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(54) Title: A NOVEL COMPLEX COMPRISING A CELL PENETRATING PEPTIDE, A CARGO AND A TLR PEPTIDE AGONIST FOR TREATMENT OF GLIOBLASTOMA

(57) Abstract: The present invention provides a novel complex for use in the prevention and/or treatment of glioma, in particular glioblastoma, the complex comprising a) a cell penetrating peptide, b) at least one antigen or antigenic epitope, and c) at least one TLR peptide agonist, wherein the components a) - c) are covalently linked. In particular, compositions for use in the prevention and/or treatment of glioma, in particular glioblastoma, such as a pharmaceutical compositions and vaccines are provided.

1

A NOVEL COMPLEX COMPRISING A CELL PENETRATING PEPTIDE, A CARGO AND A TLR PEPTIDE AGONIST FOR TREATMENT OF GLIOBLASTOMA

The present invention relates to the field of vaccination, in particular to vaccines for the prevention and/or treatment of glioma, in particular glioblastoma.

A glioma is a type of tumor that arises from glial cells, such as astrocytes and oligodendrocytes. Gliomas thus typically start in the brain or in the spine with the brain being the most common site of gliomas. Gliomas make up about 30% of all brain and central nervous system tumors and 80% of all malignant brain tumors.

The highest-grade glioma tumor is glioblastoma multiforme (GBM, also known as "glioblastoma" or "grade IV astrocytoma"). Glioblastoma is the most malignant and the most common form of astrocytoma with about 50% of relative frequency. Primary GBM represents over 90% of cases. The remaining 10%, called secondary GBM, progress gradually from a lower grades to grade IV glioma. Primary GBM occurs more frequently in the elderly people, while secondary GBM seems affecting more frequently younger population, with a slight overall preponderance in males. Finally although GBM remains rare in children, it is the first cause of cancer death in children (0-14 years) and the second one in adolescent (15-19 years) (*Cancer Statistic 2015*. Americain Cancer Society).

Glioblastoma Multiforme (GBM) is the most common primary brain tumor in adults. GBM is a malignant glioma notoriously known for its highly invasive and aggressive behavior. With an annual incidence of 2-3 per 100 000 individuals worldwide, glioblastoma is a rare disease. It is however the most common form of brain tumors and is as well one of the more lethal with a lifetime expectation reduced to 12 – 16 weeks for non-treated patients.

2

As underlined by through its name, glioblastoma is a complex multiform tumor. The histologic parameters that differentiate GBM from all other glioma grades are the presence of necrosis and hemorrhage regions. The complexity is due as well to significant genetic variations and to intratumor cell population heterogeneity. The multiplication of cell population associated

5   with genetic specificities makes GBM very difficult to treat.

Existing treatments are based on a combination of aggressive therapies. Concomitant chemotherapy and radiotherapy are generally implemented after surgical tumor resection. However, this combination of surgery, chemo and radiotherapy only gives a median survival

10   of less than 15 months (Stupp, R., et al., *Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma*. N Engl J Med, 2005. 352(10): p. 987-96). With only 3 – 5% of patients surviving longer than 3 years, due to disease recurrence, GBM remains incurable.

The gold standard treatment for GBM is referred to as the "Stupp protocol". It involves a

15   complete chirurgical resection of the tumor followed with concomitant radio- and chemotherapy with temozolomide. Within a large European clinical trial, this regimen has shown a modest improvement in the median survival from 12 months with radiotherapy alone to 14.6 months with concomitant radio- and chemotherapy (Stupp, R., et al., *Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma*. N Engl J Med, 2005. 352(10):

20   p. 987-96). More impressive was the difference in the long-term survivors, with at two years 10% in the group treated with radiotherapy and 27% for those receiving simultaneously temozolomide. This long-term benefit persists up to five years with 1.9% survival in the control group versus 9.8% in the temozolomide treated arm (Stupp, R., et al., *Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma*. N Engl J Med, 2005. 352(10):

25   p. 987-96). More recently, a genetic marker was being investigated to predict treatment outcome after temozolomide. Indeed, the methylation status of the promoter from the MGMT gene was correlated with better response to temozolomide treatment (Hegi, M.E., et al., *MGMT gene silencing and benefit from temozolomide in glioblastoma*. N Engl J Med, 2005. 352(10): p. 997-1003). In 2009, the anti-angiogenic drug Avastin received FDA approval as

30   treatment for recurrent or progressing patient. Two other treatments have also received FDA approval for the same patient population as Avastin: Gliadel and an electrical field therapy called Novocure TFF. Gliader is an implant also known as carmustine wafer. Gliadel can be

3

placed in the cavity after the surgery, resulting in a local delivery of the chemotherapy drug carmustine. In 2011, FDA approved the fourth treatment for GBM: Novocure TFF. The technology is based on an electro- magnetic field that interferes with cell division and causing cancer cell death. Since healthy brain cells rarely divide, they are unaffected. Despite these
5   treatments and with less than 30% of long-term survivors, there is still an important unmet medical to improve the prognosis of GBM patients.

An improved understanding of the interactions between the central nervous system (CNS) and the immune system, as well as advances in tumor immunology for many cancers now indicate
10   that immunotherapy for brain cancer is a rational and feasible strategy. The long-standing assumption that the brain is an immunologically privileged organ because of the blood-brain barrier and the lack of lymphatic structures is now considerably revised. Indeed, although it is conceded that immune system-CNS interactions have special features (Bechmann, I., I. Galea, and V.H. Perry, *What is the blood-brain barrier (not)?* Trends Immunol, 2007. 28(1):
15   p. 5-11; Walker, P.R., et al., *T-cell immune responses in the brain and their relevance for cerebral malignancies.* Brain Res Brain Res Rev, 2003. 42(2): p. 97-122), there is significant traffic of immunologically competent cells into and out of the brain (Hickey, W.F., B.L. Hsu, and H. Kimura, *T-lymphocyte entry into the central nervous system.* J Neurosci Res, 1991. 28(2): p. 254-60). Moreover, several studies demonstrated brain tumour-infiltrating
20   lymphocytes in patients (Abou-Ghazal, M., et al., *The incidence, correlation with tumor-infiltrating inflammation, and prognosis of phosphorylated STAT3 expression in human gliomas.* Clin Cancer Res, 2008. 14(24): p. 8228-35; Buckanovich, R.J., et al., *Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy.* Nat Med, 2008. 14(1): p. 28-36; Mittelbronn, M., et al., *Elevated HLA-E levels in*
25   *human glioblastomas but not in grade I to III astrocytomas correlate with infiltrating CD8+ cells.* J Neuroimmunol, 2007. 189(1-2): p. 50-8; Perrin, G., et al., *Astrocytoma infiltrating lymphocytes include major T cell clonal expansions confined to the CD8 subset.* Int Immunol, 1999. 11(8): p. 1337-50; Bucciero, A., et al., *Prognostic significance of lymphoid infiltration in cerebral malignant gliomas.* J Neurosurg Sci, 1990. 34(2): p. 145-8), and some evidence
30   for brain tumour specific immunity (Tang, J., et al., *Glioblastoma patients exhibit circulating tumor-specific CD8+ T cells.* Clin Cancer Res, 2005. 11(14): p. 5292-9).

4

However, the inevitable progressive growth of GBM indicates that efficacious immune control of human glioma is unlikely to occur spontaneously. Nevertheless, many studies in animal models have shown the great potential of immunotherapy for brain tumors (Mittelbronn, M., et al., *Elevated HLA-E levels in human glioblastomas but not in grade I to*

5   *III astrocytomas correlate with infiltrating CD8+ cells.* J Neuroimmunol, 2007. 189(1-2): p. 50-8; Perrin, G., et al., *Astrocytoma infiltrating lymphocytes include major T cell clonal expansions confined to the CD8 subset.* Int Immunol, 1999. 11(8): p. 1337-50; Bucciero, A., et al., *Prognostic significance of lymphoid infiltration in cerebral malignant gliomas.* J Neurosurg Sci, 1990. 34(2): p. 145-8; Tang, J., et al., *Glioblastoma patients exhibit circulating*

10 *tumor-specific CD8+ T cells.* Clin Cancer Res, 2005. 11(14): p. 5292-9; Maus, M.V., et al., *Antibody-modified T cells: CARs take the front seat for hematologic malignancies.* Blood, 2014. 123(17): p. 2625-35). This resulted in an increased number of GBM clinical trials using adoptive cell transfer (Reardon, D.A., et al., *An update on vaccine therapy and other immunotherapeutic approaches for glioblastoma.* Expert Rev Vaccines, 2013. 12(6): p. 597-

15 615), specific vaccination using antigen (Saikali, S., et al., *Expression of nine tumour antigens in a series of human glioblastoma multiforme: interest of EGFRvIII, IL-13Ralpha2, gp100 and TRP-2 for immunotherapy.* J Neurooncol, 2007. 81(2): p. 139-48; Schuster, J., et al., *A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study.* Neuro Oncol, 2015. 17(6): p. 854-61), or dendritic cell (DC) vaccines (Nakada, M., Y.

20 Hayashi, and J. Hamada, *Role of Eph/ephrin tyrosine kinase in malignant glioma.* Neuro Oncol, 2011. 13(11): p. 1163-70; Phuphanich, S., et al., *Phase I trial of a multi-epitope-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma.* Cancer Immunol Immunother, 2013. 62(1): p. 125-35).

The immune system can recognize and to some extent eliminate tumor cells, however, this

25 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.

30 Promising preclinical data and advances in clinical trials, including the recent FDA approval of the Sipuleucel-T vaccine and of the anti-CTLA-4 antibody, show that active immunization is a safe and feasible treatment modality for certain cancer types. Induction of tumor-specific

5

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

5   have reached the phase III clinical stage.

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

10   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.

15   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

20   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

25   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.

In contrast to cell-based therapy (APCs, T cells, CARs, lysates), subunits vaccines (protein or

30   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

6

of patients. Furthermore, the antigens are fully defined allowing for better immune-monitoring and reducing the risk of unwanted effects of vaccine component.

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.

For the protein-based vaccines, the results of targeting MAGE-A3 with a recombinant fusion protein-based vaccine have been enthusiastically awaited after promising phase II data in metastatic melanoma (Kruit WH, Suciu S, Dreno B, Mortier L, Robert C, Chiarion-Sileni V, et al. Selection of immunostimulant AS15 for active immunization with MAGE-A3 protein: results of a randomized phase II study of the European Organisation for Research and Treatment of Cancer Melanoma Group in Metastatic Melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2013;31(19):2413-20) and non-small cell lung cancer (NSLC)(Vansteenkiste J, Zielinski M, Linder A, Dahabreh J, Gonzalez EE, Malinowski W, et al. Adjuvant MAGE-A3 immunotherapy in resected non-small-cell lung cancer: phase II randomized study results. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2013;31(19):2396-403). However, in 2013 the phase III DERMA trial in melanoma (NCT00796445) did not meet its first co-primary endpoint, followed in 2014 with a stop of the phase III MAGRIT study in NSCL (NCT00480025). Despite these very disappointing clinical results, protein based vaccines undeniably present many advantages.

In general, 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

7

must contain molecules called antigens that are also present in the tumor and that need to be delivered to Antigen Presenting Cells (APCs), especially dendritic cells (DCs), to allow cancer immunity to be initiated. 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

5   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 (Th) 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

10   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 the specificity of the tumor antigens and the ability to deliver them efficiently to DCs.

Taken together, induction of a tumor specific immune response thus requires three main steps:

15   (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.

20   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

25   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

30   epitopes. This reduces the risk of inducing peripheral tolerance as has been 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

8

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).

5   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

10   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.

15   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.

20   To improve the efficacy of protein-based vaccine delivery, the use of cell penetrating peptides for intracellular delivery of cancer peptides into DCs has been 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 8 to 40 residues that have the ability to cross the cell membrane and enter into most cell types

25   (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

30   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

9

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
5   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-
10   dependent antitumor immunity by TAT-mediated tumor antigen delivery into dendritic cells.
J Clin Invest. 2002a; 109:1463–1470).

Moreover, subunits vaccines (peptides or proteins) are poorly immunogenic. Therefore in the
context of therapeutic cancer vaccine, a potent adjuvant is mandatory to be added to the
15   vaccine in order to increase the level of co-stimulatory molecules on DCs and therefore
augment the immune system's response to the target antigens. Adjuvants accomplish this task
by mimicking conserved microbial components that are naturally recognized by the immune
system. They include, lipopolysaccharide (LPS), components of bacterial cell walls, and
nucleic acids such as double-stranded RNA (dsRNA), single-stranded DNA (ssDNA), and
20   unmethylated CpG dinucleotide-containing DNA. Their presence together with the vaccine
can greatly increase the innate immune response to the antigen. Furthermore, this adjuvant
should promote an adaptive immune response with CTLs and type polarized Th1 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
25   include Alum, MPL (monophosphoryl lipid A) and ASO_4 (Alum and MPL) in the US, and MF59
(oil-in-water emulsion), ASO_4, 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).

30   Recently, Toll Like Receptor (TLR) ligands are emerging as promising class of adjuvants
(Baxevanis, 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

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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

5   adjuvants within human vaccines. Immunol Rev. 2011; 239:178–196). The types of signaling and cytokines produced 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

10   with Toll-like receptors. Semin Immunol. 2009; 21:185–193).

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

15   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

20   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).

However, to date the majority of cancer vaccines trials have shown limited efficacy. One

25   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

30   (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.

11

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 novel complex for glioma, in particular glioblastoma, immunotherapy applications representing a more potent vaccine,

5   having improved anti-tumor activity for use in the prevention and/or treatment of glioma, in particular glioblastoma.

This object is achieved by means of the subject-matter set out below and in the appended claims.

10

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

15   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.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be

20   combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any

25   permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

Throughout this specification and the claims which follow, unless the context requires

30   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

12

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

5   additional *e.g.*, X + Y.

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.

10   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.

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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.

20   The term "about" in relation to a numerical value x means x ± 10%.

Complexes for use according to the present invention

25   In a first aspect the present invention provides a complex comprising:

    a)   a cell penetrating peptide;

    b)   at least one antigen or antigenic epitope; and

    c)   at least one TLR peptide agonist,

    wherein the components a) – c), i.e. the cell penetrating peptide, the at least one antigen or

30   antigenic epitope and the at least one TLR peptide agonist, are covalently linked,

    for use in the prevention and/or treatment of glioma, in particular glioblastoma.

13

Such a complex 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 for use according to the present invention provides a potent vaccine, in particular having improved anti-tumor
5   activity.

Preferably, the complex 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
10   used herein means that it (here: the polypeptide or the protein) does not occur naturally. Accordingly, the complex 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.

15   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,
20   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.
25   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
30   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

14

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

5   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",

10   "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 cross-

15   linking, 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

20   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

25   lipopeptides, lipoproteins, glycopeptides, glycoproteins and the like.

However, in a particularly preferred embodiment, the complex for use according to the present invention is a "classical" peptide, polypeptide or protein, whereby a "classical" peptide, polypeptide or protein is typically composed of amino acids selected from the 20

30   amino acids defined by the genetic code, linked to each other by a normal peptide bond.

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If the complex 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.

5

Component a) – Cell penetrating peptide

The CPP allows for efficient delivery, i.e. transport and loading, in particular of at least one
10 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.

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,
15 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,
20 directed to an intracellular organelle, or further presented at the cell surface. Cell penetrating ability, or internalization, of the cell penetrating peptide or complex comprising said cell penetrating peptide, 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
25 Western blot.

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,
30 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

16

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

5   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.

Typically, cell penetrating peptides (CPPs) are peptides of 8 to 50 residues that have the ability

10   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 trans-activating 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

15   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

20   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

25   (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.

30   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

17

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

5   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,

10   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

15   as cell penetrating peptide, i.e. as component a), in the complex for use according to the present invention. In particular, the component a), i.e. the CPP, in the complex 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 for use according to the present invention may comprise Penetratin

20   having the amino acid sequence RQIKIYFQNRRMKWKK (SEQ ID NO: 1).

Various CPPs, which can be used as cell penetrating peptide, i.e. as component a), in the complex 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

25   (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 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

30   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.

18

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
5   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).

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Preferably, the cell penetrating peptide, which is comprised by the complex for use according to the present invention,

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

15   acids in total; and/or

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

20   cell penetrating ability, or a sequence variant of such a fragment.

Thereby, it is preferred that the cell penetrating peptide, which is comprised by the complex for use according to the present invention,

i)   has a length of the amino acid sequence of said peptide of 5 to 50 amino acids in

25   total, preferably of 10 to 45 amino acids in total, more preferably of 15 to 45 amino acids in total; and

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

30   acids have been substituted, deleted, and/or added without abrogating said peptide's cell penetrating ability, or a sequence variant of such a fragment.

19

Such preferred CPPs are disclosed in WO 2014/041505.

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

5   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:

10   MMDPNSTSEDVKFTPDPYQVPFVQAFDQATRVYQDLGGPSQAPLPCVLWPVLPEPLPQGQL
       TAYHVSTAPTGSWFSAPQPAPENAYQAYAAPQLFPVSDITQNQQTNQAGGEAPQPGDNST
       VQTAAAVVFACPGANQGQQLADIGVPQPAPVAAPARRTRKPQQPESLEECDSELEIKRYKNR
       VASRKCRAKFKQLLQHYREVAAAKSSENDRLRLLLKQMCPSLDVDSIIPRTPDVLHEDLLNF
       (SEQ ID NO: 3 - ZEBRA amino acid sequence (natural sequence from Epstein - Barr virus
15   (EBV)) (YP_401673))

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,

20   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

25   subsequently to promote CTL and Th cell activation and anti-tumor function. Such a CPP can thus efficiently deliver the complex for use according to the present invention to antigen presenting cells (APCs) and lead to multi-epitopic MHC class I and II restricted presentation.

In the context of the present invention, the term "MHC class I" designates one of the two

30   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

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molecules consist of two polypeptide chains, α- and β2-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
5   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).

Preferably, the sequence variant of a fragment of the minimal domain of ZEBRA as described
10   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
15   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
20   45 amino acids in total.

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.
25   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: 69. 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
30   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

21

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

5   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.

10

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

15   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

20   sequences.

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

25   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.

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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

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a mathematical algorithm. A preferred, but not limiting, 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

5   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

10   (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.

15

More preferably, the fragments of the cell penetrating peptide according to the invention 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

20   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.

25

The preferred cell penetrating peptide, which

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

30 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

23

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

5   is replaced by a residue having similar physiochemical characteristics.

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,

10   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

15   invention. Exemplary amino acid substitutions are presented in Table 1 below:

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

24

| Pro (P) | Ala, Gly |
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr, Phe
Tyr (Y)	Trp, Phe
Original residues	Examples of substitutions
Val (V)	Ile, Met, Leu, Phe, Ala

(Table 1)

Particularly preferably, the preferred cell penetrating peptide, which

i)   has a length of the amino acid sequence of said peptide of 5 to 50 amino acids in

5   total, preferably of 10 to 45 amino acids in total, more preferably of 15 to 45 amino acids in total; and/or

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

10   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.

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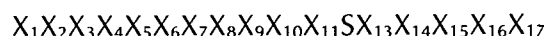
$$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}SX_{13}X_{14}X_{15}X_{16}X_{17}$$

20   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;

25   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;

5   X_9 is S or T;

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

X_{11} is K, R, or H, preferably X_{11} is K or R;

X_{13} is R, K, or H, preferably X_{13} is R or K;

X_{14} is A, V, L, I, or G, preferably X_{14} is A or G;

10   X_{15} is K, R, or H, preferably X_{15} is K or R;

X_{16} is F, L, V, I, Y, W, or M, preferably X_{16} is F, Y or W; and

X_{17} is K, R, or H, preferably X_{17} is K or R.

Preferably, such a peptide, polypeptide or protein is either (entirely) composed of L-amino

15   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)).

20

In a particular embodiment, the cell penetrating peptide according to the invention is as
generically defined above by general formula (I), wherein X_1 is K.

In a particular embodiment, the cell penetrating peptide according to the invention is as
generically defined above by general formula (I), wherein X_2 is R.

25   In a particular embodiment, the cell penetrating peptide according to the invention is as
generically defined above by general formula (I), wherein X_3 is Y.

In a particular embodiment, the cell penetrating peptide according to the invention is as
generically defined above by general formula (I), wherein X_4 is K.

In a particular embodiment, the cell penetrating peptide according to the invention is as

30   generically defined above by general formula (I), wherein X_5 is N.

In a particular embodiment, the cell penetrating peptide according to the invention is as
generically defined above by general formula (I), wherein X_6 is R.

26

In a particular embodiment, the cell penetrating peptide according to the invention is as generically defined above by general formula (I), wherein X_7 is V.

In a particular embodiment, the cell penetrating peptide according to the invention is as generically defined above by general formula (I), wherein X_8 is A.

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

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

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

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

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

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

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

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

In a particular embodiment, the cell penetrating peptide according to the invention 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).

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

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

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

27

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
5   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):
10   KRYKNRVASRKCRAKFKQLLQHYREVAAAKSSENDRLRLLLKQMC
(SEQ ID NO: 4)

CPP2 (Z12):
KRYKNRVASRKCRAKFKQLLQHYREVAAAKSSENDRLRLLLK
15   (SEQ ID NO: 5)

CPP3 (Z13):
KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSENDRLRLLLK
(SEQ ID NO: 6)
20
CPP4 (Z14):
KRYKNRVASRKSRAKFKQLLQHYREVAAAK
(SEQ ID NO: 7)

25   CPP5 (Z15):
KRYKNRVASRKSRAKFK
(SEQ ID NO: 8)

CPP6 (Z16):
30   QHYREVAAAKSSEND
(SEQ ID NO: 9)

28

CPP7 (Z17):

QLLQHYREVAAAK

(SEQ ID NO: 10)

5   CPP8 (Z18):

REVAAAKSS END RLRLLLK

(SEQ ID NO: 11)

CPP9 (Z19):

10   KRYKNRVA

(SEQ ID NO: 12)

CPP10 (Z20):

VASRKSRAKFK

15   (SEQ ID NO: 13)

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

20   (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:

25   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

30   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.

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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).

In another preferred embodiment, the cell penetrating peptide according to the invention has

5   an amino acid sequence comprising or consisting of SEQ ID NO: 7 (CPP4/Z14).

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).

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).

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

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

(i)   a nuclear localization signal (NLS). Such signals are well known to the skilled person and are described in *Nair et al. (2003, Nucleic Acids Res. 31(1): 397–399)*

20   (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?

Preferably, the cell penetrating peptide according to the invention is linked to an antigen or

25   antigenic epitope and facilitates the cellular internalization of said antigen or antigenic epitope.

The complex for use according to the present invention may comprise one single cell penetrating peptide or more than one cell penetrating peptides. Preferably, the complex for

30   use according to the present invention comprises no more than five cell penetrating peptides, more preferably the complex for use according to the present invention comprises no more than four cell penetrating peptides, even more preferably the complex for use according to

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the present invention comprises no more than three cell penetrating peptides, particularly preferably the complex for use according to the present invention comprises no more than two cell penetrating peptides and most preferably the complex for use according to the present invention comprises one single cell penetrating peptide.

5

Component b) – Antigen / antigenic epitope

The complex for use according to the present invention comprises as component b) at least
10   one antigen or antigenic epitope.

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
15   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
20   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.

25   Preferably, in the complex 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
30   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

31

in particular if more than one antigen or antigenic epitope is comprised by the inventive complex.

5   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 for use according to the present invention comprises more than one
10   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.

15   Preferably, the complex 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.

20   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.

25   It is particularly preferred that the complex 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).

30   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 tumor. For instance, tumor epitopes include glioma epitopes, in particular glioblastoma 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

5   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

10   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,

15   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 glioma, in particular glioblastoma, 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

20   neoantigenic epitopes are preferably used in a complex for use according to the present invention.

Preferably, a complex 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

25   preferably no cancer/tumor-specific epitopes). It is also preferred that a complex 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 for use according to the present invention may also preferably comprise both, (i) one or more cancer/tumor-associated epitopes and/or one or

30   more cancer/tumor-associated antigens and (ii) one or more cancer/tumor-specific epitopes and/or one or more cancer/tumor-specific antigens.

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In particular, the cancer/tumor with which the antigens or antigenic epitopes are associated or for which the antigens or antigenic epitopes are specific is a glioma, in particular glioblastoma, as described herein. Thus, the antigens are preferably glioma-associated or glioma-specific antigens and the epitopes are preferably glioma-associated or glioma-specific
5   epitopes.

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:
10 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-
15 derived epitopes, glycoprotein 100 (gp100) melanoma antigen-derived epitopes, glycoprotein 70 (gp70) antigen-derived epitopes, survivin epitopes, IEa epitopes, IL13ra2, Epha2 (ephrin type-A receptor 2), immunogenic fragments thereof, and fusions of such antigens and/or fragments. As described above, neoantigens are antigens, which are entirely absent from the normal human genome. As compared with nonmutated self-antigens, neoantigens are of
20 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.
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Specific examples of cancer/tumor-associated, in particular tumor-related, or tissue-specific antigens useful in a complex for use according to the present invention include, but are not limited to, the following antigens: Her-2/neu, SPAS-1, TRP-2, tyrosinase, Melan A/Mart-1, gplOO, BAGE, GAGE, GM2 ganglioside, kinesin 2, TATA element modulatory factor 1, tumor
30 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), MAGE-1, HOM-Mel-

34

40/SSX2, NY-ESO-1, EGFR, CEA, Epha2, Epha4, PCDGF, HAAH, Mesothelin; EPCAM; NY-ESO-1, glycoprotein MUC1 and NIUC10 mucins p5 (especially mutated versions), EGFR, 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.,

5   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

10   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 at al., 1997 Urol. Int. 58: 20-24), CAMEL, CAP-1, CASP-8,

15   CDC27/m, CDK-4/m, CEA (Huang et al., Exper Rev. Vaccines (2002)1:49-63), CT9, CT10, Cyp-B, Dek-cain, 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 at 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,

20   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-

25   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

30   al., Blood (1997) 90:2529-34), P15, p190, Pml/RARα, PRAME, PSA, PSM, PSMA, RAGE, RAS, RCAS1, RU1, RU2, SAGE, SART-1, SART-2, SART-3, SP17, SPAS-1, TEL/AML1, TPl/m, Tyrosinase, TARP, TRP-1 (gp75), TRP-2, TRP-2/INT2, WT-1, and alternatively translated NY-

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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.

Preferably, the cancer/tumor antigen or the cancer/tumor epitope is a recombinant
5   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
10 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
15 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
20 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
25 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
30 epitope sequence.

36

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,

5   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

10   fever, Dengue Fever, Diptheria, 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

15   encephalitis, Lassa Fever, Leishmaniasis, Leptospirosi, 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

20   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.

25   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

30   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

37

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.

Advantageously, the complex for use according to the invention comprises a cell penetrating
5   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.

Preferably, the complex for use according to the present invention comprises at least one
10 antigen or antigenic epitope, which is at least one CD4$^+$ epitope and/or at least one CD8$^+$ epitope.

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
15 in the groove of a MHC class II molecule. A single CD4$^+$ epitope comprised in the complex 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.

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
20 in the groove of a MHC class I molecule. A single CD8$^+$ epitope comprised in the complex 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.

25 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
30 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

38

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.

It is also preferred that the complex 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 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 for use according to the present invention may preferably comprise an Ealpha-CD4$^+$ epitope and a gp100-CD8$^+$ epitope.

Preferably, the complex 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 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 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 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.

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

Preferably, the complex 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 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 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.

For example, the complex 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 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 for use

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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 WLGVPRFAKF ASFEAQGALA
5   NIAVDKANLD VEQLESIINF EKLTEWTGS

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

For example, the complex for use according to the present invention may also comprise a
10   gp70-CD8⁺ epitope and/or a gp70-CD4⁺ epitope. In particular, the complex 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 for use according to the present invention comprises or consists of an amino acid sequence according to SEQ ID NO: 43 or sequence variants thereof as defined above:

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VTYHSPSYAYHQFERRAILNRLVQFIKDRI

SEQ ID NO: 43 (Mad8-cargo comprising a gp70-CD8⁺ and a gp70-CD4⁺ epitope)

For example, the complex for use according to the present invention may preferably comprise
20   at least one survivin epitope, such as a survivin CD8⁺ epitope and/or a survivin CD4⁺ epitope. More preferably, the complex 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 for use according to the present invention may comprise a polypeptide or protein comprising more than one survivin CD8⁺ epitope and/or more than
25   one survivin CD4⁺ epitope, such as two different survivin CD8⁺ epitopes. For example, such a polypeptide or protein comprised by the complex for use according to the present invention comprises or consists of an amino acid sequence according to SEQ ID NO: 44 or sequence variants thereof as defined above:

30   NYRIATFKNWPFLEDCAMEELTVSEFLKLDRQR

SEQ ID NO: 44 (Mad11-cargo comprising survivin CD8⁺ epitope 1 and survivin CD8⁺ epitope 2)

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For example, the complex for use according to the present invention may preferably comprise an epitope from a neoantigen. Even more preferably, the complex 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 for use according to the present invention comprises or consists of an amino acid sequence according to SEQ ID NO: 42 or sequence variants thereof as defined above:

HLELASMTNMELMSSIV

SEQ ID NO: 42 (Mad9-cargo comprising the epitope from a neoantigen as described by Yadav et al. Nature. 2014 Nov 27;515(7528):572-6).

For example, the complex 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 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 for use according to the present invention comprises or consists of an amino acid sequence according to SEQ ID NO: 63 or sequence variants thereof as defined above:

LFRAAQLANDVVLQIMEHLELASMTNMELMSSIVVISASIIVFNLLELEG

SEQ ID NO: 63 (Mad12-cargo comprising the epitope from a neoantigen as described by Yadav et al. Nature. 2014 Nov 27;515(7528):572-6).

Preferably, the at least one antigen or antigenic epitope comprised by the complex 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

42

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,

5   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,

10   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

15   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),

20   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 varizella zoster, etc., or

25   antigens or antigenic epitopes from leishmania, typanosomes, amibes, bacteria, etc., 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 %,

30   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

43

shown or described above. In this context, the definition of epitopes and variants similarly applies as defined.

Examples of antigens or antigenic epitopes in the category of peptide, polypeptide or protein

5   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 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 according to the present invention comprise different subsets

10 of all of said glioma epitopes, so that for example a vaccine according to the present invention comprising such different complexes according to the present invention comprises all of said glioma epitopes but distributed in the different complexes.

Moreover, a complex for use according to the invention may also comprise at least one

15 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.

20 In particular, the complex 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.

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

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

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44

The complex 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.

5   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)*.

10   The complex for use according to the present invention comprises at least one antigen or antigenic epitope, preferably the complex 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 for use according to the present invention comprises (at least) two or three antigens or antigenic epitopes, even more

15   preferably the complex 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 for use according to the present invention it is understood that said antigen or antigenic epitope is in particular

20   also covalently linked in the complex 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.

       The various antigens or antigenic epitopes comprised by the complex for use according to

25   the present invention may be the same or different. Preferably, the various antigens or antigenic epitopes comprised by the complex for use according to the present invention are different from each other, thus providing a multi-antigenic and/or multi-epitopic complex.

       Moreover, it is preferred that the more than one antigen or antigenic epitope, in particular 2,

30   3, 4, 5, 6, 7, 8, 9, 10 or more antigens or antigenic epitopes, are positioned consecutively in the complex 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,

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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
5   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.

Alternatively, however, the various antigens and/or antigenic epitopes may also be positioned
10   in any other way in the complex 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)
15   and/or component c).

It is understood that a number of different antigens or antigenic epitopes relating to glioma, in particular glioblastoma, may be advantageously comprised by a single complex for use according to the present invention. Alternatively, a number of different antigens or antigenic
20   epitopes relating to glioma, in particular glioblastoma, may be distributed to subsets of different antigens or antigenic epitopes, in particular subsets complementing each other in the context of glioma, in particular glioblastoma, which are comprised by different complexes according to the present invention, whereby such different complexes comprising different subsets may advantageously be administered simultaneously, e.g. in a single vaccine, to a
25   subject in need thereof.

Preferably, the complex for use according to the present invention comprises at least one tumor epitope, which is an epitope of an antigen selected from the glioma antigens disclosed by Reardon, D.A., et al., *An update on vaccine therapy and other immunotherapeutic*
30   *approaches for glioblastoma.* Expert Rev Vaccines, 2013. 12(6): p. 597-615. More preferably, the complex for use according to the present invention comprises at least one tumor epitope, which is an epitope of an antigen selected from the group consisting of CMV, EGFRvIII,

46

EphA2, gp100, Her2/neu, IL-13Rα2, survivin, hTert, TRP-2, MAGE-A1, MAGE-A3, YKL-40, brevican, neuroligin 4 and PTPRz1. Those antigens are particularly useful in the context of glioma, in particular glioblastoma. It is also preferred that the complex for use according to the present invention comprises at least one tumor antigen selected from the group consisting of CMV, EGFRvIII, EphA2, gp100, Her2/neu, IL-13Rα2, survivin, hTert, TRP-2, MAGE-A1, MAGE-A3, 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. 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.

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/).

CMV

Human cytomegalovirus (CMV), a member of the herpes virus family, persists after a usually unrecognized infection lifelong in the body. CMV is the most frequent opportunistic CNS

47

infection in severely immunocompromised patients. *Postmortem* CMV nucleic acid can be detected in the CNS of more than 20% of individuals without CNS diseases, confirming tropism of CMV to CNS cells and indicating that CMV may persist in the CNS lifelong without causing (obvious) harm. However, expression of CMV proteins and oligonucleotides was also

5   identified in a high percentage of gliomas (Cobbs CS, Harkins L, Samanta M, et al. Human cytomegalovirus infection and expression in human malignant glioma. Cancer Res. 2002;62:3347–3350).

Preferred CMV antigens are those as described in WO 2009/155535, such as a polypeptide,

10   or an immunogenic fragment thereof, selected from the group consisting of capsid polypeptides, tegument polypeptides, and envelope polypeptides; preferably selected from the group consisting of phosphoprotein unique long 83 (ppUL83; a/k/a pp65), glycoprotein UL55 (gpUL55; a/k/a gB), UL123 immediate early 1 (IEI) protein, UL122 IE2 protein, ULI I IA (a/k/a mtrll), US28, ppUL32, ppUL65, ppUL80a, ppUL82, ppUL98a, ppUL99, gpUL4 (a/k/a

15   gp48), gpULlβ, gpUL18 (a/k/a MHC), gpUL75 (a/k/a gH), gpULlOO, gpULHO (a/k/a gM), gpUL115 (a/k/a gL), pUL46, pUL48, pUL56, pUL86 (a/k/a MCP), glycoprotein unique short 10 (gpUSlO), gpUSl 1, glycoprotein complex II (gcll), gp65, and gp93. Preferably, the CMV antigen is a polypeptide, or an immunogenic fragment thereof, which is encoded by a gene corresponding to the CMV strain shown by GENBANK Accession No. BK000394.2 or Xl

20   7403.1. Moreover, WO 2009/155535 also describes preferred CMV epitopes such as the peptides comprising the amino acid sequences as described by Trivedi et al., Blood, 105:2793 (2005) and U.S. Patent Application Publication No. 2005/0019344 and other CMV epitopes as described in WO 2009/155535.

25

EGFRvIII

The epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans) is the cell-surface receptor for members of the epidermal growth factor family (EGF-family) of extracellular protein ligands. EGFRvIII is a mutated form of EGFR, which is known to play a prominent role

30   in tumorigenesis. Namely, EGFRvIII is a constitutively activated tyrosine kinase derived from a deletion mutation in EGFR. EGFRvIII is largely overexpressed by glioblastoma cells (Saikali, S., et al., *Expression of nine tumour antigens in a series of human glioblastoma multiforme:*

48

interest of EGFRvIII, IL-13Ralpha2, gp100 and TRP-2 for immunotherapy. J Neurooncol, 2007. 81(2): p. 139-48), which acquire enhanced capacity for unregulated growth, survival, invasion, and recruitment of new tumor blood vessels.

5   Several epitopes of EGFRvIII are known to the skilled person. A preferred EGFRvIII epitope, which is preferably comprised by the complex for use according to the present invention, includes the following epitope (the following epitope sequence may refer to one epitope or more than one (overlapping) epitopes):

10   LEEKKGNYVVTDHC
     [SEQ ID NO: 47]

Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 47.

15

EphA2

Ephrins and their receptors (ephrin receptors, "Ephs") belong to a subfamily of proteins involved in crucial processes occurring during embryonic development of the central nervous
20   system, including axon mapping, cell migration, and angiogenesis. Recent findings suggest that Eph/ephrin are overexpressed by glioma cells and their signaling affects glioma cell growth, migration, and invasion (Nakada, M., Y. Hayashi, and J. Hamada, *Role of Eph/ephrin tyrosine kinase in malignant glioma.* Neuro Oncol, 2011. 13(11): p. 1163-70). 16 ephrin receptors (Ephs) have been identified. The ephrin receptors are divided into two groups based
25   on the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands. EPH receptor A2 (ephrin type-A receptor 2) binds ephrin-A ligands. The amino acid sequence of EphA2 is shown in the following:

MELQAARACFALLWGCALAAAAAAQGKEVVLLDFAAAGGELGWLTHPYGKGWDLMQNIM
30   NDMPIYMYSVCNVMSGDQDNWLRTNWVYRGEAERIFIELKFTVRDCNSFPGGASSCKETFNL
     YYAESDLDYGTNFQKRLFTKIDTIAPDEITVSSDFEARHVKLNVEERSVGPLTRKGFYLAFQDIGA
     CVALLSVRVYYKKCPELLQGLAHFPETIAGSDAPSLATVAGTCVDHAVVPPGGEEPRMHCAVD

49

GEWLVPIGQCLCQAGYEKVEDACQACSPGFFKFEASESPCLECPEHTLPSPEGATSCECEEGFFR
APQDPASMPCTRPPSAPHYLTAVGMGAKVELRWTPPQDSGGREDIVYSVTCEQCWPESGEC
GPCEASVRYSEPPHGLTRTSVTVSDLEPHMNYTFTVEARNGVSGLVTSRSFRTASVSINQTEPPK
VRLEGRSTTSLSVSWSIPPPQQSRVWKYEVTYRKKGDSNSYNVRRTEGFSVTLDDLAPDTTYLV
5   QVQALTQEGQGAGSKVHEFQTLSPEGSGNLAVIGGVAVGVVLLLVLAGVGFFIHRRRKNQR
ARQSPEDVYFSKSEQLKPLKTYVDPHTYEDPNQAVLKFTTEIHPSCVTRQKVIGAGEFGEVYKG
MLKTSSGKKEVPVAIKTLKAGYTEKQRVDFLGEAGIMGQFSHHNIIRLEGVISKYKPMMIITEYM
ENGALDKFLREKDGEFSVLQLVGMLRGIAAGMKYLANMNYVHRDLAARNILVNSNLVCKVS
DFGLSRVLEDDPEATYTTSGGKIPIRWTAPEAISYRKFTSASDVWSFGIVMWEVMTYGERPYWE
10   LSNHEVMKAINDGFRLPTPMDCPSAIYQLMMQCWQQERARRPKFADIVSILDKLIRAPDSLKT
LADFDPRVSIRLPSTSGSEGVPFRTVSEWLESIKMQQYTEHFMAAGYTAIEKVVQMTNDDIKRI
GVRLPGHQKRIAYSLLGLKDQVNTVGIPI
[SEQ ID NO: 48]

15   Accordingly, a preferred complex for use according to the present invention comprises an
amino acid sequence according to SEQ ID NO: 48 or a fragment or a variant thereof as
described herein. As described above, suitable cancer/tumor epitopes of EphA2 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-
20   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:
25   http://cvc.dfci.harvard.edu/tadb/).

Gp100

Glycoprotein 100, gp100 or Melanocyte protein PMEL is 661 amino acids long and is a type
30   I transmembrane glycoprotein enriched in melanosomes, which are the melanin-producing
organelles in melanocytes. Gp100 is involved in melanosome maturation. The gp100 protein

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is a melanoma antigen i.e. a tumor-associated antigen. The amino acid sequence of gp100 is shown in the following:

MDLVLKRCLLHLAVIGALLAVGATKVPRNQDWLGVSRQLRTKAWNRQLYPEWTEAQRLDC

5   WRGGQVSLKVSNDGPTLIGANASFSIALNFPGSQKVLPDGQVIWVNNTIINGSQVWGGQPV

YPQETDDACIFPDGGPCPSGSWSQKRSFVYVWKTWGQYWQVLGGPVSGLSIGTGRAMLGT

HTMEVTVYHRRGSRSYVPLAHSSSAFTITDQVPFSVSVSQLRALDGGNKHFLRNQPLTFALQL

HDPSGYLAEADLSYTWDFGDSSGTLISRALVVTHTYLEPGPVTAQVVLQAAIPLTSCGSSPVPG

TTDGHRPTAEAPNTTAGQVPTTEVVGTTPGQAPTAEPSGTTSVQVPTTEVISTAPVQMPTAEST

10   GMTPEKVPVSEVMGTTLAEMSTPEATGMTPAEVSIVVLSGTTAAQVTTTEWVETTARELPIPEPE

GPDASSIMSTESITGSLGPLLDGTATLRLVKRQVPLDCVLYRYGSFSVTLDIVQGIESAEILQAVPS

GEGDAFELTVSCQGGLPKEACMEISSPGCQPPAQRLCQPVLPSPACQLVLHQILKGGSGTYCL

NVSLADTNSLAVVSTQLIMPGQEAGLGQVPLIVGILLVLMAVVLASLIYRRRLMKQDFSVPQLP

HSSSHWLRLPRIFCSCPIGENSPLLSGQQV

15   [SEQ ID NO: 49]

Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 49 or a fragment or a variant thereof as described herein. As described above, suitable cancer/tumor epitopes of gp100 are known from the literature or can be identified by using cancer/tumor epitope databases, e.g. from

20   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

25   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/).

30   HER-2/*neu*
Her-2 belongs to the EGFR (epidermal growth factor receptor) family. Many HLA-A epitopes are known to the skilled person. The amino acid sequence of HER2 is shown in the following:

51

MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLE
LTYLPTNASLSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLN
NTTPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNR

5   SRACHPCSPMCKGSRCWGESSEDCQSLTRTVCAGGCARCKGPLPTDCCHEQCAAGCTGPKH
SDCLACLHFNHSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPYNYLSTDVGSCTL
VCPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAF
LPESFDGDPASNTAPLQPEQLQVFETLEEITGYLYISAWPDSLPDLSVFQNLQVIRGRILHNGAY
SLTLQGLGISWLGLRSLRELGSGLALIHHNTHLCFVHTVPWDQLFRNPHQALLHTANRPEDEC

10   VGEGLACHQLCARGHCWGPGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCH
PECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQP
CPINCTHSCVDLDDKGCPAEQRASPLTSIISAVVGILLVVVLGVVFGILIKRRQQKIRKYTMRRLL
QETELVEPLTPSGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLR
ENTSPKANKEILDEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLMPYGCLLDHVRENRGRLGS

15   QDLLNWCMQIAKGMSYLEDVRLVHRDLAARNVLVKSPNHVKITDFGLARLLDIDETEYHAD
GGKVPIKWMALESILRRRFTHQSDVWSYGVTVWELMTFGAKPYDGIPAREIPDLLEKGERLPQ
PPICTIDVYMIMVKCWMIDSECRPRFRELVSEFSRMARDPQRFVVIQNEDLGPASPLDSTFYRSL
LEDDDMGDLVDAEEYLVPQQGFFCPDPAPGAGGMVHHRHRSSSTRSGGGDLTLGLEPSEEE
APRSPLAPSEGAGSDVFDGDLGMGAAKGLQSLPTHDPSPLQRYSEDPTVPLPSETDGYVAPLT

20   CSPQPEYVNQPDVRPQPPSPREGPLPAARPAGATLERPKTLSPGKNGVVKDVFAFGGAVENPE
YLTPQGGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPV
[SEQ ID NO: 50]

Accordingly, a preferred complex for use according to the present invention comprises an

25   amino acid sequence according to SEQ ID NO: 50 or a fragment or a variant thereof as described herein. As described above, suitable cancer/tumor epitopes of Her-2 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:

30   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

52

Bioinformatics Core at Cancer Vaccine Center, Dana-Farber Cancer Institute; URL: http://cvc.dfci.harvard.edu/tadb/).

5   Survivin

Survivin, also called baculoviral inhibitor of apoptosis repeat-containing 5 or BIRC5, is a member of the inhibitor of apoptosis (IAP) family. The survivin protein functions to inhibit caspase activation, thereby leading to negative regulation of apoptosis or programmed cell death. The amino acid sequence of survivin is shown in the following:

10

MGAPTLPPAWQPFLKDHRISTFKNWPFLEGCACTPERMAEAGFIHCPTENEPDLAQCFFCFKEL
EGWEPDDDPIEEHKKHSSGCAFLSVKKQFEELTLGEFLKLDRERAKNKIAKETNNKKKEFEETAKK
VRRAIEQLAAMD
[SEQ ID NO: 52]

15

Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 52 or a fragment or a variant thereof as described herein.

20   Several epitopes of survivin are known to the skilled person. A preferred survivin epitope, which is preferably comprised by the complex for use according to the present invention, includes the following epitope (the epitope sequence shown in the following is a fragment of the above survivin sequence and is, thus, shown in the above survivin sequence underlined; the following epitope sequence may refer to one epitope or more than one (overlapping)

25   epitopes):

RISTFKNWPF
[SEQ ID NO: 53]

30   Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 53.

53

Melanoma-associated antigen (MAGE)

The mammalian members of the MAGE (melanoma-associated antigen) gene family were originally described as completely silent in normal adult tissues, with the exception of male

5   germ cells and, for some of them, placenta. By contrast, these genes were expressed in various kinds of tumors. Therefore, the complex for use according to the present invention preferably comprises an antigen of the MAGE-family (a "MAGE" antigen) or an epitope thereof. Of the MAGE family, in particular MAGE-A3 and MAGE-D4 are preferred, and MAGE-A3 is particularly preferred. The normal function of MAGE-A3 in healthy cells is unknown. MAGE-

10   A3 is a tumor-specific protein, and has been identified on many tumors. The amino acid sequence of MAGE-A3 is shown in the following:

MPLEQRSQHCKPEEGLEARGEALGLVGAQAPATEEQEAASSSSTLVEVTLGEVPAAESPDPPQS
PQGASSLPTTMNYPLWSQSYEDSSNQEEEGPSTFPDLESEFQAALSRKVAELVHFLLLKYRAREP

15   VTKAEMLGSVVGNWQYFFPVIFSKAFSSLQLVFGIELMEVDPIGHLYIFATCLGLSYDGLLGDN
QIMPKAGLLIIVLAIIAREGDCAPEEKIWEELSVLEVFEGREDSILGDPKKLLTQHFVQENYLEYRQ
VPGSDPACYEFLWGPRALVETSYVKVLHHMVKISGGPHIS YPPLHEWVLREGEE
[SEQ ID NO: 59]

20   Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 59 or a fragment or a variant thereof as described herein.

Several epitopes of MAGE-A3 are known to the skilled person. A preferred MAGE-A3 epitope,

25   which is preferably comprised by the complex for use according to the present invention, includes the following epitope (the epitope sequence shown in the following is a fragment of the above MAGE-A3 sequence and is, thus, shown in the above MAGE-A3 sequence underlined; the following epitope sequence may refer to one epitope or more than one (overlapping) epitopes):

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KVAELVHFL
[SEQ ID NO: 60]

54

Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 60.

5

IL13Ralpha2

IL13Ralpha2 binds interleukin 13 (IL-13) with very high affinity (and can therefore sequester it) but does not allow IL-4 binding. It acts as a negative regulator of both IL-13 and IL-4, however the mechanism of this is still undetermined. The amino acid sequence of

10   IL13Ralpha2 is shown in the following:

MAFVCLAIGCLYTFLISTTFGCTSSSDTEIKVNPPQDFEIVDPGYLGYLYLQWQPPLSLDHFKECT
VEYELKYRNIGSETWKTIITKNLHYKDGFDLNKGIEAKIHTLLPWQCTNGSEVQSSWAETTYWIS
PQGIPETKVQDMDCVYYNWQYLLCSWKPGIGVLLDTNYNLFYWYEGLDHALQCVDYIKAD

15   GQNIGCRFPYLEASDYKDFYICVNGSSENKPIRSSYFTFQLQNIVKPLPPVYLTFTRESSCEIKLKW
SIPLGPIPARCFDYEIEIREDDTTLVTATVENETYTLKTTNETRQLCFVVRSKVNIYCSDDGIWSEW
SDKQCWEGEDLSKKTLLRFWLPFGFILILVIFVTG LLLRKPNTYPKMIPEFFCDT
[SEQ ID NO: 61]

20   Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 61 or a fragment or a variant thereof as described herein.

Several epitopes of IL13Ralpha2are known to the skilled person. A preferred IL13Ralpha2

25   epitope, which is preferably comprised by the complex for use according to the present invention, includes the following epitope (the epitope sequence shown in the following is a fragment of the above IL13Ralpha2 sequence and is, thus, shown in the above IL13Ralpha2 sequence underlined; the following epitope sequence may refer to one epitope or more than one (overlapping) epitopes):

30

LPFGFIL
[SEQ ID NO: 62]

55

Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 62.

<u>hTert</u>

5   Telomerase reverse transcriptase (abbreviated to TERT, or hTERT in humans) is a catalytic subunit of the enzyme telomerase, which, together with the telomerase RNA component (TERC), comprises the most important unit of the telomerase complex. Telomerases are part of a distinct subgroup of RNA-dependent polymerases. Telomerase lengthens telomeres in DNA strands, thereby allowing senescent cells that would otherwise become postmitotic and

10 undergo apoptosis to exceed the Hayflick limit and become potentially immortal, as is often the case with cancerous cells. The amino acid sequence of hTert is shown in the following:

MPRAPRCRAVRSLLRSHYREVLPLATFVRRLGPQGWRLVQRGDPAAFRALVAQCLVCVPWD
ARPPPAAPSFRQVSCLKELVARVLQRLCERGAKNVLAFGFALLDGARGGPPEAFTTSVRSYLPN

15 TVTDALRGSGAWGLLLRRVGDDVLVHLLARCALFVLVAPSCAYQVCGPPLYQLGAATQARPP
PHASGPRRRLGCERAWNHSVREAGVPLGLPAPGARRRGGSASRSLPLPKRPRRGAAPEPERTP
VGQGSWAHPGRTRGPSDRGFCVVSPARPAEEATSLEGALSGTRHSHPSVGRQHHAGPPSTSR
PPRPWDTPCPPVYAETKHFLYSSGDKEQLRPSFLLSSLRPSLTGARRLVETIFLGSRPWMPGTPRR
LPRLPQRYWQMRPLFLELLGNHAQCPYGVLLKTHCPLRAAVTPAAGVCAREKPQGSVAAPEE

20 EDTDPRRLVQLLRQHSSPWQVYGFVRACLRRLVPPGLWGSRHNERRFLRNTKKFISLGKHAKL
SLQELTWKMSVRDCAWLRRSPGVGCVPAAEHRLREEILAKFLHWLMSVYVVELLRSFFYVTETT
FQKNRLFFYRKSVWSKLQSIGIRQHLKRVQLRELSEAEVRQHREARPALLTSRLRFIPKPDGLRPI
VNMDYVVGARTFRREKRAERLTSRVKALFSVLNYERARRPGLLGASVLGLDDIHRAWRTFVLR
VRAQDPPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRRYAVVQKAAHGHVRKAF

25 KSHVSTLTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSGLFDVFLRFMCHHAVRIRGK
SYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRDGLLLRLVDDFLLVTPHLTHAKTFLRTLV
RGVPEYGCVVNLRKTVVNFPVEDEALGGTAFVQMPAHGLFPWCGLLLDTRTLEVQSDYSSYA
RTSIRASLTFNRGFKAGRNMRRKLFGVLRLKCHSLFLDLQVNSLQTVCTNIYKILLLQAYRFHAC
VLQLPFHQQVWKNPTFFLRVISDTASLCYSILKAKNAGMSLGAKGAAGPLPSEAVQWLCHQA

30 FLLKLTRHRVTYVPLLGSLRTAQTQLSRKLPGTTLTALEAAANPALPSDFKTILD
[SEQ ID NO: 51]

56

Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 51 or a fragment or a variant thereof as described herein. As described above, suitable cancer/tumor epitopes of hTert are known from the literature or can be identified by using cancer/tumor epitope databases, e.g. from

5   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

10   Bioinformatics Core at Cancer Vaccine Center, Dana-Farber Cancer Institute; URL: http://cvc.dfci.harvard.edu/tadb/).

TRP-2

15   Tyrosinase related protein 2 (TRP-2, also known as "L-DOPAchrome tautomerase") is a melanogenic enzyme. TRP-2 regulates a switch that controls the proportion of carboxylated subunits in the melanin biopolymer. Thus, TRP-2 is a melanosome protein involved in melanin production. It is also overexpressed in glioblastoma cells. The amino acid sequence of TRP-2 is shown in the following:

20

MSPLWWGFLLSCLGCKILPGAQGQFPRVCMTVDSLVNKECCPRLGAESANVCGSQQGRGQ
CTEVRADTRPWSGPYILRNQDDRELWPRKFFHRTCKCTGNFAGYNCGDCKFGWTGPNCER
KKPPVIRQNIHSLSPQEREQFLGALDLAKKRVHPDYVITTQHWVGLLGPNGTQPQFANCSVY
DFFVWLHYYSVRDTLLGGFFPWLKVYYYRFVIGLRVWQWEVISCKLIKRATTRQP

25   [SEQ ID NO: 54]

Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 54 or a fragment or a variant thereof as described herein. As described above, suitable cancer/tumor epitopes of TRP-2 are known

30   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:

57

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:
5   http://cvc.dfci.harvard.edu/tadb/).

YKL-40

YKL-40 (also known as human cartilage glycoprotein-39 (HC gp-39) and as Chitinase-3-like
10   protein 1 (CHI3L1)), is a secreted glycoprotein and a member of family 18 glycosyl hydrolases. The name YKL-40 is derived from the three N-terminal amino acids present on the secreted form and its molecular mass. YKL-40 is secreted by various cell types, including chondrocytes, synovial cells, macrophages and some types of cancer cells. The amino acid sequence of YKL-40 is shown in the following:

15

MGVKASQTGFVVLVLLQCCSAYKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISN
DHIDTWEWNDVTLYGMLNTLKNRNPNLKTLLSVGGWNFGSQRFSKIASNTQSRRTFIKSVPP
FLRTHGFDGLDLAWLYPGRRDKQHFTTLIKEMKAEFIKEAQPGKKQLLLSAALSAGKVTIDSSY
DIAKISQHLDFISIMTYDFHGAWRGTTGHHSPLFRGQEDASPDRFSNTDYAVGYMLRLGAPA
20   SKLVMGIPTFGRSFTLASSETGVGAPISGPGIPGRFTKEAGTLAYYEICDFLRGATVHRILGQQVP
YATKGNQWVGYDDQESVKSKVQYLKDRQLAGAMVWALDLDDFQGSFCGQDLRFPLTNAI
KDALAAT
[SEQ ID NO: 55]

25   Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 55 or a fragment or a variant thereof as described herein. As described above, suitable cancer/tumor epitopes of YKL-40 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-
30   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

58

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/).

5

Brevican

Brevican core protein is a protein that in humans is encoded by the *BCAN* gene. Brevican is a member of the lectican protein family. Brevican is localised to the surface of neurons in the brain. In melanocytic cells, BCAN gene expression may be regulated by MITF. The amino acid sequence of brevican is shown in the following:

MAQLFLPLLAALVLAQAPAALADVLEGDSSEDRAFRVRIAGDAPLQGVLGGALTIPCHVHYLR
PPPSRRAVLGSPRVKWTFLSRGREAEVLVARGVRVKVNEAYRFRVALPAYPASLTDVSLALSELR
PNDSGIYRCEVQHGIDDSSDAVEVKVKGVVFLYREGSARYAFSFSGAQEACARIGAHIATPEQL
YAAYLGGYEQCDAGWLSDQTVRYPIQTPREACYGDMDGFPGVRNYGVVDPDDLYDVYCY
AEDLNGELFLGDPPEKLTLEEARAYCQERGAEIATTGQLYAAWDGGLDHCSPGWLADGSVRY
PIVTPSQRCGGGLPGVKTLFLFPNQTGFPNKHSRFNVYCFRDSAQPSAIPEASNPASNPASDGL
EAIVTVTETLEELQLPQEATESESRGAIYSIPIMEDGGGGSSTPEDPAEAPRTLLEFETQSMVPPTGF
SEEEGKALEEEEKYEDEEEKEEEEEEEEVEDEALWAWPSELSSPGPEASLPTEPAAQEESLSQAPARA
VLQPGASPLPDGESEASRPPRVHGPPTETLPTPRERNLASPSPSTLVEAREVGEATGGPELSGVPR
GESEETGSSEGAPSLLPATRAPEGTRELEAPSEDNSGRTAPAGTSVQAQPVLPTDSASRGGVAV
VPASGDCVPSPCHNGGTCLEEEEGVRCLCLPGYGGDLCDVGLRFCNPGWDAFQGACYKHF
STRRSWEEAETQCRMYGAHLASISTPEEQDFINNRYREYQWIGLNDRTIEGDFLWSDGVPLLYE
NWNPGQPDSYFLSGENCVVMVWHDQGQWSDVPCNYHLSYTCKMGLVSCGPPPELPLAQ
VFGRPRLRYEVDTVLRYRCREGLAQRNLPLIRCQENGRWEAPQISCVPRRPARALHPEEDPEGR
QGRLLGRWKALLIPPSSPMPGP
[SEQ ID NO: 56]

30   Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 56 or a fragment or a variant thereof as described herein. As described above, suitable cancer/tumor epitopes of brevican are known

59

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/).

Neuroligin 4

Neuroligin (NLGN), a type I membrane protein, is a cell adhesion protein on the postsynaptic membrane that mediates the formation and maintenance of synapses between neurons. Neuroligins act as ligands for β-Neurexins, which are cell adhesion proteins located presynaptically. Neuroligins also affect the properties of neural networks by specifying synaptic functions, and they mediate signalling by recruiting and stabilizing key synaptic components. Neuroligins interact with other postsynaptic proteins to localize neurotransmitter receptors and channels in the postsynaptic density as the cell matures. The amino acid sequence of neuroligin 4 is shown in the following:

MSRPQGLLWLPLLFTPVCVMLNSNVLLWLTALAIKFTLIDSQAQYPVVNTNYGKIRGLRTPLPN
EILGPVEQYLGVPYASPPTGERRFQPPEPPSSWTGIRNTTQFAAVCPQHLDERSLLHDMLPIWF
TANLDTLMTYVQDQNEDCLYLNIYVPTEDDIHDQNSKKPVMVYIHGGSYMEGTGNMIDGSI
LASYGNVIVITINYRLGILGFLSTGDQAAKGNYGLLDQIQALRWIEENVGAFGGDPKRVTIFGS
GAGASCVSLLTLSHYSEGLFQKAIIQSGTALSSWAVNYQPAKYTRILADKVGCNMLDTTDMVE
CLRNKNYKELIQQTITPATYHIAFGPVIDGDVIPDDPQILMEQGEFLNYDIMLGVNQGEGLKFV
DGIVDNEDGVTPNDFDFSVSNFVDNLYGYPEGKDTLRETIKFMYTDWADKENPETRRKTLVA
LFTDHQWVAPAVATADLHAQYGSPTYFYAFYHHCQSEMKPSWADSAHGDEVPYVFGIPMI
GPTELFSCNFSKNDVMLSAVVMTYWTNFAKTGDPNQPVPQDTKFIHTKPNRFEEVAWSKYNP
KDQLYLHIGLKPRVRDHYRATKVAFWLELVPHLHNLNEIFQYVSTTTKVPPPDMTSFPYGTRRS
PAKIWPTTKRPAITPANNPKHSKDPHKTGPEDTTVLIETKRDYSTELSVTIAVGASLLFLNILAFAA

60

LYYKKDKRRHETHRRPSPQRNTTNDIAHIQNEEIMSLQMKQLEHDHECESLQAHDTLRLTCPP
DYTLTLRRSPDDIPLMTPNTIT MIPNTLTGMQPLHTFNTFSGGQNSTNLPHGHSTTRV
[SEQ ID NO: 57]

5   Accordingly, a preferred complex for use according to the present invention comprises an
       amino acid sequence according to SEQ ID NO: 57 or a fragment or a variant thereof as
       described herein. As described above, suitable cancer/tumor epitopes of neuroligin 4 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-
10   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:
15   http://cvc.dfci.harvard.edu/tadb/).

       PTPRz1
       Receptor-type tyrosine-protein phosphatase zeta also known as phosphacan is a single-pass
20   type I membrane protein with two cytoplasmic tyrosine-protein phosphatase domains, an
       alpha-carbonic anhydrase domain and a fibronectin type III domain and belongs to the
       receptor tyrosine phosphatase family. Both, the protein and transcript are overexpressed in
       glioblastoma cells, promoting their haptotactic migration. The amino acid sequence of
       PTPRz1 is shown in the following:

25

       MRILKRFLACIQLLCVCRLDWANGYYRQQRKLVEEIGWSYTGALNQKNWGKKYPTCNSPKQ
       SPINIDEDLTQVNVNLKKLKFQGWDKTSLENTFIHNTGKTVEINLTNDYRVSGGVSEMVFKASK
       ITFHWGKCNMSSDGSEHSLEGQKFPLEMQIYCFDADRFSSFEEAVKGKGKLRALSILFEVGTEEN
       LDFKAIIDGVESVSRFGKQAALDPFILLNLLPNSTDKYYIYNGSLTSPPCTDTVDWIVFKDTVSISE
30   SQLAVFCEVLTMQQSGYVMLMDYLQNNFREQQYKFSRQVFSSYTGKEEIHEAVCSSEPENVQ
       ADPENYTSLLVTWERPRVVYDTMIEKFAVLYQQLDGEDQTKHEFLTDGYQDLGAILNNLLPN
       MSYVLQIVAICTNGLYGKYSDQLIVDMPTDNPELDLFPELIGTEEIIKEEEEGKDIEEGAIVNPGRD

61

SATNQIRKKEPQISTTTHYNRIGTKYNEAKTNRSPTRGSEFSGKGDVPNTSLNSTSQPVTKLATE

KDISLTSQTVTELPPHTVEGTSASLNDGSKTVLRSPHMNLSGTAESLNTVSITEYEEESLLTSFKLDT

GAEDSSGSSPATSAIPFISENISQGYIFSSENPETITYDVLIPESARNASEDSTSSGSEESLKDPSMEG

NVWFPSSTDITAQPDVGSGRESFLQTNYTEIRVDESEKTTKSFSAGPVMSQGPSVTDLEMPHYS

5   TFAYFPTEVTPHAFTPSSRQQDLVSTVNVVYSQTTQPVYNGETPLQPSYSSEVFPLVTPLLLDNQ

ILNTTPAASSSDSALHATPVFPSVDVSFESILSSYDGAPLLPFSSASFSSELFRHLHTVSQILPQVTSA

TESDKVPLHASLPVAGGDLLLEPSLAQYSDVLSTTHAASETLEFGSESGVLYKTLMFSQVEPPSSD

AMMHARSSGPEPSYALSDNEGSQHIFTVSYSSAIPVHDSVGVTYQGSLFSGPSHIPIPKSSLITPT

ASLLQPTHALSGDGEWSGASSDSEFLLPDTDGLTALNISSPVSVAEFTYTTSVFGDDNKALSKSEI

10 IYGNETELQIPSFNEMVYPSESTVMPNMYDNVNKLNASLQETSVSISSTKGMFPGSLAHTTTKVF

DHEISQVPENNFSVQPTHTVSQASGDTSLKPVLSANSEPASSDPASSEMLSPSTQLLFYETSASFS

TEVLLQPSFQASDVDTLLKTVLPAVPSDPILVETPKVDKISSTMLHLIVSNSASSENMLHSTSVPVF

DVSPTSHMHSASLQGLTISYASEKYEPVLLKSESSHQVVPSLYSNDELFQTANLEINQAHPPKGR

HVFATPVLSIDEPLNTLINKLIHSDEILTSTKSSVTGKVFAGIPTVASDTFVSTDHSVPIGNGHVAIT

15 AVSPHRDGSVTSTKLLFPSKATSELSHSAKSDAGLVGGGEDGDTDDDGDDDDDRGSDGLS

IHKCMSCSSYRESQEKVMNDSDTHENSLMDQNNPISYSLSENSEEDNRVTSVSSDSQTGMDRS

PGKSPSANGLSQKHNDGKEENDIQTGSALLPLSPESKAWAVLTSDEESGSGQGTSDSLNENET

STDFSFADTNEKDADGILAAGDSEITPGFPQSPTSSVTSENSEVFHVSEAEASNSSHESRIGLAEGL

ESEKKAVIPLVIVSALTFICLVVLVGILIYWRKCFQTAHFYLEDSTSPRVISTPPTPIFPISDDVGAIPIK

20 HFPKHVADLHASSGFTEEFETLKEFYQEVQSCTVDLGITADSSNHPDNKHKNRYINIVAYDHSR

VKLAQLAEKDGKLTDYINANYVDGYNRPKAYIAAQGPLKSTAEDFWRMIWEHNVEVIVMITN

LVEKGRRKCDQYWPADGSEEYGNFLVTQKSVQVLAYYTVRNFTLRNTKIKKGSQKGRPSGRV

VTQYHYTQWPDMGVPEYSLPVLTFVRKAAYAKRHAVGPVVVHCSAGVGRTGTYIVLDSMLQ

QIQHEGTVNIFGFLKHIRSQRNYLVQTEEQYVFIHDTLVEAILSKETEVLDSHIHAYVNALLIPGP

25 AGKTKLEKQFQLLSQSNIQQSDYSAALKQCNREKNRTSSIIPVERSRVGISSLSGEGTDYINASY

MGYYQSNEFIITQHPLLHTIKDFWRMIWDHNAQLVVMIPDGQNMAEDEFVYWPNKDEPIN

CESFKVTLMAEEHKCLSNEEKLIIQDFILEATQDDYVLEVRHFQCPKWPNPDSPISKTFELISVIKEE

AANRDGPMIVHDEHGGVTAGTFCALTTLMHQLEKENSVDVYQVAKMINLMRPGVFADIEQ

YQFLYKVIL SLVSTRQEENPSTSLDSNGAALPDGNIAESLESLV

30 [SEQ ID NO: 58]

62

Accordingly, a preferred complex for use according to the present invention comprises an amino acid sequence according to SEQ ID NO: 58 or a fragment or a variant thereof as described herein. As described above, suitable cancer/tumor epitopes of PTPRz1 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/).

Preferably, the complex for use according to the present invention comprises at least one tumor epitope, which is an epitope of an antigen selected from the group consisting of EGFRvIII, EphA2, Her2/neu, IL-13Rα2, survivin, TRP-2, brevican, neuroligin 4 and PTPRz1, such as an epitope according to any of SEQ ID NOs 47, 53 and 62; more preferably the at least one tumor epitope is an epitope of an antigen selected from the group consisting of EGFRvIII, EphA2, IL-13Rα2, TRP-2 and brevican, such as an epitope according to any of SEQ ID NOs 47 and 62; and even more preferably the at least one tumor epitope is an epitope of an antigen selected from the group consisting of EGFRvIII, EphA2 and brevican, such as an epitope according to SEQ ID NO 47.

It is also preferred that the complex for use according to the present invention comprises

— one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

— one or more epitopes of EphA2 or functional sequence variants thereof;

— one or more epitopes of Her2/neu or functional sequence variants thereof;

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof;

— one or more epitopes of survivin (such as the epitope according to SEQ ID NO: 53) or functional sequence variants thereof;

63

— one or more epitopes of TRP-2 or functional sequence variants thereof;

— one or more epitopes of brevican or functional sequence variants thereof;

— one or more epitopes of neuroligin 4 or functional sequence variants thereof; and/or

— one or more epitopes of PTPRz1 or functional sequence variants thereof.

5   As described above, further epitopes of those antigens (in addition to the exemplified epitopes) can easily be retrieved 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/, or from

10   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/).

A „sequence variant" is as defined above, namely a sequence variant has an (amino acid)

15   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 epitope, that the function as an epitope is not impaired or abolished. Preferably, however, the amino acid sequence of an epitope of a cancer/tumor antigen as

20   described herein is not mutated and, thus, identical to the reference epitope sequence.

It is also preferred that the complex for use according to the present invention comprises

— a fragment of EGFRvIII comprising one or more epitopes or a functional sequence variant thereof;

25   — a fragment of EphA2 (such as a fragment of the polypeptide according to SEQ ID NO: 48) comprising one or more epitopes or a functional sequence variant thereof;

— a fragment of Her2/neu (such as a fragment of the polypeptide according to SEQ ID NO: 50) comprising one or more epitopes or a functional sequence variant thereof;

— a fragment of IL-13Rα2 (such as a fragment of the polypeptide according to SEQ ID

30   NO: 61) comprising one or more epitopes or a functional sequence variant thereof;

— a fragment of survivin (such as a fragment of the polypeptide according to SEQ ID NO: 52) comprising one or more epitopes or a functional sequence variant thereof;

64

— a fragment of TRP-2 (such as a fragment of the polypeptide according to SEQ ID NO: 54) comprising one or more epitopes or a functional sequence variant thereof;

— a fragment of brevican (such as a fragment of the polypeptide according to SEQ ID NO: 56) comprising one or more epitopes or a functional sequence variant thereof;

5   — a fragment of neuroligin 4 (such as a fragment of the polypeptide according to SEQ ID NO: 57) comprising one or more epitopes or a functional sequence variant thereof; and/or

— a fragment of PTPRz1 (such as a fragment of the polypeptide according to SEQ ID NO: 58) comprising one or more epitopes or a functional sequence variant thereof.

10

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

15   antigen. Accordingly, a fragment of EGFRvIII comprises at least 10 consecutive amino acids of EGFRvIII, preferably at least 15 consecutive amino acids of EGFRvIII, more preferably at least 20 consecutive amino acids of EGFRvIII, even more preferably at least 25 consecutive amino acids of EGFRvIII, and most preferably at least 30 consecutive amino acids of EGFRvIII; a fragment of survivin comprises at least 10 consecutive amino acids of survivin (SEQ ID NO:

20   52), preferably at least 15 consecutive amino acids of survivin (SEQ ID NO: 52), more preferably at least 20 consecutive amino acids of survivin (SEQ ID NO: 52), even more preferably at least 25 consecutive amino acids of survivin (SEQ ID NO: 52) and most preferably at least 30 consecutive amino acids of survivin (SEQ ID NO: 52); a fragment of EphA2 comprises at least 10 consecutive amino acids of EphA2 (SEQ ID NO: 48), preferably

25   at least 15 consecutive amino acids of EphA2 (SEQ ID NO: 48), more preferably at least 20 consecutive amino acids of EphA2 (SEQ ID NO: 48), even more preferably at least 25 consecutive amino acids of EphA2 (SEQ ID NO: 48) and most preferably at least 30 consecutive amino acids of EphA2 (SEQ ID NO: 48); a fragment of Her2/neu comprises at least 10 consecutive amino acids of Her2/neu (SEQ ID NO: 50), preferably at least 15

30   consecutive amino acids of Her2/neu (SEQ ID NO: 50), more preferably at least 20 consecutive amino acids of Her2/neu (SEQ ID NO: 50), even more preferably at least 25 consecutive amino acids of Her2/neu (SEQ ID NO: 50) and most preferably at least 30

65

consecutive amino acids of Her2/neu (SEQ ID NO: 50); a fragment of IL-13Rα2 comprises at least 10 consecutive amino acids of IL-13Rα2 (SEQ ID NO: 61), preferably at least 15 consecutive amino acids of IL-13Rα2 (SEQ ID NO: 61), more preferably at least 20 consecutive amino acids of IL-13Rα2 (SEQ ID NO: 61), even more preferably at least 25

5   consecutive amino acids of IL-13Rα2 (SEQ ID NO: 61) and most preferably at least 30 consecutive amino acids of IL-13Rα2 (SEQ ID NO: 61); a fragment of TRP-2 comprises at least 10 consecutive amino acids of TRP-2 (SEQ ID NO: 54), preferably at least 15 consecutive amino acids of TRP-2 (SEQ ID NO: 54), more preferably at least 20 consecutive amino acids of TRP-2 (SEQ ID NO: 54), even more preferably at least 25 consecutive amino

10   acids of TRP-2 (SEQ ID NO: 54) and most preferably at least 30 consecutive amino acids of TRP-2 (SEQ ID NO: 54); a fragment of brevican comprises at least 10 consecutive amino acids of brevican (SEQ ID NO: 56), preferably at least 15 consecutive amino acids of brevican (SEQ ID NO: 56), more preferably at least 20 consecutive amino acids of brevican (SEQ ID NO: 56), even more preferably at least 25 consecutive amino acids of brevican (SEQ ID NO:

15   56) and most preferably at least 30 consecutive amino acids of brevican (SEQ ID NO: 56); a fragment of neuroligin 4 comprises at least 10 consecutive amino acids of neuroligin 4 (SEQ ID NO: 57), preferably at least 15 consecutive amino acids of neuroligin 4 (SEQ ID NO: 57), more preferably at least 20 consecutive amino acids of neuroligin 4 (SEQ ID NO: 57), even more preferably at least 25 consecutive amino acids of neuroligin 4 (SEQ ID NO: 57) and

20   most preferably at least 30 consecutive amino acids of neuroligin 4 (SEQ ID NO: 57); and a fragment of PTPRz1 comprises at least 10 consecutive amino acids of PTPRz1 (SEQ ID NO: 58), preferably at least 15 consecutive amino acids of PTPRz1 (SEQ ID NO: 58), more preferably at least 20 consecutive amino acids of PTPRz1 (SEQ ID NO: 58), even more preferably at least 25 consecutive amino acids of PTPRz1 (SEQ ID NO: 58) and most

25   preferably at least 30 consecutive amino acids of PTPRz1 (SEQ ID NO: 58).

A functional sequence variant of such a fragment 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%

30   identical to the reference sequence, and the epitope function of at least one, preferably all, epitope(s) comprised by the fragment is maintained.

66

Preferably, such a complex comprises

— one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

— one or more epitopes of EphA2 or functional sequence variants thereof;

5   — one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof;

— one or more epitopes of TRP-2 or functional sequence variants thereof; and/or

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of CMV, gp100, Her2/neu,

10   survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

More preferably, such a complex comprises

— one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

15   — one or more epitopes of EphA2 or functional sequence variants thereof;

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof;

— one or more epitopes of TRP-2 or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

20   Such a complex does preferably not comprise any epitope of CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of EphA2 or functional sequence variants thereof;

25   — one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof;

— one or more epitopes of TRP-2 or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvIII, CMV, gp100,

30   Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

67

It is also more preferred that such a complex comprises

— one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof;

— one or more epitopes of TRP-2 or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EphA2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

— one or more epitopes of EphA2 or functional sequence variants thereof;

— one or more epitopes of TRP-2 or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of IL-13Ralpha2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

— one or more epitopes of EphA2 or functional sequence variants thereof;

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of TRP-2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

68

— one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

— one or more epitopes of EphA2 or functional sequence variants thereof;

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof; and

— one or more epitopes of TRP-2 or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of brevican, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

— one or more epitopes of EphA2 or functional sequence variants thereof; and

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of TRP-2, brevican, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

— one or more epitopes of EphA2 or functional sequence variants thereof; and

— one or more epitopes of TRP-2 or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of brevican, IL-13Ralpha2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

— one or more epitopes of EphA2 or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

69

Such a complex does preferably not comprise any epitope of IL-13Ralpha2, TRP-2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

5   — one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

       — one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof; and

       — one or more epitopes of TRP-2 or functional sequence variants thereof.

10   Such a complex does preferably not comprise any epitope of EphA2, brevican, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

       — one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47)
15   or functional sequence variants thereof;

       — one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof; and

       — one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EphA2, TRP-2, CMV, gp100,
20   Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

       — one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof;

25   — one or more epitopes of TRP-2 or functional sequence variants thereof; and

       — one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EphA2, IL-13Ralpha2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

30   It is also more preferred that such a complex comprises

       — one or more epitopes of EphA2 or functional sequence variants thereof;

70

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof; and

— one or more epitopes of TRP-2 or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvlll, brevican, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of EphA2 or functional sequence variants thereof;

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvlll, TRP-2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of EphA2 or functional sequence variants thereof;

— one or more epitopes of TRP-2 or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvlll, IL-13Ralpha2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof;

— one or more epitopes of TRP-2 or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvlll, EphA2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

71

- one or more epitopes of EGFRvlll (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof; and

- one or more epitopes of EphA2 or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of IL-13Ralpha2, brevican, TRP-2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

- one or more epitopes of EGFRvlll (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof; and

- one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EphA2, brevican, TRP-2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

- one or more epitopes of EGFRvlll (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof; and

- one or more epitopes of TRP-2 or functional sequence variants thereof; and

Such a complex does preferably not comprise any epitope of EphA2, IL-13Ralpha2, brevican, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

- one or more epitopes of EGFRvlll (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof; and

- one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EphA2, IL-13Ralpha2, TRP-2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

72

It is also more preferred that such a complex comprises

— one or more epitopes of EphA2 or functional sequence variants thereof; and

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof;

5 Such a complex does preferably not comprise any epitope of EGFRvIII, TRP-2, brevican, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

10 — one or more epitopes of EphA2 or functional sequence variants thereof; and

— one or more epitopes of TRP-2 or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvIII, IL-13Ralpha2, brevican, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

15

It is also more preferred that such a complex comprises

— one or more epitopes of EphA2 or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvIII, IL-13Ralpha2, TRP-2,

20 CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof; and

25 — one or more epitopes of TRP-2 or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvIII, EphA2, brevican, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

30

It is also more preferred that such a complex comprises

73

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvIII, EphA2, TRP-2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of TRP-2 or functional sequence variants thereof; and

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvIII, EphA2, IL-13Ralpha2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of EGFRvIII (such as the epitope according to SEQ ID NO: 47) or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EphA2, TRP-2, brevican, IL-13Ralpha2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of EphA2 or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvIII, IL-13Ralpha2, TRP-2, brevican, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

It is also more preferred that such a complex comprises

— one or more epitopes of IL-13Rα2 (such as the epitope according to SEQ ID NO: 62) or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvIII, EphA2, TRP-2, brevican, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

5   It is also more preferred that such a complex comprises

— one or more epitopes of TRP-2 or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvIII, EphA2, IL-13Ralpha2, brevican, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4 or PTPRz1.

10

It is also more preferred that such a complex comprises

— one or more epitopes of brevican or functional sequence variants thereof.

Such a complex does preferably not comprise any epitope of EGFRvIII, EphA2, IL-13Ralpha2, TRP-2, CMV, gp100, Her2/neu, survivin, hTert, MAGE-A1, MAGE-A3, YKL-40, neuroligin 4

15 or PTPRz1.

Component c) – TLR peptide agonist

In the complex for use according to the present invention, the TLR peptide agonist allows an

20 increased targeting of the vaccine towards dendritic cells along with self-adjuvancity. Physical linkage of a TLR peptide agonist to the CPP and the at least one antigen or antigenic epitope according to the present invention in the complex 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

25 antigen(s).

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

30 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

75

amino acids, particularly preferably from 25 to 110 amino acids, and most preferably from 30 to 100 amino acids.

5   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

10   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

15   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.

20

Preferably, the TLR peptide agonist comprised by the complex 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

25   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.

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However, TLR2, 4, and 5 are constitutively expressed at the surface of DCs. Accordingly, the TLR peptide agonist comprised by the complex for use according to the present invention is

76

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

5   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,

10   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

15   (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).

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

20   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 lipomannas from *M. tuberculosis*). Certain viral

25   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

30   bacterial lipopeptides, include MALP-2 and it's synthetic analogue di-palmitoyl-S-glyceryl cysteine (Pam_2Cys) or tri-palmitoyl-S-glyceryl cysteine (Pam_3Cys).

77

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

5   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

10   described for example in WO 2013/120073 (A1).

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 for use according

15   to the present invention.

Examples of TLR peptide agonists thus include the TLR2 lipopeptide agonists MALP-2, Pam$_2$Cys and Pam$_3$Cys 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

20   flagellin.

However, it is preferred that the TLR peptide agonist comprised by the complex for use according to the present invention is neither a lipopeptide nor a lipoprotein, neither a glycopeptide nor a glycoprotein, more preferably, the TLR peptide agonist comprised by the

25   complex for use according to the present invention is a classical peptide, polypeptide or protein as defined herein.

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,

30   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.

78

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
5   complex for use according to the present invention.

STVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE
SEQ ID NO: 15 (TLR2 peptide agonist Anaxa)

10   Regarding TLR4, TLR peptides agonists are particularly preferred, which 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 cellular glycoprotein Fibronectin (FN) has multiple isoforms generated from a single gene by alternative splicing of three exons. One of these isoforms is the extra domain
15   A (EDA), which interacts with TLR4.

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
20   A1. Thereby, a TLR4 peptide agonist comprising or consisting of an amino acid sequence according to SEQ ID NO: 45 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 for use according to the present invention.

25   NIDRPKGLAFTDVDVDSIKIAWESPQGGQVSRYRVTYSSPEDGIRELFPAPDGEDDTAELQGLRP
GSEYTVSVVALHDDMESQPLIGIQST
SEQ ID NO: 45 (TLR4 peptide agonist EDA)

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

79

The complex for use according to the present invention comprises at least one TLR peptide agonist, preferably the complex 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 for use according to the present invention comprises

5   (at least) two or three TLR peptide agonists, even more preferably the complex 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 for use according to the present invention it is understood that said TLR peptide agonist is in particular also covalently linked in the complex for use according to the present invention, e.g. to another TLR peptide

10   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.

In a particularly preferred embodiment, the complex for use according to the present invention comprises one single TLR peptide agonist. In particularly, in this particularly

15   preferred embodiment, the complex 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.

The various TLR peptide agonists comprised by the complex for use according to the present

20   invention may be the same or different. Preferably, the various TLR peptide agonists comprised by the complex for use according to the present invention are different from each other.

Moreover, it is preferred that the more than one antigen or antigenic epitope, in particular 2,

25   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 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

30   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

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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.

5   Alternatively, however, the various TLR peptide agonists may also be positioned in any other way in the complex 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).

10

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 for use according to the present invention. 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

15   activating the same or different TLR receptors, which are comprised by different complexes according to the present invention, whereby such different complexes comprising different subsets may advantageously be administered simultaneously, e.g. in a single vaccine, to a subject in need thereof.

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Linkage of components a), b), and c) in the complex for use according to the present invention

In the complex 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

25   the complex for use according to the present invention is a covalent linkage. Preferably, two out of the three components a), b), and c) of the complex for use according to the present invention 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

30   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).

81

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

5   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 for use

10 according to the present invention may also be referred to as "compound", in particular it may be referred to as "molecule".

Preferably, in the complex for use according to the present invention, components a), b), and

15 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

20 components of the complex for use according to the present invention 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

25 is described e.g. by Rose *et al.* (1994), JACS 116, 30.

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

30 group may be used, if just one cystein residue is present in a certain component a), b), or c) of complex 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

82

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

5   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

10   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 for use according to the present invention. When a cysteine

15   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 cystein.

20   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, DICI, 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-

25   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;

30   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 bismaleimidohexane (BMH). BMH contains two maleimide functional

83

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-

5   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),

10   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

15   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

20   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 for use according to the present invention 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

25   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

30   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

84

complex for use according to the present invention can be retrieved from: Wong, Chemistry of Protein Conjugation and Cross-Linking, CRC Press (1991).

5   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
10   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,
15   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-
20   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.

25   Examples of crosslinkers in general, which can be used in the complex 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*-Maleimmidyl-2,3-dihydroxy-butane, *Bis*-Maleimidohexane, *Bis*-Maleimidoethane, *N* -(β-Maleimidopropionic acid)hydrazide*TFA,   *N*   -(β-Maleimidopropyloxy)succinimide   ester,   1,8-*Bis*-
30   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*

85

(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(succimidylpropionate) (Lomant's

5   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-

10 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-[*N*2-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-

15 *N*6-(6-biotinamidocaproyl)-L-lysinyl]ethylmethanethiosulfate, 2-{*N*2-[*N*6-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-*N*6-(6-biotinamidocaproyl)-L-lysinyl]}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-

20 tetraoxatetradecane-*N*-hydroxysuccinimide, 2-pyridyldithiol-tetraoxaoctatriacontane-*N*-hydroxysuccinimide, *N*-(*p*-Maleimidophenyl)isocyanate, Succinimdyl 3-(bromoacetamido)propionate, NHS-Diazirine, NHS-SS-Diazirine, *N*-succinimidyl iodoacetate, *N*-Succinimidyl(4-iodoacetyl)aminobenzoate, Succinimidyl 4-(*N*-maleimido-methyl)cyclohexane-1-carboxylate, NHS-PEG2-Maliemide, NHS-PEG4-Maliemide, NHS-

25 PEG6-Maliemide, NHS-PEG8-Maliemide, NHS-PEG12-Maliemide, NHS-PEG24-Maliemide, 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-

30 Maleimidocaproyloxy)sulfosuccinimide ester, *N*-(γ-Maleimidobutryloxy)sulfosuccinimide ester, *N*-(κ-Maleimidoundecanoyloxy)sulfosuccinimide ester, Sulfo-NHS-LC-Diazirine, Sulfosuccinimidyl 6-(3'-[2-pyridyldithio]propionamido)hexanoate, *m*-Maleimidobenzoyl-*N*-

86

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-iodo-acetyl)aminobenzoate,   Sulfosuccinimidyl   4-(*N*-

5   maleimidomethyl)cyclohexane-1-carboxylate,   Sulfosuccinimidyl   4-(*p*-

maleimidophenyl)butyrate,   *Tris*-(2-Maleimidoethyl)amine   (Trifunctional),   and   *Tris*-

(succimimidyl aminotricetate) (Trifunctional).

The linkage between two out of the three components a), b), and c) of the complex for use

10   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.

A direct linkage may be realized preferably by an amide bridge, if the components to be

15   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,

20   wherein both (protein or peptide) components are preferably synthesized in one step. Such

protein synthesis methods include e.g., without being limited thereto, 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

25   hexafluorophosphate) based solid-phase peptide synthesis, etc.. Alternatively, ester or ether

linkages are preferred.

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

30   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

5   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.

The complex 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

10   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

15   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

20   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 for use according to the present invention or to design a linker to be used in the complex for use according to the present invention.

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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),

30   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

88

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:

5   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

10   or GlyGlyCys, etc. 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.

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

In particular, the complex 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

20   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.

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

25   the antigens or antigenic epitopes and/or each of the TLR peptide agonists comprised in the complex of the invention 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 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

30   possible that some antigens or antigenic epitopes and/or some TLR peptide agonists comprised by the complex of the invention 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.

For example, two successive antigens or antigenic epitopes or two successive TLR peptide
5   agonists comprised in the complex of the invention 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 consists of up to about 8 amino acids corresponding
10   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 present invention, the spacer used to link a first antigen/antigenic epitope or a first TLR peptide agonist ("antigen/epitope/TLR
15   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.
20   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
25   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
30   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

90

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 invention in the

5   situation where a spacer has less or more than 8 amino acids.

In another particular illustration of 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,

10   ("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

15   for use according to the present invention.

The technics 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

20   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.

25   Preferably, the at least one antigen or antigenic epitope comprised by the complex for use according to the present invention as well as any optional spacer or linker comprised by the complex for use according to the present invention are of peptidic nature. More preferably, all components of the complex 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,

30   polypeptide or protein, the at least one TLR peptide agonist and any optional peptidic linker or spacer are linked in the complex for use according to the present invention by a peptide

91

bond. Most preferably, the complex 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.

In this context, a complex comprising or consisting of an amino acid sequence according to
5   SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 46 or SEQ ID NO: 69 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:
10   27, SEQ ID NO: 28, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 46 or SEQ ID NO: 69 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: 33, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41 or SEQ ID NO: 69 or a complex comprising or consisting of an amino acid sequence
15   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: 33, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41 or SEQ ID NO: 69 is more preferred; a complex comprising or consisting of an amino acid sequence according to SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 40,
20   SEQ ID NO: 41 or SEQ ID NO: 69 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: 37, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41 or SEQ ID NO: 69 is even more preferred; and a complex comprising or consisting of an amino acid sequence according to
25   SEQ ID NO: 28, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41 or SEQ ID NO: 69 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: 39, SEQ ID NO: 40, SEQ ID NO: 41 or SEQ ID NO: 69 is particularly preferred.

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SEQ ID NO: 26:

92

MHHHHHHHNID   RPKGLAFTDV   DVDSIKIAWE   SPQGQVSRYR   VTYSSPEDGI
RELFPAPDGEDDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQSTKRY KNRVASRKSR
AKFKQLLQHY REVAAAKSSE NDRLRLLLKE SLKISQAVHA AHAEINEAGR EVVGVGALKV
PRNQDWLGVP RFAKFASFEA QGALANIAVD KANLDVEQLE SIINFEKLTE WTGS

SEQ ID NO: 27:
MHHHHHHHSTV HEILCKLSLE GDHSTPPSAY GSVKPYTNFD AEKRYKNRVA SRKSRAKFKQ
LLQHYREVAA AKSSENDRLR LLLKESLKIS QAVHAAHAEI NEAGREVVGV GALKVPRNQD
WLGVPRFAKF ASFEAQGALA NIAVDKANLD VEQLESIINF EKLTEWTGS

SEQ ID NO: 28:
MHHHHHHHKRYKNRVA   SRKSRAKFKQ   LLQHYREVAA   AKSSENDRLR   LLLKESLKIS
QAVHAAHAEI   NEAGREVVGV   GALKVPRNQD   WLGVPRFAKF   ASFEAQGALA
NIAVDKANLD VEQLESIINF EKLTEWTGSS TVHEILCKLS LEGDHSTPPS AYGSVKPYTN FDAE

SEQ ID NO: 33:
MHHHHHHHKRY KNRVASRKSR AKFKQLLQHY REVAAAKESL KISQAVHAAH AEINEAGREV
VGVGALKVPR NQDWLGVPRF AKFASFEAQG ALANIAVDKA NLDVEQLESI INFEKLTEWT
GSSTVHEILC KLSLEGDHST PPSAYGSVKP YTNFDAE

SEQ ID NO: 34:
MHHHHHHHREV AAAKSSENDR LRLLLKESLK ISQAVHAAHA EINEAGREVV GVGALKVPRN
QDWLGVPRFA KFASFEAQGA LANIAVDKAN LDVEQLESII NFEKLTEWTG SSTVHEILCK
LSLEGDHSTP PSAYGSVKPY TNFDAE

SEQ ID NO: 37:
MHHHHHHHNID RPKGLAFTDV DVDSIKIAWE SPQGQVSRYR VTYSSPEDGI RELFPAPDGE
DDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQSTKRY KNRVASRKSR AKFKQLLQHY
REVAAAKESL KISQAVHAAH AEINEAGREV VGVGALKVPR NQDWLGVPRF AKFASFEAQG
ALANIAVDKA NLDVEQLESI INFEKLTEWT GS

SEQ ID NO: 38:

93

MHHHHHHNID RPKGLAFTDV DVDSIKIAWE SPQGQVSRYR VTYSSPEDGI RELFPAPDGE DDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQSTREV AAAKSSENDR LRLLLKESLK ISQAVHAAHA EINEAGREVV GVGALKVPRN QDWLGVPRFA KFASFEAQGA LANIAVDKAN LDVEQLESII NFEKLTEWTG S

5

SEQ ID NO: 39:
KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSENDRLRLLLKVTYHSPSYAYHQFERRAILNRLV QFIKDRISVVQALVLTSTVHEILCKLSLEGDHSTPPSAYGSVKPYTN FDAE

10   SEQ ID NO: 40:
KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSENDRLRLLLKNYRIATFKNWPFLEDCAMEELT VSEFLKLDRQRSTVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE

SEQ ID NO: 41:
15   KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSENDRLRLLLKHLELASMTNMELMSSIVSTVHEI LCKLSLEGDHSTPPSAYGSVKPYTNFDAE

SEQ ID NO: 46:
RKKRRQRRRRVKRISQAVHAAHAEINEAGRRVKRKVPRNQDWLRVKRASFEAQGALANIAVD
20   KARVKRSIINFEKLRVKRSTVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE

SEQ ID NO: 69:
KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSENDRLRLLLKLFRAAQLANDVVLQIMEHLELA SMTNMELMSSIVVISASIIVFNLLELEGSTVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE
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Arrangement of components a), b), and c) in the complex for use according to the present invention

30   The components a), b), and c) may be arranged in the complex for use according to the present invention in any way.

94

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 for use according to the present invention, the more than one cell penetrating peptide may be positioned in a non-consecutive manner, i.e. at least one antigen or antigenic epitope

5   (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

10   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

15   (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.

       However, it is preferred that the more than one cell penetrating peptide is positioned in the

20   complex 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 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 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

25   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

30   each other for example by a spacer or linker as described herein, which is not of the other two components.

95

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

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.

In the complex 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 for use according to the present invention, namely:

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

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 for use according to the present invention, 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 for use

96

according to the present invention, optionally together with further components, for example linker(s), spacer(s), etc.. 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

5   → 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);

10   (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
15   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).

20

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

25   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.

Even more preferably, the at least one antigen or antigenic epitope is positioned C-terminally
30   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

97

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 for use according to the present invention is a recombinant polypeptide or a

5   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

10   a spacer.

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

15   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

20   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

25   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).

30   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:

98

(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);

5   (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
10   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).

15 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);

20 (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
25   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) –
30   component a) (cell penetrating peptide) – component b) (at least one antigen or
        antigenic epitope).

99

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).

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

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, a spacer etc.:

(1) cell penetrating peptide (a) – antigen/antigenic epitope (b); or

(2) antigen/antigenic epitope (b) – cell penetrating peptide (a).

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:

100

(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;

5   (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;

(iii) component c) may be linked – optionally via a spacer or a linker – via side-chain/side-

10   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;

(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

15   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;

(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

20   chain of the at least one antigen or antigenic epitope;

(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;

25   (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);

30   (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

101

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

(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).

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, a spacer etc.:

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

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

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;

102

(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).

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, a spacer etc.:

103

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

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

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-
5   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-
10   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
15   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
20   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;

25   (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-
30   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;

104

(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).

Alternatively, it is also conceivable that in the complex 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.

Glioma, in particular glioblastoma

The present invention provides the complex as described above for use in the prevention and/or treatment of glioma, in particular glioblastoma.

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 complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the complex as described herein; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine

105

may be used in conjunction with existing modalities for glioma, in particular (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.

5   Gliomas are classified by cell type, by grade, and/or by location.

Gliomas are named according to the specific type of cell with which they share histological features, but not necessarily from which they originate. The main types of gliomas include (i) ependymomas (ependymal cells); (ii) astrocytomas (astrocytes); (iii) oligodendrogliomas

10   (oligodendrocytes); (iv) brainstem glioma (developing in the brain stem); (v) optic nerve glioma (developing in or around the optic nerve); and (vi) mixed gliomas (containing cells from different types of glia), such as oligoastrocytomas. Accordingly, the glioma to be treated and/or prevented by a complex for use according to the present invention is preferably selected from the group consisting of (i) ependymomas (ependymal cells); (ii) astrocytomas

15   (astrocytes); (iii) oligodendrogliomas (oligodendrocytes); (iv) brainstem glioma (developing in the brain stem); (v) optic nerve glioma (developing in or around the optic nerve); and (vi) mixed gliomas (containing cells from different types of glia), such as oligoastrocytomas.

Gliomas are further categorized according to their grade, which is determined by pathologic

20   evaluation of the tumor. Low-grade gliomas [WHO grade II] are well-differentiated (not anaplastic); these tend to exhibit benign tendencies and portend a better prognosis for the patient. However, they have a uniform rate of recurrence and increase in grade over time so should be classified as malignant. High-grade [WHO grade III–IV] gliomas are undifferentiated or anaplastic; these are malignant and carry a worse prognosis. Of numerous

25   grading systems in use, the most common is the World Health Organization (WHO) grading system for astrocytoma, under which tumors are graded from I (least advanced disease — best prognosis) to IV (most advanced disease — worst prognosis). The tumors are classified based on their microscopic appearances. The grade indicates the level of malignancy. The grading system considers aspects such as: mitotic index (growth rate), vascularity (blood supply),

30   presence of a necrotic center, invasive potential (border distinctness), and similarity to normal cells. As malignant tumors may contain several grades of cells, it is the most malignant grade of cell found that determines the grade for the entire tumor.

106

The World Health Organization grading system is defining four different grades:

Grade I

The grade 1 tumors are the least malignant. These tumors grow slowly and microscopically

5   appear almost normal; surgery alone may be effective. Even if grade 1 tumors should be considered as a life-threatening agent, grade 1 tumors are often associated with long-term survival.

Grade II

10   Grade II tumors, such as astrocytoma, grow slightly faster than grade I tumors and have a slightly abnormal microscopic appearance. These tumors may invade surrounding normal tissue, and may recur as a grade II or higher tumor.

Grade III

15   Anaplastic astrocytomas as any other grade III tumors are malignant. These tumors contain actively reproducing abnormal cells and invade surrounding normal tissue. Grade III tumors frequently recur, often as grade IV tumors, because their tendency to spread into surrounding tissue makes them difficult to completely remove surgically.

20   *Grade IV*

Glioblastoma multiforme is the most common grade IV tumors. These tumors are the most malignant and invade wide areas of surrounding normal tissue. These tumors reproduce rapidly, appear very unusual microscopically and are necrotic (have dead cells) in the center. Grade IV tumors cause new blood vessels to form, to help maintain their rapid growth.

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Whereas grade I and II tumors are considered as low grade, grade III and IV are called high-grade glioma. Patients with low-grade glioma have a better prognosis with a median survival of 5-10 years. However, 50-75% of these patients have tumors, which tend to evolve continuously to a high-grade glioma (Shaw, E.G., et al., *Recurrence following neurosurgeon-*

30   *determined gross-total resection of adult supratentorial low-grade glioma: results of a prospective clinical trial.* J Neurosurg, 2008. 109(5): p. 835-41).

107

Preferably, the glioma to be treated and/or prevented by a complex for use according to the present invention is a grade I glioma, such as a grade I astrocytoma, a grade I oligodendroglioma, a grade I ependymoma or a grade I oligoastrocytoma; a grade II glioma, such as a grade II astrocytoma, a grade II oligodendroglioma, a grade II ependymoma or a

5   grade II oligoastrocytoma; a grade III glioma, such as a grade III astrocytoma, a grade III oligodendroglioma, a grade III ependymoma or a grade III oligoastrocytoma; and/or a grade IV glioma, such as a grade IV astrocytoma, a grade IV oligodendroglioma, a grade IV ependymoma or a grade IV oligoastrocytoma.

10   More preferably the glioma to be treated and/or prevented by a complex for use according to the present invention is a grade II glioma, such as a grade II astrocytoma, a grade II oligodendroglioma, a grade II ependymoma or a grade II oligoastrocytoma; a grade III glioma, such as a grade III astrocytoma, a grade III oligodendroglioma, a grade III ependymoma or a grade III oligoastrocytoma; and/or a grade IV glioma, such as a grade IV astrocytoma, a grade

15   IV oligodendroglioma, a grade IV ependymoma or a grade IV oligoastrocytoma.

Even more preferably the glioma to be treated and/or prevented by a complex for use according to the present invention is a grade III glioma, such as a grade III astrocytoma, a grade III oligodendroglioma, a grade III ependymoma or a grade III oligoastrocytoma; and/or

20   a grade IV glioma, such as a grade IV astrocytoma, a grade IV oligodendroglioma, a grade IV ependymoma or a grade IV oligoastrocytoma.

Most preferably the glioma to be treated and/or prevented by a complex for use according to the present invention is a grade IV astrocytoma, a grade IV oligodendroglioma, a grade IV

25   ependymoma or a grade IV oligoastrocytoma. A grade IV astrocytoma, namely a glioblastoma (GBM) is particularly preferred.

Additionally, gliomas can be classified according to whether they are above or below a membrane in the brain called the tentorium. The tentorium separates the cerebrum (above)

30   from the cerebellum (below). The supratentorial is above the tentorium, in the cerebrum, and mostly found in adults (70%). The infratentorial is below the tentorium, in the

108

cerebellum, and mostly found in children (70%). The pontine is located in the pons of the brainstem. Preferably, the the glioma to be treated and/or prevented by a complex for use according to the present invention is a supratentorial glioma. Preferably, the the glioma to be treated and/or prevented by a complex for use according to the present

5   invention is an infratentorial glioma. Preferably, the the glioma to be treated and/or prevented by a complex for use according to the present invention is a pontine glioma.

Nucleic acid encoding the peptides and protein complexes

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In another aspect the present invention provides a nucleic acid encoding the complex as described herein, wherein the complex is a polypeptide or a protein, for use in the prevention and/or treatment of glioma, in particular glioblastoma. In particular, the present invention provides polynucleotides for use in the prevention and/or treatment of glioma, in particular

15   glioblastoma, said polynucleotides encoding the complex as defined above.

In this context, nucleic acids preferably comprise single stranded, double stranded or partially double stranded nucleic acids, preferably selected from genomic DNA, cDNA, RNA, siRNA, antisense DNA, antisense RNA, ribozyme, complimentary RNA/DNA sequences with or

20   without expression elements, a mini-gene, gene fragments, regulatory elements, promoters, and combinations thereof.

Preferably, the invention relates to a nucleic acid for use according to the present invention, said nucleic acid encoding a complex, which is in particular a polypeptide or protein, said

25   complex comprising a cell penetrating peptide, at least one antigen or antigenic epitope, which is a polypeptide or protein, and at least one TLR peptide agonist, wherein the cell penetrating peptide, the at least one antigen or antigenic epitope, and the at least one TLR peptide agonist are covalently linked, optionally with peptidic spacer(s) or linker(s) as described herein. If more than one antigen or antigenic epitope, which is a polypeptide or

30   protein, is comprised by said complex, the more than one antigens or antigenic epitopes are also covalently linked, optionally with peptidic spacer(s) or linker(s) as described herein.

109

Similarly, if more than one TLR peptide agonist is comprised by said complex, the more than one TLR peptide agonists are also covalently linked, optionally with peptidic spacer(s) or linker(s) as described herein.

5   Particularly preferably the nucleic acid for use according to the present invention encodes a complex which is a (recombinant) fusion protein comprising (a) a cell penetrating peptide as described above, (b) at least one, preferably at least two, more preferably at least three, even more preferably at least four, particularly preferably at least five, most preferably at least six antigens or antigenic epitopes as described above, preferably arranged in a consecutive

10   manner as described above and (c) at least one TLR agonist as described above.

Production and purification of the complexes

15   According to a further aspect the present invention provides a vector for use in the prevention and/or treatment of glioma, in particular glioblastoma,, in particular a recombinant vector, comprising a nucleic acid as described above.

The term "vector", as used in the context of the present invention, refers to a nucleic acid

20   molecule, preferably to an artificial 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 etc. A storage vector is a vector which allows the convenient storage of a nucleic acid molecule. Thus, the vector may

25   comprise a sequence corresponding, e.g., to a desired antibody or antibody fragment thereof according to the present invention. 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

30   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

110

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

5 the vector, such as an origin of replication. Preferably, a vector in the context of the present application is a plasmid vector. Preferably, a vector in the context of the present application is an expression vector.

Cells transformed with a vector as described above for use in the prevention and/or treatment

10 of glioma, in particular glioblastoma, are also included within the scope of the invention. Examples of such cells include, but are not limited to, bacterial cells, e.g. E. coli, and eukaryotic cells, e.g., yeast cells, animal cells or plant cells. In one embodiment the cells are mammalian, e.g., human, CHO, HEK293T, PER.C6, NS0, myeloma or hybridoma cells. Accordingly, the present invention also relates to a cell expressing the antibody, or the antigen

15 binding fragment thereof, for use according to the present invention; or comprising the vector for use according to the present invention.

In particular, a cell may be transfected with a vector as described above, preferably with an expression vector. The term "transfection" refers to the introduction of nucleic acid

20 molecules, such as DNA or RNA (e.g. mRNA) molecules, into cells, preferably into eukaryotic cells. In the context of the present invention, the term "transfection" encompasses any method known to the skilled person for introducing nucleic acid molecules into cells, preferably into eukaryotic cells, such as into mammalian cells. Such methods encompass, for example, electroporation, lipofection, e.g. based on cationic lipids and/or liposomes, calcium

25 phosphate precipitation, nanoparticle based transfection, virus based transfection, or transfection based on cationic polymers, such as DEAE-dextran or polyethylenimine etc. Preferably, the introduction is non-viral.

Numerous expression systems can be used, including without limitation chromosomes,

30 episomes, and derived viruses. More particularly, the vector as described above, in particular the recombinant vector used, can be derived from bacterial plasmids, transposons, yeast episomes, insertion elements, yeast chromosome elements, viruses such as baculovirus,

111

papilloma viruses such as SV40, vaccinia viruses, adenoviruses, fox pox viruses, pseudorabies viruses, retroviruses.

For example, such vectors, in particular recombinant vectors, can equally be cosmid or
5   phagemid derivatives. The nucleotide sequence, in particular the nucleic acid according to the present invention, may be inserted in the recombinant expression vector by methods well known to a person skilled in the art such as, for example, those described in MOLECULAR CLONING: A LABORATORY MANUAL, Sambrook et al., 4th Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001.
10

The vector, in particular the recombinant vector, may also include nucleotide sequences that control the regulation of the expression, in particular of the nucleic acid for use according to the present invention, as well as nucleotide sequences permitting the expression, the transcription, and the translation, in particular of the nucleic acid for use according to the
15   present invention. Typically, these sequences are selected according to the host cells used.

Thus, for example, an appropriate secretion signal can be integrated in the vector for use according to the present invention, in particular in a recombinant vector, so that the polypeptide or protein encoded by the nucleic acid for use according to the present invention,
20   will be directed, for example towards the lumen of the endoplasmic reticulum, towards the periplasmic space, on the membrane or towards the extracellular environment. The choice of an appropriate secretion signal may facilitate subsequent protein purification.

In yet another aspect the present invention provides a host cell for use in the prevention and/or
25   treatment of glioma, in particular glioblastoma,, the host cell comprising a vector, in particular a recombinant vector, as described herein.

The introduction of the vector, in particular the recombinant vector, into a host cell can be carried out according to methods that are well known to a person skilled in the art, such as
30   those described in BASIC METHODS IN MOLECULAR BIOLOGY, Davis et al., 2nd ed., McGraw-Hill Professional Publishing, 1995, and MOLECULAR CLONING: A LABORATORY MANUAL, supra, including for example transfection as described above, e.g. by calcium

112

phosphate, by DEAE dextran, or by cationic lipids; microinjection, electroporation, transduction or infection.

The host cell can be, for example, bacterial cells such as *E. coli*, cells of fungi such as yeast cells and cells of *Aspergillus, Streptomyces*, insect cells, and/or any cell line, e.g. Chinese Hamster Ovary cells (CHO), C127 mouse cell line, BHK cell line of Syrian hamster cells, Human Embryonic Kidney 293 (HEK 293) cells. Preferably, the host cell for use according to the present invention is mammalian, *e.g.*, human, CHO, HEK293T, PER.C6, NS0, myeloma or hybridoma cells. Dendritic cells and dendritic cell lines are particularly preferred as a host cell. Typically, the choice of a culture medium depends in particular on the choice of the cell type and/or the cell line, whereby the skilled person is aware of suitable culture media, which are appropriate for a selected cell type and/or cell line.

The host cells can be used, for example, to express a polypeptide or protein, in particular the complex for use according to the present invention, on the basis of the vector and/or the nucleic acid as described herein. After purification by standard methods, the expressed polypeptide or protein, in particular the complex for use according to the present invention, can be used as described herein.

Accordingly, the present invention also provides a method for preparing the complex as defined herein, in particular wherein the complex is a polypeptide or protein. Said method comprises the steps of:

(i) cultivating a host cell as described above in a culture medium; and

(ii) separating the complex as defined herein from the culture medium or separating the complex as defined herein from the host cell lysate after host cell lysis.

Thus, the complex obtained by such a method according to the present invention is preferably a complex for use according to the present invention as described herein.

For protein extraction commercially available kits and/or reagents may be used, for example BugBuster™ from Novagen.

113

Preferably, the method for preparing the complex as defined herein further comprises the following step:

> (iii) solubilization of the complex as defined herein , e.g. by resuspension in solutions containing urea or guanidine hydrochloride (GuHCl),

5   wherein step (iii) follows step (ii) as described above.

Moreover, it is preferred that the method for preparing the complex as defined herein further comprises the following step:

> (iv) purification of the complex as defined herein , preferably by one-step affinity
10   chromatography,

wherein step (iv) follows step (ii), or, if present, step (iii) as described above.

In addition, the complex as defined herein may also be prepared by synthetic chemistry methods, for example by solid-phase peptide synthesis.

15

Purification of those peptides or proteins may be carried out by means of any technique known in the art for protein/peptide purification. Exemplary techniques include ion-exchange chromatography, hydrophobic interaction chromatography, and immunoaffinity methods.

20   Thus, the present invention also provides a method for preparing the complex as defined herein comprising the steps of:

> (i)   chemically synthesizing said complex; and
>
> (ii) purifying said complex.

25   Preferably, in the method for preparing a complex as defined herein, the complex chemically synthesized in step (i) and purified in step (ii) comprises an amino acid sequences as described herein for a cell penetrating peptide, an amino acid sequence as described herein for a TLR peptide agonist, and, optionally if the at least one antigen and/or antigenic epitope is a peptide or a protein, an amino acid sequence as described herein for an antigen or antigenic epitope.

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Alternatively, the present invention also provides a method for preparing the complex as defined herein, wherein

114

(i)   the cell penetrating peptide, the at least one antigen or antigenic fragment and/or
         the at least one TLR peptide agonist are synthesized separately;

(ii)   optionally, the cell penetrating peptide, the at least one antigen or antigenic
         fragment and/or the at least one TLR peptide agonist are purified; and

5   (iii)   the cell penetrating peptide, the at least one antigen or antigenic fragment and/or
         the at least one TLR peptide agonist are covalently linked as described above,
         optionally by a spacer or linker or by a cross-linking agent as described above.

10   *Cells loaded with the complexes according to the invention*

In yet another aspect the present invention relates to a cell loaded with the complex as defined
herein for use in the prevention and/or treatment of glioma, in particular glioblastoma,. For
example, the cells loaded with the complex as defined herein are cells from a subject to be
15   treated, in particular isolated cells from a subject to be treated, i.e. cells isolated from a subject
to be treated.

As used in the context of the present invention, the term "subject" refers in particular to
mammals. For example, mammals contemplated by the present invention include human,
20   primates, domesticated animals such as cattle, sheep, pigs, horses, laboratory rodents and the
like. More preferably, the term "subject" refers to a human subject.

As used in the context of the present invention, "treatment" and "treating" and the like
generally mean obtaining a desired pharmacological and physiological effect. The effect may
25   be prophylactic in terms of preventing or partially preventing a disease, a symptom or a
condition thereof and/or may be therapeutic in terms of a partial or complete cure of a disease,
a condition, a symptom or an adverse effect attributed to the disease. The term "treatment"
as used herein covers any treatment of a disease in a mammal, in particular in a human, and
includes: (a) preventing the disease from occurring in a subject who may be predisposed to
30   the disease but the outbreak of the disease has not yet occurred and/or the disease has not
yet been diagnosed in this subject, for example a preventive early asymptomatic intervention;
(b) inhibiting the disease, i.e., arresting or slowing down its development; or (c) relieving the

115

disease, i.e., causing an at least partial regression of the disease and/or of at least one of its symptoms or conditions such as improvement or remediation of damage. In particular, the methods, uses, formulations and compositions according to the invention are useful in the treatment of cancers or infectious diseases and/or in the prevention of evolution of cancers

5   into an advanced or metastatic stage in subjects with early stage cancer, thereby improving the staging of the cancer. When applied to cancers, prevention of a disease or disorder includes the prevention of the appearance or development of a cancer in an individual identified as at risk of developing said cancer, for instance due to past occurrence of said cancer in the circle of the individual's relatives, and prevention of infection with tumor

10   promoting pathogens such as, for example, Epstein-Barr virus (EBV), Human papillomavirus (HPV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human Herpes virus 8 (HHV8), human T-cell leukemia virus type 1 (HTLV-1), Merkel cell polyomavirus (MCV) and Helicobacter pylori. Also covered by the terms "prevention/treatment" of a cancer is the stabilization or delay of an already diagnosed cancer in an individual. By "stabilization", it is

15   meant the prevention of evolution of cancer into advanced or metastatic stage in subjects with early stage cancer.

Preferably, the cell loaded with the complex as defined herein is an antigen-presenting cell (APC). Preferably, the antigen presenting cell is selected from the group consisting of a

20   dendritic cell (DC), a macrophage and a B-cell. Dendritic cells, in particular dendritic cells (conventional and/or plasmacytoid) isolated from a subject to be treated, are more preferred.

Methods to isolate antigen-presenting cells, in particular dendritic cells, from a subject are known to the skilled person. They include harvesting monocytes or hematopoietic stem cells

25   from bone marrow, cord blood, or peripheral blood. They also include the use of embryonic stem (ES) cells and induced pluripotent stem cells (iPS). Antigen presenting cells, in particular dendritic cells or their precursors, can be enriched by methods including elutriation and magnetic bead based separation, which may involve enrichment for $CD14^+$ precursor cells.

30   Methods to load the complex as defined herein into the cells, preferably into the above-mentioned antigen presenting cells, more preferably into dendritic cells, and further to prepare such cells before administration to a subject are known to one skilled in the art. For

116

example, preparation of dendritic cells can include their culture or differentiation using cytokines that may include for example GM-CSF and IL-4. Dendritic cell lines may also be employed. Loading of the complex of the invention into the cells, preferably into APC, more preferably into the dendritic cells, can involve co-incubation of the complex of the invention

5   with the cells in culture, making use of the intrinsic properties of the cell penetrating peptide comprised by the complex as defined herein (i.e. its internalization ability). Further culture of the cells, e.g. the dendritic cells, thus loaded to induce efficient maturation can include addition of cytokines including IL-1β, IL-6, TNFα, PGE2, IFNα, and adjuvants which may include poly-IC, poly-ICLC (i.e. a synthetic complex of carboxymethylcellulose, polyinosinic-

10   polycytidylic acid, and poly-L-lysine double-stranded RNA), and further TLR agonists and NLR (nucleotide-binding oligomerization domain-like receptors) agonists.

A method for preparing cells, in particular antigen presenting cells, loaded with the complex

15   as defined herein   may comprise the steps of:

    (i)   transducing or transfecting said cells with the complex of the invention;

    (ii)   cultivating said cells in a culture medium; and

    (iii)   separating said cells from the culture medium.

20   Preferably, the cells are loaded with a complex as defined herein, wherein the complex is a polypeptide or a protein and used in the prevention and/or treatment of glioma, in particular glioblastoma.

Preferably, the cells loaded with a complex(es) according as defined herein and used in the

25   prevention and/or treatment of glioma, in particular glioblastoma, present the at least one antigen or antigenic epitope comprised by said complex at the cell surface in an MHC class I context and/or in an MHC class II context.

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117

Compositions and kits according to the present invention

According to another aspect, the invention provides a composition for use in the prevention and/or treatment of glioma, in particular glioblastoma, the composition comprising at least

5   one component selected from:

(i)   a complex as described above,

(ii)   a nucleic acid as described above,

(iii)   a vector as described above,

(iv)   a host cell as described above, and

10   (v)   a cell loaded with a complex as defined herein as described above.

Preferably, the composition according to the present invention comprises the complex as defined herein.

The composition for use according to the present invention may also comprises more than

15   one of the above components (i) to (v). For example, the composition for use according to the present invention may comprise at least two different complexes under (i), at least two different nucleic acids under (ii), at least two different vectors under (iii), at least two different host cells under (iv), and/or at least two different cells under (v); e.g., the composition for use according to the invention may comprise at least two different complexes (i) and/or at least

20   two different nucleic acids (ii).

For example, the different complexes (i) comprised by the composition as described above may differ in either component a), i.e. in the cell penetrating peptides, in component b), i.e. in the antigens or antigenic epitopes or in the subsets of more than one antigen or antigenic

25   epitope, or in component c), i.e. in the TLR peptide agonist or in the subset of more than one TLR peptide agonist; or the different complexes (i) comprised by the composition as described above may differ in two out of the three components a), b), and c); or the different complexes (i) comprised by the composition as described above may differ in all three components a), b), and c) of the complex. Accordingly, the different nucleic acids (ii) comprised by the

30   composition as described above may differ in that they encode such different complexes; the different vectors (iii) comprised by the composition as described above may differ in that they comprise such different nucleic acids; the different host cells (iv) comprised by the

118

composition as described above may differ in that they comprise such different vectors; and the different cells loaded with a complex (v) comprised by the composition as described above may differ in that they are loaded with such different complexes.

5   The present invention also provides a vaccine for use in the prevention and/or treatment of glioma, in particular glioblastoma, the vaccine comprising at least one component selected from:

      (i)   a complex as described above,

      (ii)   a nucleic acid as described above,

10   (iii)   a vector as described above,

      (iv)   a host cell as described above, and

      (v)   a cell loaded with a complex as described above.

Preferably, the vaccine for use according to the present invention comprises the complex as defined herein.

15

Thereby, the above details described for the composition for use according to the present invention regarding more than one of the components (i) to (v), also apply for the vaccine for use according to the present invention.

20   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 defined herein typically leads to or supports an

25   adaptive immune response in the patient to be treated, and the TLR peptide agonist of the complex as defined herein may lead to or support an innate immune response.

The inventive composition, in particular the inventive vaccine, may also comprise a pharmaceutically acceptable carrier, adjuvant, and/or vehicle as defined below for the

30   inventive pharmaceutical composition. In the specific context of the inventive composition, in particular of the inventive vaccine, the choice of a pharmaceutically acceptable carrier is determined in principle by the manner in which the inventive composition, in particular the

119

inventive vaccine, is administered. The inventive composition, in particular the inventive vaccine, can be administered, for example, systemically or locally. Routes for systemic administration in general include, for example, transdermal, oral, parenteral routes, including subcutaneous, intravenous, intramuscular, intraarterial, intradermal and intraperitoneal injections and/or intranasal administration routes. Routes for local administration in general include, for example, topical administration routes but also intradermal, transdermal, subcutaneous, or intramuscular injections or intralesional, intracranial, intrapulmonal, intracardial, intranodal and sublingual injections. More preferably, inventive composition, in particular the vaccines, may be administered by an intradermal, subcutaneous, intranodal or intramuscular route. Even more preferably, the inventive composition, in particular the vaccine, may be administered by subcutaneous, intranodal or intramuscular route. Particularly preferably, the inventive composition, in particular the vaccines, may be administered by subcutaneous or intranodal route. Most preferably, the inventive composition, in particular the vaccines may be administered by subcutaneous route. Inventive composition, in particular the inventive vaccines, are therefore preferably formulated in liquid (or sometimes in solid) form.

The suitable amount of the inventive composition, in particular the inventive vaccine, to be administered can be determined by routine experiments with animal models. Such models include, without implying any limitation, 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 and the like. If the inventive composition, in particular the inventive vaccine, is to be administered orally, tablets, capsules and the like 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.

120

The inventive composition, in particular the inventive 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

5   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, TNF-alpha or CD40 ligand, 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,

10   etc.) 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,

15   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.

Further additives which may be included in the inventive vaccine are emulsifiers, such as, for

20   example, Tween®; wetting agents, such as, for example, sodium lauryl sulfate; colouring agents; taste-imparting agents, pharmaceutical carriers; tablet-forming agents; stabilizers; antioxidants; preservatives.

The inventive composition, in particular the inventive vaccine, can also additionally contain

25   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.

30   Another class of compounds, which may be added to an inventive composition, in particular to an inventive 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),

121

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

5   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

10   present in methylated form.

The present invention also provides a pharmaceutical composition for use in the prevention and/or treatment of glioma, in particular glioblastoma, in particular a vaccine composition as described above, and a method for treating a subject, preferably a mammalian subject, and

15   most preferably a human subject, who is suffering from glioma, in particular glioblastoma.

In particular, the present invention provides a pharmaceutical composition for use in the prevention and/or treatment of glioma, in particular glioblastoma, comprising at least one complex as defined herein or at least one cell loaded with a complex as defined herein, and

20   optionally a pharmaceutically acceptable carrier and/or vehicle, or any excipient, buffer, stabilizer or other materials well known to those skilled in the art, in particular the pharmaceutical composition comprising at least one complex as defined herein or at least one cell loaded with a complex as defined herein and a pharmaceutically acceptable carrier.

25   As a further ingredient, the inventive 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 inventive pharmaceutical composition. If the inventive pharmaceutical composition is provided in liquid form, the carrier will typically be pyrogen-free water; isotonic saline or

30   buffered (aqueous) solutions, e.g phosphate, citrate etc. buffered solutions. Particularly for injection of the inventive inventive pharmaceutical composition, water or preferably a buffer, more preferably an aqueous buffer, may be used, containing a sodium salt, preferably at least

122

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
5   hydroxides, carbonates, hydrogen carbonates, or sulfates, etc. Without being limited thereto, examples of sodium salts include e.g. NaCl, NaI, NaBr, Na_2CO_3, $NaHCO_3$, Na_2SO_4, examples of the optional potassium salts include e.g. KCl, KI, KBr, K_2CO_3, $KHCO_3$, K_2SO_4, and examples of calcium salts include e.g. $CaCl_2$, CaI_2, $CaBr_2$, $CaCO_3$, $CaSO_4$, $Ca(OH)_2$. Furthermore, organic anions of the aforementioned cations may be contained in the buffer. According to
10   a more preferred embodiment, the buffer suitable for injection purposes as defined above, may contain salts selected from sodium chloride (NaCl), calcium chloride ($CaCl_2$) and optionally potassium chloride (KCl), wherein further anions may be present additional to the chlorides. $CaCl_2$ 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
15   least 1 mM potassium chloride (KCl) and at least 0,05 mM calcium chloride ($CaCl_2$). 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
20   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.

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However, one or more compatible solid or liquid fillers or diluents or encapsulating compounds may be used as well for the inventive 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 inventive pharmaceutical composition are capable of
30   being mixed with the complex as defined herein as defined above in such a manner that no interaction occurs which would substantially reduce the pharmaceutical effectiveness of the inventive pharmaceutical composition under typical use conditions. Pharmaceutically

123

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;

5   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,

10   polypropylene glycol, glycerol, sorbitol, mannitol and polyethylene glycol; alginic acid.

The inventive 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-

15   articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, intranodal and sublingual injection or infusion techniques. Preferably, the inventive pharmaceutical composition may be administered intradermally, intramuscularly, intranodally or subcutaneously. More preferably the inventive pharmaceutical composition may be

20   administered intramuscularly, intranodally or subcutaneously. Even more preferably the inventive pharmaceutical composition may be administered intranodally or subcutaneously. Most preferably, the inventive pharmaceutical composition may be administered subcutaneously.

25   Preferably, the inventive 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, intranodal and sublingual injection or via infusion techniques. Sterile injectable forms of the inventive

30   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

124

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.

5   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

10   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 inventive

15   pharmaceutical composition.

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

20   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,

25   administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be), 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.

30   The inventive pharmaceutical composition as defined above may also be administered orally in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include

125

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

5   emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

The inventive pharmaceutical composition may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical

10   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 inventive 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

15   topical administration include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the inventive pharmaceutical composition can be formulated in a suitable lotion or cream. In the context of the present invention, suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax,

20   cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

In this context, prescription of treatment, e.g. decisions on dosage etc. 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

25   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.

Accordingly, the inventive pharmaceutical composition typically comprises a "safe and

30   effective amount" of the components of the inventive pharmaceutical composition, in particular of the complex as defined herein as defined above and/or cells loaded with said complex. As used herein, a "safe and effective amount" means an amount of the complex as

126

defined herein that is sufficient to significantly induce a positive modification of a disease or disorder, i.e. an amount of the complex as defined herein or cells loaded with said complex, that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought. An effective amount may be a "therapeutically effective amount" for the

5   alleviation of the symptoms of the disease or condition being treated and/or a "prophylactically effective amount" for prophylaxis of the symptoms of the disease or condition being prevented. The term also includes the amount of active complex 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

10   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 "safe and effective amount" is 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 "safe and effective amount" of the

15   components of the inventive pharmaceutical composition, particularly of the complex as defined herein as defined above, 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 a), b), and c) of the complex as

20   defined herein as defined above, 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. The inventive pharmaceutical composition may be used for human and also for veterinary medical purposes, preferably for human medical purposes, as a pharmaceutical composition

25   in general or as a vaccine.

Pharmaceutical compositions, in particular vaccine compositions, or formulations according to the invention may be administered as a pharmaceutical formulation which can contain a complex as defined herein in any form described herein.

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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

127

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.

5   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 or a method 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
10   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
15   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
20   pathogen), correlating with pathogen specific antibodies and/or T cell immune responses.

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 a complex according to the invention in any form
25   described herein.

The vaccine and/or the composition for use 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
30   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

128

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.

In the context of the present invention, in particular in the context of a pharmaceutical
5   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
10   amount of the active ingredient commensurate with the intended daily dosage range to be employed.

Examples of suitable adjuvants and/or immunomodulatory materials in the context of the present invention include MPL® (Corixa), aluminum-based minerals including aluminum
15   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
20   component antibacterial peptides with synthetic oligodeoxynucleotides (e.g. IC31), Imiquimod, Resiquimod, Immunostimulatory sequences (ISS), monophosphoryl lipid A (MPLA), Fibroblast-stimulating lipopeptide (FSL1), and anti-CD40 antibodies.

Compositions, in particular pharmaceutical compositions and vaccines, for use according to
25   the present invention may be liquid formulations including, but not limited to, 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, but not limited to, suspending agents, emulsifying agents, non-aqueous vehicles and preservatives. Suspending agents
30   include, but are not limited to, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminum stearate gel, and hydrogenated edible fats. Emulsifying agents include, but are not limited to, lecithin, sorbitan monooleate,

129

and acacia. Preservatives include, but are not limited to, methyl or propyl p-hydroxybenzoate and sorbic acid. Dispersing or wetting agents include but are not limited to poly(ethylene glycol), glycerol, bovine serum albumin, Tween®, Span®.

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

Compositions, in particular pharmaceutical compositions and vaccines, for use according to
10   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, but not limited to, binding agents, fillers, lubricants, disintegrants and wetting agents. Binding agents include, but are not limited to, syrup, accacia, gelatin, sorbitol, tragacanth, mucilage of starch and
15   polyvinylpyrrolidone. Fillers include, but are not limited to, lactose, sugar, microcrystalline cellulose, maizestarch, calcium phosphate, and sorbitol. Lubricants include, but are not limited to, magnesium stearate, stearic acid, talc, polyethylene glycol, and silica. Disintegrants include, but are not limited to, potato starch and sodium starch glycollate. Wetting agents include, but are not limited to, sodium lauryl sulfate. Tablets may be coated
20   according to methods well known in the art.

Compositions, in particular pharmaceutical compositions and vaccines, for use according to the present invention may also be administered in sustained release forms or from sustained release drug delivery systems.
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Moreover, the compositions, in particular pharmaceutical compositions and vaccines, for use according to the present invention may be adapted for delivery by repeated administration.

30   Further materials as well as formulation processing techniques and the like, which are useful in the context of compositions, in particular pharmaceutical compositions and vaccines, for use according to the present invention or in the context of their preparation are set out in

130

"Part 5 of Remington's "The Science and Practice of Pharmacy", 22nd Edition, 2012, University of the Sciences in Philadelphia, Lippincott Williams & Wilkins".

In a further aspect, the present invention also relates to a kit-of-parts for use in the prevention and/or treatment of glioma, in particular glioblastoma, the kit of parts comprising at least one of:

(i)   a complex as described above,

(ii)   a nucleic acid as described above,

(iii)   a vector as described above,

(iv)   a host cell as described above, and

(v)   a cell loaded with a complex as described above.

In particular, the kit-of-parts of the invention may comprise more than one component (i) to (v). For example, the kit-of-parts according to the present invention may comprise at least two different complexes under (i), at least two different nucleic acids under (ii), at least two different vectors under (iii), at least two different host cells under (iv), and/or at least two different cells under (v); e.g., the kit-of-parts of the invention may comprise at least two different complexes (i) and/or at least two different nucleic acids (ii).

For example, the different complexes (i) comprised by the kit-of-parts as described above may differ in either component a), i.e. in the cell penetrating peptides, in component b), i.e. in the antigens or antigenic epitopes or in the subsets of more than one antigen or antigenic epitope, or in component c), i.e. in the TLR peptide agonist or in the subset of more than one TLR peptide agonist; or the different complexes (i) comprised by the kit-of-parts as described above may differ in two out of the three components a), b), and c); or the different complexes (i) comprised by the kit-of-parts as described above may differ in all three components a), b), and c) of the complex. Accordingly, the different nucleic acids (ii) comprised by the kit-of-parts as described above may differ in that they encode such different complexes; the different vectors (iii) comprised by the kit-of-parts as described above may differ in that they comprise such different nucleic acids; the different host cells (iv) comprised by the kit-of-parts as described above may differ in that they comprise such different vectors; and the different cells loaded with a complex (v) comprised by the kit-of-parts as described above may differ in that they are loaded with such different complexes.

131

The various components of the kit-of-parts 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. The kit may also comprise additional reagents including, for instance, preservatives,

5   growth media, and/or buffers for storage and/or reconstitution of the above-referenced components, washing solutions, and the like. In addition, the kit-of-parts according to the present invention may optionally contain instructions of use.

Moreover, the present invention also provides a vaccination kit for treating, preventing and/or

10   stabilizing glioma, in particular glioblastoma, comprising the pharmaceutical composition as described herein or a vaccine as described herein and instructions for use of said pharmaceutical composition or of said vaccine in the prevention and/or treatment of glioma, in particular glioblastoma.

15   Thus, the present invention also provides a kit comprising the complex as described herein, the cell as described herein, the composition as described herein, the vaccine as described herein, and/or the pharmaceutical composition as described herein.

Preferably, such a kit further comprises a package insert or instruction leaflet with directions

20   to treat glioma, in particular glioblastoma, by using the complex for use according to the present invention as described herein, the cell as described herein, the composition as described herein, the vaccine as described herein, and/or the pharmaceutical composition as described herein.

25

Use and methods according to the invention

In another aspect, the present invention provides the use of any one of: (i) a complex as described herein, and/or (ii) cells, such as antigen-presenting cells, loaded with a complex as

30   described herein, (for the preparation of a medicament) for the prevention, treatment or stabilization of glioma, in particular glioblastoma. Accordingly, the present invention provides any one of: (i) a complex as described herein, and/or (ii) cells, such as antigen-

132

presenting cells, loaded with a complex as described herein, for use in the prevention, treatment or stabilization of glioma, in particular glioblastoma.

5   The present invention also provides a complex for use according to the present invention, which allows the transport and presentation of the at least one antigen or antigenic epitope comprised by the complex at the cell surface of antigen presenting cells in an MHC class I and/or MHC class II context, for use in vaccination and/or immunotherapy.

10   According to another aspect, the present invention provides a method of preventing, treating or repressing glioma, in particular glioblastoma, wherein said method comprises administering any one of: (i) a complex of the invention, (ii) cells, such as antigen-presenting cells, loaded with a complex of the invention, or (iii) a pharmaceutical formulation of (i) to (ii), to said subject.

15   Moreover, the present invention provides a method for eliciting or improving, in a subject, an immune response against one or multiple epitopes that is dependent on CD4$^+$ helper T cells and/or CD8$^+$ cytotoxic T cells, wherein said method comprises administering any one of: (i) a complex for use according to the present invention, and/or (ii) cells, such as antigen-presenting cells, loaded with said complex, or (iii) a pharmaceutical formulation of (i) to (ii),
20   to said subject.

An immune response that is dependent on CD4$^+$ and/or CD8$^+$ response can be determined by evaluating an inflammatory response, a pro-inflammatory cytokine response, including an increase in the expression of one or more of IFN-γ, TNF-α and IL-2 mRNA or protein relative
25   to the level before administration of the compounds of the invention. It can also be measured by an increase in the frequency or absolute number of antigen-specific T cells after administration of the compounds of the invention, measured by HLA-peptide multimer staining, ELISPOT assays, and delayed type hypersensitivity tests. It can also be indirectly measured by an increase in antigen-specific serum antibodies that are dependent on antigen-
30   specific T helper cells.

133

The present invention also provides a method for eliciting or improving, in a subject, an immune response against one or multiple antigens or antigenic epitopes that is restricted by multiple MHC class I molecules and/or multiple MHC class II molecules, wherein said method comprises administering any one of: (i) a complex for use according to the present

5   invention, and/or (ii) cells, such as antigen-presenting cells, loaded with said complex, or (iii) a pharmaceutical formulation of (i) to (ii), to said subject.

A method for eliciting or improving, in a subject, an immune response against multiple epitopes as described herein, that is restricted by multiple MHC class I molecules and/or

10   multiple MHC class II molecules can be determined by evaluating a cytokine response, including an increase in the expression of one or more of IFN-γ, TNF-α and IL-2 mRNA or protein relative to the level before administration of the compounds of the invention, after in vitro stimulation of T cells with individual peptides binding to discrete MHC class I and class II molecules on antigen presenting cells. Restriction to different MHC molecules can also be

15   validated by using antigen presenting cells expressing different MHC molecules, or by using MHC blocking antibodies. It can also be measured by an increase in the frequency or absolute number of antigen-specific T cells after administration of the compounds of the invention, measured by HLA-peptide multimer staining, which uses multimers assembled with discrete MHC molecules.

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Preferably, in the methods for eliciting or improving an immune response against one or multiple antigens or antigenic epitopes according to the present invention, the immune response is directed against one or multiple epitopes of a tumor-associated antigen or a tumor-specific antigen as, for instance, a combination of glioma epitopes such as those described in

25   *Novellino et al. (2005, Cancer Immunol Immunother, 54(3):187-207)* and *Vigneron et al. (2013, Cancer Immun.13:15)* and those described herein.

Alternatively or additionally, the immune response may be directed against multiple epitopes of an antigenic protein from a pathogen.

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134

The methods according to the present invention as described herein, may be for eliciting or improving, in a subject, an immune response against one or multiple epitopes that is restricted by MHC class I molecules and/or MHC class II molecules.

5   In particular, the present invention thus provides a method for preventing and/or treating glioma, in particular glioblastoma, or initiating, enhancing or prolonging an anti-tumor-response in a subject in need thereof comprising administering to the subject a complex comprising:

            a cell penetrating peptide;

10   at least one antigen or antigenic epitope; and

            at least one TLR peptide agonist,

            wherein the components a) – c) are covalently linked.

In such a method it is preferred that the complex for use according to the present invention

15   as described herein, the cell as described herein, the composition as described herein, the vaccine as described herein, and/or the pharmaceutical composition as described herein is administered to the subject.

Preferably, the subject has glioma, in particular glioblastoma, and/or was diagnosed with

20   glioma, in particular glioblastoma.

In another aspect, the present invention provides the use of any one of: (i) a complex as described herein, and/or (ii) cells, such as antigen-presenting cells, loaded with the complex as described herein, for the preparation of an imaging composition for imaging techniques in the context of (diagnosis of) glioma, in particular glioblastoma, or for the preparation of a

25   diagnosis composition ("diagnostic compositions") for diagnosing glioma, in particular glioblastoma. . A diagnostic composition for diagnosing glioma, in particular glioblastoma, according to the present invention comprises at least one component selected from:

    (i)   a complex as described above,

    (ii)   a nucleic acid as described above,

30   (iii)   a vector as described above,

    (iv)   a host cell as described above, and

    (v)   a cell loaded with a complex as described above.

135

Preferably, the diagnostic composition according to the present invention comprises the complex as described above.

In particular, the complex for use according to the present invention, the cell, such as antigen-
5   presenting cell, loaded with the complex for use according to the present invention, the inventive composition, the inventive pharmaceutical composition or the inventive vaccine or, most preferably, the inventive diagnostic composition may be utilized in diagnosis as a diagnostic tool, e.g. in (*in vivo* or *in vitro*) assays, e.g. in immunoassays, to detect, prognose, diagnose, or monitor glioma, in particular glioblastoma.
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As an example, (*in vitro*) assays may be performed by delivering the complex for use according to the present invention, the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention, the inventive composition, the inventive pharmaceutical composition or the inventive vaccine or, most preferably, the inventive
15   diagnostic composition to target cells typically selected from e.g. cultured animal cells, human cells or micro-organisms, and to monitor the cell response by biophysical methods typically known to a skilled person. The target cells typically used therein may be cultured cells (*in vitro*), e.g. cells isolated from human or animal body, such as blood cells isolated from human or animal body, or *in vivo* cells, i.e. cells composing the organs or tissues of
20   living animals or humans, or microorganisms found in living animals or humans. Particularly preferable in this context are so called markers or labels, which may be contained in the complex for use according to the present invention and, in particular, in the diagnostic composition according to the present invention.

25   According to a further aspect, the invention provides a method of diagnosing glioma, in particular glioblastoma, in a subject, wherein said method comprises administering any one of: (i) a complex of the invention, (ii) cells, such as antigen-presenting cells, loaded with the complex of the invention, or (iii) a pharmaceutical formulation of (i) to (ii), to said subject or to said subject's sample *ex vivo*.
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Preferably, uses and methods according to the present invention comprise administration of a complex for use according to the invention.

136

Moreover, uses and methods according to the present invention comprise administration of more than one complex, cells, or pharmaceutical formulation according to the invention. For example, in the uses and methods according to the present invention, at least two different

5   complexes are used or administered, wherein each complex comprises at least one antigen or antigenic epitope and said antigen or antigenic epitope or (if more than one antigen or antigenic epitope is comprised by said complex) said subset of antigens or antigenic epitopes are different between the two complexes.

10   For example, the different complexes (i) comprised by the composition as described above may differ in either component a), i.e. in the cell penetrating peptides, in component b), i.e. in the antigens or antigenic epitopes or in the subsets of more than one antigen or antigenic epitope, or in component c), i.e. in the TLR peptide agonist or in the subset of more than one TLR peptide agonist; or the different complexes (i) comprised by the composition as described

15   above may differ in two out of the three components a), b), and c); or the different complexes (i) comprised by the composition as described above may differ in all three components a), b), and c) of the complex. Accordingly, the different nucleic acids (ii) comprised by the composition as described above may differ in that they encode such different complexes; the different vectors (iii) comprised by the composition as described above may differ in that they

20   comprise such different nucleic acids; the different host cells (iv) comprised by the composition as described above may differ in that they comprise such different vectors; and the different cells loaded with a complex (v) comprised by the composition as described above may differ in that they are loaded with such different complexes.

25   Moreover, in the uses and methods according to the present invention, the cells according to the present invention may be antigen presenting cells, in particular dendritic cells, more preferably dendritic cells from the subject to be treated.

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137

Mode of administration

The complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive

5   composition; the inventive pharmaceutical composition or the inventive vaccine may be administered in any manner as described above, including enterally, such as orally or rectally, and parenterally, such as intravenously or combinations thereof. Parenteral administration includes, but is not limited to, intravenous, intra-arterial, intra-peritoneal, subcutaneous, intradermal and intramuscular. Preferably, the complex for use according to the present

10   invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition and/or the inventive vaccine are administered via systemic route of administration or via administration directly into the CNS, such as intraventricular, intracerebral, intracranial or intracerebroventricular administration. Administration directly

15   into the CNS, such as intraventricular, intracerebral, intracranial or intracerebroventricular administration is preferred, since the complex for use according to the present invention does not have to cross the blood-brain barrier when it is administered directly to the CNS. However, systemic administration is also preferred, since it is more convenient for the patient and, thus, compliance is increased. In systemic administration, parental routes of

20   administration are preferred, such as intravenous, intraarterial, subcutaneous or intradermal administration. Intranodal administration is also preferred. The complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine may also be preferably administered

25   via topical, intratumoral, intradermal, subcutaneous, intramuscular, intranasal, or intranodal route. The complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine may also be administered in the form of an implant, which allows slow release of the

30   compositions as well as a slow controlled i.v. infusion. For example, the complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the

138

complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine may be administered subcutaneously.

The administration of complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine may require multiple successive injections/administrations. Thus, the administration may be repeated at least two times, for example once as primary immunization injections/administration and, later, as booster injections/administration.

In particular, the complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine may be administered repeatedly or continuously. The complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine 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.

Moreover, the cell penetrating peptide, components a), b), and c), i.e. the at least one antigen or antigenic epitope and the at least one TLR peptide agonist, composing the complex for use according to the present invention may be contained in separate compositions which are mixed just before administration or which are administered simultaneously to the subject in need thereof.

According to one approach, the complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine may be administered directly to a patient using the administration routes as described above, in particular for pharmaceutical compositions. Alternatively, the complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive composition; the

139

inventive pharmaceutical composition or the inventive vaccine may be administered to a patient using an *ex vivo* approach, e.g. by introducing the pharmaceutical composition, the vaccine or the inventive transporter cargo conjugate molecule as defined above into cells, preferably autologous cells, i.e. cells derived from the patient to be treated, and transplanting

5   these cells into the site of the patient to be treated, optionally subsequent to storing and/or culturing these cells prior to treatment.

The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factors, including pharmacokinetic properties, subject conditions and

10   characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired.

Typically, for cancer treatment, the therapeutically effective dose of a complex for use according to the present invention is from about 0.01 mg to 5 mg per injection, in particular

15   from about 0.1mg 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 µmol to 1 mmol per injection.

Typically, for cancer treatment, the therapeutically effective dose of an antigen presenting

20   cell loaded with a complex for use according to the present invention is from about 0.2 million cells to 2 million cells per injection.

Combination therapy

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The administration of the complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine in the methods and uses according to the invention can be carried out

30   alone or in combination with a co-agent useful for treating and/or stabilizing glioma, in particular glioblastoma.

140

For instance, in the case of treatment, prevention, or stabilization of a glioma, in particular glioblastoma, the administration of the pharmaceutical compositions in the methods and uses according to the invention can be carried out in combination with substances used in conventional chemotherapy directed against solid tumors and for control of establishment of

5   metastases or any other molecule that act by triggering programmed cell death e.g. for example a co-agent selected from Tumor Necrosis Family Members including, but not limited, to Fas Ligand and tumor necrosis factor (TNF)-related apoptosis inducing (TRAIL) ligand. According to a further embodiment, the administration of the complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the

10   complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine in the methods and uses according to the present invention can be carried out in parallel of radiotherapy.

The invention encompasses the administration of the complex for use according to the present

15   invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine, wherein it is administered to a subject prior to, simultaneously or sequentially with other therapeutic regimens or co-agents useful for treating, and/or stabilizing a glioma, in particular glioblastoma, and/or preventing glioma, in

20   particular glioblastoma, relapsing (e.g. multiple drug regimens), in a therapeutically effective amount. Said complex, cell, composition, vaccine or pharmaceutical composition, that is administered simultaneously with said co-agents can be administered in the same or different composition(s) and by the same or different route(s) of administration.

25   Said other therapeutic regimens or co-agents may be selected from the group consisting of radiation therapy, chemotherapy, surgery, targeted therapy (including small molecules, peptides and monoclonal antibodies), and anti-angiogenic therapy. Anti-angiogenic therapy is defined herein as the administration of an agent that directly or indirectly targets tumor-associated vasculature.

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Accordingly, the present invention also provides a combination of

        (i) a complex as defined herein; and

141

(ii) a chemotherapeutic agent, a targeted drug and/or an immunotherapeutic
     agent, such as an immune checkpoint modulator,

for use in the prevention and/or treatment of glioma, in particular glioblastoma.

5   Traditional chemotherapeutic agents are cytotoxic, i.e. they act by killing cells that divide
     rapidly, one of the main properties of most cancer cells. Preferred chemotherapeutic agents
     for combination with the complex as defined herein are such chemotherapeutic agents known
     to the skilled person for treatment of glioma, in particular glioblastoma. A preferred
     chemotherapeutic agent for combination is in particular temozolomide (TMZ).

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     Targeted drugs (also referred to as targeted agents) for combination with the complex as
     defined herein for treatment of glioma, in particular glioblastoma, include VEGF-targeted
     drugs, EGFR-targeted drugs, PDGFR-targeted drugs, mTOR-targeted drugs, PKC-targeted
     drugs, RAF-MEK-ERK-targeted drugs and drugs targeting integrins. Preferred examples of
15   VEGF-targeted drugs include Bevacizumab (Avastin®), VEGF trap, Vatalinib, Vandetanib,
     Cediranib, Sunitinib and Pazopanib, with Bevacizumab (Avastin®) being particularly
     preferred. Preferred examples of EGFR-targeted drugs include Cetuximab (Erbitux®),
     Gefitinib, Erlotinib, Lapatinib, Canertinib, Pelitinib and BIBW-2992. Preferred examples of
     PDGFR-targeted drugs include Imitanib mesylate and Tandutinib. Preferred examples of
20   mTOR-targeted drugs include Sirolimus, Temsirolimus, Everolimus and AP23573. Preferred
     examples of PKC-targeted drugs include Enzastaurin. Preferred examples of RAF-MEK-ERK-
     targeted drugs include Tipifarnib, Lonafarnib and Sorafenib. Preferred examples of drugs
     targeting integrins include in particular Cilengitide. Preferred targeted drugs include VEGF-
     targeted drugs and EGFR-targeted drugs.

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     Immunotherapeutic agents for combination with the complex as defined herein for treatment
     of glioma, in particular glioblastoma, include vaccines, chimeric antigen receptors (CARs),
     checkpoint modulators and oncolytic virus therapies.

30   Preferred vaccines for combination with the complex as defined herein for treatment of
     glioma, in particular glioblastoma, include CDX-110, HSPPC-96, DCVax®-Brain, ICT-107,
     GMB-Vax, ERC1671, TVI-Brain-1, SL-701, ICT-121, NeoVax, ADU-623 and IMA950.

142

Artificial T cell receptors (also known as chimeric T cell receptors, chimeric immunoreceptors, chimeric antigen receptors (CARs)) are engineered receptors, which graft an arbitrary specificity onto an immune effector cell. Artificial T cell receptors (CARs) are preferred in the context of adoptive cell transfer. To this end, T cells are removed from a patient and modified so that they express receptors specific to glioma, in particular glioblastoma. The T cells, which can then recognize and kill the cancer cells, are reintroduced into the patient. Preferred CARs target Her-2 or EGFRvIII.

As used herein, 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 inhibitor".

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

143

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:

5   10.1093/jjco/hyv201 [Epub ahead of print]; in particular Table 1).

Preferably, the immune checkpoint modulator for combination with the complex as defined herein for treatment of glioma, in particular glioblastoma,is an activator or an inhibitor of one or more immune checkpoint point molecule(s) selected from CD27, CD28, CD40, CD122,

10   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.

More preferably, the immune checkpoint modulator is an activator of a (co-)stimulatory

15   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.

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Even 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,

25   CEACAM1, GARP, PS, CSF1R, CD94/NKG2A, TDO, TNFR and/or DcR3 or of a ligand thereof.

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

30   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.

144

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

5   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.

10   Accordingly, the checkpoint modulator for combination with the complex as defined herein for treatment of glioma, in particular glioblastoma, may be selected from known modulators of the CD40 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

15   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 h409All,

20   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

25   Lambrolizumab (e.g. disclosed as hPD109A and its humanized derivatives h409All, h409A16 and h409A17 in WO2008/156712; Hamid O. et al., 2013; N. Engl. J. Med. 369: 134-144).

It is also preferred that the immune checkpoint modulator for combination with the complex as defined herein for treatment of glioma, in particular glioblastoma, is selected from the

30   group consisting of Pembrolizumab, Ipilimumab, Nivolumab and MEDI4736.

145

Oncolytic viruses are engineered to cause cell lysis by replicating in tumors, thus activating an antitumor immune response. An oncolytic virus therapy for combination with the complex as defined herein for treatment of glioma, in particular glioblastoma, is preferably selected from the group consisting of Toca 511 (Toca FC, retrovirus), AdV-tk (adenovirus), ParvOryx

5   (autonomous parvovirus), DNX-2401 (adenovirus), INXN-2001 (adenovirus), M032 (NSC 733972, Herpes simplex virus), HSV-1716 (Herpes simplex virus), G207 (Herpes simplex virus), MV-CEA (Measles virus), PVSRIPO (poliovirus) and wild-type reovirus.

Preferably, (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or the

10   immunotherapeutic agent, such as an immune checkpoint modulator, are administered at about the same time.

"At about the same time", as used herein, means in particular simultaneous administration or that directly after administration of (i) the chemotherapeutic agent, the targeted drug and/or

15   the immunotherapeutic agent, such as an immune checkpoint modulator, (ii) the complex is administered or directly after administration of (i) the complex (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an 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

20   exposing and disinfecting the location for the second administration as well as appropriate preparation of the "administration device" (e.g., syringe, pump, etc.). Simultaneous administration also includes if the periods of administration of (i) the complex and of (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, overlap or if, for example, one component is administered

25   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 is administered at some time during such a long period. Administration of (i) the complex and of (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, at about the same time is in particular preferred if different routes of administration and/or different administration

30   sites are used.

146

It is also preferred that (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, are administered consecutively. This means that (i) the complex is administered before or after (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such

5   as an immune checkpoint modulator. In consecutive administration, the time between administration of the first component and administration of the second component 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 (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or

10   the immunotherapeutic agent, such as an immune checkpoint modulator, are administered at the same day with the time between administration of the first component and administration of the second component 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.

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Preferably, (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, are administered via the same route of administration. It is also preferred that (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an

20   immune checkpoint modulator, are administered via distinct routes of administration.

Moreover, (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, are preferably provided in distinct compositions. Alternatively, (i) the complex and (ii) the chemotherapeutic agent,

25   the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, are preferably provided in the same composition.

Accordingly, the present invention provides a pharmaceutical formulation comprising a complex for use according to the invention or a cell for use according to the invention, in

30   particular an antigen-presenting cell for use according to the invention, combined with at least one co-agent useful for treating and/or stabilizing a glioma and/or preventing a glioma relapsing, and at least one pharmaceutically acceptable carrier.

147

Moreover, the complex for use according to the present invention; the cell, such as antigen-presenting cell, loaded with the complex for use according to the present invention; the inventive composition; the inventive pharmaceutical composition or the inventive vaccine

5   can be administered after surgery where solid tumors have been removed as a prophylaxis against relapsing and/or metastases.

Moreover, the administration of the imaging or diagnosis composition in the methods and uses according to the invention can be carried out alone or in combination with a co-agent

10   useful for imaging and/or diagnosing glioma, in particular glioblastoma.

Subjects

The present invention can be applied to any subject suffering from glioma, in particular

15   glioblastoma, or at risk to develop glioma, in particular glioblastoma. In particular, the therapeutic effect of said 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.It is also preferred that subjects according to the invention have been

20   subjected to a surgical removal of a tumor.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein

25   will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications fall within the scope of the appended claims.

All references cited herein are herewith incorporated by reference.

30   Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can

148

be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods,

5   and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE FIGURES

10   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 expression of activation marker CD40 by human blood
15   monocyte-derived dendritic cells (DCs) from one single buffy. The DCs were
                    stimulated with 300nM of EDAZ13Mad5, Z13Mad5, Mad5 or 25ng/ml of LPS
                    during 48h. Isotype staining for each condition was also performed (isotype is
                    not shown in the Fig. 1) (one experiment).

20   Figure 2   shows for Example 1 expression of activation marker CD86 by human blood
                    monocyte-derived dendritic cells (DCs) from one single buffy. The DCs were
                    stimulated with 300nM of EDAZ13Mad5, Z13Mad5, Mad5 or 25ng/ml of LPS
                    during 48h. Isotype staining for each condition was also performed (isotype is
                    not shown in the Fig. 2) (one experiment).

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     Figure 3   shows for Example 1 expression of activation marker HLADR by human blood
                    monocyte-derived dendritic cells (DCs) from one single buffy. The DCs were
                    stimulated with 300nM of EDAZ13Mad5, Z13Mad5, Mad5 or 25ng/ml of LPS
                    during 48h. Isotype staining for each condition was also performed (isotype is
30   not shown in the Fig. 3) (one experiment).

149

Figure 4   shows for Example 1 expression of activation marker CD83 by human blood
                  monocyte-derived dendritic cells (DCs) from one single buffy. The DCs were
                  stimulated with 300nM of EDAZ13Mad5, Z13Mad5, Mad5 or 25ng/ml of LPS
                  during 48h. Isotype staining for each condition was also performed (isotype is
5   not shown in the Fig. 4) (one experiment).

Figure 5   shows for Example 2 functional MHC class I-restricted cross-presentation in a
                  murine *in an vitro* system using bone marrow derived dendritic cells (BMDCs)
                  and splenocytes from different TCR transgenic mice. To this end, BMDCs were
10   loaded overnight with 300nM of EDAZ13Mad5, EDAMad5 or Mad5. Efficient
                  MHC class I-restricted presentation of OVACD8 epitope and gp100 epitope
                  was monitored after 4 days with CFSE-labeled OT1 cells and P-Mel cells
                  respectively. Efficient MHC class II-restricted presentation of OVACD4 epitope
                  was monitored after 4 days with CFSE-labeled OT2 cells. As control, BMDCs
15   were pulsed for 1h with 5uM peptide (one experiment representative of 2
                  individual experiments).

Figure 6   shows the results for the 2 nmol groups for Example 3. C57BL/6 mice were
                  vaccinated twice (Wk0 and Wk2) with 2 nmol of EDAMad5 or EDAZ13Mad5.
20   Positive control group was vaccinated with Mad5 and MPLA (equimolar to
                  EDA). Mice were bled 7 days after last vaccination and pentamer staining was
                  performed (3-4 mice per group, one experiment).

Figure 7   shows the results for the 10 nmol groups for Example 3. C57BL/6 mice were
25   vaccinated twice (Wk0 and Wk2) with 10 nmol of EDAMad5 or
                  EDAZ13Mad5. Positive control group was vaccinated with Mad5 and MPLA
                  (equimolar to EDA). Mice were bled 7 days after last vaccination and pentamer
                  staining was performed (3-4 mice per group, one experiment).

30   Figure 8   shows for Example 3 the percentage of pentamer positive CD8+ T cells for all
                  groups tested. C57BL/6 mice were vaccinated twice (Wk0 and Wk2) with 2
                  nmol or 10 nmol of EDAMad5 or EDAZ13Mad5. Positive control group was

150

vaccinated with Mad5 and MPLA (equimolar to EDA). Mice were bled 7 days after last vaccination and pentamer staining was performed (one experiment with 3-4 mice per group).

5   Figure 9   shows for Example 4 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 $3x10^5$ 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.

10   in the right flank. Tumor size was measured with a caliper.

       Figure 10   shows for Example 4 individual tumor growth curves (7 individual mice per group). C57BL/6 mice were implanted s.c. with $3x10^5$ EG7-OVA tumor cells in the left flank and vaccinated twice (d5 and d13) by subcutaneous injection

15   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 11   shows for Example 4 (A) the survival curve of 7 mice per group; *, p<0.05 EDAZ13Mad5 versus control group (Log-rank test) and (B) the tumor-free

20   progression curve of 7 mice per group; *, p<0.05 EDAZ13Mad5 versus control group (Log-rank test).

       Figure 12   shows for Example 5 the number of metastasis for every experimental group. C57BL/6 mice were implanted i.v. with $1x10^5$ B16-OVA melanoma tumor

25   cells and vaccinated twice (d0 and d9) by subcutaneous injection of 2 nmol of EDAZ13Mad5, EDAMad5 or Z13Mad5 + MPLA (equimolar to EDA) or MPLA alone s.c. in the right flank. Mice were euthanized at day 13 and lung recovered. Number of metastasis foci was counted for each lung. **, p<0.01; ****, p<0.0001 (Unpaired T test).

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       Figure 13   shows for Example 6 the number of metastasis for every experimental group. C57BL/6 mice were vaccinated twice (d-21 and d-7) by subcutaneous

151

injection of 2nmoles of EDAZ13Mad5, EDAMad5 or Z13Mad5 + MPLA (equimolar to EDA) s.c. in the right flank. At day 0, mice were implanted i.v. with 1×10^5 B16-OVA melanoma tumor cells. Mice were euthanized at day 14 and lung recovered. Number of metastasis foci was counted for each lung. *, $p < 0.05$. ***, $p < 0.001$ (Unpaired T test).

Figure 14:   shows the results for Example 8. HEK-hTLR2 cell lines were seeded in flat 96-well plate in culture medium, stimulated with 0.3 μM, 1 μM or 3 μM of AnaxaZ13Mad5 or Z13Mad5Anaxa and incubated at 37°C for 24h. Positive control was performed with 500ng/ml of Pam3CSK4. (A) Twenty microliters of supernatant were added to QuantiBlue® detection medium and incubated at 37°C for 1h before OD reading (620nm). (B) Quantification of IL-8 secretion (by ELISA) in the supernatant.

Figure 15:   shows the results for Example 9. C57BL/6 mice were vaccinated twice (Wk0 and Wk2) with 2nmoles of Z13Mad5Anaxa or AnaxaZ13Mad5. Mice were bled 7 days after last vaccination and pentamer staining was performed (one experiment).

Figure 16:   shows the results for Example 9. C57BL/6 mice were vaccinated twice (Wk0 and Wk2) with 2nmoles Z13Mad5Anaxa or AnaxaZ13Mad5. Mice were bled 7 days after last vaccination and pentamer staining was performed (one experiment with 4 mice per group). *, $p < 0.05$.

Figure 17:   shows for Example 10 the tumor growth of 7 mice per group (mean ± 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. *, $p < 0.05$; ***, $p < 0.001$, ****, $p < 0.0001$.

152

Figure 18:   shows for Example 10 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 19:   shows for Example 10 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 20:   shows for Example 11 the tumor growth of 7 mice per group (mean ± 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 21:   shows for Example 11 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 22:   shows for Example 11 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).

153

Figure 23:   shows for Example 12 the tumor growth of 7 mice per group (mean±SEM); ****, p<0.0001 (Log-rank test). C57BL/6 mice were implanted s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and vaccinated twice (once at d5 and once at d13) by subcutaneous injection of either 0.5 nmol, 2 nmol or 10 nmol of Z13Mad5Anaxa in the right flank. Tumor size was measured with a caliper.

Figure 24:   shows for Example 13 the SIINFEKL-specific CD8 T cell responses detected in the blood of C57BL/6 mice vaccinated three times (once at Wk0, once at Wk2 and once at Wk4) s.c., i.d. or i.m. with 0.5 nmol (A) or 2nmol (B) of Z13Mad5Anaxa. Blood was obtained from mice 7 days after the 2nd and the 3rd vaccination and multimer staining was performed (one experiment with 4 mice per group). *, p<0.05.

Figure 25:   shows for Example 13 KLRG1 expression (A) and PD-1 expression (B), which were analyzed on multimer-positive CD8 T cells (one experiment with 4 mice per group). Briefly, C57BL/6 mice were vaccinated three times (once at Wk0, once at Wk2 and once at Wk4) s.c., i.d. or i.m. with 2nmol of Z13Mad5Anaxa. Blood was obtained from mice 7 days after the 2nd and the 3rd vaccination and FACS staining was performed.

Figure 26:   shows for Example 14 SIINFEKL-specific CD8 T cell responses in C57BL/6 mice vaccinated two times (once at Wk0 and once at Wk2) intranodally with 0.5 nmol of Z13Mad5Anaxa. Blood was obtained from mice 7 days after the 2nd vaccination and multimer staining was performed (3 mice per group).

Figure 27:   shows for Example 15 the percentage of pentamer-positive cells among CD8 T cells (A and B; *, p<0.05) and KLRG1 geomean of pentamer-positive CD8 T cells (C and D). Briefly, C57BL/6 mice were vaccinated 3 times (A and C: Wk0, Wk2 and Wk4; B and D: Wk0, Wk2 and Wk8) s.c. with 2 nmol of Z13Mad5Anaxa. Mice were bled 7 days after last vaccination and pentamer staining was performed (one experiment with 4 mice per group).

154

Figure 28:   shows for Example 15 the percentage of multimer-positive cells among CD8 T cells (A and D); KLRG1 geomean of multimer-positive CD8 T cells (B and E) and PD1 geomean of multimer-positive CD8 T cells (C and F). A-C, C57BL/6 mice were vaccinated 3 times at Day0, Day3 and Day7 and bled at Day7 and Day14. D-F, C57BL/6 mice were vaccinated 3 times at Day0, Day7 and Day14 and bled at Day14 and Day21. Vaccination was performed s.c. with 0.5 nmol of Z13Mad5Anaxa. Multimer staining was performed on blood samples (one experiment with 4 mice per group).

Figure 29:   shows for Example 16 the IL-6 secretion indicating the APC activation after incubation of BMDCs with various constructs as indicated in the Figure. Briefly, BMDCs were seeded in flat 96-well plate in culture medium, stimulated with 1 μM of Z13Mad5Anaxa, Mad5Anaxa, Z13Mad5, EDAZ13Mad5 or EDAMad5 and incubated for 24h at 37°C. IL-6 secretion was quantified by ELISA in the supernatant. Mean ± SEM of 2 to 3 individual experiments.

Figure 30:   shows for Example 16 the TNF-α secretion indicating the APC activation after incubation of Raw 264.7 cells with various constructs as indicated in the Figure. Briefly, Raw 264.7 cells were seeded in flat 96-well plate in culture medium, stimulated with 1 μM of Z13Mad5Anaxa, Mad5Anaxa or Z13Mad5 and incubated for 24h at 37°C. TNF-α secretion was quantified by ELISA in the supernatant. Mean ± SEM of 2 to 3 individual experiments.

Figure 31:   shows for Example 17 the IL-8 secretion indicating TLR4 binding after incubation of HEK-hTLR4 cells with various constructs as indicated in the Figure. Briefly, HEK-hTLR4 were seeded in flat 96-well plate in culture medium, stimulated with 1 μM of Z13Mad5Anaxa, Mad5Anaxa, Z13Mad5, EDAZ13Mad5 or EDAMad5 and incubated 24h at 37°C. IL-8 secretion was quantified by ELISA in the supernatant. Mean ± SEM of 2 individual experiments.

155

Figure 32:　shows for Example 18 the number of metastasis in a lung metastasis model with semitherapeutic settings. Briefly, C57BL/6 mice were implanted i.v. with 1×10^5 B16-OVA melanoma tumor cells and vaccinated twice (d0 and d9) by subcutaneous injection of 2 nmol of EDAZ13Mad5, Z13Mad5 + MPLA (equimolar to EDA) or MPLA alone s.c. in the right flank. Mice were euthanized at day 13 and lung recovered. Number of metastasis foci was counted for each lung. **, $p<0.01$ (One-way Anova with Tukey's multiple comparisons test).

Figure 33:　shows for Example 19 the number of metastasis in a lung metastasis model with semitherapeutic settings. Briefly, C57BL/6 mice were implanted i.v. with 1×10^5 B16-OVA melanoma tumor cells and vaccinated twice (d0 and d9) by subcutaneous injection of 0.5 nmol of Z13Mad5Anaxa, Mad5Anaxa or Z13Mad5 + Pam3CSK4 (equimolar to Anaxa) s.c. in the right flank. Mice were euthanized at day 21 and lung recovered. Number of metastasis foci was counted for each lung. *, $p<0.05$; **, $p<0.01$ (Unpaired t-test).

Figure 34:　shows for Example 20 the quantification of SIINFEKL-specific CD8 T cells in a Quad-Gl261 glioblastoma model. Briefly, C57BL/6 mice were implanted i.c. with 5×10^5 Gl261-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 CDS T cells were quantified in blood and in BILs at d28 by multimer staining (5-8 mice per group).

Figure 35:　shows for Example 20 the cytokine secretion. Briefly, C57BL/6 mice were implanted i.c. with 5×10^5 Gl261-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 (5-8 mice per group).

156

Figure 36:   shows for Example 21 the effect of Z13Mad5Anaxa on survival in the Quad-
              Gl261 glioblastoma model. Briefly, C57BL/6 mice were implanted i.c. with
              5×10^5 Gl261-Quad tumor cells and vaccinated three times (d7, d21 and d35)
              by s.c. injection of 2 nmol of Z13Mad5Anaxa. Mice were weight daily and
              euthanized when weight loss reached more than 15%.

Figure 37:   shows for Example 22 the effect of Z13Mad5Anaxa on tumor growth and
              survival in subcutaneous EG7-OVA tumor model in a prophylactic setting.
              Briefly, C57BL/6 mice were vaccinated twice (d-21 and d-7) by s.c. injection
              of 0.5 nmol of Z13Mad5Anaxa in the right flank and then implanted at day0
              s.c. with 3×10^5 EG7-OVA tumor cells in the left flank. Tumor size was
              measured with a caliper. (A) Tumor growth of 7 mice per group (mean ± SEM);
              ****, $p < 0.0001$ (2-way Anova test at day 30). (B) Survival curve of 7 mice per
              group. Median survival is indicated on the graph (m.s.). ***, $p < 0.001$ (Log-
              rank test).

Figure 38:   shows for Example 23 the effect of Z13Mad5Anaxa on tumor growth and
              survival in subcutaneous B16-OVA tumor model in a therapeutic setting on an
              established tumor. Briefly, C57BL/6 mice were implanted s.c. with 1×10^5 B16-
              OVA tumor cells in the left flank and vaccinated twice (d14 and d21) by s.c.
              injection of 0.5 nmol of Z13Mad5Anaxa in the right flank. (A) Tumor growth
              of 7 mice per group (mean± SEM); *, $p < 0.05$ (2-way Anova test at day 32). (B)
              Survival curve of 7 mice per group. Median survival is indicated on the graph
              (m.s.).

Figure 39:   shows for Example 24 the effect of the CPP in Z13Mad5Anaxa on tumor
              growth and survival in subcutaneous EG7-OVA tumor model. Briefly, C57BL/6
              mice were implanted at day0 s.c. with 3×10^5 EG7-OVA tumor cells in the left
              flank and then vaccinated twice (d5 and d13) by s.c. injection of 0.5 nmol of
              Z13Mad5Anaxa or Mad5Anaxa in the right flank. Tumor size was measured
              with a caliper. (A) Tumor growth of 7 mice per group (mean ± SEM); ****,

157

p<0.0001. (B) Survival curve of 7 mice per group. Median survival is indicated on the graph (m.s.). **, $p<0.01$; ***, $p<0.001$.

Figure 40:   shows for Example 25 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 41:   shows for Example 26 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 42:   shows for Example 26 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 43:   shows for Example 26 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.

158

Figure 44:   shows for Example 27 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
5   s.c. injection of 0.5 nmol of Z13Mad5Anaxa or Z14Mad5Anaxa in the right
              flank. (A) Tumor growth of 7 mice per group (mean ± 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).

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Figure 45:   shows for Example 28 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
15   staining was performed (one experiment with 4 mice per group). *, $p < 0.05$

Figure 46:   shows for Example 29 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
20   of 2nmoles of EDAZ14Mad5 in the right flank. Left panel: Tumor growth of 7
              mice per group (mean ± 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.).

25   Figure 47:   shows for Example 30 the quantification of SIINFEKL-specific CD8 T cells in a
              Quad-Gl261 glioblastoma model. Briefly, C57BL/6 mice were implanted i.c.
              with 5×10^5 Gl261-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
30   d28 by multimer staining (7-16 mice per group).

159

Figure 48:   shows for Example 30 the cytokine secretion. Briefly, C57BL/6 mice were
             implanted i.c. with 5×10^5 Gl261-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
5   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 49:   shows for Example 31 the effect of Z13Mad8Anaxa on T cells in spleen (CD8
10   T cell response (A) and CD4 T cell response (B)). C57BL/6 mice were
             vaccinated four times (Wk0, Wk2, Wk4 and Wk6) s.c. with 2 nmol of
             Z13Mad8Anaxa. (A) one week after the 4th vaccination, Elispot assay was
             performed on spleen cells stimulated gp70CD8 peptide. (B) one week after the
             4th vaccination, Elispot assay was performed on spleen cells stimulated with
15   gp70CD4 peptide.

Figure 50:   shows for Example 32 the effect of Z13Mad11Anaxa on the number of
             metastasis in the B16 lung metastasis model (A) and on the T cell response in
             spleen (B). C57BL/6 mice were vaccinated two times (day0, day10) s.c. with 1
20   nmol of Z13Mad11Anaxa.

Figure 51:   shows for Example 33 the effect of Z13Mad9Anaxa on T cells in spleen (CD8
             T cell response. C57BL/6 mice were vaccinated four times (Wk0, Wk2, Wk4
             and Wk6) s.c. with 2 nmol of Z13Mad9Anaxa. One week after the 4th
25   vaccination, Elispot assay was performed on spleen cells stimulated with
             adpgk peptide.

Figure 52:   shows for Example 34 the effect of complexes having different CPPs on the
             immune response. C57BL/6 mice were vaccinated two times (Wk0 and Wk2)
30   s.c. with 2 nmol of either Z13Mad5Anaxa or TatFMad5Anaxa. Mice were bled
             7 days after the 2nd vaccination and multimer staining was performed (one
             experiment with 8 mice per group).

160

Figure 53:   shows for Example 35 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 54:   shows for Example 36 the effect of Z13Mad12Anaxa on T cells in blood (CD8 T cell response). C57BL/6 mice were vaccinated twice (Wk0 and Wk2) s.c. with 2 nmol of Z13Mad12Anaxa. One week after the 2nd vaccination, multimer staining for the neoantigen reps1 was performed on blood cells.

Figure 55:   shows for Example 37 expression of activation marker HLA-DR, CD83, CD80 and CD86 (from left to right) by human blood monocyte-derived dendritic cells (DCs) from one single buffy. The DCs were stimulated with 300nM of Z13Mad5Anaxa (lower panels) or Z13Mad5 (upper panels) during 48h. Isotype staining for each condition was also performed as shown.

161

EXAMPLES

In the following, particular examples illustrating various embodiments and aspects of the invention are presented. However, the present invention shall not to be limited in scope by

5   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,

10   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. All such modifications fall within the scope of the appended claims.

15

Example 1:   In vitro human dendritic cell maturation

The goal of this study was to investigate the capacity of a complex for use according to the present invention to induce maturation of dendritic cells. In the present study, the complex

20   for use according to the present invention 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.

25

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:

30   EDAZ13Mad5

Sequence:

162

MHHHHHH*NID   RPKGLAFTDV   DVDSIKIAWE   SPQGQVSRYR   VTYSSPEDGI*
RELFPAPDGEDDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQST<u>KRY KNRVASRKSR</u>
<u>AKFKQLLQHY REVAAAKSSE NDRLRLLLKE</u> SLKISQAVHA AHAEINEAGR EVVGVGALKV
PRNQDWLGVP RFAKFASFEA QGALANIAVD KANLDVEQLE SIINFEKLTE WTGS

5   [SEQ ID NO: 26]

Molecular weight: 25'057 Da

Characteristics:

10   — 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-
15   Arginine, pH 8

        — Endotoxin level: < 0.01EU/ug

<u>Z13Mad5</u>

20

Sequence:

MHHHHHH<u>KRY KNRVASRKSR AKFKQLLQHY REVAAAKSSE NDRLRLLLKE</u> SLKISQAVHA
AHAEINEAGR EVVGVGALKV PRNQDWLGVP RFAKFASFEA QGALANIAVD KANLDVEQLE
SIINFEKLTE WTGS

25   [SEQ ID NO: 29]

Molecular weight: 15'196 Da

Characteristics:

30   — Mad5 cargo contains OVACD4, gp100CD8, EalphaCD4 and OVACD8 epitopes

        — Storage buffer: 50 mM Tris-HCl, , 150 mM NaCl, 10% Glycerol, 2 mM DTT, 1 M L-
        Arginine, pH 9

163

— Endotoxin level:

  o Batch 1: 0.32EU/mg

  o Batch 2: 0.44EU/mg

5 <u>Mad5</u>

Sequence:

MHHHHHHE SLKISQAVHA AHAEINEAGR EVVGVGALKV PRNQDWLGVP RFAKFASFEA

QGALANIAVD KANLDVEQLE SIINFEKLTE WTGS

10 [SEQ ID NO: 30]

Molecular weight: 10'154.6 Da

Characteristics:

15 — 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

20 The EDAZ13Mad5, Z13Mad5 and Mad5 proteins were investigated for their capacity to induce human dendritic cell (DC) maturation. After incubation during 48h with 300nM of protein, activation markers expression (CD86, CD40, CD83 and HLA-DR) was assessed on the human DCs by FACS (Figures 1 - 4). Specific buffers of each protein were used as negative controls.

25

Results are shown for CD40 in Fig. 1, for CD86 in Fig. 2, for HLADR in Fig. 3, and for CD83 in Fig. 4. Whereas EDAZ13Mad5 induced maturation of human DCs, shown by the up-regulation of CD86, HLADR and CD83, Z13Mad5 and Mad5 proteins were not able to activate human DCs. These results indicate that the EDA portion of the protein is responsible

30 for the up-regulation of the activation markers on the human DCs.

164

Example 2:   In vitro epitope presentation (MHC I)

The goal of this study was to assess functional MHC class I-restricted cross-presentation in a murine *in an vitro* system using bone marrow derived dendritic cells (BMDCs) and
5   splenocytes from different TCR transgenic mice. To this end, the constructs EDAZ13Mad5 and Mad5 (described above in Example 1) and the construct EDAMad5 were used:

EDAMad5

10   Sequence

MHHHHHH*NID   RPKGLAFTDV   DVDSIKIAWE   SPQGQVSRYR   VTYSSPEDGI RELFPAPDGEDDTAELQGLR   PGSEYTVSVV   ALHDDMESQP   LIGIQSTE   SLKISQAVHA AHAEINEAGR EVVGVGALKV PRNQDWLGVP RFAKFASFEA QGALANIAVD KANLDVEQLE SIINFEKLTE WTGS*

15   [SEQ ID NO: 31]

Molecular weight: 20'017 Da

Characteristics:
20   — 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

25

BMDCs were loaded overnight with 300nM of with the EDAMad5, EDAZ13Mad5 and Mad5 proteins containing OVACD8, OVACD4 and gp100 epitopes. Processing and presentation of these MHC I-restricted OVACD8 and gp100 epitopes were monitored by measuring the *in vitro* proliferation of naïve OVA$_{257-264}$-specific CD8$^+$ T cells from OT-1 T cell receptor (TCR)
30   transgenic mice and gp100-specific CD8$^+$ T cells from P-mel T cell TCR transgenic mice respectively. Accordingly, efficient MHC class I-restricted presentation of OVACD8 epitope and gp100 epitope was monitored after 4 days with CFSE-labeled OT1 cells and P-Mel cells

165

respectively. Processing and presentation of MHC II-restricted OVACD4 epitope was monitored by measuring the *in vitro* proliferation of naïve $OVA_{323\text{-}339}$-specific $CD4^+$ T cells from OT-2 T cell receptor (TCR) transgenic mice. Accordingly, efficient MHC class II-restricted presentation of OVACD4 epitope was monitored after 4 days with CFSE-labeled

5   OT2 cells. As control, BMDCs were pulsed for 1h with 5uM peptide (one experiment representative of 2 individual experiments).

Results are shown in Fig. 5. Similar cross-presentation and processing capacity of all assessed Mad5-based proteins were observed.

10

Example 3:   CD8 T cell immune response

To investigate the efficacy of EDA-conjugated proteins in inducing polyclonal $CD8^+$ T cell

15   response, C57BL/6 mice were vaccinated twice (Wk0 and Wk2), by subcutaneous injection of either 2 nmol or 10 nmol of the constructs EDAZ13Mad5 or EDAMad5 (described in Examples 1 and 2). Positive control group was vaccinated with Mad5 and the TLR4 agonist MPLA (equimolar to EDA). Two doses were assessed 2 nmol of the construct (Fig. 6) and 10 nmol of the construct (Fig. 7). 3 – 4 mice were used per group.

20

Seven days after the last vaccination, mice were bled and pentamer staining was performed to monitor the OVA-specific immune response in the blood. In Fig. 8, the percentage of pentamer positive CD8+ T cells is shown for all groups and both doses tested.

25   These data show that interestingly the immune response is lower at 10nmol compared to 2nmol. At both doses, 2nmol and 10nmol, the vaccine mediated immune response was observed more consistently in the EDAZ13Mad5 group in contrast to the EDAMad5 group. Moreover, there is an increased immune response when the TLR4 agonist is conjugated with the vaccine.

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166

Example 4:   Vaccine efficacy on tumor growth in a benchmark EG.7-OVA tumor model

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): EDAZ13Mad5, EDAMad5, Mad5, or Mad5 and MPLA (equimolar to EDA) s.c. in the right flank. Tumor size was measured with a caliper.

Fig. 9 shows the tumor growth of 7 mice per group (mean ± SEM); *, p<0.05 EDAZ13Mad5 versus control group (2-way Anova test). Fig. 10 shows individual tumor growth curves (7 individual mice per group). Fig. 11 A shows the survival curve of 7 mice per group; *, p<0.05 EDAZ13Mad5 versus control group (Log-rank test). Fig. 11 B shows the tumor-free progression curve of 7 mice per group; *, p<0.05 EDAZ13Mad5 versus control group (Log-rank test).

The results show that in a therapeutic setting, EDAZ13Mad5 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.

The results therefore suggest that the construct protein EDAZ13Mad5 is a highly potent vaccine for controlling the tumor growth in a therapeutic setting.

Example 5:   Vaccine efficacy on tumor growth in a melanoma metastasis model

To assess the efficacy in a lung metastasis model using B16-OVA tumor cells in a semi-therapeutic setting, different construct proteins were used: EDAMad5, EDAZ13Mad5, Z13Mad5 + MPLA (cf. Examples 1 and 2 for design of the constructs), and MPLA alone. C57BL/6 mice were implanted i.v. with 1×10^5 B16-OVA melanoma tumor cells and at the same time (d0) 2 nmol of the vaccine (EDAMad5, EDAZ13Mad5, Z13Mad5 + MPLA, MPLA alone) was administered by subcutaneous injection in the right flank. Nine days later, mice were vaccinated a second time with the same dose. Further control groups were vaccinated

167

with 2 nmol of Z13Mad5 and the TLR4 agonist MPLA (equimolar to EDA) or MPLA alone. Mice were euthanized at day 13 and lung recovered. Number of metastasis foci was counted for each lung. The results are shown in Fig. 12.

5   The results show that the conjugate EDAZ13Mad5 is as potent as Z13Mad5 + MPLA to inhibit tumor metastasis in the lung. Furthermore, EDA-Mad5 is less potent than EDAZ13Mad5, indicating a crucial role of Z13 in vaccine efficacy.

10   **Example 6:   Vaccine efficacy on tumor growth in a melanoma metastasis model – prophylactic setting**

Furthermore, the efficacy of the different construct proteins EDAMad5, EDAZ13Mad5, and Z13Mad5 + MPLA (cf. Examples 1 and 2 for design of the constructs) was assessed in a lung
15   metastasis model in a prophylactic setting. C57BL/6 mice were vaccinated 21 and 7 days before implantation of tumor cells (d-21 and d-7) by subcutaneous injection of 2 nmol of EDAZ13Mad5, EDAMad5 or Z13Mad5 + MPLA (equimolar to EDA) s.c. in the right flank. At day 0, mice were implanted i.v. with 1×10^5 B16-OVA melanoma tumor cells. Mice were euthanized at day 14 and lung recovered. Results are shown in Fig. 13.
20

Example 7:   Design of further constructs comprising a TLR2 peptide agonist

Herein, the complex for use according to the present invention is again a fusion protein,
25   comprising the cell-penetrating peptide "Z13", the protein "MAD5", which consists of different CD8$^+$ and CD4$^+$ epitopes from various antigens, and the TLR2 peptide agonist "Anaxa". Accordingly, fused proteins with the Anaxa peptide at the C-terminal or N-terminal position were designed.

30   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 "Anaxa" is shown in italics:

AnaxaZ13Mad5

Sequence:

MHHHHHH*STV HEILCKLSLE GDHSTPPSAY GSVKPYTNFD AE*KRYKNRVA SRKSRAKFKQ
LLQHYREVAA AKSSENDRLR LLLKESLKIS QAVHAAHAEI NEAGREVVGV GALKVPRNQD
WLGVPRFAKF ASFEAQGALA NIAVDKANLD VEQLESIINF EKLTEWTGS
[SEQ ID NO: 27]

Molecular weight: 18973 Da

Characteristics:

— Mad5 cargo contains OVACD4, gp100CD8, EalphaCD4 and OVACD8 epitopes

— Contains the 35-mer peptide of Annexin

— Storage buffer: 50 mM Tris-HCl, , 150 mM NaCl, 10% Glycerol, 2 mM DTT, 0.5 M
   L-Arginine, pH 8

— Endotoxin level: 5.17 EU/mg

Z13Mad5Anaxa

Sequence:

MHHHHHHKRYKNRVA   SRKSRAKFKQ   LLQHYREVAA   AKSSENDRLR   LLLKESLKIS
QAVHAAHAEI   NEAGREVVGV   GALKVPRNQD   WLGVPRFAKF   ASFEAQGALA
NIAVDKANLD VEQLESIINF EKLTEWTGS*S TVHEILCKLS LEGDHSTPPS AYGSVKPYTN FDAE*
[SEQ ID NO: 28]

Molecular weight: 18973 Da

Characteristics:

— Mad5 cargo contains OVACD4, gp100CD8, EalphaCD4 and OVACD8 epitopes

— Contains the 35-mer peptide of Annexin

169

—   Storage buffer: 50 mM Tris-HCl, , 150 mM NaCl, 10% Glycerol, 2 mM DTT, 0.5 M
        L-Arginine, pH 8

Endotoxin level: 3.1 EU/mg

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Example 8:   TLR2 binding (HEK-hTLR2 cell lines)

The goal of this study was to assess whether the Z13Mad5Anaxa and AnaxaZ13Mad5
construct proteins (cf. Example 7 for design of these construct proteins) were able to bind

10   TLR2 as an agonist. HEK-Blue™ hTLR2 were seeded in flat 96-well plate in culture medium,
stimulated with 0.3 µM, 1 µM or 3 µM of AnaxaZ13Mad5 or Z13Mad5Anaxa and incubated
at 37°C for 24h. Positive control was performed with 500ng/ml of Pam3CSK4, a TLR2 agonist.

To monitor the activation of NF-κB/AP1, twenty microliters of the supernatant were added to

15   QuantiBlue® detection medium and incubated at 37°C for 1h before OD reading (620nm).
Results are shown in Figure 14 A.

The secretion of IL-8 in the supernatant was quantified by ELISA. Results are shown in Figure
14 B.

20

Results (Fig. 14 A, B) showed that Z13Mad5Anaxa and AnaxaZ13Mad5 are similarly able to
bind to TLR2 in a dose dependent manner.

25   ### Example 9:   *In vivo* induction of specific CD8^{+} T cells

To investigate the efficacy of the Anaxa-conjugated proteins of Example 7 in the induction of
CD8^{+} T cell responses, C57BL/6 mice were vaccinated twice (Wk0 and Wk2), by
subcutaneous injection of 2 nmol of AnaxaZ13Mad5 or 2 nmol of Z13Mad5Anaxa. Seven

30   days after the last vaccination, mice were bled and to monitor the OVA-specific immune
response in the blood, pentamer staining was performed (one experiment with 4 mice per
group). Results are shown in Figures 15 and 16.

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These data indicate that both, the Z13Mad5Anaxa vaccine and the AnaxaZ13Mad5 construct, elicit a strong immune response.

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Example 10:   Therapeutic effect on tumor growth

To evaluate the effect of the Anaxa-conjugated construct proteins designed in Example 7 on tumor growth control, a benchmark tumor model was used, namely the s.c. implantation of EG.7-OVA thymoma cells.

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). In order to compare the effect to a protein mixed with an external adjuvant, a control group was vaccinated with Z13Mad5 and Pam3CSK4 (equimolar to Anaxa). Tumor size was measured with a caliper. Results are shown in Fig. 17 – 19.

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 Pam3CSK showing a significant better survival curve. In particular, Z13Mad5Anaxa and AnaxaZ13Mad5 demonstrate significantly higher efficacy than Z13Mad5 administrated 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.

171

Example 11:   Therapeutic effect on tumor growth – comparison of constructs with different
               TLR agonists

The goal of this study was to compare the efficacy of the different construct protein vaccines
conjugated to different TLR agonist, namely EDAZ13Mad5 and Z13Mad5Anaxa of Example
1 and 7, 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 in Example 10. 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).

Results are shown in Figures 20, 21 and 22. In this experimental setting, Z13Mad5Anaxa,
EDAZ13Mad5, and Z13Mad5+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 12:   Dose effect of Z13Mad5Anaxa on tumor growth control

To identify the optimal dose of the conjugate vaccine, three different doses (0.5 nmol, 2 nmol
and 10 nmol) of Z13Mad5Anaxa (cf. Example 7) were assessed for their ability to control
tumor growth. The dose effect of Z13Mad5Anaxa construct was evaluated in the s.c. model
of EG.7-OVA thymoma cells as described previously in Example 10. After tumor implantation,
mice were vaccinated twice (at day 5 and at day 13 after tumor implantation) in a therapeutic
setting at 0.5, 2 or 10 nmol of Z13Mad5Anaxa.

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 either 0.5 nmol, 2 nmol or 10
nmol of Z13Mad5Anaxa in the right flank. Tumor size was measured with a caliper.

172

The tumor growth of 7 mice per group is depicted in Figure 23. Those data show that the doses of 0.5 and 2 nmol are at least as efficacious as 10 nmol for controlling tumor growth.

5   **Example 13:   Effect of different routes of administration of Z13Mad5Anaxa**

This study was based on the previous Examples demonstrating the efficacy of Z13Mad5Anaxa conjugate vaccine (cf. Example 7), which is able to elicit specific immune responses and is efficacious for controlling tumor growth in the subcutaneous tumor model EG7 as shown

10   above.

To investigate the effect of subcutaneous, intramuscular and intradermal routes of administration, immune responses elicited by subcutaneous, intramuscular and intradermal injection were compared. Intradermal injections were performed using the PLEASE® device

15   from Pantec Biosolutions.

Mice were vaccinated three times every two weeks (Wk0, Wk2 and Wk4) with 0.5 or 2 nmol of Z13Mad5Anaxa (cf. Example 7). In order to target several lymph nodes, the 1st and the 3rd vaccinations were performed in the right flank whereas the 2nd was done in the left flank.

20   SIINFEKL-specific CD8+ T cell response was analyzed 1 week after the 2nd and the 3rd vaccination in the blood. Figure 24 shows the SIINFEKL-specific CD8 T cell responses after each vaccination detected in the blood of C57BL/6 mice vaccinated three times (Wk0, Wk2 and Wk4) s.c., i.d. or i.m. with 0.5 nmol (Figure 24 A) or 2nmol (Figure 24 B) of Z13Mad5Anaxa. Blood was obtained from mice 7 days after the 2nd and the 3rd vaccination

25   and multimer staining was performed (one experiment with 4 mice per group).

The results indicate that at the two doses assessed (0.5 and 2nmol), (i) all routes of administration tested elicited a SIINFEKL-specific CD8 immune response and (ii) the subcutaneous vaccination elicited the strongest SIINFEKL-specific CD8 immune response. For

30   subcutaneous administration, the maximum response was reached after the 3nd vaccination and still maintained after the 3rd vaccination. The SIINFEKL-specific CD8 immune response

173

after the 2nd vaccination elicited by intradermal and intramuscular vaccinations is lower compared to subcutaneous vaccination and is not enhanced after the 3rd vaccination.

Next, the effector function and the exhaustion status of SIINFEKL-specific CD8 T cells was
5   evaluated by analyzing KLRG 1 (Killer cell lectin-like receptor subfamily G member 1) and PD-1 respectively.

To this end, C57BL/6 mice were vaccinated three times (Wk0, Wk2 and Wk4) s.c., i.d. or i.m. with 2nmol of Z13Mad5Anaxa (cf. Example 7). Blood was obtained from mice 7 days after
10   the 2nd and the 3rd vaccination and FACS staining was performed. KLRG1 and PD-1 expression were analyzed on multimer-positive CD8 T cells (one experiment with 4 mice per group). Results are shown in Figure 25.

These data indicate that the expression of KLRG 1 is strongly increasing on SIINFEKL-specific
15   CD8 T cells after subcutaneous vaccination. After i.d. or i.m. vaccination, the observed effects were lower. The percentage of KLRG 1-positive cells among SIINFEKL-specific CD8 T cells is also enhanced after s.c. vaccination (data not shown).

In contrast to KLRG 1, PD-1 expression is decreasing with the time and the vaccinations, for
20   subcutaneous and intramuscular vaccination routes. This suggests that SIINFEKL-specific CD8 T cells are not exhausted. The percentage of PD1-positive cells among SIINFEKL-specific CD8 T cells is also reduced after s.c. and i.m. vaccination (data not shown). It is important to note that PD-1 expression is higher after the 2nd vaccination when mice were vaccinated subcutaneously, reflecting the early activation status of specific T cells (Keir, M.E., et al., *PD-*
25   *1 and its ligands in tolerance and immunity.* Annu Rev Immunol, 2008. 26: p. 677-704).

The expression of the late exhaustion marker Tim-3 was also analyzed. A very low expression as observed for all groups.

30   Taken together, results indicate that subcutaneous vaccination elicits the best specific CD8 immune response compared to intramuscular or intradermal injections.

174

Example 14:   Intranodal route of administration

Based on the previous experiments (Example 13), the intranodal route of administration was additionally investigated. To this end, the immune response elicited by intranodal injection of Z13Mad5Anaxa (cf. Example 7) was investigated.

For this purpose, mice were first injected with Evans Blue subcutaneously in order to allow easily visualizing the lymph nodes for injection and inject intranodally without invasive surgery, for example as described in Jewell, C.M., S.C. Lopez, and D.J. Irvine, *In situ engineering of the lymph node microenvironment via intranodal injection of adjuvant-releasing polymer particles.* Proc Natl Acad Sci US A, 2011. 108(38): p. 15745-50.

C57BL/6 mice were vaccinated two times every two weeks (Wk0 and Wk2) intranodally with 0.5 nmol of Z13Mad5Anaxa (cf. Example 7). The 1st vaccination was performed in the right inguinal lymph node, whereas the second vaccination was done in the left inguinal lymph node. Blood was obtained from mice 7 days after the 2nd vaccination and multimer staining was performed (3 mice per group). In other words, SIINFEKL-specific CD8+ T cell response was analyzed one week after the 2nd vaccination in the blood. Figure 26 shows the SIINFEKL-specific CD8 T cell responses. Those data indicate that also intranodal injection was able to elicit SIINFEKL-specific CD8 T cells.

Example 15:   Vaccination schedule

The vaccination schedule evaluation work was initiated with the objective to identify the impact of the third vaccination using the same Z13Mad5Anaxa construct as described above (cf. Example 7). The subcutaneous route was chosen given the previous results.

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In the experiment first two vaccinations were performed at wk0 and wk2 with a 3rd vaccination either at wk4 (Figure 27 A) or at wk8 (Figure 27B). Thus, C57BL/6 mice were vaccinated three times (Figure 27 A and C: Wk0, Wk2 and Wk4 and Figure 27 B and D: Wk0, Wk2 and Wk8) s.c. with 2 nmol of Z13Mad5Anaxa. Blood was obtained from mice 7 days

5   after last vaccination and pentamer staining was performed (one experiment with 4 mice per group). Accordingly, SIINFEKL-specific CD8+ T cell response was analyzed 1 week after the 2nd and the 3rd vaccination (Figure 27 A and B). Additionally, the effector function of SIINFEKL-specific T cells was evaluated by analyzing the expression of KLRG 1 on specific CD8 T cells (Figure 27C and D).

10

The data indicate that compared to control the percentage of SIINFEKL-specific CD8 T cells was significantly increased at all time points tested (Vac2 and Vac3) as well as in both vaccination schedules (Figure 27 A and B).

15   Interestingly, the third vaccination at Wk4 allowed to most prominently increasing the percentage of SIINFEKL-specific CD8 T cells (Figure 27 A). The same cells also demonstrate an improved effector function through higher KLRG 1 expression (Figure 27 C). In contrast, with a third vaccination performed at Wk8 no improvement from the second to the third vaccination could be observed in the SIINFEKL-specific immune response and in the KLRG 1

20   expression.

Taken together, these results indicate that the CD8 immune response could be increased by shorten the delay between the second and the third vaccination.

25   Given that an earlier third vaccination seems to increase immune response, in the next study two short schedules of vaccination were investigated:
i) three vaccinations at day 0, day 3 and day 7 and
ii) three vaccinations at day 0, day 7 and day 14.

30   Again, C57BL/6 mice were used and vaccination was performed s.c. with 0.5 nmol of Z13Mad5Anaxa (cf. Example 7). Multimer staining was performed on blood samples obtained one week after the 2nd and the 3rd vaccination (one experiment with 4 mice per group).

176

Thus, SIINFEKL-specific CD8+ T cell response was analyzed one week after the 2nd and the 3rd vaccination (Figure 28A and D). Additionally, the effector function of SIINFEKL-specific T cells was evaluated by analyzing the expression of KLRG 1 on specific CD8 T cells (Figure 28B and 28E) and the exhaustion status by analyzing the PD-1 expression of specific T cells (Figure 28C and 28F).

The data indicate that – similarly to the first study regarding the vaccination schedule described above – compared to control the percentage of SIINFEKL-specific CD8 T cells was increased at all time points tested (Vac2 and Vac3) as well as in both vaccination schedules (Figure 28 A and B).

However, compared to the schedule wk0-wk2-wk4, a schedule with vaccinations at Day0, Day3 and Day7 did not elicit such a high SIINFEKL-specific CD8 T cell immune response. Concerning the schedule with vaccinations at Day0, Day7 and Day14, the SIINFEKL-specific CD8 T cell immune response elicited is better compared to the previous schedule (d0-d3-d7) but is not maintained after the 3rd vaccination.

Taken together, vaccination schedule data set indicates that the Wk0-Wk2-Wk4 vaccination schedule is the best vaccination schedule for inducing potent OVA-specific CD8 immune response with high effector function.

Example 16:   Capacity of TLR agonist-CPP conjugate constructs to activate murine antigen-presenting cells (APCs)

To investigate the effect of both, the CPP component and the TLR agonist component in a complex for use according to the present invention, again the fusion proteins as described above (cf. Examples 1, 2 and 7) were used.

In addition, a further "control peptide" was designed, which is also a fusion protein and which comprises the protein "MAD5", which consists of different CD8+ and CD4+ epitopes from

177

various antigens, and the TLR2 peptide agonist "Anaxa" (i.e. without cell penetrating peptide). Accordingly, the following control construct was additionally designed:

5   Mad5Anaxa

Sequence:
MHHHHHHESL   KISQAVHAAH   AEINEAGREV   VGVGALKVPR   NQDWLGVPRF
AKFASFEAQG   ALANIAVDKA   NLDVEQLESI   INFEKLTEWT   GSSTVHEILC   KLSLEGDHST
10   PPSAYGSVKP   YTNFDAE
[SEQ ID NO: 32]

Molecular weight: 13933 Da

15   Characteristics:
        — Mad5 cargo contains OVACD4, gp100CD8, EalphaCD4 and OVACD8 epitopes
        — Contains the 35-mer peptide of Annexin in C-terminal position
        — Storage buffer: 50 mM Tris-HCl, 150 mM NaCl, 10% Glycerol, 2 mM DTT, 0.5 M L-
          Arginine, pH 8
20   — Endotoxin level: Batch 1 – 12.15 EU/mg

The aim of this study was to evaluate the capacity of two exemplary complexes according to the present invention, namely EDAZ13Mad5 (cf. Example 1) and Z13Mad5Anaxa (cf. Example 7), to promote antigen-presenting cells activation in comparison to reference
25   complexes lacking either the cell penetrating peptide component Z13 (Mad5Anaxa, cf. above; EDAMad5, cf. Example 2) or the TLR agonist (Z13Mad5, cf. Example 1).

To this end, the capacity of the above mentioned constructs to promote antigen-presenting cells (APC) activation was assessed in bone marrow-derived dendritic cells (BMDCs), which
30   express all TLRs except TLR7.

178

BMDCs were seeded in flat 96-well plate in culture medium, stimulated with 1 μM of either Z13Mad5Anaxa (cf. Example 7), Mad5Anaxa (cf. above), Z13Mad5 (cf. Example 1), EDAZ13Mad5 (cf. Example 1) or EDAMad5 (cf. Example 2) and incubated for 24h at 37°C.

5   The APC activation was investigated by monitoring the secretion of IL-6 in the culture supernatant of BMDCs. IL-6 secretion was quantified by ELISA in the supernatant.

The results are shown in Figure 29. These data clearly show that Z13Mad5Anaxa was able to activate BMDCs, whereas no such activation was observed when the cells were cultured in
10   presence of Z13Mad5 or Mad5Anaxa. This suggests that not only the TLR agonist (Anaxa or EDA) is critical for the activation of macrophages and dendritic cells, but that the CPP is also needed. Also the presence of the CPP without the TLR agonist is not sufficient, but indeed both, CPP and TLR agonist are critical for the activation of macrophages and dendritic cells.

15   Those results were confirmed by using another cell line, namely in the Raw 264.7 mouse macrophage cell line, which expresses all TLRs except TLR5 (Applequist, S.E., R.P. Wallin, and H.G. Ljunggren, *Variable expression of Toll-like receptor in murine innate and adaptive immune cell lines.* Int Immunol, 2002. 14(9): p. 1065-74).

20   Raw 264.7 cells were seeded in flat 96-well plate in culture medium, stimulated with 1 μM of either Z13Mad5Anaxa (cf. Example 7), Mad5Anaxa (cf. above) or Z13Mad5 (cf. Example 1) and incubated for 24h at 37°C.

In Raw 264.7 cells the APC activation was investigated by monitoring the secretion of TNF-α
25   in the culture supernatant of Raw 264.7. TNF-α secretion was quantified by ELISA in the supernatant. The results are shown in Figure 30.

It is thought that the CPP may facilitate the entry of the molecule into the cells, allowing a better targeting of intracellular TLR.

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179

Taken together, the data reveal the critical role of both, CPP and TLR agonist, within the conjugate constructs to activate APC. This effect may be due to helping the entry of the construct into the cells, therefore resulting in an optimal targeting of the intracellular TLR.

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Example 17:   Ability of the conjugate constructs to bind to human TLR4

It was recently shown that the Anaxa peptide owns an adjuvant activity by signaling through TLR2 (WO 2012/048190 A1), whereas the EDA peptide is a natural ligand for TLR4
10   (Okamura, Y., et al., *The extra domain A of fibronectin activates Toll-like receptor 4.* J Biol Chem, 2001. 276(13): p. 10229-33).

Moreover, as shown above in Example 8 and Figure 14, a complex for use according to the present invention comprising the Anaxa peptide as TLR agonist, for example Z13Mad5Anaxa,
15   is able to bind to human TLR2 and to promote the secretion of IL-8 by HEK-hTLR2 cells (cf. Example 8, Fig. 14).

In the present study, the ability of complexes according to the present invention comprising either the Anaxa peptide as TLR agonist or the EDA peptide as TLR agonist to bind to human
20   TLR4 was evaluated. To this end, HEK cells transfected with human TLR4 (HEK-hTLR4) were seeded in flat 96-well plate in culture medium, stimulated with 1 µM of either Z13Mad5Anaxa (cf. Example 7), Mad5Anaxa (cf. above), Z13Mad5 (cf. Example 1), EDAZ13Mad5 (cf. Example 1) or EDAMad5 (cf. Example 2) and incubated for 24h at 37°C. IL-8 secretion was quantified by ELISA in the supernatant.
25
Results are shown in Figure 31. As expected, incubation of HEK-hTLR4 with EDAZ13Mad5 resulted in remarkable IL-8 secretion, indicating binding of EDAZ13Mad5 to TLR4. In line with the results obtained in Example 16, the IL-8 secretion of EDAMad5 (without the CPP) was remarkably lower as compared to EDAZ13Mad5, showing the effect of the presence of a
30   CPP. The Z13Mad5 construct, which does not comprise a TLR agonist, showed no IL-8 secretion, indicating – as expected – a lack of binding to TLR4.

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Interestingly, incubation of HEK-hTLR4 with the construct Z13Mad5Anaxa resulted in the most pronounced IL-8 secretion, indicating binding of Z13Mad5Anaxa to TLR4. This is astonishing, since Anaxa was previously hypothesized to be a TLR2 agonist. Again, the same construct but without the CPP (Mad5Anaxa) resulted in remarkably lower IL-8 secretion,

5   confirming the results obtained in Example 16.

Taken together, these data (i) confirm the results obtained in Example 16, (ii) confirm that EDA is indeed a TLR4 agonist, and (iii) show surprisingly that the Anaxa peptide is also a TLR4 agonist (in addition to being a TLR2 agonist, cf. Example 8 and Fig. 14).

10

Example 18:   Vaccine efficacy on tumor growth in a lung metastasis model – semi-therapeutic setting: TLR agonist EDA

15   This study is based on Example 6, showing the efficacy of a complex for use according to the present invention, namely EDAZ13Mad5, in a melanoma lung metastasis model in a prophylactic setting (cf. Figure 13).

In the present study the same lung metastasis model was used as well as the construct proteins

20   EDAZ13Mad5 and Z13Mad5 + MPLA (cf. Examples 1 and 2 for design of the constructs). However, in the semi-therapeutic setting, C57BL/6 mice were vaccinated at the same time as tumor cells were implanted (d0) and, for a second time, at nine days after implantation (d9). Vaccination was performed by subcutaneous injection of 2 nmol of EDAZ13Mad5, Z13Mad5 + MPLA (equimolar to EDA) or MPLA s.c. in the right flank. At day 0, mice were implanted

25   i.v. with 1×10^5 B16-OVA melanoma tumor cells and vaccinated twice (d0 and d9) by subcutaneous injection of 2 nmol of EDAZ13Mad5, Z13Mad5 + MPLA (equimolar to EDA) or MPLA alone s.c. in the right flank. Mice were euthanized at day 13 and lung recovered. Results are shown in Fig. 32.

30   The results show that EDAZ13Mad5 is slightly more potent than Z13Mad5 + MPLA to inhibit the growth of melanoma metastasis. In addition, no adjuvant effect was observed in mice injected with MPLA only.

181

Both, EDAZ13Mad5 and Z13Mad5 + MPLA, significantly inhibit the growth of melanoma metastasis in the lung in prophylactic and semitherapeutic settings.

5

Example 19: Vaccine efficacy on tumor growth in a lung metastasis model – semitherapeutic setting: TLR agonist Anaxa

This study is based on Example 18 with the same model (semitherapeutic settings) and
10   experimental schedule. However, the effect of complexes according to the present invention comprising the "Anaxa" peptide as TLR agonist were investigated – instead of the EDA TLR agonist as in Example 18.

To this end, C57BL/6 mice were implanted i.v. with 1×10^5 B16-OVA melanoma tumor cells
15   and vaccinated twice (d0 and d9) by subcutaneous injection of 0.5 nmol of Z13Mad5Anaxa, Mad5Anaxa or Z13Mad5 + Pam3CSK4 (equimolar to Anaxa) s.c. in the right flank. Mice were euthanized at day 21 and the lung was recovered. Number of metastasis foci was counted for each lung. The results are shown in Figure 33.

20   The results show that Z13Mad5Anaxa is sensibly more potent than Z13Mad5 + Pam3CSK4 to inhibit the growth of melanoma metastasis. In contrast, Mad5Anaxa was not able to control metastasis growth in the lung, underlining again the importance of CPP.

Altogether, the B16-OVA lung metastasis experiment showed that Z13Mad5Anaxa was highly
25   efficacious in inhibiting the growth of melanoma metastasis in the lung.

Example 20: Vaccine efficacy in a glioblastoma model

30   In this study, another cancer model was used, namely a glioblastoma model. Glioma is the most frequent form of primary brain tumors in adults, with glioblastoma multiforme (GBM) being the most lethal. This tumor is notorious for its highly invasive and aggressive behavior.

182

Currently, the best treatment against GBM is a regimen involving a combination of surgery, chemotherapy and radiotherapy, which has a median survival period of only 14.6 months. There is an urgent, unmet medical need for new treatment modalities that improve the prognosis of glioma patients. T-cell mediated immunotherapy is a conceptually attractive
5   treatment option to use in conjunction with existing modalities for glioma, in particular highly invasive GBM.

The Gl261 glioma is a carcinogen-induced mouse glioma model. This model represents one of the very few brain tumor models developed in immunocompetent animals, that has growth
10   characteristics similar to human GBM (Newcomb, E. and D. Zagzag, *The murine GL261 glioma experimental model to assess novel brain tumor treatments,* in *CNS Cancer Models, Markers, Prognostic, Factors, Targets, and Therapeutic Approaches,* E.G. Van Meir, Editor. 2009, Humana Press: Atlanta. p. 227-241; Jacobs, V.L., et al., *Current review of in vivo GBM rodent models: emphasis on the CNS-1 tumour model.* ASN Neuro, 2011. 3(3): p. e00063).
15   Low numbers of intracranially transplanted Gl261 cells formed intracranial tumors in C57BL/6 mice (Zhu, X., et al., *Poly-/CLC promotes the infiltration of effector T cells into intracranial gliomas via induction of CXCL10 in IFN-alpha and IFN-gamma dependent manners.* Cancer Immunol Immunother, 2010. 59(9): p. 1401-9; Zhu, X., et al., *Toll like receptor-3 ligand poly-/CLC promotes the efficacy of peripheral vaccinations with tumor*
20   *antigen-derived peptide epitopes in murine CNS tumor models.* J Transl Med, 2007. 5: p. 10). The cells are moderately immunogenic: they are able to elicit tumor-specific immune response at the tumor site. However, the tumor-specific immune cells are not capable of complete tumor clearance.

25   Recently, M. Ollin generated a new Gl261 model (Ohlfest, J.R., et al., *Vaccine injection site matters: qualitative and quantitative defects in CDB T cells primed as a function of proximity to the tumor in a murine glioma model.* J Immunol, 2013. 190(2): p. 613-20) by transfecting Gl261 cell line with the "Quad Cassette" expressing four peptides presented by H-2b class I or II molecules: human gp100$_{25-33}$, chicken OVA$_{257-264}$, chicken OVA$_{323-339}$, and mouse I-Ea$_{52-}$
30   $_{68}$. The Quad-Gl261 cell line also stably expresses luciferase, which allows the follow-up of tumor growth by bioluminescence.

183

The goal of this study was to assess the efficacy of a complex for use according to the present invention in the Quad-Gl261 glioblastoma model.

5   The effect of a complex for use according to the present invention, namely Z13Mad5Anaxa (cf. Example 7) was evaluated in the above described glioblastoma model. T cell homing at the tumor site was therefore analyzed in Gl261-Quad tumor-bearing mice vaccinated twice (Wk1 and Wk3) with Z13Mad5Anaxa vaccine. A group vaccinated with Z13Mad5 and Anaxa (equimolar to Z13Mad5Anaxa) administrated separately was used as control. Briefly, C57BL/6 mice were implanted i.c. (intracranially) with 5x10^5 Gl261-Quad tumor cells and vaccinated
10   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 Wk4, 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 (5-8 mice per group). Results are shown in Figure 34.

15

In general, 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. In all groups, there was a sensibly stronger accumulation of SIINFEKL-specific CD8 T cells in the BILs.

20

After two vaccinations with Z13Mad5Anaxa, the frequency of SIINFEKL-specific cells CD8+ T cells in the BILs was 2-fold higher (24%) than with Z13Mad5 + Anaxa (12%).

Next, cytokine secretion was assessed. To this end, C57BL/6 mice were implanted i.c. with
25   5x10^5 Gl261-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. Results are shown in Figure 35.

30   Despite heterogeneity, a high level of cytokine secretion was observed for brain-infiltrating CD8 T cells from mice vaccinated with Z13Mad5Anaxa. These results demonstrate that

184

Z13Mad5Anaxa vaccine was able to elicit a stronger SIINFEKL specific CD8 T cell immune response in the brain of tumor-bearing mice with potent effector function.

The results obtained are indicating that Z13Mad5Anaxa is efficacious for eliciting high brain

5   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:   Vaccine efficacy on survival in the Gl261-Quad glioblastoma model

10

In an independent experiment, the survival of control and Z13Mad5Anaxa-vaccinated mice was monitored. The therapeutic settings were three consecutive vaccinations with 2 nmol of Z13Mad5Anaxa at day 7, 21 and 35, post i.c. tumor implantation.

15   C57BL/6 mice were implanted i.c. with $5x10^5$ Gl261-Quad tumor cells and vaccinated three times (d7, d21 and d35) by s.c. injection of 2 nmol of Z13Mad5Anaxa. Mice were weight daily and euthanized when weight loss reached more than 15%. Results are shown in Figure 36.

20   The results show that Z13Mad5Anaxa therapeutic vaccination is more efficacious than the control group with a median survival prolonged by 10 days.

Example 22:   Vaccine efficacy in a subcutaneous tumor model – prophylactic setting

25

This study is based on the results obtained in Example 10 as shown in Figures 17 – 19.

To evaluate the effect of the Anaxa-conjugated construct proteins designed in Example 7 on tumor growth control, a benchmark tumor model was used, namely the s.c. implantation of

30   EG.7-OVA thymoma cells. In contrast to Example 10, wherein vaccination was performed on days 5 and 13, in the present study a prophylactic setting was evaluated , wherein mice were vaccinated 21 and 7 days before tumor implantation.

185

C57BL/6 mice were vaccinated twice (d-21 and d-7) by s.c. injection of 0.5 nmol of Z13Mad5Anaxa in the right flank and then implanted at day0 s.c. with 3×10^5 EG7-OVA tumor cells in the left flank and. Tumor size was measured with a caliper.

5

The results are shown in Figure 37 with tumor volume (Fig. 37 A) and survival rate (Fig. 37 B). The data is showing that prophylactic vaccination with Z13Mad5Anaxa is highly efficacious for controlling tumor growth and survival rate. The volume of the tumor is highly significantly decreased in mice treated with Z13Mad5Anaxa as compared to control mice. 10 The survival rate is highly significantly increased in mice treated with Z13Mad5Anaxa as compared to control mice.

Example 23: **Vaccine efficacy in a subcutaneous tumor model – therapeutic setting with**
15 **established tumor**

This study is based on the results obtained in Example 10 as shown in Figures 17 – 19 and on the results obtained in Example 22 shown in Figure 37. It was the goal of this study to evaluate the effect of Z13Mad5Anaxa (cf. Example 7) on an established tumor.

20

For this purpose, the s.c. model of B16-OVA melanoma cells was used. In this model tumor cells are spreading slowly, therefore allowing a bigger vaccination time window.

The first vaccination with the low dose of 0.5 nmol of Z13Mad5Anaxa was performed once 25 the tumor was established and visible i.e. at day 14 after tumor cell implantation. A second vaccination was done at day 21.

Thus, C57BL/6 mice were implanted s.c. with 1×10^5 B16-OVA tumor cells in the left flank and vaccinated twice (d14 and d21) by s.c. injection of 0.5 nmol of Z13Mad5Anaxa in the 30 right flank. Tumor growth and survival curves were monitored. Results are shown in Fig. 38.

186

The results indicate that Z13Mad5Anaxa efficaciously controls the growth of an established and visible tumor. Moreover, despite an established and visible tumor survival rates increased in mice treated with Z13Mad5Anaxa as compared to controls.

5

Example 24:   Vaccine efficacy in a subcutaneous tumor model – therapeutic setting: effect
                     of the CPP

The protocol of this study corresponds to the study described in Example 10, with the
10   difference that an additional group "Mad5Anaxa" (cf. Example 16) was evaluated.

Briefly, a benchmark tumor model was used, namely the s.c. implantation of EG.7-OVA thymoma cells. C57BL/6 mice were implanted s.c. with $3x10^5$ EG7-OVA tumor cells in the left flank. After tumor implantation, groups of 7 mice each were vaccinated s.c. in the right
15   flank at day 5 and 13 by subcutaneous injection of 0.5 nmol of either Z13Mad5Anaxa (group 1) or Mad5Anaxa (group 2) and compared to a control group. Tumor size was measured with a caliper. Results are shown in Figure 39.

The results show that the mice treated with Z13Mad5Anaxa show a significantly decreased
20   tumor volume and a significantly increased survival rate compared to both, control mice and mice treated with Mad5Anaxa, i.e. a construct without CPP. These results indicate that the presence of a CPP results in significantly decreased tumor volume and a significantly increased survival rate, i.e. in increased efficiency of vaccination. Therefore, the results indicate – together with the results obtained in Example 10 – that the presence of a CPP and
25   the TLR agonist exert a synergic effect on tumor growth and survival rate.

Example 25:   Comparison of the kinetic of immune responses with complexes having
                     different cell penetrating peptides
30

To investigate the effect of different CPPs in the complex for use according to the present invention the fusion protein Z13Mad5Anaxa as described above (cf. Example 7) was used.

187

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

Sequence:
MHHHHHHKRY KNRVASRKSR AKFKQLLQHY REVAAAKESL KISQAVHAAH AEINEAGREV VGVGALKVPR NQDWLGVPRF AKFASFEAQG ALANIAVDKA NLDVEQLESI INFEKLTEWT GSSTVHEILC KLSLEGDHST PPSAYGSVKP YTNFDAE
(SEQ ID NO: 33)

Z18Mad5Anaxa

Sequence:
MHHHHHHREV AAAKSSENDR LRLLLKESLK ISQAVHAAHA EINEAGREVV GVGALKVPRN QDWLGVPRFA KFASFEAQGA LANIAVDKAN LDVEQLESII NFEKLTEWTG SSTVHEILCK LSLEGDHSTP PSAYGSVKPY TNFDAE
(SEQ ID NO: 34)

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).

The results are shown in Figure 40. All groups vaccinated with Z13Mad5Anaxa, Z14Mad5Anaxa or Z18Mad5Anaxa showed an increased percentage of multimer-positive

188

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.

5

Example 26:   Comparison of T cell immune responses with complexes having different cell penetrating peptides

To investigate the CD8 T cell immune responses in more detail, C57BL/6 mice were assigned

10   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 (cf. Example 7) or Z14Mad5Anaxa (cf. Example 25). Nine days after the 5th

15   vaccination, mice were euthanized, organs recovered and multimer staining was performed to identify the percentage of SIINFEKL-specific CD8 T cells in the spleen, bone marrow and draining lymph nodes (inguinal and axillary).

The results are shown in Figure 41. Mice vaccinated with Z13Mad5Anaxa or with

20   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.

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

25   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.

The results are shown in Figure 42A. Mice vaccinated with Z13Mad5Anaxa showed a significant increase in IFN-γ producing cells compared to naïve mice. Mice vaccinated with

30   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).

189

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.

5

The results are shown in Figure 42B. 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).

10

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 43. Mice vaccinated with Z13Mad5Anaxa or with Z14Mad5Anaxa showed a similar increase in CD107a$^+$IFN-γ$^+$TNF-α$^+$ cells.

15

Example 27: Comparison of the effect of complexes having different cell penetrating peptides on tumor growth and survival in the EG.7-OVA s.c. model

20

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 3x10^5 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.

25

Results are shown in Figure 44. Vaccination with Z13Mad5Anaxa or with Z14Mad5Anaxa resulted in significantly decreased tumor volumes compared to control mice (Figure 44 A) as well as to significantly increased survival rates compared to control mice (Figure 44 B). Those

30

190

results indicate that both complexes, Z13Mad5Anaxa and Z14Mad5Anaxa, are able to significantly decrease tumor growth and to significantly prolong survival.

5   Example 28:   Comparison of the immune responses after vaccination with complexes
                  having different cell penetrating peptides

In this experiment the effect of different CPPs in the complex for use according to the present invention was investigated by using a complex with the TLR agonist "EDA". Therefore, the

10 fusion protein EDAZ13Mad5 as described above (cf. Example 1) was used.

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

15 antigens, and the TLR4 peptide agonist "EDA". Accordingly, the following constructs were additionally designed:

EDAZ14Mad5

20 Sequence:
    MHHHHHHNID RPKGLAFTDV DVDSIKIAWE SPQGQVSRYR VTYSSPEDGI RELFPAPDGE
    DDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQSTKRY KNRVASRKSR AKFKQLLQHY
    REVAAAKESL KISQAVHAAH AEINEAGREV VGVGALKVPR NQDWLGVPRF AKFASFEAQG
    ALANIAVDKA NLDVEQLESI INFEKLTEWT GS
25 (SEQ ID NO: 37)

EDAZ18Mad5

Sequence:
30 MHHHHHHNID RPKGLAFTDV DVDSIKIAWE SPQGQVSRYR VTYSSPEDGI RELFPAPDGE
    DDTAELQGLR PGSEYTVSVV ALHDDMESQP LIGIQSTREV AAAKSSENDR LRLLLKESLK

ISQAVHAAHA EINEAGREVV GVGALKVPRN QDWLGVPRFA KFASFEAQGA LANIAVDKAN
LDVEQLESII NFEKLTEWTG S
(SEQ ID NO: 38)

5   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
10   staining was performed (one experiment with 4 mice per group).

     The results are shown in Figure 45. All groups vaccinated with EDAZ13Mad5, EDAZ14Mad5
     or EDAZ18Mad5 showed an increased percentage of multimer-positive cells compared to the
     control group. These results indicate that complexes according to the present invention
15   having different cell penetrating peptides are able to elicit an immune response at different
     doses.

     ### Example 29:   Effect of EDAZ14Mad5 on tumor growth and survival in the EG.7-OVA s.c.
20   model

     To investigate the effect of EDAZ14Mad5 on tumor growth and survival the EG.7-OVA s.c.
     model was used (cf. Example 4 and Figures 9 – 11 for the effect of EDAZ13Mad5 in the same
     model).
25
     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.
30
     Results are shown in Figure 46. Similarly to EDAZ13Mad5 (cf. Example 4, Figures 9 – 11)
     vaccination with EDAZ14Mad5 resulted in significantly decreased tumor volumes compared

192

to control mice (Figure 46 A) as well as to significantly increased survival rates compared to control mice (Figure 46 B). Those results indicate that EDAZ14Mad5 is able to significantly decrease tumor growth and to significantly prolong survival – similarly to EDAZ13Mad5 (cf. Example 4, Figures 9 – 11).

5

Example 30:   Superior efficacy of Z13Mad5Anaxa fusion construct compared to Z13Mad5 and Anaxa in a glioblastoma model

10   To investigate the efficacy of a complex according to the present invention the glioblastoma model was chosen (cf. Example 20). Namely, Z13Mad5Anaxa (cf. Example 7; SEQ ID NO: 28) was administered to one group of mice, whereas Z13Mad5 (SEQ ID NO: 29) and Anaxa (SEQ ID NO: 15) were administered (both together) to another group of mice.

15   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 20   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).

25   Results are shown in Figure 47. 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. 47A). 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. 47B, p = 0.0539).

30

Next, cytokine secretion was assessed. To this end, C57BL/6 mice were implanted i.c. with 5×10^5 Gl261-Quad tumor cells and vaccinated twice (d7 and 21) by s.c. injection of 2 nmol

193

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.

5   Results are shown in Figure 48. 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.

10

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.

15   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.

20   **Example 31:   Effect of another antigenic cargo in the complex according to the present invention**

To investigate the effect of a different antigenic cargo ("Mad8"), another complex comprising a cell penetrating peptide, different antigens and a TLR peptide agonist was designed
25   ("Z13Mad8Anaxa"). Z13Mad8Anaxa differs from Z13Mad5Anaxa (described in Example 7) 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
30   underlined and the TLR peptide agonist "Anaxa" shown in italics:

194

KRYKNRVASR KSRAKFKQLL QHYREVAAAK SSENDRLRLLLK VTYHSPSYAY HQFERRAILN
RLVQFIKDRI SVVQALVLT*S TVHEILCKLS LEGDHSTPPS AYGSVKPYTN FDAE*
(SEQ ID NO: 39)

5   Naïve Balb/c mice (4 mice per group) were vaccinated four times s.c. (week0, week2, week4
     and week6 with 2 nmol of Z13Mad8Anaxa.

     To investigate the CD4 T cell responses after vaccination, one week after the 4th vaccination,
     mice were euthanized; organs recovered and *ex vivo* Elispot assay was performed on spleen
10   cells stimulated with gp70CD4 peptide (SEQ ID NO: 64) or gp70CD8 peptide (SEQ ID NO:
     65) in order to quantify IFN-γ-producing epitope-specific CD4 and CD8 T cells.

     The results are shown in Figure 49. Mice vaccinated with Z13Mad8Anaxa showed a
     significant increase in IFN-γ-producing cells compared to naïve mice. These data show that
15   Z13Mad8Anaxa vaccine was able to elicit potent epitope-specific CD8 and CD4 T cell
     immune response and thus that the complex according to the present invention is able to
     elicit self-antigen immune response.

20   **Example 32:   Effect of another antigenic cargo in the complex according to the present
                      invention**

     To investigate the effect of a further different antigenic cargo ("Mad11"), another complex
     comprising a cell penetrating peptide, different antigens and a TLR peptide agonist was
25   designed ("Z13Mad11Anaxa"). Z13Mad11Anaxa differs from Z13Mad5Anaxa (described in
     Example 7) in the antigenic cargoes. In particular, "Z13Mad11Anaxa" is a fusion protein
     comprising the cell-penetrating peptide "Z13", the antigenic cargo "MAD11" comprising two
     CD8 epitopes of surviving as described in Derouazi M, Wang Y, Marlu R, et al. Optimal
     epitope composition after antigen screening using a live bacterial delivery vector: Application
30   to TRP-2. *Bioengineered Bugs*. 2010;1(1):51-60. doi:10.4161/bbug.1.1.9482, and the TLR
     peptide agonist "Anaxa". In the following, the amino acid sequence of Z13Mad11Anaxa is

195

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

5   KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSENDRLRLLLKNYRI<u>ATFKNWPFL</u>EDCAMEEL<u>T</u>
    <u>VSEFLKL</u>DRQRSTVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE
    (SEQ ID NO: 40)

Naïve C57BL/6 mice (5 mice per group) were implanted i.v. with 1×10^5 B16 melanoma tumor cells and vaccinated twice (d0 and d10) by subcutaneous injection of 1 nmol of
10 Z13Mad11Anaxa. On day18 mice were euthanized, organs recovered and *ex vivo* Elispot assay was performed on spleen cells stimulated with survivin peptides survivin20-28 (SEQ ID NO: 67) and survivin97-104: (SEQ ID NO: 68) in order to quantify IFN-γ producing survivin-specific T cells.

15 The results are shown in Figure 50. Mice vaccinated with Z13Mad11Anaxa showed less metastasis compared to naïve mice (Fig. 50A). Moreover, in the spleen of mice vaccinated with Z13Mad11Anaxa significantly higher numbers of IFN-γ producing survivin-specific T cells were observed (Fig. 49B).

20 The results obtained show that Z13Mad11Anaxa is efficacious for reducing the number of metastasis and Z13Mad11Anaxa is able to promote the secretion of cytokines by antigen-specific CD8 T cells in the spleen.

25 **Example 33:   Effect of another antigenic cargo in the complex according to the present invention**

To investigate the effect of a further different antigenic cargo ("Mad9"), another complex comprising a cell penetrating peptide, a different antigen and a TLR peptide agonist was
30 designed ("Z13Mad9Anaxa"). Z13Mad9Anaxa differs from Z13Mad5Anaxa (described in Example 7) in the antigenic cargo. In particular, "Z13Mad9Anaxa" is a fusion protein comprising the cell-penetrating peptide "Z13", the antigenic cargo "Mad9" comprising the

196

neoantigen 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 Z13Mad9Anaxa is shown with the cell-penetrating peptide "Z13" shown underlined and the TLR peptide agonist "Anaxa" shown in italics:

KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSENDRLRLLLKHLELASMTNMELMSSIVSTVHEI
LCKLSLEGDHSTPPSAYGSVKPYTNFDAE
(SEQ ID NO: 41)

Naïve C57BL/6 mice (4 mice per group) were vaccinated four times s.c. (week0, week2, week4 and week6 with 2 nmol of Z13Mad9Anaxa. To investigate the CD8 T cell responses after vaccination, one week after the 4th vaccination, mice were euthanized, organs recovered and Elispot assay was performed on spleen cells after a 7-day in vitro restimulation with stimulated with adpgk peptide (SEQ ID NO: 66) in order to quantify to quantify IFN-γ-producing epitope-specific CD8 T cells .

The results are shown in Figure 51. Mice vaccinated with Z13Mad9Anaxa showed a significant increase in effector neoantigen-specific CD8 T cells compared to naïve mice.

Example 34:   Comparison of the immune responses after vaccination with complexes
              having different cell penetrating peptides

In this experiment the effect of a further different CPP in the complex according to the present invention was investigated by using a complex with the TLR agonist "Anaxa". Therefore, the fusion protein Z13Mad5Anaxa as described above (cf. Example 7, SEQ ID NO: 28) was used.

In addition, a further fusion protein was designed, which comprise the TAT CPP combined to furin linkers as described in Lu et al., Multiepitope trojan antigen peptide vaccines for the induction of antitumor CTL and Th immune responses J. Immunol., 172 (2004), pp. 4575–4582. That fusion protein also comprises the protein "MAD5", which consists of different

197

CD8$^+$ and CD4$^+$ epitopes from various antigens, and the TLR4 peptide agonist "Anaxa". Accordingly, the following construct was additionally designed:

TatFMad5Anaxa

5

Sequence:
RKKRRQRRRVKRISQAVHAAHAEINEAGRRVKRKVPRNQDWLRVKRASFEAQGALANIAVD
KARVKRSIINFEKLRVKRSTVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE

10   (SEQ ID NO: 46)

C57BL/6 mice were assigned to three different groups (8 mice per group): one group receiving 2 nmol of Z13Mad5Anaxa, one group receiving 2 nmol of TatFMad5Anaxa and a respective control. The mice were vaccinated two times (Week0 and Week2) s.c. with either 2 nmol of
15   Z13Mad5Anaxa or 2 nmol of TatFMad5Anaxa. Mice were bled 7 days after the 2nd vaccination and multimer staining was performed (8 mice per group).

The results are shown in Figure 52. Mice vaccinated with Z13Mad5Anaxa or TatFMad5Anaxa showed an increased percentage of multimer-positive cells compared to the control group.
20   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. However, the CPP derived from ZEBRA (Z13) was better than the TAT CPP.

25   Example 35:   Superior efficacy of Z13Mad5Anaxa fusion construct compared to Z13Mad5
                     and Anaxa in naïve mice

Next, the efficacy of a complex according to the present invention was investigated in naïve mice. Namely, Z13Mad5Anaxa (cf. Example 7; SEQ ID NO: 28) was administered to one
30   group of mice, whereas Z13Mad5 (SEQ ID NO: 29) and Anaxa (SEQ ID NO: 15) were administered (both together) to another group of mice.

198

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

5   group).

Results are shown in Figure 53. 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. 53).

10

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.

15

Example 36:   Effect of another antigenic cargo in the complex according to the present invention

To investigate the effect of a further different antigenic cargo ("Mad12"), another complex

20   comprising a cell penetrating peptide, a different antigen and a TLR peptide agonist was designed ("Z13Mad12Anaxa"). Z13Mad12Anaxa differs from Z13Mad5Anaxa (described in Example 7) in the antigenic cargo. In particular, "Z13Mad12Anaxa" is a fusion protein comprising the 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

25   MC-38 tumor cell line, and the TLR peptide agonist "Anaxa". In the following, the amino acid sequence of Z13Mad12Anaxa is shown:

KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSENDRLRLLLKLFRAAQLANDVVLQIMEHLELA
SMTNMELMSSIVVISASIIVFNLLELEGSTVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE

30

(SEQ ID NO: 69)

199

Naïve C57BL/6 mice (4 mice per group) were vaccinated twice s.c. (week0, week2) with 2 nmol of Z13Mad12Anaxa. To investigate the CD8 T cell responses after vaccination, one week after the 2nd vaccination, the blood was analyzed, whereby neoantigen reps1-specific CD8 T cells were quantified in blood by multimer staining (4 mice per group).

5

The results are shown in Figure 54. Mice vaccinated with Z13Mad12Anaxa showed a significant increase in effector neoantigen-specific CD8 T cells compared to naïve mice.

10   Example 37:   In vitro human dendritic cell maturation

The goal of this study was to investigate the capacity of a complex for use according to the present invention ("Z13Mad5Anaxa", SEQ ID NO: 28, cf. Example 7) to induce maturation of dendritic cells in comparison to a complex lacking a TLR peptide agonist ("Z13Mad5",

15   SEQ ID NO: 29, cf. Example 1).

The Z13Mad5Anaxa polypeptide and the Z13Mad5 polypeptide were investigated for their capacity to induce human dendritic cell (DC) maturation. After incubation over night with 300nM of protein, activation markers expression (CD86, CD80, CD83 and HLA-DR) was

20   assessed on the human DCs by FACS (Figure 55). Same buffer volumes of each protein were used as negative controls.

Results are shown in Fig. 55. Whereas Z13Mad5Anaxa induced maturation of human DCs, shown by the up-regulation of CD86, HLADR and CD83, Z13Mad5 was not able to activate

25   human DCs. These results indicate that the Anaxa portion of the protein is responsible for the up-regulation of the activation markers on the human DCs.

200

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	MMDPNSTSEDVKFTPDPYQVPFVQAFDQATRV YQDLGGPSQAPLPCVLWPVLPEPLPQGQLTAY HVSTAPTGSWFSAPQPAPENAYQAYAAPQLFP VSDITQNQQTNQAGGEAPQPGDNSTVQTAA AVVFACPGANQGQQLADIGVPQPAPVAAPAR RTRKPQQPESLEECDSELEIKRYKNRVASRKCRAK FKQLLQHYREVAAAKSSENDRLRLLLKQMCPSL DVDSIIPRTPDVLHEDLLNF	ZEBRA amino acid sequence (natural sequence from Epstein - Barr virus (EBV)) (YP_401673)
SEQ ID NO: 4	KRYKNRVASRKCRAKFKQLLQHYREVAAAKSSE NDRLRLLLKQMC	CPP1 (Z11)
SEQ ID NO: 5	KRYKNRVASRKCRAKFKQLLQHYREVAAAKSSE NDRLRLLLK	CPP2 (Z12)
SEQ ID NO: 6	KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSE NDRLRLLLK	CPP3 (Z13)
SEQ ID NO: 7	KRYKNRVASRKSRAKFKQLLQHYREVAAAK	CPP4 (Z14)
SEQ ID NO: 8	KRYKNRVASRKSRAKFK	CPP5 (Z15)
SEQ ID NO: 9	QHYREVAAAKSSEND	CPP6 (Z16)
SEQ ID NO: 10	QLLQHYREVAAAK	CPP7 (Z17)
SEQ ID NO: 11	REVAAAKSSENDRLRLLLK	CPP8 (Z18)
SEQ ID NO: 12	KRYKNRVA	CPP9 (Z19)
SEQ ID NO: 13	VASRKSRAKFK	CPP10 (Z20)
SEQ ID NO: 14	ESLKISQAVHAAHAEINEAGREVVGVGAL KVPRNQDWLGVPRFAKFASFEAQGALA NIAVDKANLDVEQLESIINFEKLTEWTGS	MAD5 cargo
SEQ ID NO: 15	STVHEILCKLSLEGDHSTPPSAYGSVKPYTNFDAE	TLR2 peptide agonist Anaxa
SEQ ID NO: 16	DDDK	enterokinase target site
SEQ ID NO: 17	IEDGR	factor Xa target site
SEQ ID NO: 18	LVPRGS	thrombin target site
SEQ ID NO: 19	ENLYFQG	protease TEV target site
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 WESPQGQVSRYRVTYSSPEDGIRELFPAP DGEDDTAELQGLRPGSEYTVSVVALHDD MESQPLIGIQSTKRYKNRVASRKSRAKFKQ LLQHYREVAAAKSSENDRLRLLLKESLKISQ AVHAAHAEINEAGREVVGVGALKVPRN QDWLGVPRFAKFASFEAQGALANIAVDK ANLDVEQLESIINFEKLTEWTGS	EDAZ13Mad5
SEQ ID NO: 27	MHHHHHHSTVHEILCKLSLEGDHSTPPSA YGSVKPYTNFDAEKRYKNRVASRKSRAKF KQLLQHYREVAAAKSSENDRLRLLLKESLKI SQAVHAAHAEINEAGREVVGVGALKVPR NQDWLGVPRFAKFASFEAQGALANIAVD KANLDVEQLESIINFEKLTEWTGS	AnaxaZ13Mad5
SEQ ID NO: 28	MHHHHHHKRYKNRVASRKSRAKFKQLL QHYREVAAAKSSENDRLRLLLKESLKISQA VHAAHAEINEAGREVVGVGALKVPRNQD WLGVPRFAKFASFEAQGALANIAVDKANL DVEQLESIINFEKLTEWTGSSTVHEILCKLSL EGDHSTPPSAYGSVKPYTNFDAE	Z13Mad5Anaxa
SEQ ID NO: 29	MHHHHHHKRYKNRVASRKSRAKFKQLL QHYREVAAAKSSENDRLRLLLKESLKISQAV HAAHAEINEAGREVVGVGALKVPRNQD WLGVPRFAKFASFEAQGALANIAVDKANL DVEQLESIINFEKLTEWTGS	Z13Mad5
SEQ ID NO: 30	MHHHHHHESLKISQAVHAAHAEINEAGREV VGVGALKVPRNQDWLGVPRFAKFASFEAQ GALANIAVDKANLDVEQLESIINFEKLTEWTG S	Mad5
SEQ ID NO: 31	MHHHHHHNIDRPKGLAFTDVDVDSIKIA WESPQGQVSRYRVTYSSPEDGIRELFPAP DGEDDTAELQGLRPGSEYTVSVVALHDD MESQPLIGIQSTESLKISQAVHAAHAEINE AGREVVGVGALKVPRNQDWLGVPRFAK FASFEAQGALANIAVDKANLDVEQLESIIN FEKLTEWTGS	EdaMad5

202

SEQ ID NO: 32	MHHHHHHESLKISQAVHAAHAEINEAG REVVGVGALKVPRNQDWLGVPRFAKFAS FEAQGALANIAVDKANLDVEQLESIINFEK LTEWTGSSTVHEILCKLSLEGDHSTPPSAY GSVKPYTNFDAE	Mad5Anaxa
SEQ ID NO: 33	MHHHHHHKRYKNRVASRKSRAKFKQLL QHYREVAAAKESLKISQAVHAAHAEINE AGREVVGVGALKVPRNQDWLGVPRFA KFASFEAQGALANIAVDKANLDVEQLESI INFEKLTEWTGSSTVHEILCKLSLEGDHST PPSAYGSVKPYTNFDAE	Z14 Mad5Anaxa
SEQ ID NO: 34	MHHHHHHREVAAAKSSENDRLRLLLKES LKISQAVHAAHAEINEAGREVVGVGALKV PRNQDWLGVPRFAKFASFEAQGALANIA VDKANLDVEQLESIINFEKLTEWTGSSTVH EILCKLSLEGDHSTPPSAYGSVKPYTNFDA E	Z18 Mad5Anaxa
SEQ ID NO: 35	SIINFEKL	SIINFEKL OVACD8
SEQ ID NO: 36	ISQAVHAAHAEINEAGR	OVACD4 peptide
SEQ ID NO: 37	MHHHHHHHNIDRPKGLAFTDVDVDSIKIA WESPQGQVSRYRVTYSSPEDGIRELFPAP DGEDDTAELQGLRPGSEYTVSVVALHDD MESQPLIGIQSTKRYKNRVASRKSRAKFKQ LLQHYREVAAAKESLKISQAVHAAHAEIN EAGREVVGVGALKVPRNQDWLGVPRFA KFASFEAQGALANIAVDKANLDVEQLESII NFEKLTEWTGS	EDAZ14Mad5
SEQ ID NO: 38	MHHHHHHHNIDRPKGLAFTDVDVDSIKIA WESPQGQVSRYRVTYSSPEDGIRELFPAP DGEDDTAELQGLRPGSEYTVSVVALHDD MESQPLIGIQSTREVAAAKSSENDRLRLLL KESLKISQAVHAAHAEINEAGREVVGVGA LKVPRNQDWLGVPRFAKFASFEAQGALA NIAVDKANLDVEQLESIINFEKLTEWTGS	EDAZ18Mad5
SEQ ID NO: 39	KRYKNRVASRKSRAKFKQLLQHYREVAAA KSSENDRLRLLLKVTYHSPSYAYHQFERRA ILNRLVQFIKDRISVVQALVLTSTVHEILCK LSLEGDHSTPPSAYGSVKPYTN FDAE	Z13Mad8Anaxa
SEQ ID NO: 40	KRYKNRVASRKSRAKFKQLLQHYREVAAA KSSENDRLRLLLKNYRIATFKNWPFLEDCA MEELTVSEFLKLDRQRSTVHEILCKLSLEGD HSTPPSAYGSVKPYTNFDAE	Z13Mad11Anaxa

SEQ ID NO: 41	KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSEN DRLRLLLKHLELASMTNMELMSSIVSTVHEILCKLS LEGDHSTPPSAYGSVKPYTNFDAE	Z13Mad9Anaxa
SEQ ID NO: 42	HLELASMTNMELMSSIV	Mad9
SEQ ID NO: 43	VTYHSPSYAYHQFERRAILN	Mad8
SEQ ID NO: 44	NYRIATFKNWPFLEDCAMEELTVSEFLKLD	Mad11
SEQ ID NO: 45	NIDRPKGLAFTDVDVDSIKIAWESPQGQVSRYR VTYSSPEDGIRELFPAPDGEDDTAELQGLRPGSEY TVSVVALHDDMESQPLIGIQST	EDA
SEQ ID NO: 46	RKKRRQRRRRVKRISQAVHAAHAEINEAGRRVK RKVPRNQDWLRVKRASFEAQGALANIAVDKAR VKRSIINFEKLRVKRSTVHEILCKLSLEGDHSTPPSA YGSVKPYTNFDAE	TatFMad5Anaxa
SEQ ID NO: 47	LEEKKGNYVVTDHC	EGFRvIII epitope
SEQ ID NO: 48	MELQAARACFALLWGCALAAAAAAQGKEVVLL DFAAAGGELGWLTHPYGKGWDLMQNIMND MPIYMYSVCNVMSGDQDNWLRTNWVYRGEA ERIFIELKFTVRDCNSFPGGASSCKETFNLYYAESD LDYGTNFQKRLFTKIDTIAPDEITVSSDFEARHVK LNVEERSVGPLTRKGFYLAFQDIGACVALLSVRV YYKKCPELLQGLAHFPETIAGSDAPSLATVAGTC VDHAVVPPGGEEPRMHCAVDGEWLVPIGQCL CQAGYEKVEDACQACSPGFFKFEASESPCLECPE HTLPSPEGATSCECEEGFFRAPQDPASMPCTRPPS APHYLTAVGMGAKVELRWTPPQDSGGREDIVY SVTCEQCWPESGECGPCEASVRYSEPPHGLTRTS VTVSDLEPHMNYTFTVEARNGVSGLVTSRSFRTA SVSINQTEPPKVRLEGRSTTSLSVSWSIPPPQQSR VWKYEVTYRKKGDSNSYNVRRTEGFSVTLDDLA PDTTYLVQVQALTQEGQGAGSKVHEFQTLSPE GSGNLAVIGGVAVGVVLLLVLAGVGFFIHRRRK NQRARQSPEDVYFSKSEQLKPLKTYVDPHTYEDP NQAVLKFTTEIHPSCVTRQKVIGAGEFGEVYKG MLKTSSGKKEVPVAIKTLKAGYTEKQRVDFLGEA GIMGQFSHHNIIRLEGVISKYKPMMIITEYMENG ALDKFLREKDGEFSVLQLVGMLRGIAAGMKYLA NMNYVHRDLAARNILVNSNLVCKVSDFGLSRVL EDDPEATYTTSGGKIPIRWTAPEAISYRKFTSASD VWSFGIVMWEVMTYGERPYWELSNHEVMKAIN DGFRLPTPMDCPSAIYQLMMQCWQQERARRP KFADIVSILDKLIRAPDSLKTLADFDPRVSIRLPSTS GSEGVPFRTVSEWLESIKMQQYTEHFMAAGYTAI EKVVQMTNDDIKRIGVRLPGHQKRIAYSLLGLK DQVNTVGIPI	EphA2

204

SEQ ID NO: 49	MDLVLKRCLLHLAVIGALLAVGATKVPRNQDW LGVSRQLRTKAWNRQLYPEWTEAQRLDCWRG GQVSLKVSNDGPTLIGANASFSIALNFPGSQKVL PDGQVIWVNNTIINGSQVWGGQPVYPQETDD ACIFPDGGPCPSGSWSQKRSFVYVWKTWGQY WQVLGGPVSGLSIGTGRAMLGTHTMEVTVYHR RGSRSYVPLAHSSSAFTITDQVPFSVSVSQLRALD GGNKHFLRNQPLTFALQLHDPSGYLAEADLSYT WDFGDSSGTLISRALVVTHTYLEPGPVTAQVVL QAAIPLTSCGSSPVPGTTDGHRPTAEAPNTTAG QVPTTEVVGTTPGQAPTAEPSGTTSVQVPTTEVI STAPVQMPTAESTGMTPEKVPVSEVMGTTLAEM STPEATGMTPAEVSIVVLSGTTAAQVTTTEWVET TARELPIPEPEGPDASSIMSTESITGSLGPLLDGTAT LRLVKRQVPLDCVLYRYGSFSVTLDIVQGIESAEIL QAVPSGEGDAFELTVSCQGGLPKEACMEISSPG CQPPAQRLCQPVLPSPACQLVLHQILKGGSGTY CLNVSLADTNSLAVVSTQLIMPGQEAGLGQVPL IVGILLVLMAVVLASLIYRRRLMKQDFSVPQLPHS SSHWLRLPRIFCSCPIGENSPLLSGQQV	Gp100

205

| SEQ ID NO: 50 | MELAALCRWGLLLALLPPGAASTQVCTGTDMKL RLPASPETHLDMLRHLYQGCQVVQGNLELTYLP TNASLSFLQDIQEVQGYVLIAHNQVRQVPLQRL RIVRGTQLFEDNYALAVLDNGDPLNNTTPVTGA SPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTI LWKDIFHKNNQLALTLIDTNRSRACHPCSPMCK GSRCWGESSEDCQSLTRTVCAGGCARCKGPLPT DCCHEQCAAGCTGPKHSDCLACLHFNHSGICE LHCPALVTYNTDTFESMPNPEGRYTFGASCVTA CPYNYLSTDVGSCTLVCPLHNQEVTAEDGTQRC EKCSKPCARVCYGLGMEHLREVRAVTSANIQEFA GCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQV FETLEEITGYLYISAWPDSLPDLSVFQNLQVIRGRI LHNGAYSLTLQGLGISWLGLRSLRELGSGLALIH HNTHLCFVHTVPWDQLFRNPHQALLHTANRPE DECVGEGLACHQLCARGHCWGPGPTQCVNCS QFLRGQECVEECRVLQGLPREYVNARHCLPCHP ECQPQNGSVTCFGPEADQCVACAHYKDPPFCV ARCPSGVKPDLSYMPIWKFPDEEGACQPCPINC THSCVDLDDKGCPAEQRASPLTSIISAVVGILLVV VLGVVFGILIKRRQQKIRKYTMRRLLQETELVEPL TPSGAMPNQAQMRILKETELRKVKVLGSGAFGT VYKGIWIPDGENVKIPVAIKVLRENTSPKANKEIL DEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLM PYGCLLDHVRENRGRLGSQDLLNWCMQIAKG MSYLEDVRLVHRDLAARNVLVKSPNHVKITDFG LARLLDIDETEYHADGGKVPIKWMALESILRRRFT HQSDVWSYGVTVWELMTFGAKPYDGIPAREIP DLLEKGERLPQPPICTIDVYMIMVKCWMIDSECR PRFRELVSEFSRMARDPQRFVVIQNEDLGPASPL DSTFYRSLLEDDDMGDLVDAEEYLVPQQGFFCP DPAPGAGGMVHHRHRSSSTRSGGGDLTLGLEP SEEEAPRSPLAPSEGAGSDVFDGDLGMGAAKGL QSLPTHDPSPLQRYSEDPTVPLPSETDGYVAPLT CSPQPEYVNQPDVRPQPPSPREGPLPAARPAGA TLERPKTLSPGKNGVVKDVFAFGGAVENPEYLTP QGGAAPQPHPPPAFSPAFDNLYYWDQDPPER GAPPSTFKGTPTAENPEYLGLDVPV | Her2/neu |

SEQ ID NO: 51	MPRAPRCRAVRSLLRSHYREVLPLATFVRRLGPQ GWRLVQRGDPAAFRALVAQCLVCVPWDARPP PAAPSFRQVSCLKELVARVLQRLCERGAKNVLAF GFALLDGARGGPPEAFTTSVRSYLPNTVTDALRG SGAWGLLLRRVGDDVLVHLLARCALFVLVAPSC AYQVCGPPLYQLGAATQARPPPHASGPRRRLG CERAWNHSVREAGVPLGLPAPGARRRGGSASRS LPLPKRPRRGAAPEPERTPVGQGSWAHPGRTRG PSDRGFCVVSPARPAEEATSLEGALSGTRHSHPS VGRQHHAGPPSTSRPPRPWDTPCPPVYAETKHF LYSSGDKEQLRPSFLLSSLRPSLTGARRLVETIFLGS RPWMPGTPRRLPRLPQRYWQMRPLFLELLGNH AQCPYGVLLKTHCPLRAAVTPAAGVCAREKPQ GSVAAPEEEDTDPRRLVQLLRQHSSPWQVYGFV RACLRRLVPPGLWGSRHNERRFLRNTKKFISLGK HAKLSLQELTWKMSVRDCAWLRRSPGVGCVPA AEHRLREEILAKFLHWLMSVYVVELLRSFFYVTETT FQKNRLFFYRKSVWSKLQSIGIRQHLKRVQLREL SEAEVRQHREARPALLTSRLRFIPKPDGLRPIVNM DYVVGARTFRREKRAERLTSRVKALFSVLNYERAR RPGLLGASVLGLDDIHRAWRTFVLRVRAQDPPP ELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTY CVRRYAVVQKAAHGHVRKAFKSHVSTLTDLQP YMRQFVAHLQETSPLRDAVVIEQSSSLNEASSGL FDVFLRFMCHHAVRIRGKSYVQCQGIPQGSILST LLCSLCYGDMENKLFAGIRRDGLLLRLVDDFLLV TPHLTHAKTFLRTLVRGVPEYGCVVNLRKTVVNF PVEDEALGGTAFVQMPAHGLFPWCGLLLDTRTL EVQSDYSSYARTSIRASLTFNRGFKAGRNMRRKL FGVLRLKCHSLFLDLQVNSLQTVCTNIYKILLLQA YRFHACVLQLPFHQQVWKNPTFFLRVISDTASL CYSILKAKNAGMSLGAKGAAGPLPSEAVQWLC HQAFLLKLTRHRVTYVPLLGSLRTAQTQLSRKLP GTTLTALEAAANPALPSDFKTILD	hTert
SEQ ID NO: 52	MGAPTLPPAWQPFLKDHRISTFKNWPFLEGCAC TPERMAEAGFIHCPTENEPDLAQCFFCFKELEGW EPDDDPIEEHKKHSSGCAFLSVKKQFEELTLGEFL KLDRERAKNKIAKETNNKKKEFEETAKKVRRAIEQ LAAMD	survivin
SEQ ID NO: 53	RISTFKNWPF	survivin epitope

SEQ ID NO: 54	MSPLWWGFLLSCLGCKILPGAQGQFPRVCMTV DSLVNKECCPRLGAESANVCGSQQGRGQCTEV RADTRPWSGPYILRNQDDRELWPRKFFHRTCKC TGNFAGYNCGDCKFGWTGPNCERKKPPVIRQ NIHSLSPQEREQFLGALDLAKKRVHPDYVITTQH WVGLLGPNGTQPQFANCSVYDFFVWLHYYSV RDTLLGGFFPWLKVYYYRFVIGLRVWQWEVISC KLIKRATTRQP	TRP-2
SEQ ID NO: 55	MGVKASQTGFVVLVLLQCCSAYKLVCYYTSWS QYREGDGSCFPDALDRFLCTHIIYSFANISNDHI DTWEWNDVTLYGMLNTLKNRNPNLKTLLSVG GWNFGSQRFSKIASNTQSRRTFIKSVPPFLRTHG FDGLDLAWLYPGRRDKQHFTTLIKEMKAEFIKEA QPGKKQLLLSAALSAGKVTIDSSYDIAKISQHLDF ISIMTYDFHGAWRGTTGHHSPLFRGQEDASPDR FSNTDYAVGYMLRLGAPASKLVMGIPTFGRSFTL ASSETGVGAPISGPGIPGRFTKEAGTLAYYEICDFL RGATVHRILGQQVPYATKGNQWVGYDDQESV KSKVQYLKDRQLAGAMVWALDLDDFQGSFCG QDLRFPLTNAIKDALAAT	YKL-40

208

| SEQ ID NO: 56 | MAQLFLPLLAALVLAQAPAALADVLEGDSSEDR
AFRVRIAGDAPLQGVLGGALTIPCHVHYLRPPPS
RRAVLGSPRVKWTFLSRGREAEVLVARGVRVKV
NEAYRFRVALPAYPASLTDVSLALSELRPNDSGIY
RCEVQHGIDDSSDAVEVKVKGVVFLYREGSARY
AFSFSGAQEACARIGAHIATPEQLYAAYLGGYEQ
CDAGWLSDQTVRYPIQTPREACYGDMDGFPG
VRNYGVVDPDDLYDVYCYAEDLNGELFLGDPP
EKLTLEEARAYCQERGAEIATTGQLYAAWDGGL
DHCSPGWLADGSVRYPIVTPSQRCGGGLPGVK
TLFLFPNQTGFPNKHSRFNVYCFRDSAQPSAIPE
ASNPASNPASDGLEAIVTVTETLEELQLPQEATESE
SRGAIYSIPIMEDGGGGSSTPEDPAEAPRTLLEFET
QSMVPPTGFSEEEGKALEEEEKYEDEEEKEEEEEEE
VEDEALWAWPSELSSPGPEASLPTEPAAQEESLSQ
APARAVLQPGASPLPDGESEASRPPRVHGPPTET
LPTPRERNLASPSPSTLVEAREVGEATGGPELSGV
PRGESEETGSSEGAPSLLPATRAPEGTRELEAPSED
NSGRTAPAGTSVQAQPVLPTDSASRGGVAVVP
ASGDCVPSPCHNGGTCLEEEEGVRCLCLPGYGG
DLCDVGLRFCNPGWDAFQGACYKHFSTRRSW
EEAETQCRMYGAHLASISTPEEQDFINNRYREYQ
WIGLNDRTIEGDFLWSDGVPLLYENWNPGQPD
SYFLSGENCVVMVWHDQGQWSDVPCNYHLS
YTCKMGLVSCGPPPELPLAQVFGRPRLRYEVDTV
LRYRCREGLAQRNLPLIRCQENGRWEAPQISCVP
RRPARALHPEEDPEGRQGRLLGRWKALLIPPSSP
MPGP | brevican |

209

| SEQ ID NO: 57 | MSRPQGLLWLPLLFTPVCVMLNSNVLLWLTALAI KFTLIDSQAQYPVVNTNYGKIRGLRTPLPNEILGP VEQYLGVPYASPPTGERRFQPPEPPSSWTGIRNT TQFAAVCPQHLDERSLLHDMLPIWFTANLDTLM TYVQDQNEDCLYLNIYVPTEDDIHDQNSKKPV MVYIHGGSYMEGTGNMIDGSILASYGNVIVITIN YRLGILGFLSTGDQAAKGNYGLLDQIQALRWIE ENVGAFGGDPKRVTIFGSGAGASCVSLLTLSHYS EGLFQKAIIQSGTALSSWAVNYQPAKYTRILADK VGCNMLDTTDMVECLRNKNYKELIQQTITPATY HIAFGPVIDGDVIPDDPQILMEQGEFLNYDIML GVNQGEGLKFVDGIVDNEDGVTPNDFDFSVSN FVDNLYGYPEGKDTLRETIKFMYTDWADKENPE TRRKTLVALFTDHQWVAPAVATADLHAQYGSP TYFYAFYHHCQSEMKPSWADSAHGDEVPYVFG IPMIGPTELFSCNFSKNDVMLSAVVMTYWTNFA KTGDPNQPVPQDTKFIHTKPNRFEEVAWSKYNP KDQLYLHIGLKPRVRDHYRATKVAFWLELVPHL HNLNEIFQYVSTTTKVPPPDMTSFPYGTRRSPAKI WPTTKRPAITPANNPKHSKDPHKTGPEDTTVLIE TKRDYSTELSVTIAVGASLLFLNILAFAALYYKKDK RRHETHRRPSPQRNTTNDIAHIQNEEIMSLQMK QLEHDHECESLQAHDTLRLTCPPDYTLTLRRSPD DIPLMTPNTITMIPNTLTGMQPLHTFNTFSGGQ NSTNLPHGHSTTRV | Neuroligin 4 |

210

SEQ ID NO: 58	MRILKRFLACIQLLCVCRLDWANGYYRQQRKLV	PTPRz1
	EEIGWSYTGALNQKNWGKKYPTCNSPKQSPINI	
	DEDLTQVNVNLKKLKFQGWDKTSLENTFIHNT	
	GKTVEINLTNDYRVSGGVSEMVFKASKITFHWG	
	KCNMSSDGSEHSLEGQKFPLEMQIYCFDADRFS	
	SFEEAVKGKGKLRALSILFEVGTEENLDFKAIIDGV	
	ESVSRFGKQAALDPFILLNLLPNSTDKYYIYNGSL	
	TSPPCTDTVDWIVFKDTVSISESQLAVFCEVLTM	
	QQSGYVMLMDYLQNNFREQQYKFSRQVFSSYT	
	GKEEIHEAVCSSEPENVQADPENYTSLLVTWERP	
	RVVYDTMIEKFAVLYQQLDGEDQTKHEFLTDGY	
	QDLGAILNNLLPNMSYVLQIVAICTNGLYGKYS	
	DQLIVDMPTDNPELDLFPELIGTEEIIKEEEEGKDIE	
	EGAIVNPGRDSATNQIRKKEPQISTTTHYNRIGT	
	KYNEAKTNRSPTRGSEFSGKGDVPNTSLNSTSQP	
	VTKLATEKDISLTSQTVTELPPHTVEGTSASLNDG	
	SKTVLRSPHMNLSGTAESLNTVSITEYEEESLLTSFK	
	LDTGAEDSSGSSPATSAIPFISENISQGYIFSSENPE	
	TITYDVLIPESARNASEDSTSSGSEESLKDPSMEGN	
	VWFPSSTDITAQPDVGSGRESFLQTNYTEIRVDE	
	SEKTTKSFSAGPVMSQGPSVTDLEMPHYSTFAYF	
	PTEVTPHAFTPSSRQQDLVSTVNVVYSQTTQPV	
	YNGETPLQPSYSSEVFPLVTPLLLDNQILNTTPAA	
	SSSDSALHATPVFPSVDVSFESILSSYDGAPLLPFSS	
	ASFSSELFRHLHTVSQILPQVTSATESDKVPLHASL	
	PVAGGDLLLEPSLAQYSDVLSTTHAASETLEFGSE	
	SGVLYKTLMFSQVEPPSSDAMMHARSSGPEPSY	
	ALSDNEGSQHIFTVSYSSAIPVHDSVGVTYQGSL	
	FSGPSHIPIPKSSLITPTASLLQPTHALSGDGEWSG	
	ASSDSEFLLPDTDGLTALNISSPVSVAEFTYTTSVF	
	GDDNKALSKSEIIYGNETELQIPSFNEMVYPSEST	
	VMPNMYDNVNKLNASLQETSVSISSTKGMFPGS	
	LAHTTTKVFDHEISQVPENNFSVQPTHTVSQAS	
	GDTSLKPVLSANSEPASSDPASSEMLSPSTQLLFYE	
	TSASFSTEVLLQPSFQASDVDTLLKTVLPAVPSDPI	
	LVETPKVDKISSTMLHLIVSNSASSENMLHSTSVP	
	VFDVSPTSHMHSASLQGLTISYASEKYEPVLLKSES	
	SHQVVPSLYSNDELFQTANLEINQAHPPKGRHV	
	FATPVLSIDEPLNTLINKLIHSDEILTSTKSSVTGKV	
	FAGIPTVASDTFVSTDHSVPIGNGHVAITAVSPH	
	RDGSVTSTKLLFPSKATSELSHSAKSDAGLVGGG	
	EDGDTDDDGDDDDDDRGSDGLSIHKCMSCSS	
	YRESQEKVMNDSDTHENSLMDQNNPISYSLSEN	
	SEEDNRVTSVSSDSQTGMDRSPGKSPSANGLSQ	
	KHNDGKEENDIQTGSALLPLSPESKAWAVLTSDE	
	ESGSGQGTSDSLNENETSTDFSFADTNEKDADGI	
	LAAGDSEITPGFPQSPTSSVTSENSEVFHVSEAEAS	
	NSSHESRIGLAEGLESEKKAVIPLVIVSALTFICLVV	
	LVGILIYWRKCFQTAHFYLEDSTSPRVISTPPTPIFP	

	ISDDVGAIPIKHFPKHVADLHASSGFTEEFETLKEF YQEVQSCTVDLGITADSSNHPDNKHKNRYINIV AYDHSRVKLAQLAEKDGKLTDYINANYVDGYN RPKAYIAAQGPLKSTAEDFWRMIWEHNVEVIVM ITNLVEKGRRKCDQYWPADGSEEYGNFLVTQKS VQVLAYYTVRNFTLRNTKIKKGSQKGRPSGRVV TQYHYTQWPDMGVPEYSLPVLTFVRKAAYAKR HAVGPVVVHCSAGVGRTGTYIVLDSMLQQIQH EGTVNIFGFLKHIRSQRNYLVQTEEQYVFIHDTL VEAILSKETEVLDSHIHAYVNALLIPGPAGKTKLEK QFQLLSQSNIQQSDYSAALKQCNREKNRTSSIIP VERSRVGISSLSGEGTDYINASYMGYYQSNEFIT QHPLLHTIKDFWRMIWDHNAQLVVMIPDGQN MAEDEFVYWPNKDEPINCESFKVTLMAEEHKCLS NEEKLIIQDFILEATQDDYVLEVRHFQCPKWPNP DSPISKTFELISVIKEEAANRDGPMIVHDEHGGVT AGTFCALTTLMHQLEKENSVDVYQVAKMINLM RPGVFADIEQYQFLYKVILSLVSTRQEENPSTSLDS NGAALPDGNIAESLESLV	
SEQ ID NO: 59	MPLEQRSQHCKPEEGLEARGEALGLVGAQAPAT EEQEAASSSSTLVEVTLGEVPAAESPDPPQSPQGA SSLPTTMNYPLWSQSYEDSSNQEEEGPSTFPDLES EFQAALSRKVAELVHFLLLKYRAREPVTKAEMLGS VVGNWQYFFPVIFSKAFSSLQLVFGIELMEVDPIG HLYIFATCLGLSYDGLLGDNQIMPKAGLLIIVLAII AREGDCAPEEKIWEELSVLEVFEGREDSILGDPKK LLTQHFVQENYLEYRQVPGSDPACYEFLWGPRA LVETSYVKVLHHMVKISGGPHIS YPPLHEWVLREGEE	MAGE-A3
SEQ ID NO: 60	KVAELVHFL	MAGE-A3 epitope
SEQ ID NO: 61	MAFVCLAIGCLYTFLISTTFGCTSSSDTEIKVNPPQ DFEIVDPGYLGYLYLQWQPPLSLDHFKECTVEYE LKYRNIGSETWKTIITKNLHYKDGFDLNKGIEAKI HTLLPWQCTNGSEVQSSWAETTYWISPQGIPET KVQDMDCVYYNWQYLLCSWKPGIGVLLDTNY NLFYWYEGLDHALQCVDYIKADGQNIGCRFPY LEASDYKDFYICVNGSSENKPIRSSYFTFQLQNIV KPLPPVYLTFTRESSCEIKLKWSIPLGPIPARCFDYEI EIREDDTTLVTATVENETYTLKTTNETRQLCFVVR SKVNIYCSDDGIWSEWSDKQCWEGEDLSKKTLL RFWLPFGFILILVIFVTGLLLRKPNTYPKMIPEFFCD T	IL13Ralpha2
SEQ ID NO: 62	LPFGFIL	IL13Ralpha2 epitope
SEQ ID NO: 63	LFRAAQLANDVVLQIMEHLELASMTNMELMSSI VVISASIIVFNLLELEG	Mad12
SEQ ID NO: 64	LVQFIKDRISVVQA	gp70CD4 peptide

212

SEQ ID NO: 65	SPSYVYHQF	gp70CD8 peptide
SEQ ID NO: 66	ASMTNMELM	adpgk peptide
SEQ ID NO: 67	ATKNWPFL	survivin20-28
SEQ ID NO: 68	TVSEFLKL	survivin97-104
SEQ ID NO: 69	KRYKNRVASRKSRAKFKQLLQHYREVAAAKSSEN DRLRLLLKLFRAAQLANDVVLQIMEHLELASMTN MELMSSIVVISASIIVFNLLELEGSTVHEILCKLSLEG DHSTPPSAYGSVKPYTNFDAE	Z13Mad12Anaxa

213

CLAIMS

1. A complex comprising:

   a)   a cell penetrating peptide;

   b)   at least one antigen or antigenic epitope; and

   c)   at least one TLR peptide agonist,

   wherein the components a) – c) are covalently linked,

   for use in the prevention and/or treatment of glioma, in particular glioblastoma.

2. The complex for use according to claim 1, wherein the complex is a recombinant polypeptide or a recombinant protein.

3. The complex for use according to claim 1 or 2, wherein the cell penetrating peptide

   (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

   (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 thereof.

4. The complex for use according to claim 3, wherein the cell penetrating peptide has an amino acid sequence comprising a Ser (S) at the equivalent of position 189 of the ZEBRA amino acid sequence according to SEQ ID NO: 3.

5. The complex for use according to claim 3 or 4, wherein the cell penetrating peptide has an amino acid sequence comprising:

   (i)   a sequence according to the following general formula (I):

214

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

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;

X_{11} is K, R, or H, preferably X_{11} is K or R;

X_{13} is R, K, or H, preferably X_{13} is R or K;

X_{14} is A, V, L, I, or G, preferably X_{14} is A or G;

X_{15} is K, R, or H, preferably X_{15} is K or R;

X_{16} is F, L, V, I, Y, W, or M, preferably X_{16} is F, Y or W; and

X_{17} is K, R, or H, preferably X_{17} is K or R.

6. The complex for use according to claim 3 or 4, wherein the cell penetrating peptide has an amino acid sequence comprising an amino acid sequence selected from the group consisting of amino acid sequences according to SEQ ID NOs: 4 – 13, or sequence variants thereof without abrogating said peptide's cell penetrating ability, in particular sequence variants thereof sharing at least 70% sequence identity, preferably at least 80% sequence identity and more preferably at least 90% sequence identity without abrogating said peptide's cell penetrating ability.

7. The complex for use according to claim 6, wherein 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

215

(CPP5/Z15), or SEQ ID NO: 11 (CPP8/Z18), or sequence variants thereof without abrogating said peptide's cell penetrating ability, in particular sequence variants thereof sharing at least 70% sequence identity, preferably at least 80% sequence identity and more preferably at least 90% sequence identity without abrogating said peptide's cell penetrating ability.

8. The complex for use according to any of claims 1 to 7, wherein 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, (v) a glycolipid, (vi) a nucleic acid, and (vii) a small molecule drug or a toxin.

9. The complex for use according to any of claims 1 to 8, wherein the at least one antigen or antigenic epitope comprises or consists of at least one tumor epitope, preferably of at least one glioma epitope.

10. The complex for use according to any of claims 1 to 9, wherein the at least one antigen or antigenic epitope is at least one $CD4^+$ epitope and/or at least one $CD8^+$ epitope.

11. The complex for use according to any of claims 1 to 10, wherein the at least one antigen or antigenic epitope is a peptide, a polypeptide, or a protein.

12. The complex for use according to any of claims 1 to 11, wherein the complex 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.

13. The complex for use according to claim 12, wherein 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.

14. The complex for use according to any of claims 9 to 13, wherein the at least one tumor epitope is an epitope of an antigen selected from the group consisting of CMV,

216

EGFRvIII, EphA2, gp100, Her2/neu, IL-13Rα2, survivin, hTert, TRP-2, MAGE-A1, MAGE-A3, YKL-40, brevican, neuroligin 4 and PTPRz1.

15. The complex for use according to claim 14, wherein the at least one tumor epitope is an epitope of an antigen selected from the group consisting of EGFRvIII, EphA2, Her2/neu, IL-13Rα2, survivin, TRP-2, brevican, neuroligin 4 and PTPRz1, preferably the at least one tumor epitope is an epitope of an antigen selected from the group consisting of EGFRvIII, EphA2, IL-13Rα2, TRP-2 and brevican, more preferably the at least one tumor epitope is an epitope of an antigen selected from the group consisting of EGFRvIII, EphA2 and brevican.

16. The complex for use according to any of claims 12 to 15, wherein the complex comprises

a) one or more epitopes of EGFRvIII or functional sequence variants thereof;

b) one or more epitopes of EphA2 or functional sequence variants thereof;

c) one or more epitopes of Her2/neu or functional sequence variants thereof;

d) one or more epitopes of IL-13Rα2 or functional sequence variants thereof;

e) one or more epitopes of survivin or functional sequence variants thereof;

f) one or more epitopes of TRP-2 or functional sequence variants thereof;

g) one or more epitopes of brevican or functional sequence variants thereof;

h) one or more epitopes of neuroligin 4 or functional sequence variants thereof; and/or

i) one or more epitopes of PTPRz1 or functional sequence variants thereof.

17. The complex for use according to claim 16, wherein the complex comprises

a)   a fragment of EGFRvIII comprising one or more epitopes or a functional sequence variant thereof;

b)   a fragment of EphA2 comprising one or more epitopes or a functional sequence variant thereof;

c)   a fragment of Her2/neu comprising one or more epitopes or a functional sequence variant thereof;

d)   a fragment of IL-13Rα2 comprising one or more epitopes or a functional sequence variant thereof;

217

e)   a fragment of survivin comprising one or more epitopes or a functional sequence variant thereof;

f)   a fragment of TRP-2 comprising one or more epitopes or a functional sequence variant thereof;

g)   a fragment of neuroligin 4 comprising one or more epitopes or a functional sequence variant thereof;

h)   a fragment of brevican comprising one or more epitopes or a functional sequence variant thereof; and/or

i)   a fragment of PTPRz1 comprising one or more epitopes or a functional sequence variant thereof.

18. The complex for use according to claim 16 or 17, wherein the complex comprises

a)   one or more epitopes of EGFRvIII or functional sequence variants thereof;

b)   one or more epitopes of EphA2 or functional sequence variants thereof;

c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof;

d)   one or more epitopes of TRP-2 or functional sequence variants thereof; and/or

e)   one or more epitopes of brevican or functional sequence variants thereof.

19. The complex for use according to claim 18, wherein the complex comprises

a)   one or more epitopes of EGFRvIII or functional sequence variants thereof;

b)   one or more epitopes of EphA2 or functional sequence variants thereof;

c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof;

d)   one or more epitopes of TRP-2 or functional sequence variants thereof; and

e)   one or more epitopes of brevican or functional sequence variants thereof.

20. The complex for use according to claim 18, wherein the complex comprises

b)   one or more epitopes of EphA2 or functional sequence variants thereof;

c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof;

d)   one or more epitopes of TRP-2 or functional sequence variants thereof; and

e)   one or more epitopes of brevican or functional sequence variants thereof.

21. The complex for use according to claim 18, wherein the complex comprises

218

    a) one or more epitopes of EGFRvIII or functional sequence variants thereof;

    c) one or more epitopes of IL-13Rα2 or functional sequence variants thereof;

    d) one or more epitopes of TRP-2 or functional sequence variants thereof; and

    e) one or more epitopes of brevican or functional sequence variants thereof.

22. The complex for use according to claim 18, wherein the complex comprises

    a) one or more epitopes of EGFRvIII or functional sequence variants thereof;

    b) one or more epitopes of EphA2 or functional sequence variants thereof;

    d) one or more epitopes of TRP-2 or functional sequence variants thereof; and

    e) one or more epitopes of brevican or functional sequence variants thereof.

23. The complex for use according to claim 18, wherein the complex comprises

    a) one or more epitopes of EGFRvIII or functional sequence variants thereof;

    b) one or more epitopes of EphA2 or functional sequence variants thereof;

    c) one or more epitopes of IL-13Rα2 or functional sequence variants thereof; and

    e) one or more epitopes of brevican or functional sequence variants thereof.

24. The complex for use according to claim 18, wherein the complex comprises

    a)   one or more epitopes of EGFRvIII or functional sequence variants thereof;

    b)   one or more epitopes of EphA2 or functional sequence variants thereof;

    c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof; and

    d)   one or more epitopes of TRP-2 or functional sequence variants thereof.

25. The complex for use according to claim 18, wherein the complex comprises

    a)   one or more epitopes of EGFRvIII or functional sequence variants thereof;

    b)   one or more epitopes of EphA2 or functional sequence variants thereof; and

    c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof.

26. The complex for use according to claim 18, wherein the complex comprises

    a)   one or more epitopes of EGFRvIII or functional sequence variants thereof;

    b)   one or more epitopes of EphA2 or functional sequence variants thereof; and

    d)   one or more epitopes of TRP-2 or functional sequence variants thereof.

219

27. The complex for use according to claim 18, wherein the complex comprises

   a)   one or more epitopes of EGFRvIII or functional sequence variants thereof;

   b)   one or more epitopes of EphA2 or functional sequence variants thereof; and

   e)   one or more epitopes of brevican or functional sequence variants thereof.

28. The complex for use according to claim 18, wherein the complex comprises

   a)   one or more epitopes of EGFRvIII or functional sequence variants thereof;

   c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof; and

   d)   one or more epitopes of TRP-2 or functional sequence variants thereof.

29. The complex for use according to claim 18, wherein the complex comprises

   a)   one or more epitopes of EGFRvIII or functional sequence variants thereof;

   c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof; and

   e)   one or more epitopes of brevican or functional sequence variants thereof.

30. The complex for use according to claim 18, wherein the complex comprises

   a)   one or more epitopes of EGFRvIII or functional sequence variants thereof;

   d)   one or more epitopes of TRP-2 or functional sequence variants thereof; and

   e)   one or more epitopes of brevican or functional sequence variants thereof.

31. The complex for use according to claim 18, wherein the complex comprises

   b)   one or more epitopes of EphA2 or functional sequence variants thereof;

   c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof; and

   d)   one or more epitopes of TRP-2 or functional sequence variants thereof.

32. The complex for use according to claim 18, wherein the complex comprises

   b)   one or more epitopes of EphA2 or functional sequence variants thereof;

   c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof; and

   e)   one or more epitopes of brevican or functional sequence variants thereof.

33. The complex for use according to claim 18, wherein the complex comprises

220

b)   one or more epitopes of EphA2 or functional sequence variants thereof;

d)   one or more epitopes of TRP-2 or functional sequence variants thereof; and

e)   one or more epitopes of brevican or functional sequence variants thereof.

34. The complex for use according to claim 18, wherein the complex comprises

c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof;

d)   one or more epitopes of TRP-2 or functional sequence variants thereof; and

e)   one or more epitopes of brevican or functional sequence variants thereof.

35. The complex for use according to claim 18, wherein the complex comprises

a)   one or more epitopes of EGFRvIII or functional sequence variants thereof; and

b)   one or more epitopes of EphA2 or functional sequence variants thereof.

36. The complex for use according to claim 18, wherein the complex comprises

a)   one or more epitopes of EGFRvIII or functional sequence variants thereof; and

c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof.

37. The complex for use according to claim 18, wherein the complex comprises

a)   one or more epitopes of EGFRvIII or functional sequence variants thereof; and

d)   one or more epitopes of TRP-2 or functional sequence variants thereof.

38. The complex for use according to claim 18, wherein the complex comprises

a)   one or more epitopes of EGFRvIII or functional sequence variants thereof; and

e)   one or more epitopes of brevican or functional sequence variants thereof.

39. The complex for use according to claim 18, wherein the complex comprises

b)   one or more epitopes of EphA2 or functional sequence variants thereof; and

c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof.

40. The complex for use according to claim 18, wherein the complex comprises

b)   one or more epitopes of EphA2 or functional sequence variants thereof; and

d)   one or more epitopes of TRP-2 or functional sequence variants thereof.

221

41. The complex for use according to claim 18, wherein the complex comprises

   b)   one or more epitopes of EphA2 or functional sequence variants thereof; and

   e)   one or more epitopes of brevican or functional sequence variants thereof.

42. The complex for use according to claim 18, wherein the complex comprises

   c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof; and

   d)   one or more epitopes of TRP-2 or functional sequence variants thereof.

43. The complex for use according to claim 18, wherein the complex comprises

   c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof; and

   e)   one or more epitopes of brevican or functional sequence variants thereof.

44. The complex for use according to claim 18, wherein the complex comprises

   d)   one or more epitopes of TRP-2 or functional sequence variants thereof; and

   e)   one or more epitopes of brevican or functional sequence variants thereof.

45. The complex for use according to claim 18, wherein the complex comprises

   a)   one or more epitopes of EGFRvIII or functional sequence variants thereof.

46. The complex for use according to claim 18, wherein the complex comprises

   b)   one or more epitopes of EphA2 or functional sequence variants thereof.

47. The complex for use according to claim 18, wherein the complex comprises

   c)   one or more epitopes of IL-13Rα2 or functional sequence variants thereof.

48. The complex for use according to claim 18, wherein the complex comprises

   d)   one or more epitopes of TRP-2 or functional sequence variants thereof.

49. The complex for use according to claim 18, wherein the complex comprises

   e)   one or more epitopes of brevican or functional sequence variants thereof.

222

50. The complex for use according to any of claims 9 to 13, wherein the at least one tumor epitope is an epitope of a neoantigen, preferably an epitope of a glioma-specific neoantigen.

51. The complex for use according to any of claims 1 to 50, wherein the at least one TLR peptide agonist is a TLR2, TLR4 and/or TLR5 peptide agonist, preferably a TLR2 peptide agonist and/or a TLR4 peptide agonist.

52. The complex for use according to claim 51, wherein the at least one TLR peptide agonist comprises or consists of an amino acid sequence according to SEQ ID NO: 15 or a sequence variant thereof, in particular a sequence variant thereof sharing at least 70% sequence identity, preferably at least 80% sequence identity and more preferably at least 90% sequence identity without abrogating said peptide's TLR agonist ability.

53. The complex for use according to any of claims 1 to 52, wherein the complex comprises more than one TLR peptide agonists, in particular 2, 3, 4, 5, 6, 7, 8, 9, 10 or more TLR peptide agonists.

54. The complex for use according to any of claims 1 to 53, wherein 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.

55. The complex for use according to claim 54, wherein 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.

223

56. The complex for use according to any of claims 2 to 55, wherein said complex is a recombinant polypeptide or a recombinant protein and the components a) to c) are positioned in N-terminal → C-terminal 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.

57. A nucleic acid encoding the complex as defined in any of claims 1 to 56 for use in the prevention and/or treatment of glioma, in particular glioblastoma, wherein the complex is a polypeptide or a protein.

58. A vector comprising the nucleic acid as defined in claim 57 for use in the prevention and/or treatment of glioma, in particular glioblastoma.

59. A host cell comprising the vector as defined in claim 58 for use in the prevention and/or treatment of glioma, in particular glioblastoma.

60. A method of preparing the complex as defined in any of claims 1 to 56, the complex being a polypeptide or a protein, comprising cultivating a host cell as defined in claim 59 in a culture medium and separating said complex from the culture medium or from the host cell lysate after host cell lysis.

61. A cell loaded with a complex as defined in any one of claims 1 to 56 for use in the prevention and/or treatment of glioma, in particular glioblastoma.

62. A cell for use according to claim 61, wherein said cell is an antigen presenting cell, preferably a dendritic cell.

63. A composition comprising at least one of:

(i)   a complex as defined in any one of claims 6 to 56;

224

    (ii)   a nucleic acid as defined in claim 57;

    (ii)   a vector as defined in claim 58;

    (iv)   a host cell as defined in claim 59; or

    (v)   a cell loaded with a complex as defined in claim 61 or 62

5   for use in the prevention and/or treatment of glioma, in particular glioblastoma.

    64. A vaccine comprising at least one of:

        (i)   a complex as defined in any one of claims 6 to 56;

        (ii)   a nucleic acid as defined in claim 57;

10   (iii)   a vector as defined in claim 58;

        (iv)   a host cell as defined in claim 59; or

        (v)   a cell loaded with a complex as defined in claim 61 or 62

        for use in the prevention and/or treatment of glioma, in particular glioblastoma.

15 65. A pharmaceutical composition comprising at least one complex as defined in any one
        of claims 1 to 56 or at least one cell as defined in claim 61 or 62, and a
        pharmaceutically acceptable carrier for use in the prevention and/or treatment of
        glioma, in particular glioblastoma.

20 66. The pharmaceutical composition for use according to claim 65, wherein said
        composition comprises at least two different complexes.

    67. A diagnostic composition comprising a complex as defined in any one of claims 1 to
        56.

25

    68. A combination of

        (i)   a complex as defined in any of claims 1 to 56; and

        (ii)   a chemotherapeutic agent, a targeted drug and/or an immunotherapeutic
                 agent, such as an immune checkpoint modulator

30   for use in the prevention and/or treatment of glioma, in particular glioblastoma.

225

69. The combination for use according to claim 68, wherein (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, are administered at about the same time.

70. The combination for use according to claim 68 or 69, wherein (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, are administered consecutively.

71. The combination for use according to any of claims 68 to 70, wherein (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, are administered via the same route of administration.

72. The combination for use according to any of claims 68 to 70, wherein (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, are administered via distinct routes of administration.

73. The combination for use according to any of claims 68 to 70, wherein (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, are provided in distinct compositions.

74. The combination for use according to any of claims 68 to 70, wherein (i) the complex and (ii) the chemotherapeutic agent, the targeted drug and/or the immunotherapeutic agent, such as an immune checkpoint modulator, are provided in the same composition.

75. A kit comprising the complex as defined in any of claims 1 to 56, the cell as defined in claim 61 or 62, the composition as defined in claim 63, the vaccine as defined in claim 64, and/or the pharmaceutical composition as defined in claim 65 or 66 for use in the prevention and/or treatment of glioma, in particular glioblastoma.

226

76. The kit for use according to claim 75, wherein the kit further comprises a package insert or instruction leaflet with directions to prevent or to treat glioma, in particular glioblastoma, by using the complex as defined in any of claims 1 to 56, the cell as defined in claim 61 or 62, the composition as defined in claim 63, the vaccine as defined in claim 64, and/or the pharmaceutical composition as defined in claim 65 or 66.

77. A method for preventing and/or treating glioma, in particular glioblastoma, or initiating, enhancing or prolonging an anti-tumor-response in a subject in need thereof comprising administering to the subject a complex comprising:

    a)   a cell penetrating peptide;

    b)   at least one antigen or antigenic epitope; and

    c)   at least one TLR peptide agonist,

wherein the components a) – c) are covalently linked.

78. The method of claim 77, wherein the complex as defined in any of claims 1 to 56, the cell as defined in claim 61 or 62, the composition as defined in claim 63, the vaccine as defined in claim 64, and/or the pharmaceutical composition as defined in claim 65 or 66 is administered to the subject.

79. The method of claim 77 or 78, wherein the subject has glioma, in particular glioblastoma.

1/55
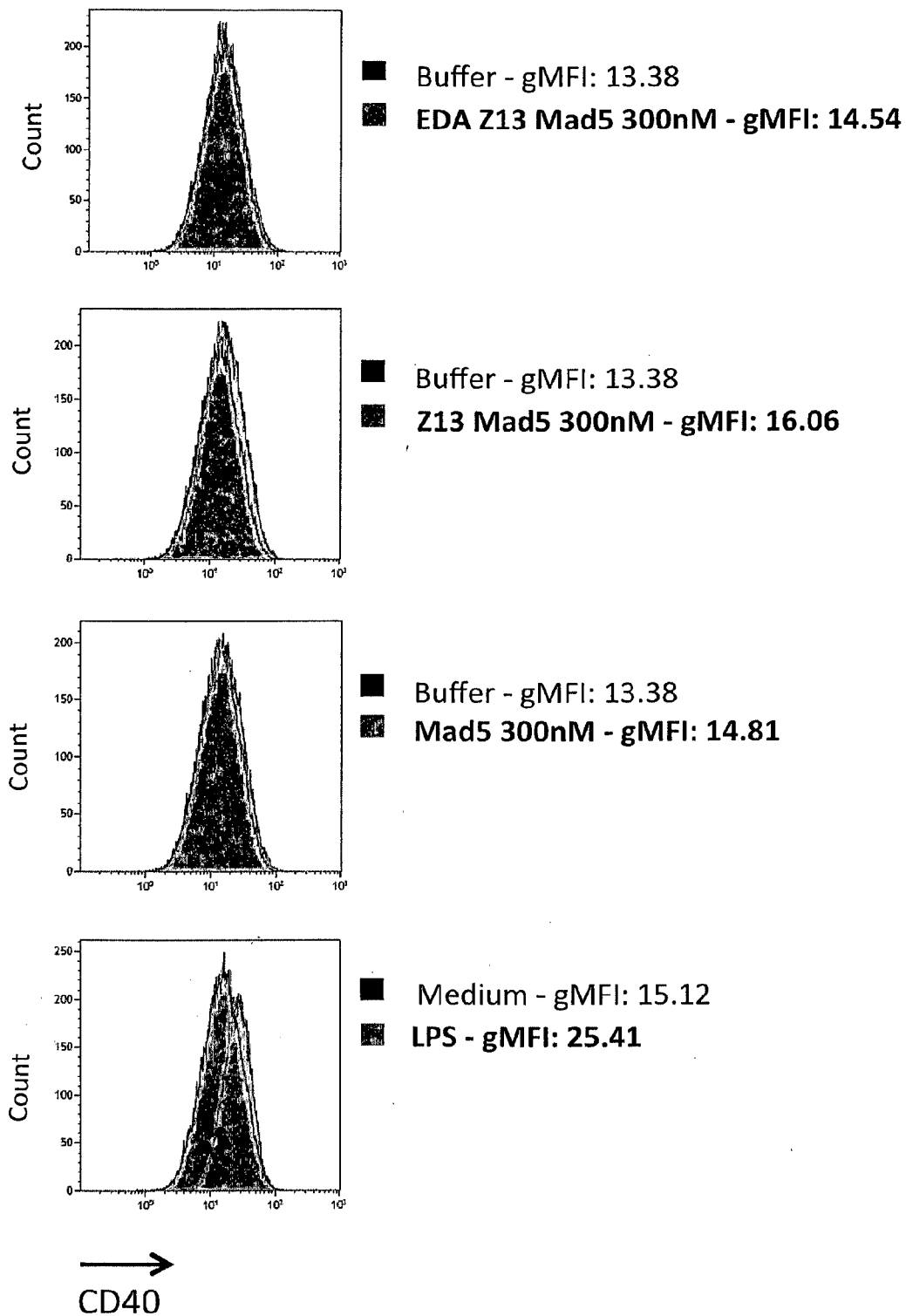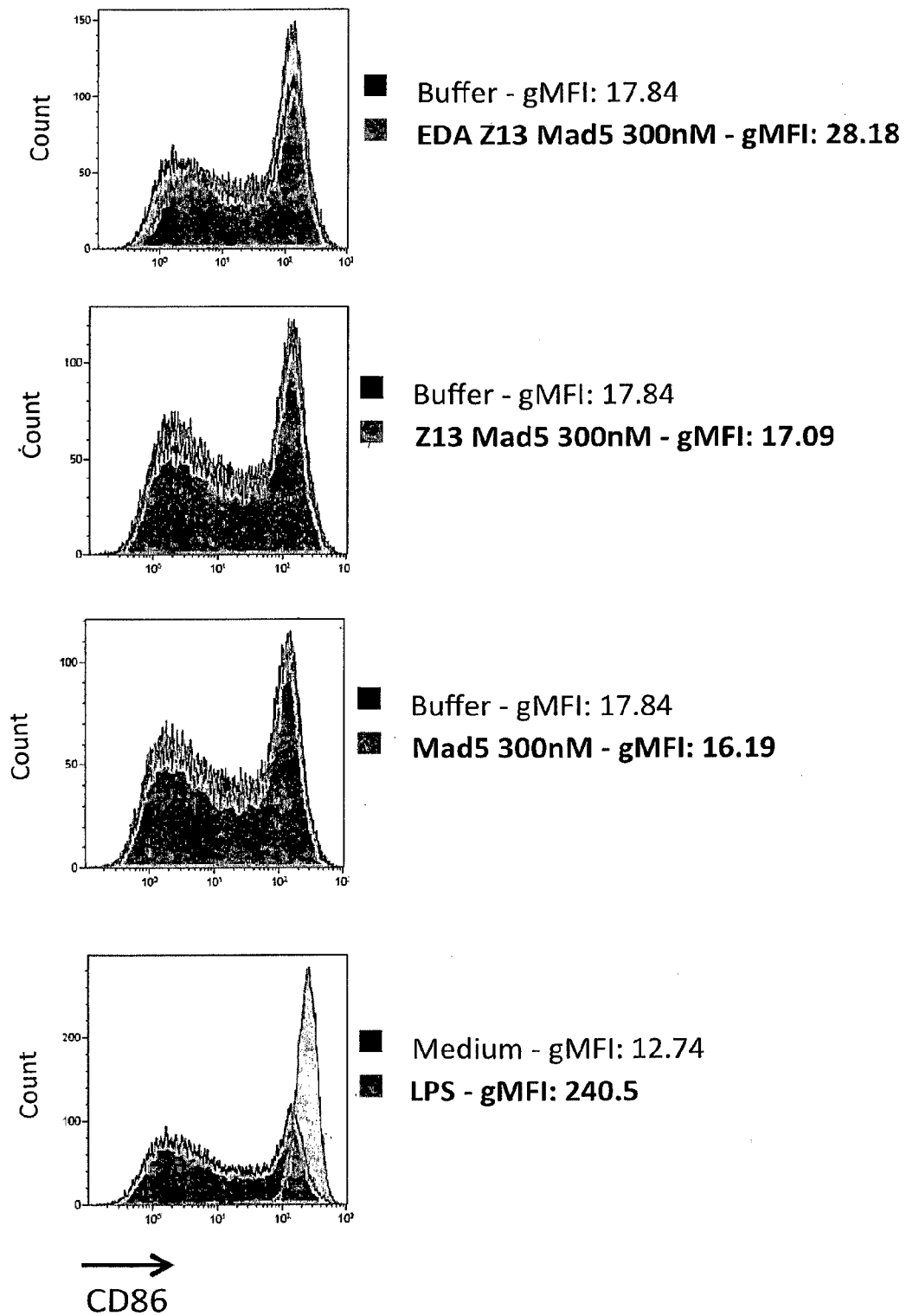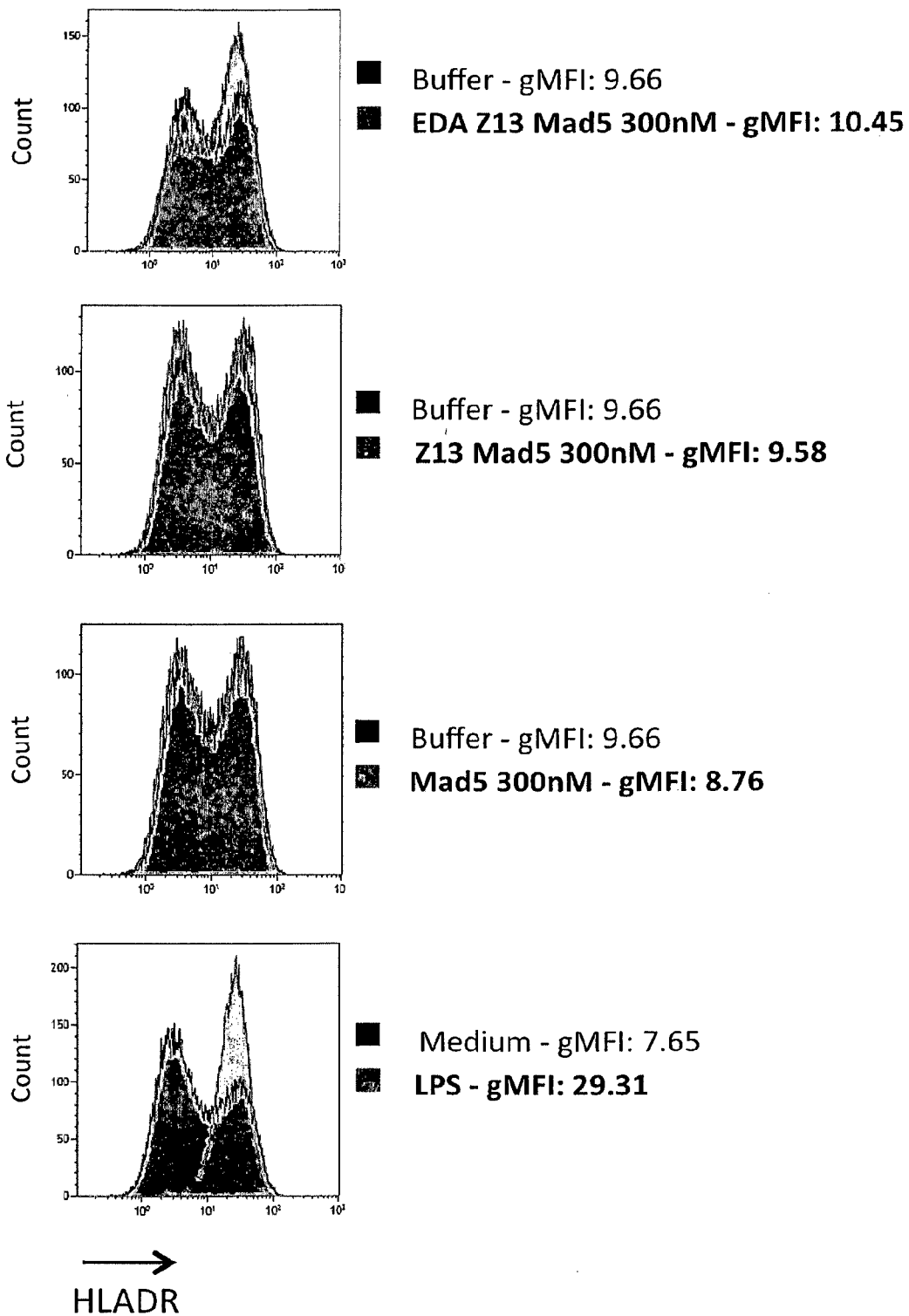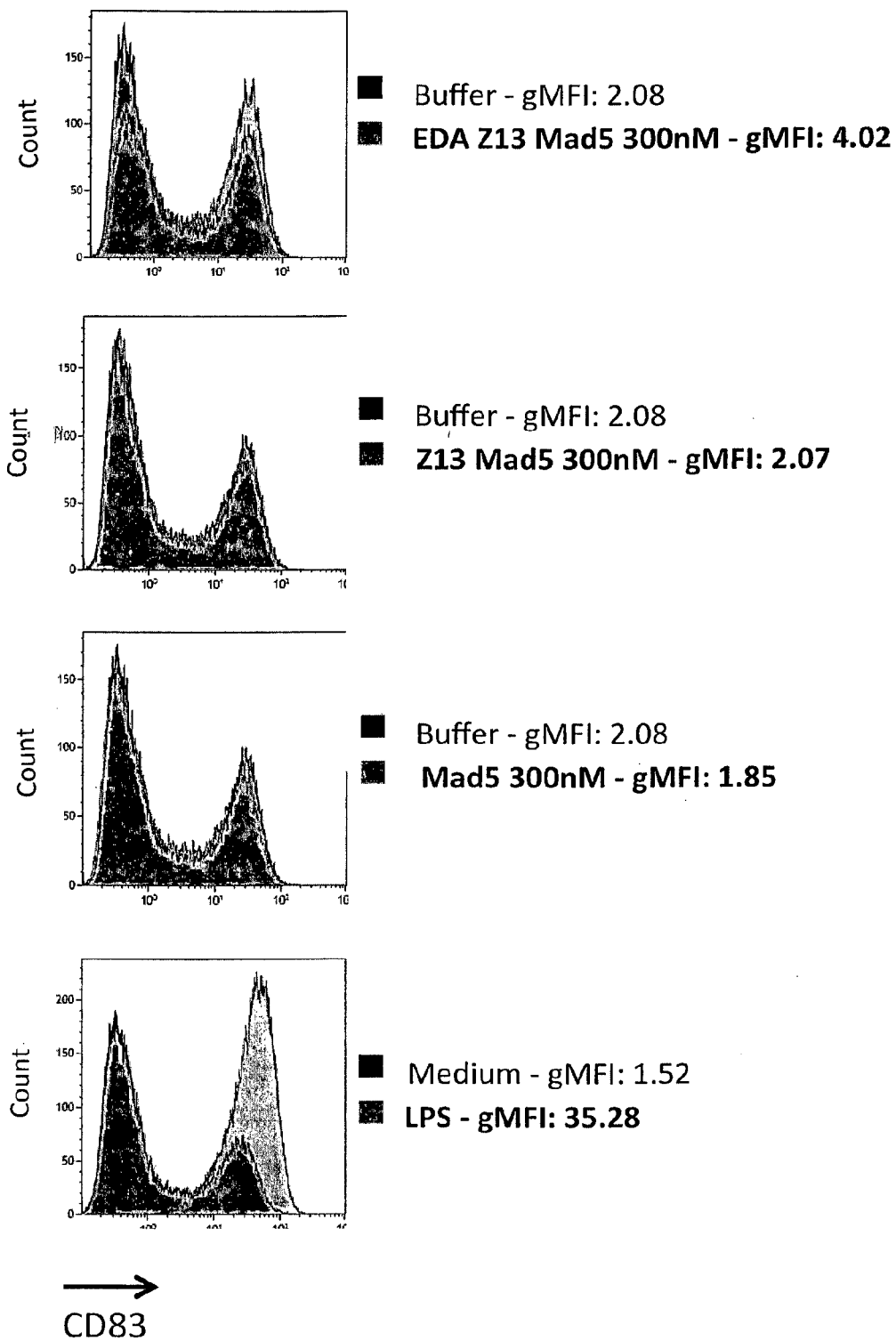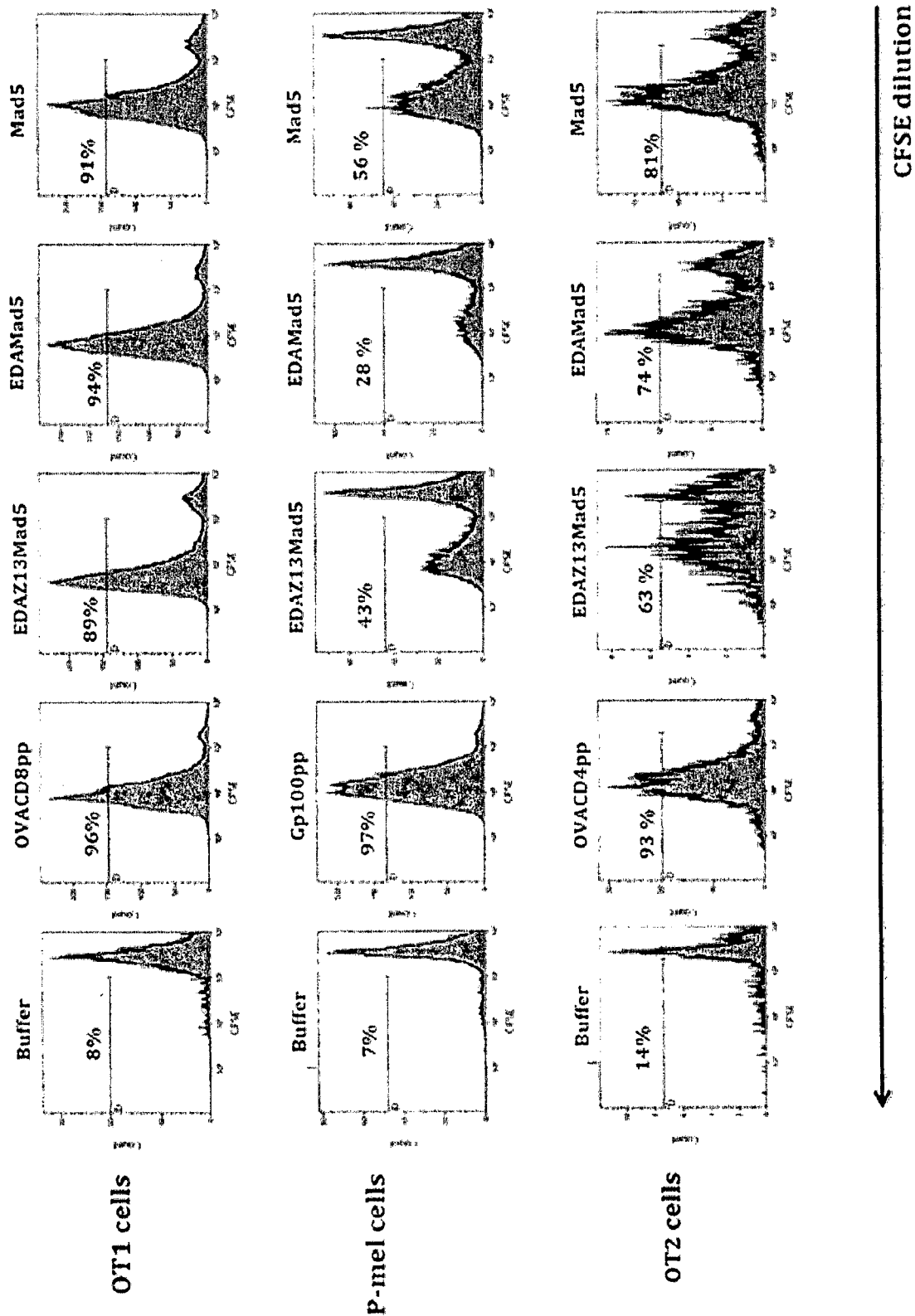
Buffer - gMFI: 13.38
EDA Z13 Mad5 300nM - gMFI: 14.54

Buffer - gMFI: 13.38
Z13 Mad5 300nM - gMFI: 16.06

Buffer - gMFI: 13.38
Mad5 300nM - gMFI: 14.81

Medium - gMFI: 15.12
LPS - gMFI: 25.41

CD40

Fig. 1

Fig. 2

3/55

Buffer - gMFI: 9.66
EDA Z13 Mad5 300nM - gMFI: 10.45

Buffer - gMFI: 9.66
Z13 Mad5 300nM - gMFI: 9.58

Buffer - gMFI: 9.66
Mad5 300nM - gMFI: 8.76

Medium - gMFI: 7.65
LPS - gMFI: 29.31

HLADR

Fig. 3

4/55

Fig. 4

Fig. 5

2 nmol of protein injected
EDA-MAD5

EDA-Z13-MAD5

MAD5+MPLA

Naive

Fig. 6

10 nmol of protein injected

EDA-MAD5

EDA-Z13-MAD5

MAD5+MPLA

Naïve

Fig. 7

Fig. 8

Days after tumor implantation

Fig. 9

Fig. 10

A

B

Fig. 11

Fig. 12

13/55

Fig. 13

A

HEK-hTLR2

B

IL8 release HEK hTLR2
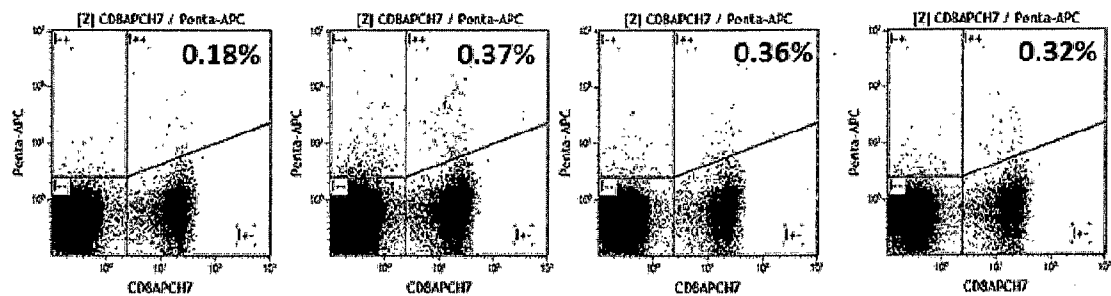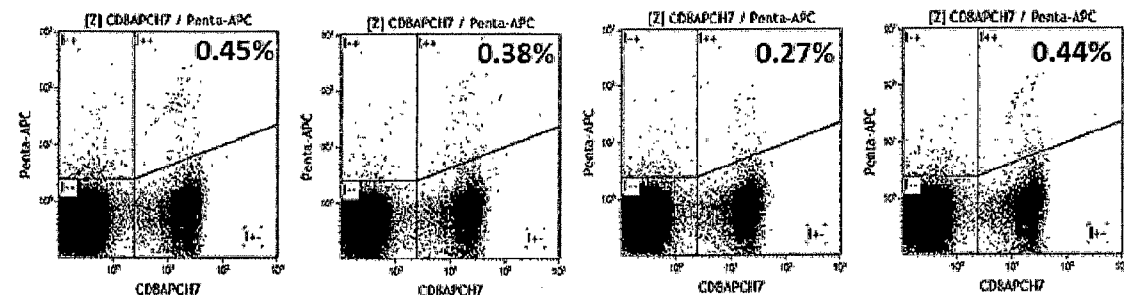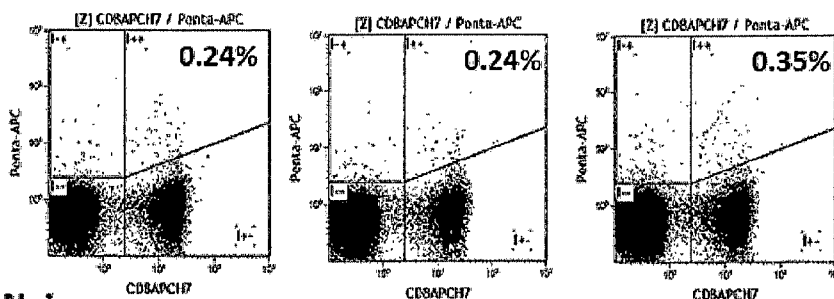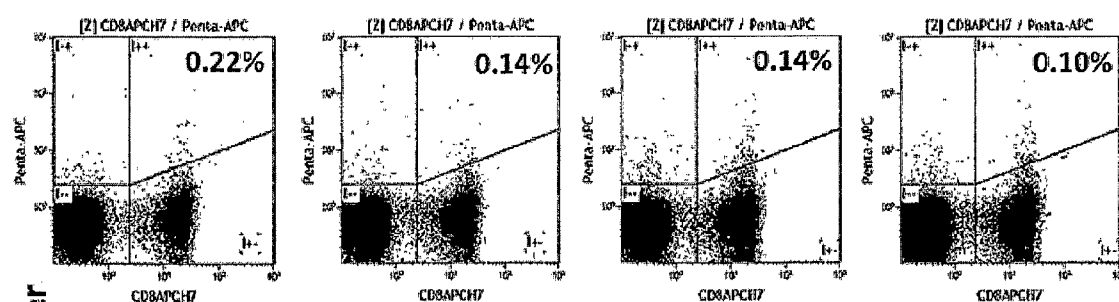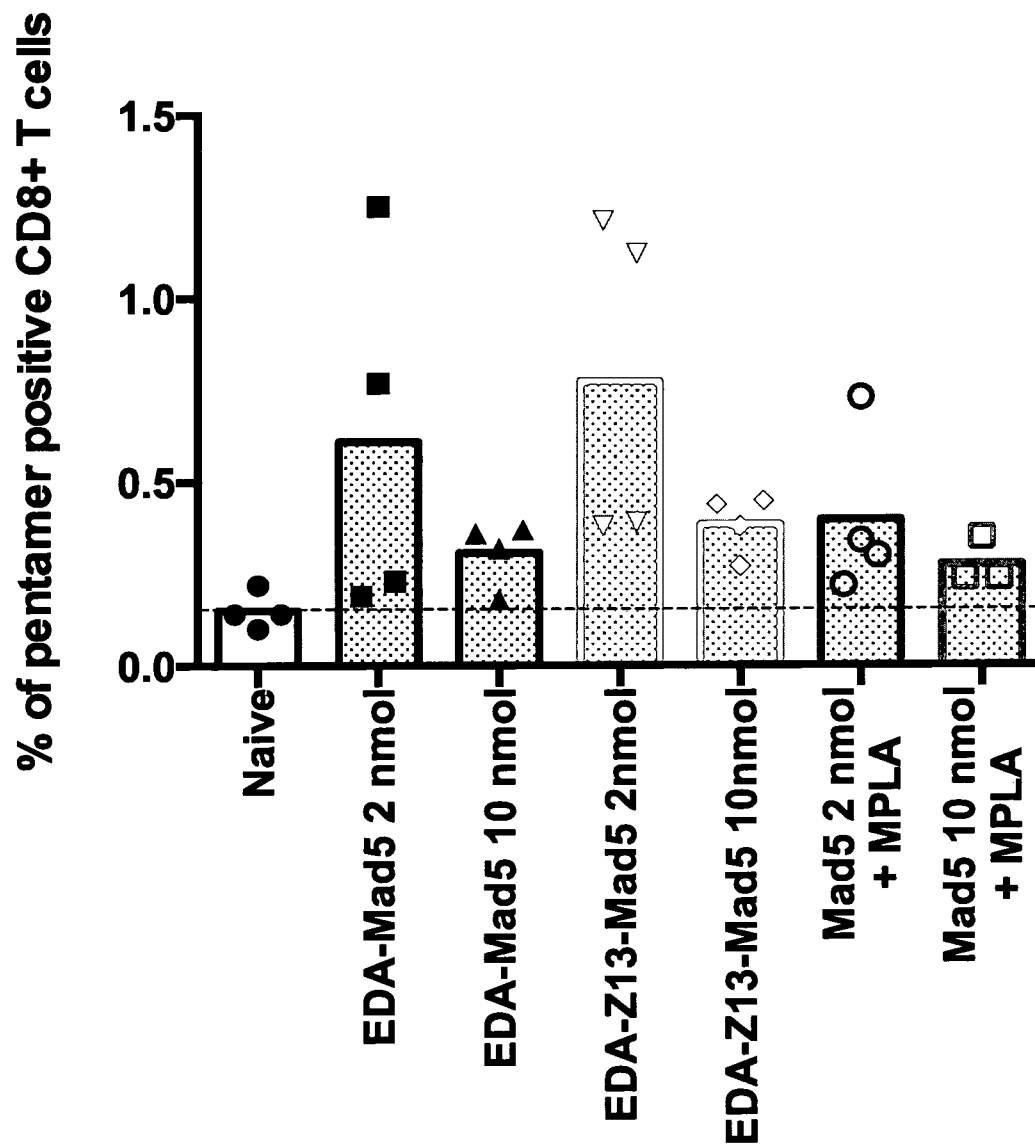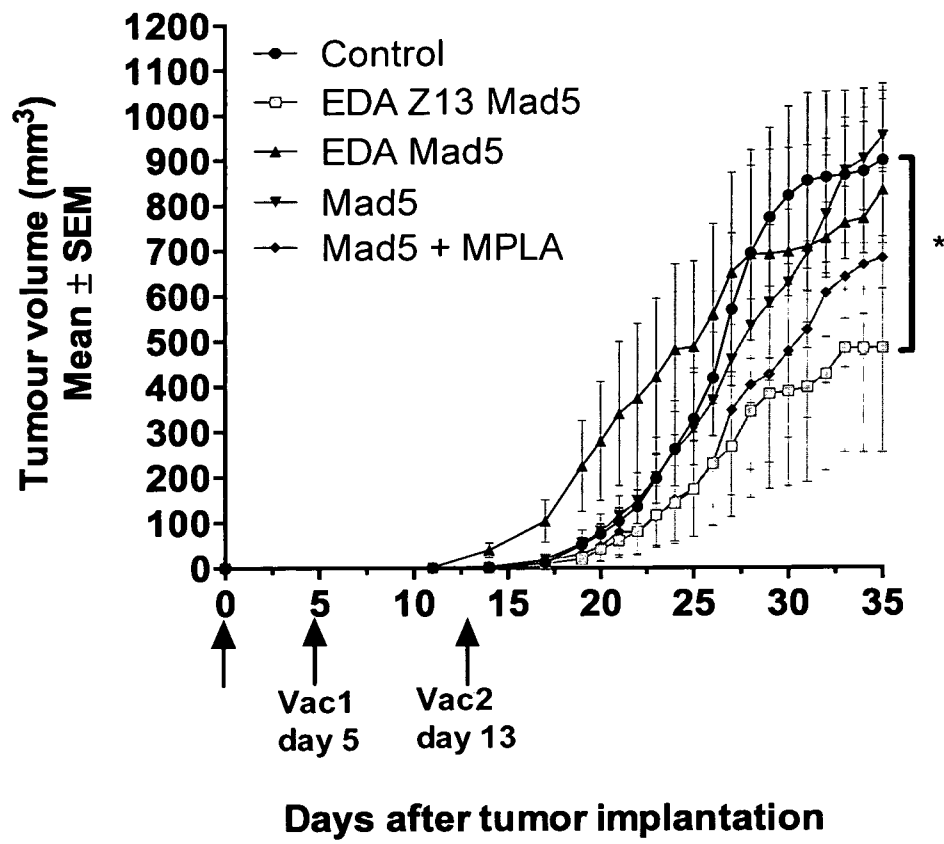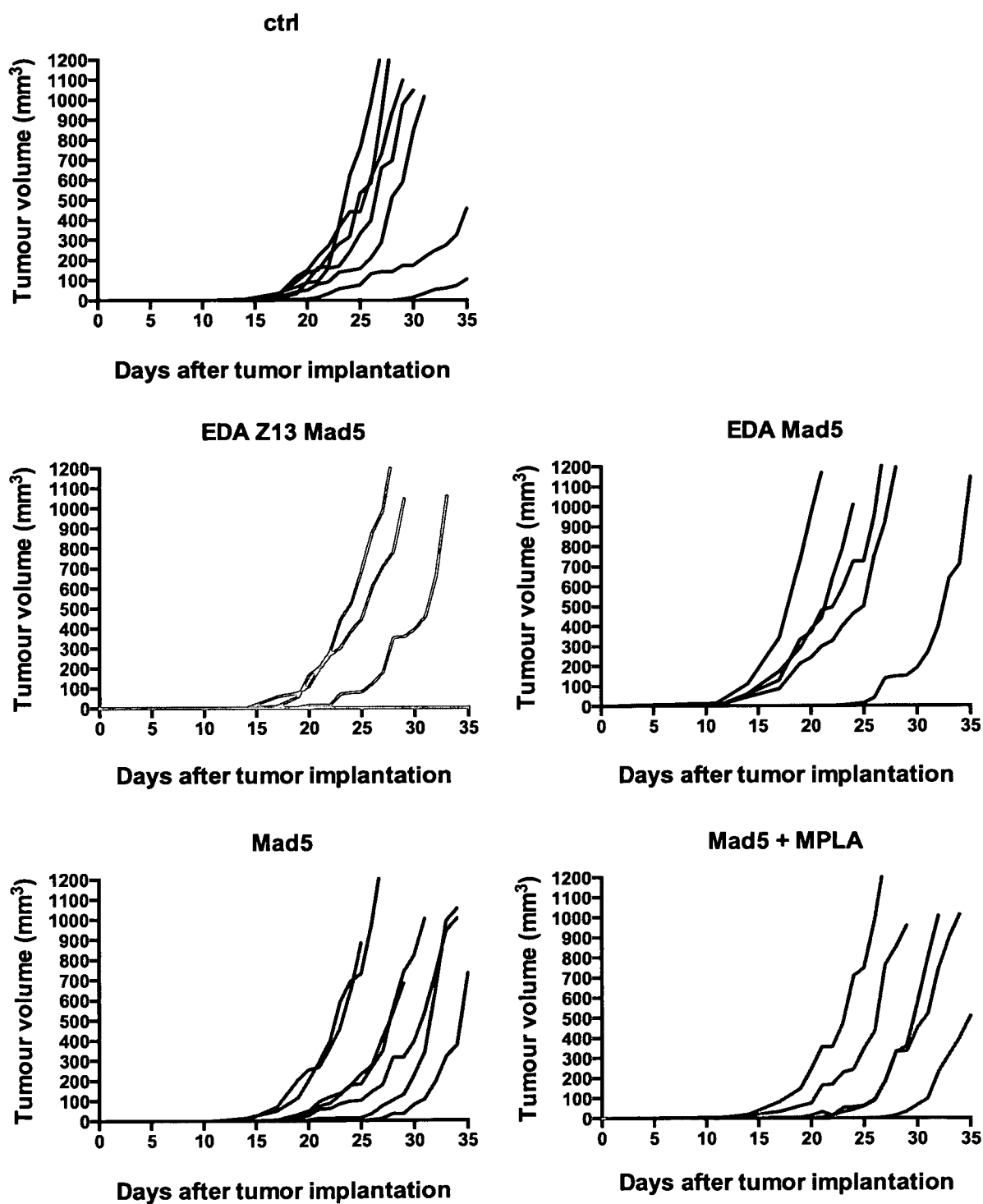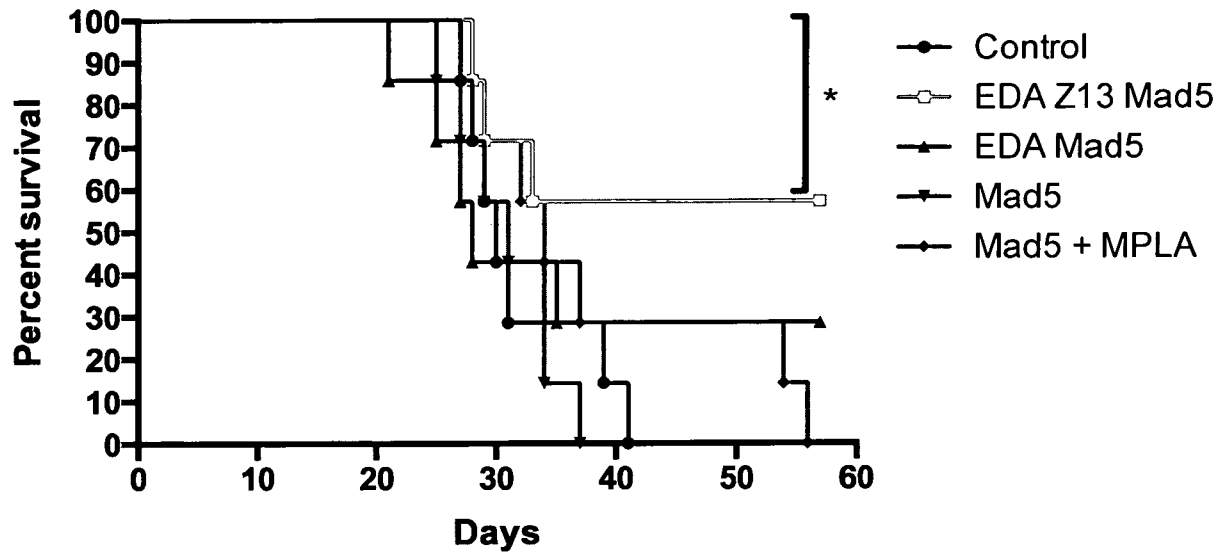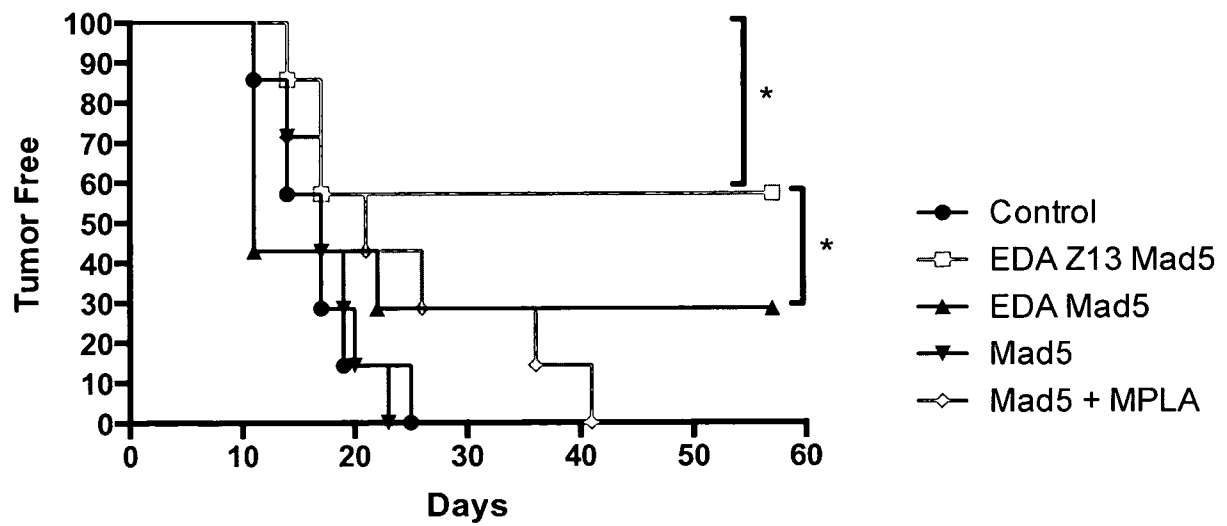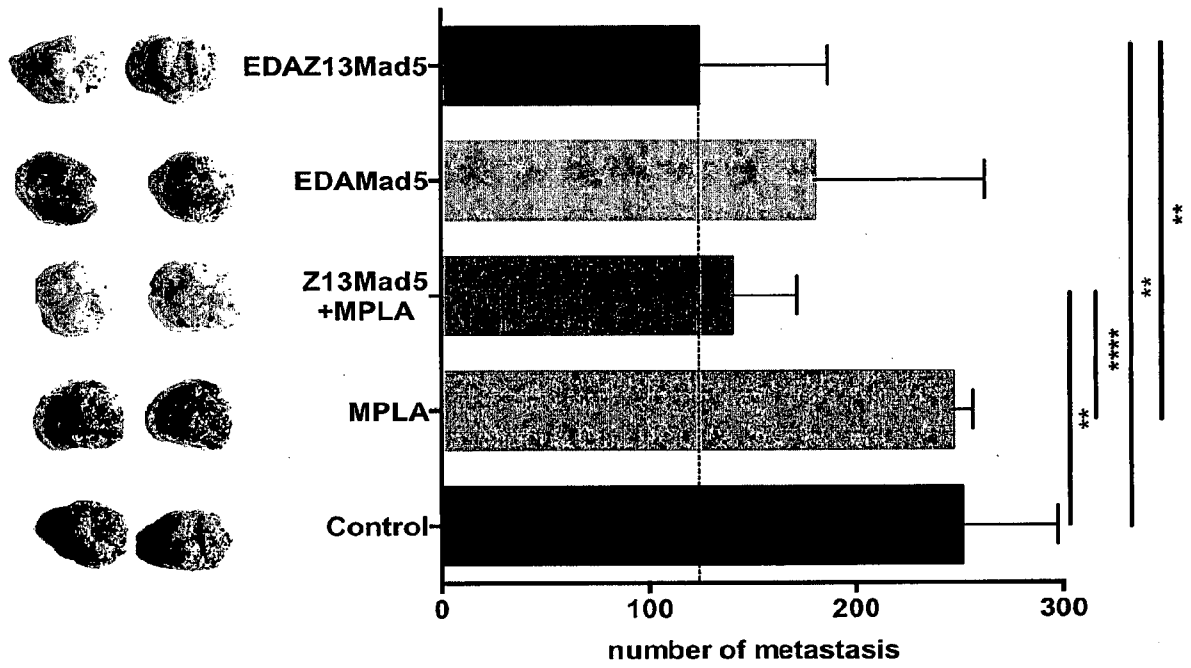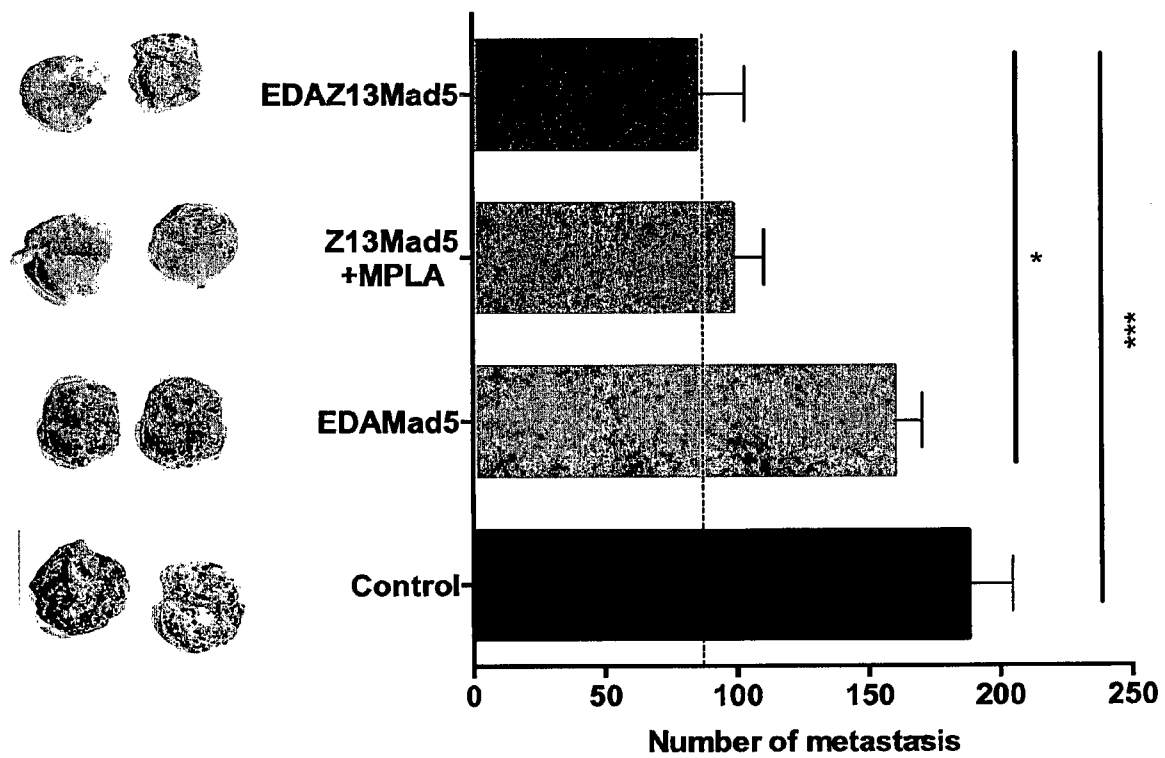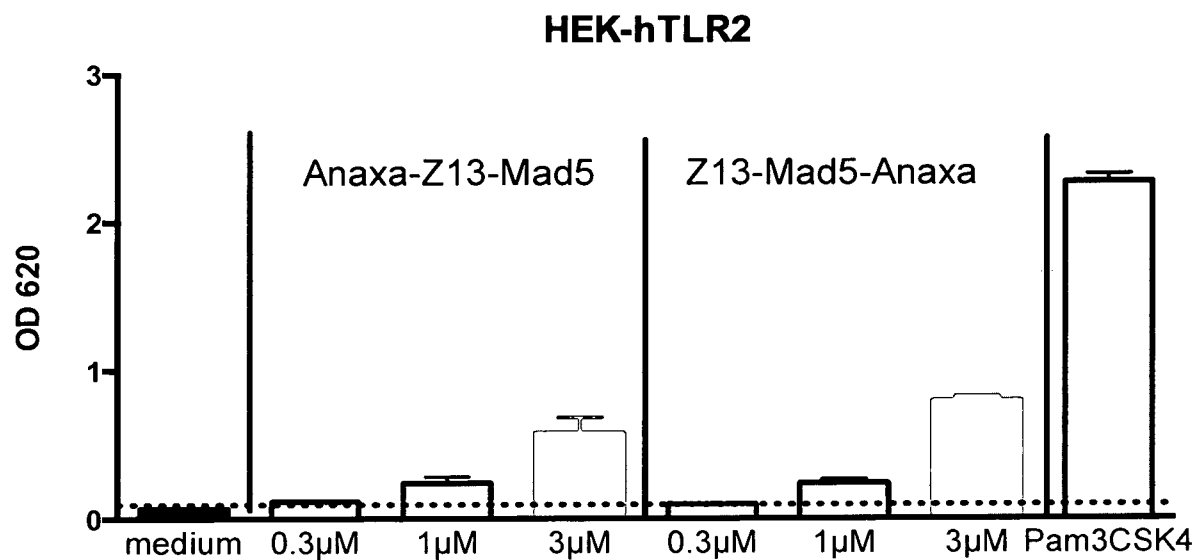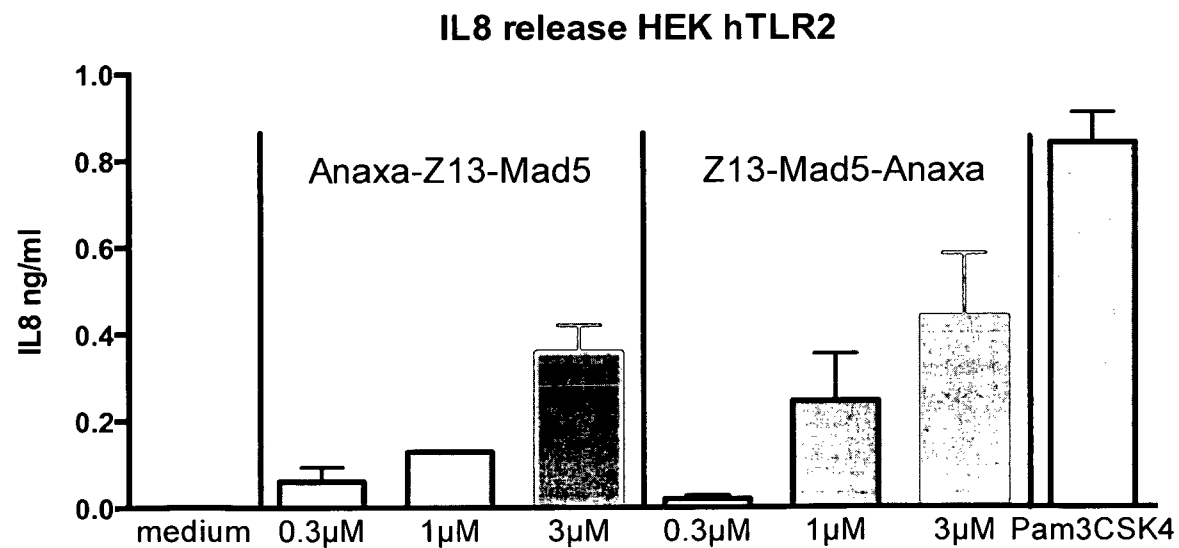
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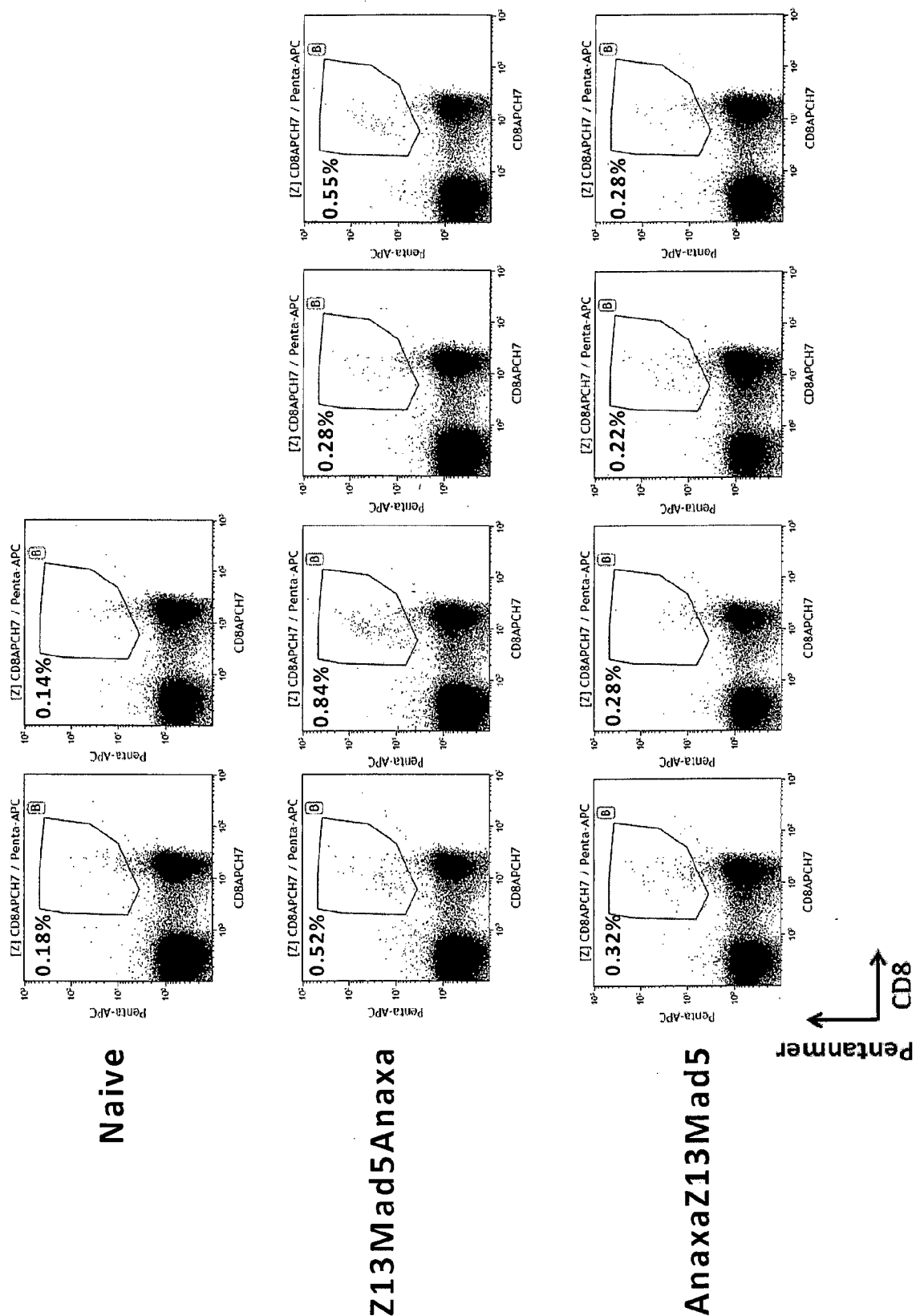
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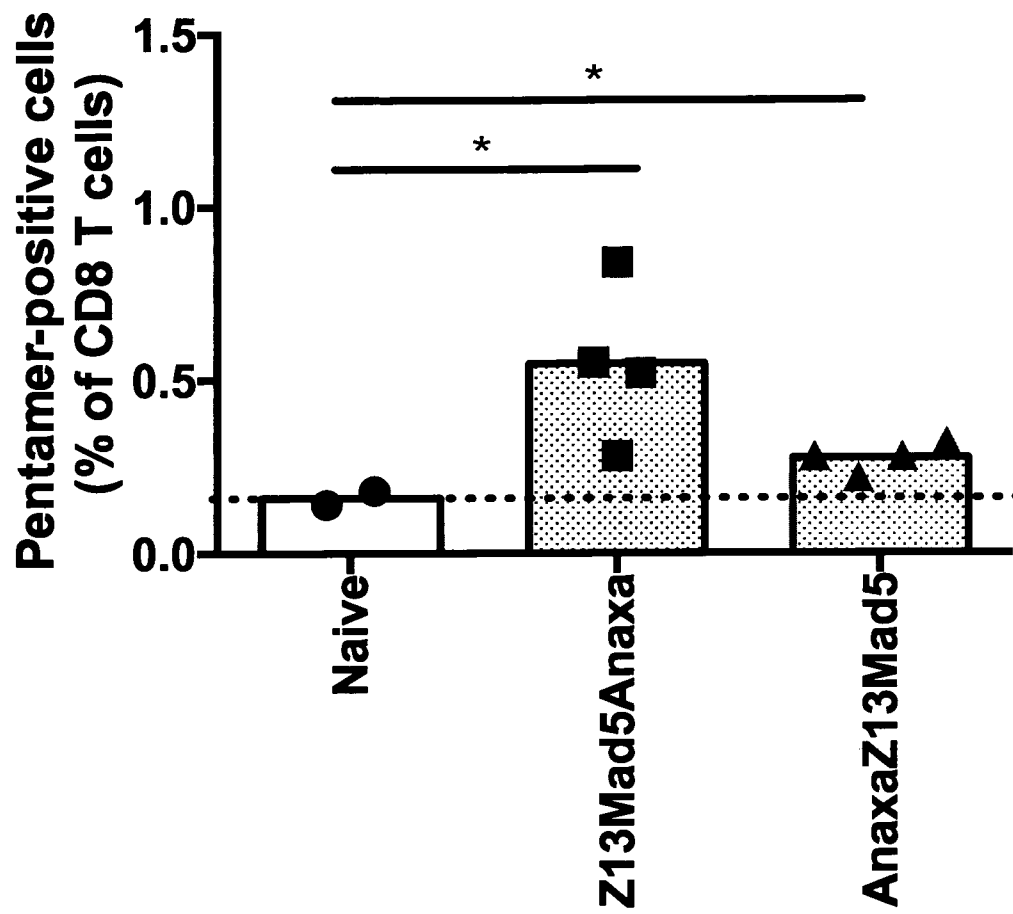
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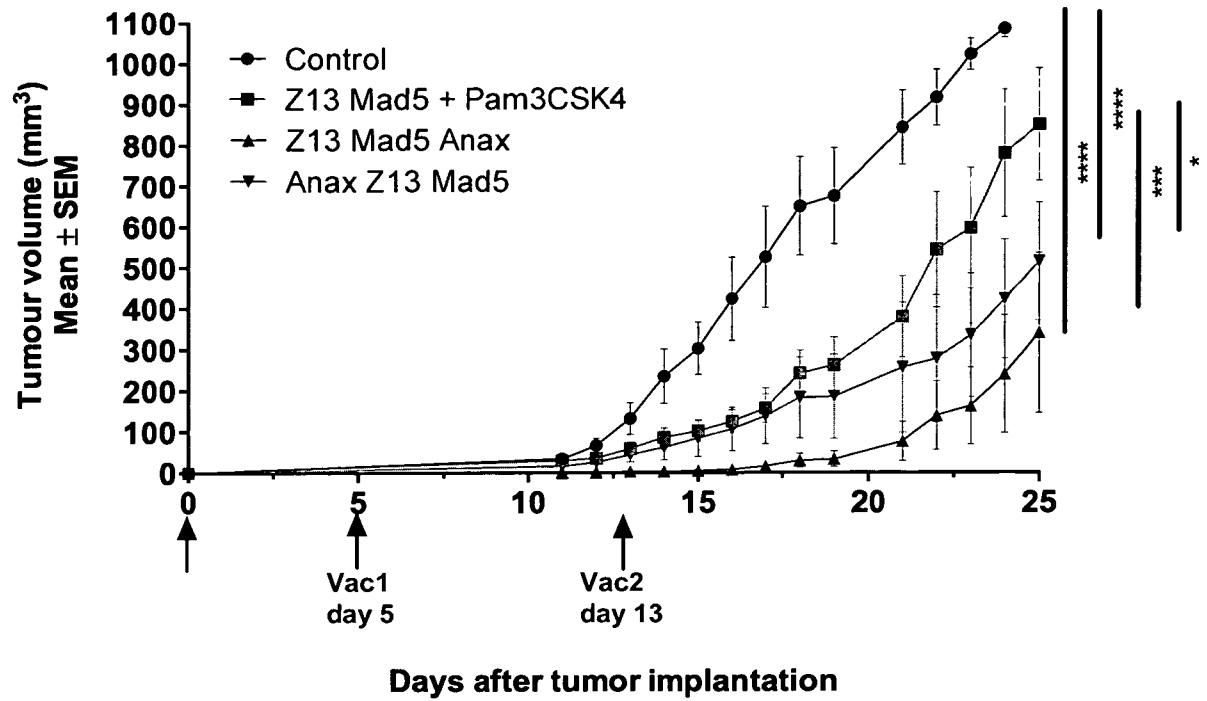
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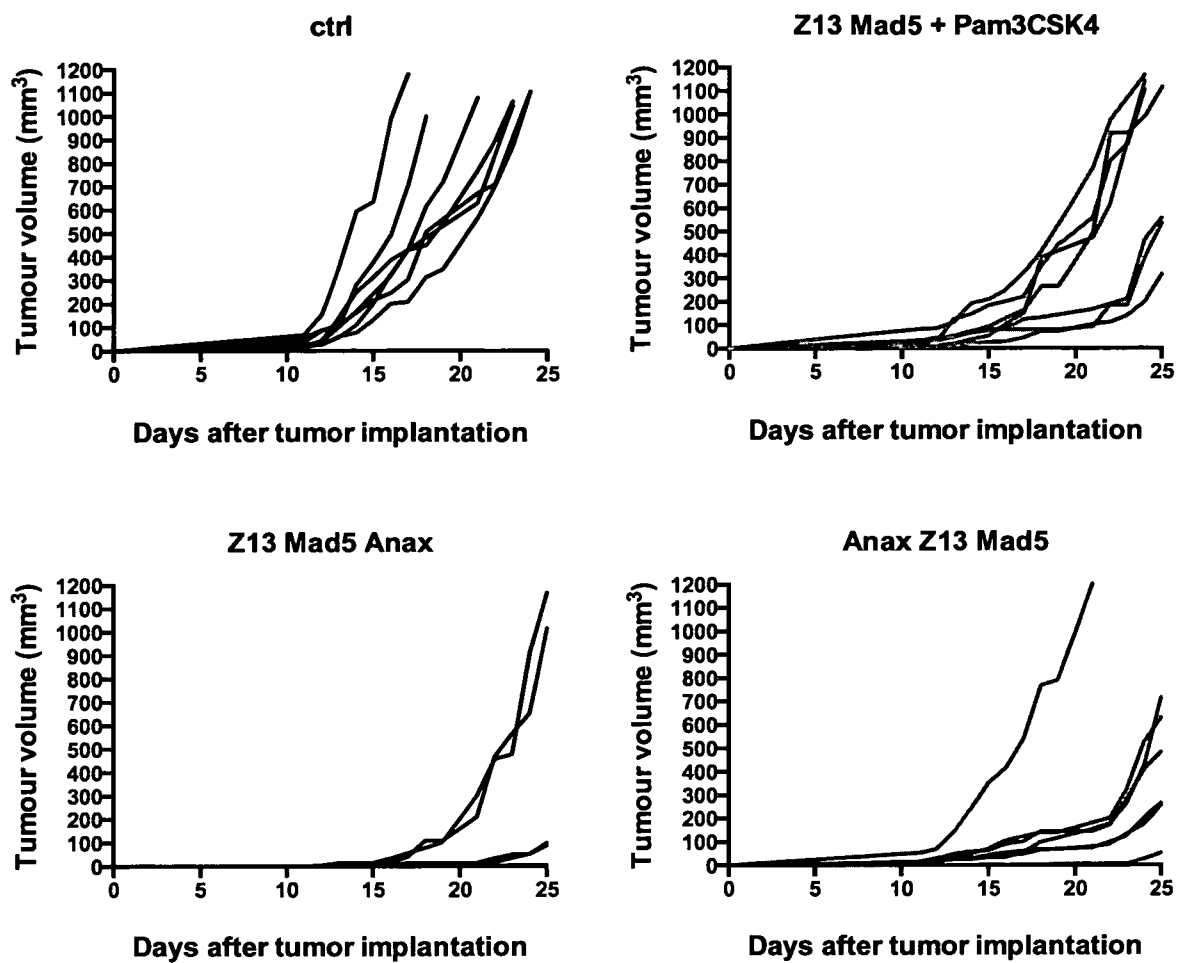
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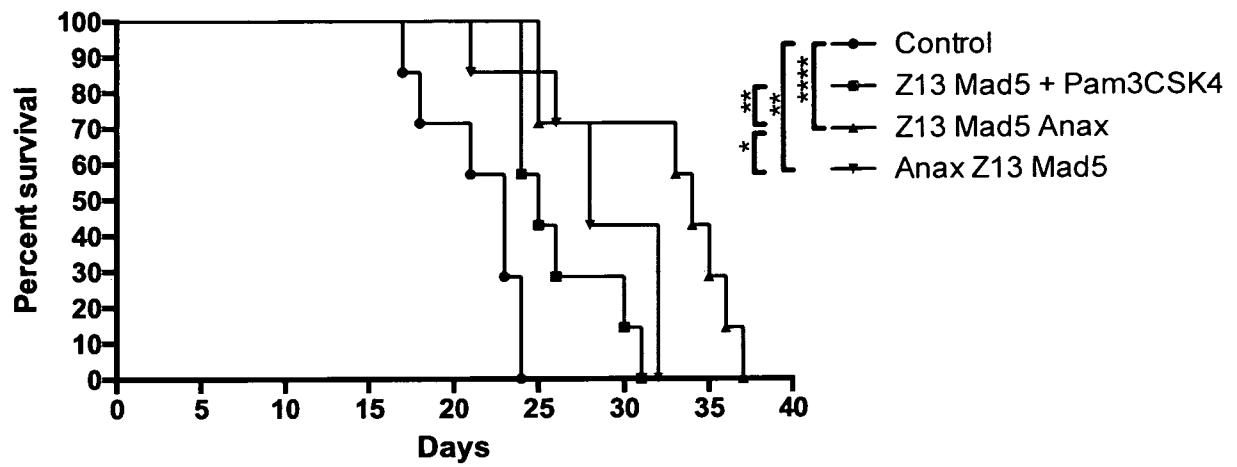
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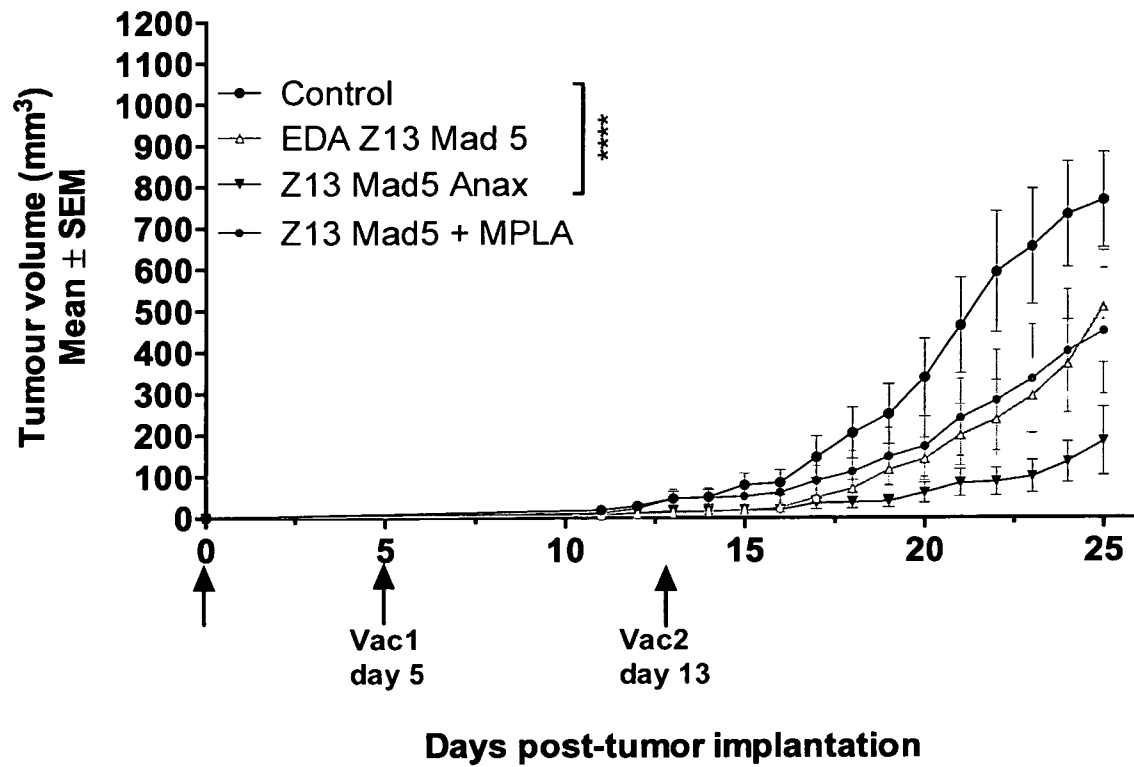
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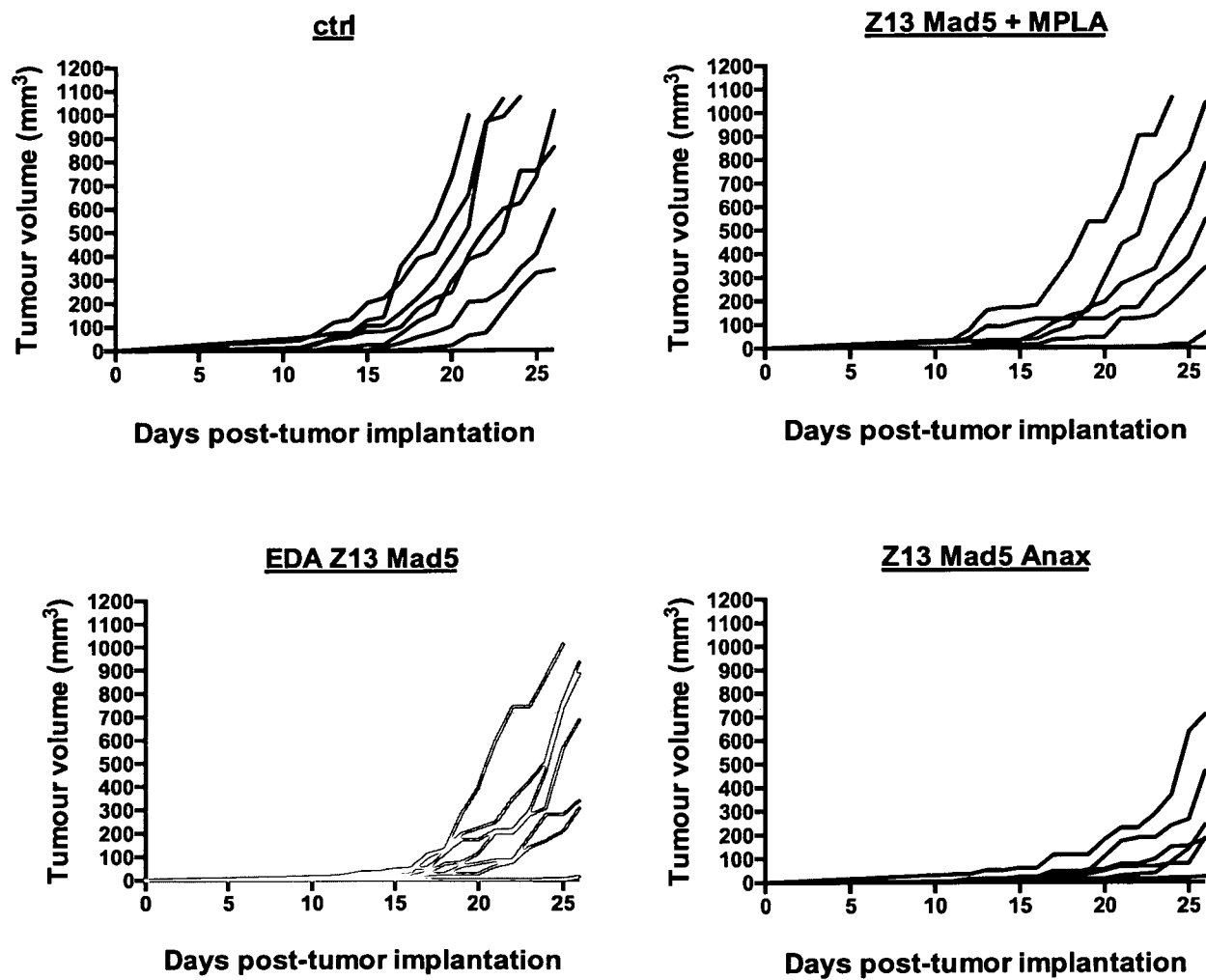
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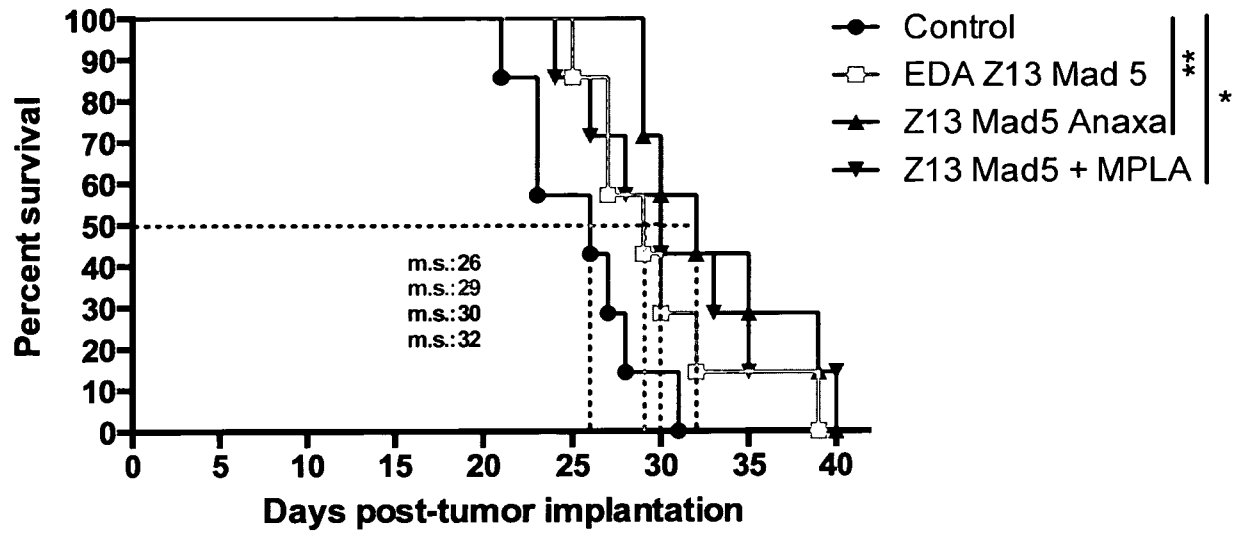
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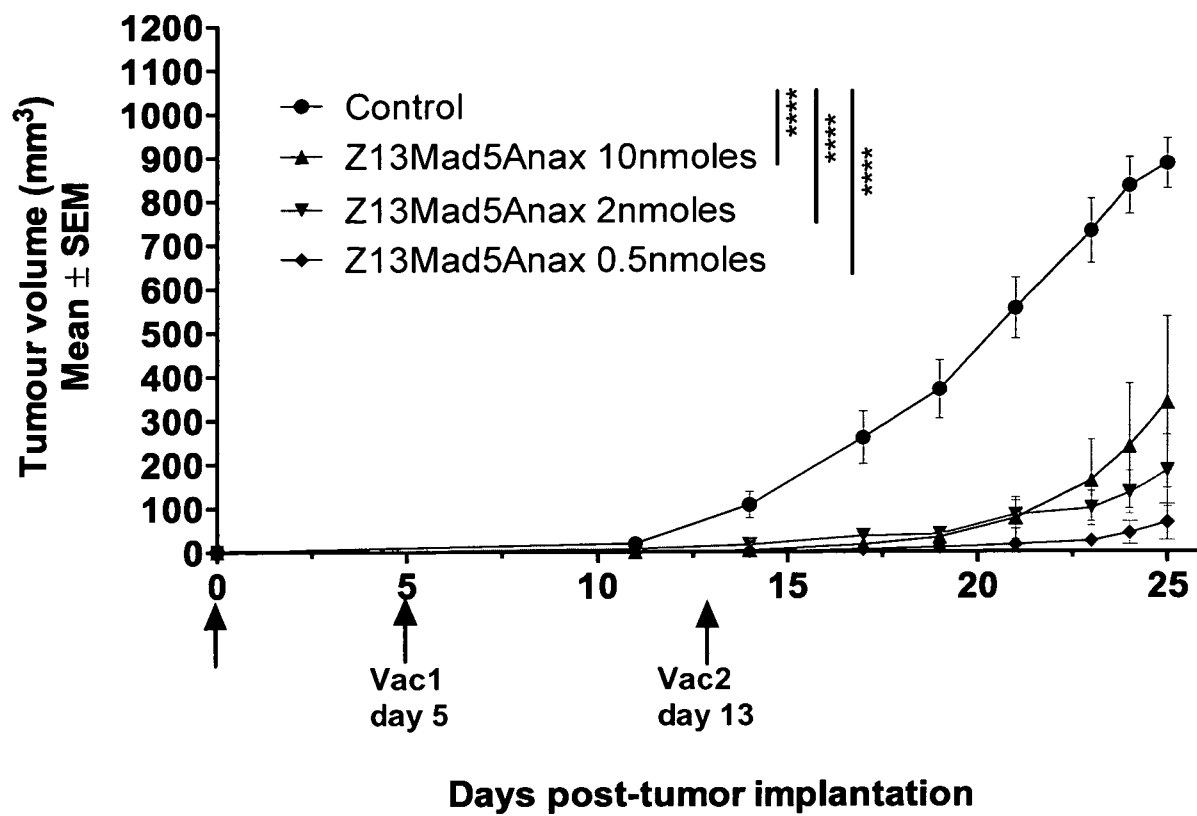
Fig. 23

A

0.5nmoles

B

2nmoles

Fig. 24

A

KLRG1

B

PD-1

Fig. 25

Fig. 26

Fig. 27

Fig. 28

Fig. 29

Fig. 30

Fig. 31

Fig. 32

Fig. 33

Fig. 34

Fig. 35

Fig. 36

A

B

Fig. 37

A

B

Fig. 38

A

B

Fig. 39

A   2 nmol

B   0.5 nmol

Fig. 40

A

Draining Lymph nodes

B

Spleen

C

Bone Marrow

Fig. 41

A

B

Fig. 42

Fig. 43

A

B

Fig. 44

Fig. 45

A

B

Fig. 46

A

B

Fig. 47

48/55

Fig. 48

A

B

Fig. 49

A

B

Fig. 50

Fig. 51

Fig. 52

Fig. 53

Fig. 54

Fig. 55