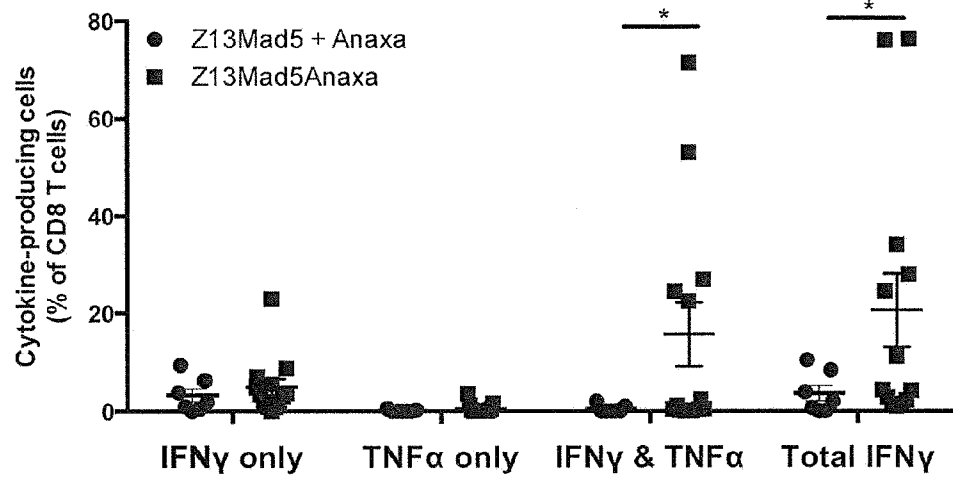


Fig. 35

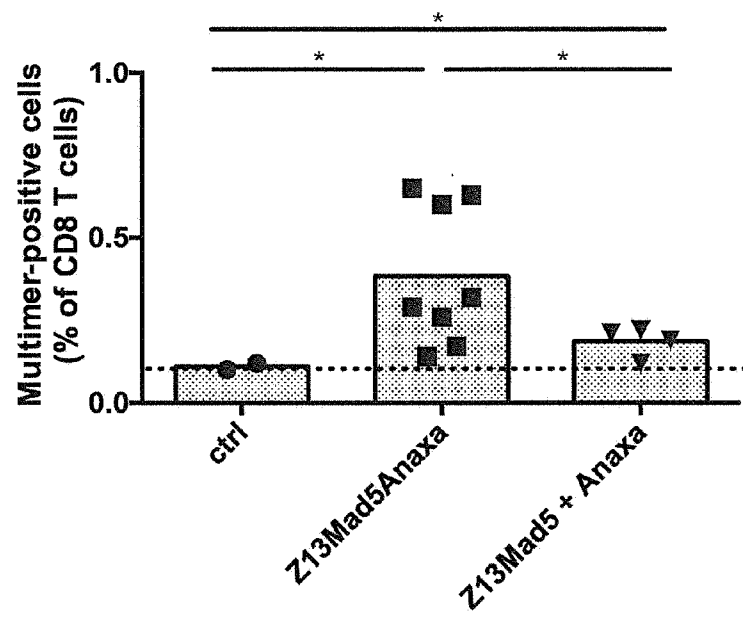
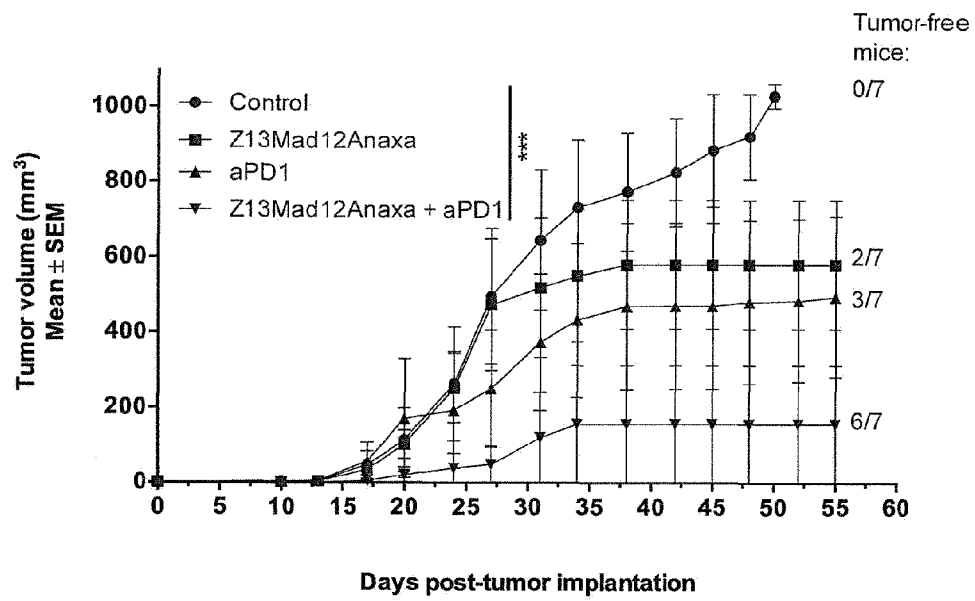


Fig. 36

A



B

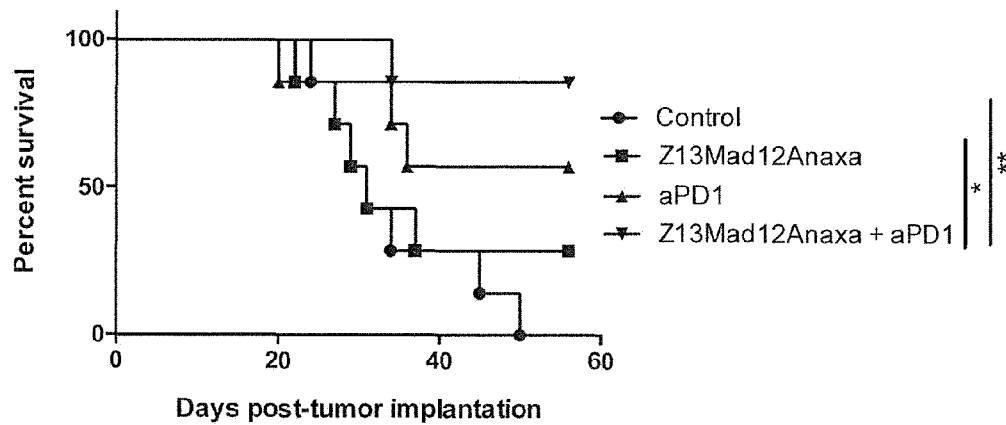


Fig. 37

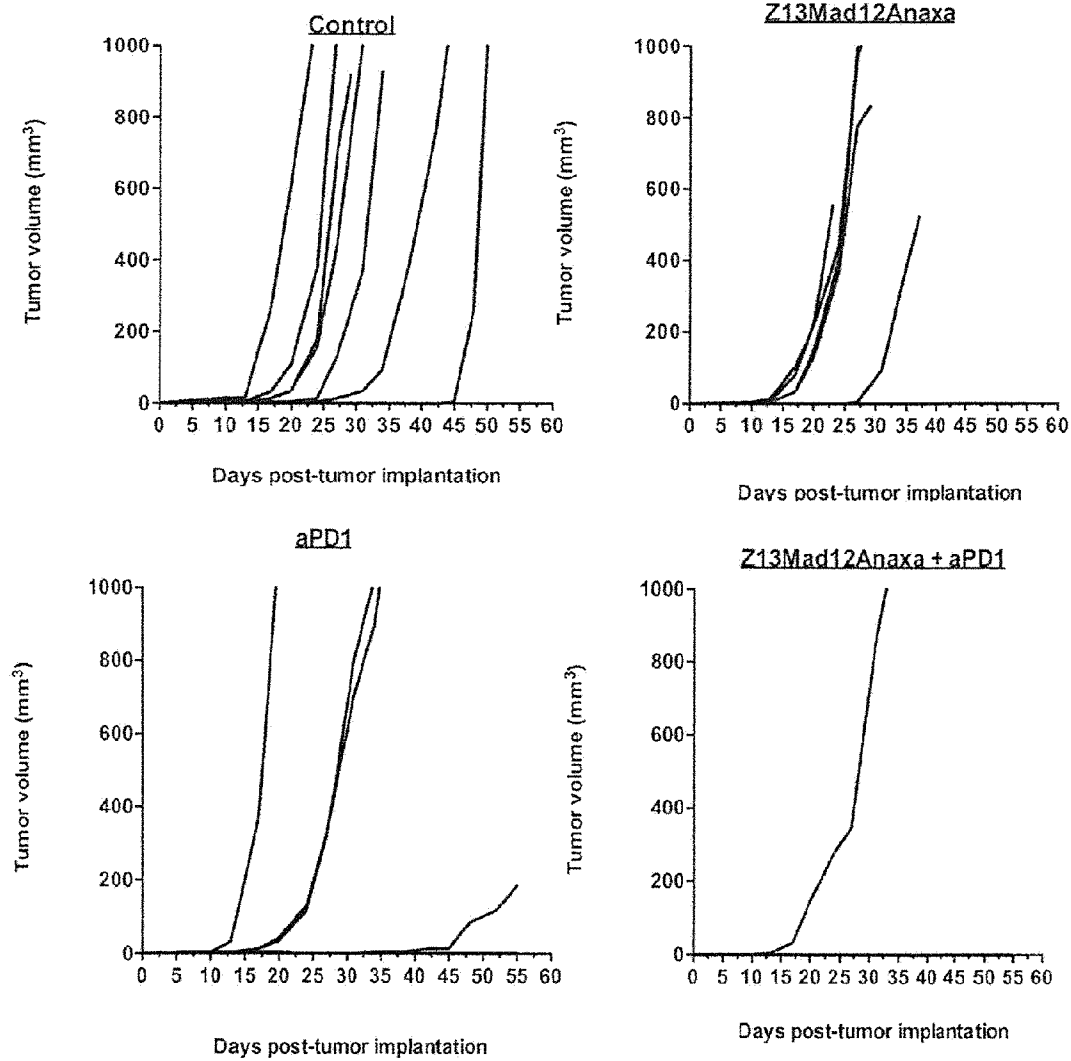
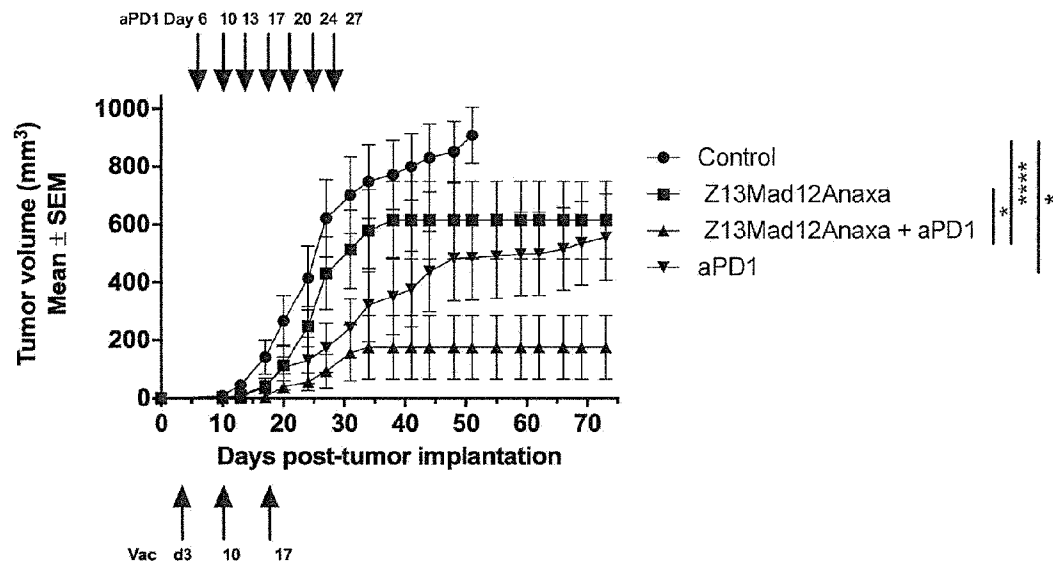


Fig. 38

A



B

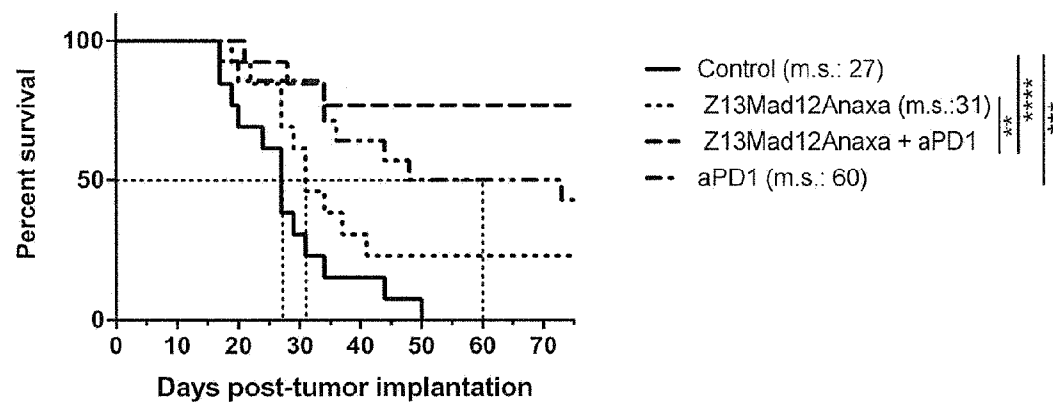


Fig. 39

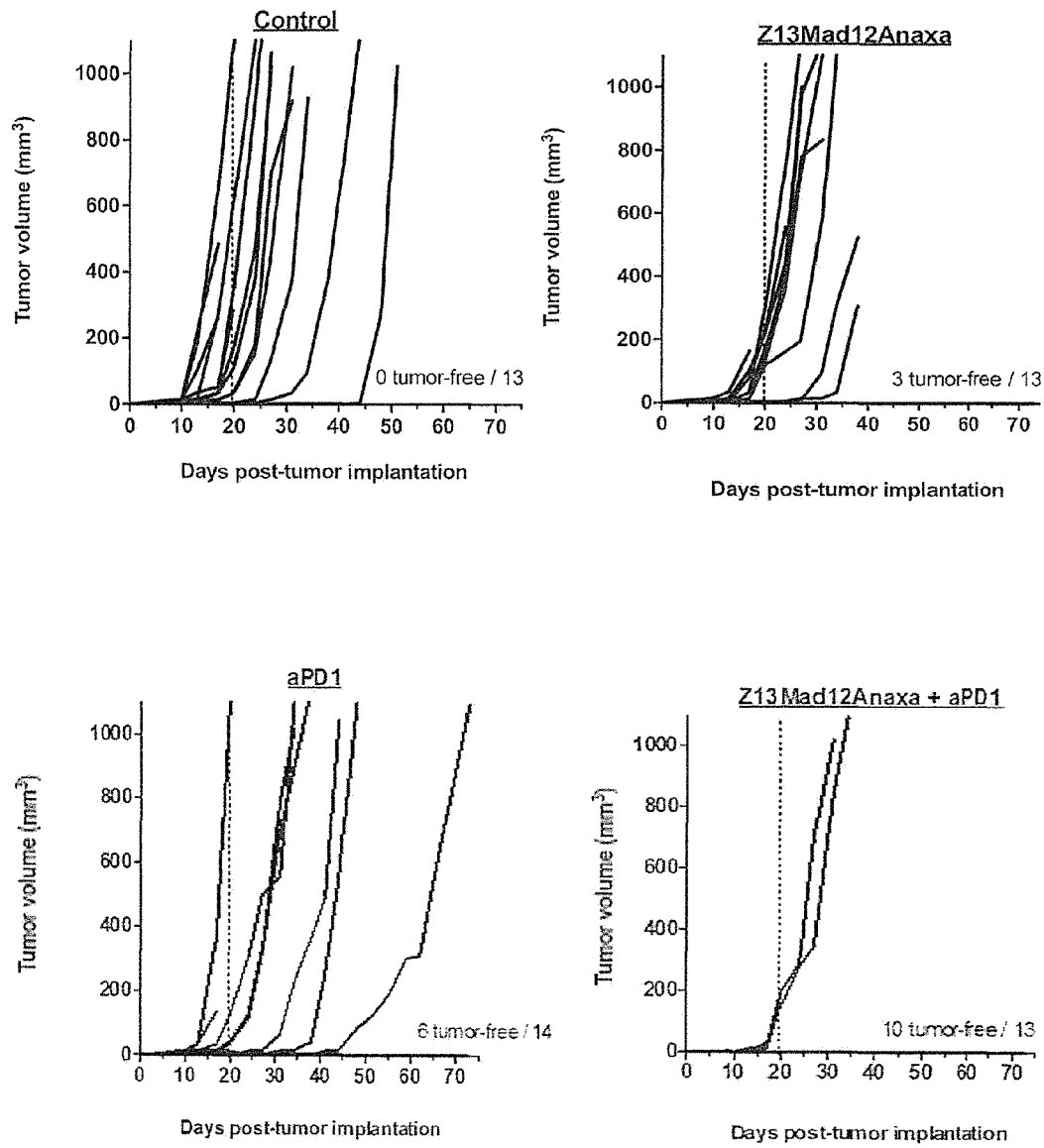


Fig. 40

SEKVENSLISTE

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