Fig. 5: Efficacy of GAd vaccines in animals with high tumour burden requires targeting many neo-antigens and the combination with anti-PD1. Mice were inoculated s.c. with CT26 cells. One week later, mice were randomized according to tumor volume (mean of 70-100 mm^3). Treatment with GAds vaccine started at day 0. **A)** Tumor growth in individual mice over time is shown in mice vaccinated with GAd-CT26-31 versus cntr (untreated) mice. **B)** Efficacy of anti-PD1 and combination of anti-PD1 with GAd-CT26-5 or GAd-CT26-31. Vaccine was administered at day 0 (im), while anti-PD1 was given twice per week until day 16 (ip). Shown is tumor growth in individual mice over time. Statistics are calculated by a Chi square test evaluating the number of cured mice (responders) versus non-responder mice.

Fig. 6: neoAg-specific T cell responses measured by IFN-γ ELISpot in tumor bearing mice receiving GAd-CT26-31 and anti-PD1 treatment (day30). Responses are measured on splenocytes stimulated in presence of synthetic peptides corresponding to the immunogenic neoAgs and expressed as number of T cells producing IFN-γ per millions of splenocytes.

Fig. 7: Tumor growth in tumor bearing mice treated with GAd-CT26-31 and anti-PD1 in the presence of a CD4+ T cell (CD4 depleted) or CD8+ T cell (CD8 depleted) depleting antibody or in an undepleted T cell control group. Data represent at least 2 independent experiments. Statistical significance is indicated by *($P < 0.05$ by Fisher exact test) or by NS (not significant).

Fig. 8: Significant difference (onesided Wilcoxon test) of intra-tumor TCR diversity (number of clonotypes) between mice from the combined treatment (left) and mice from only anti-PD1 treatment (right). Combined treatment responder mice (left, filled circles), combined treatment non-responder mice (left, filled boxes), anti-PD1 only treatment responder mice (right, triangles pointing upwards), anti-PD1 only treatment non-responder mice (right, triangles pointing downwards).

SUMMARY ~~OF THE INVENTION~~

In one aspect, the present disclosure relates to a polypeptide comprising at least 31 tumor-specific neo-antigens and at least one T cell enhancer amino acid sequence, wherein the neo-antigens independently of each other have a length of 8 to 50 amino acids, wherein the T cell enhancer amino acid sequence is an invariant chain or a leader sequence of tissue-type plasminogen activator (TPA) and wherein the T cell enhancer amino acid sequence is placed N-terminally within the polypeptide. In a first ~~aspect~~example the present ~~invention~~disclosure relates

to a polypeptide comprising at least 25 different tumor-specific neo-antigens and at least one T cell enhancer amino acid sequence.

In ~~a second~~another ~~aspect~~example the present ~~invention~~disclosure relates to a nucleic acid encoding the polypeptide of the first ~~aspect~~example of the invention.

In another~~a third aspect~~ example the present ~~invention~~disclosure relates to a vector comprising the nucleic acid ~~of the second aspect of the present invention~~ as described herein operatively linked to an expression control sequence.

In yet another aspect, the present disclosure relates to a collection of one or more expression vectors each comprising a nucleic acid as described herein, wherein each expression vector is selected from the group consisting of a plasmid; a cosmid; an RNA; an RNA-formulated with an adjuvant; an RNA formulated in liposomal particles; a self-amplifying RNA (SAM); a SAM formulated with an adjuvant; a SAM formulated in liposomal particles; a viral vector; or an alphavirus vector, a venezuelan equine encephalitis (VEE) virus vector, a sindbis (SIN) virus vector, a semliki forest virus (SFV) virus vector, a simian or human cytomegalovirus (CMV) vector, a Lymphocyte choriomeningitis virus (LCMV) vector, a retroviral or lentiviral vector, or a replication competent or incompetent Great Apes derived adenoviral vector derived from chimpanzee or bonobo or gorilla, a poxvirus vector, a vaccinia virus vector or a modified vaccinia ankara (MVA) vector. In an ~~fourthaspect~~example the present ~~invention~~disclosure relates to a collection of one or more expression vectors each comprising a nucleic acid as described herein~~according to the second aspect of the invention~~, wherein each expression vector is selected from the group consisting of a plasmid; a cosmid; an RNA; an RNA-formulated with an adjuvant; an RNA formulated in liposomal particles; a self-amplifying RNA (SAM); a SAM formulated with an adjuvant; a SAM formulated in liposomal particles; a viral vector; preferably an alphavirus vector, a venezuelan equine encephalitis (VEE) virus vector, a sindbis (SIN) virus vector, a semliki forest virus (SFV) virus vector, a simian or human cytomegalovirus (CMV) vector, a Lymphocyte choriomeningitis virus (LCMV) vector, a retroviral or lentiviral vector. P preferably a replication competent or incompetent Great Apes derived adenoviral vector preferably derived from chimpanzee or bonobo or gorilla, a poxvirus vector, a vaccinia virus vector or a modified vaccinia ankara (MVA) vector.

In yet another aspect, the present disclosure relates to a use of the composition comprising a vaccine comprising the polypeptide as described herein, the nucleic acid as described herein, the vector as described herein or a collection of vectors as described herein and at least one modulator of a checkpoint molecule or immunomodulator or a nucleic acid encoding the modulator or immunomodulator or a vector comprising the nucleic acid encoding the modulator or immunomodulator in the manufacture of a medicament for preventing or treating a proliferative disease in a subject. In a fifth ~~aspect~~example the present ~~invention~~disclosure relates to a composition comprising a vaccine comprising the polypeptide as described herein~~of the first aspect~~, the nucleic acid as described herein~~of the second aspect of the invention~~, the vector as described herein ~~of claim the third aspect of the invention~~ or a collection of vectors as described herein~~according to the fourth aspect of the invention~~ and at least one modulator of a checkpoint molecule or a nucleic acid encoding the modulator or a vector comprising the nucleic acid encoding the modulator for use in preventing or treating a proliferative disease in a subject.

In a ~~sixth~~ further ~~aspect~~example the present ~~invention~~disclosure relates to a vaccination kit comprising in separate packaging:

(i) a vaccine comprising the polypeptide as described herein~~of the first aspect of the invention~~, the nucleic acid as described herein~~of the second aspect of the invention~~, the vector as described herein ~~of the third aspect of the invention~~ or a collection of vectors as described herein~~according to the fourth aspect of the invention~~; and

(ii) at least one modulator of a checkpoint molecule or a nucleic acid encoding the modulator or a vector comprising the nucleic acid encoding the modulator.

DETAILED DESCRIPTION OF THE INVENTION

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

Claims

1. A polypeptide comprising at least ~~25~~ 31 ~~different~~ tumor-specific neo-antigens and at least one T cell enhancer amino acid sequence, wherein the neo-antigens independently of each other have a length of 8 to 50 amino acids, wherein the T cell enhancer amino acid sequence is an invariant chain or a leader sequence of tissue-type plasminogen activator (TPA) and wherein the T cell enhancer amino acid sequence is placed N-terminally within the polypeptide.

~~2. The polypeptide of claim 1, comprising at least 31 tumor-specific neo-antigens.~~

~~3~~2. The polypeptide of claim 1 ~~or 2~~, wherein each tumor-specific neo-antigens independently of each other have a length of ~~8 to 50 amino acids, preferably a length of~~ 15 to 35, ~~more~~ or ~~preferably of~~ 25 amino acids.

~~4~~3. The polypeptide of ~~any of~~ claim~~s~~ 1 ~~to 3~~or 2, wherein each tumor-specific neo-antigen is independently selected from the group consisting of a single amino acid mutant peptide, a frame-shift peptide, a read-through mutation peptide, and a splice site mutant peptide.

~~5~~4. The polypeptide of any of claims 1 to ~~4~~3, wherein at least 4 of the tumor-specific neo-antigens elicits a T cell response in the patient.

~~6~~5. The polypeptide of any of claims 1 to ~~5~~4, wherein the tumor-specific neo-antigens are linked directly to each other.

~~7. The polypeptide of any of claims 1 to 6, wherein the T cell enhancer amino acid sequence is selected from the group consisting of an invariant chain; a leader sequence of tissue-type plasminogen activator (TPA); a PEST sequence; a cyclin destruction box; an ubiquitination signal; and a SUMOylation signal.~~

~~8~~6. The polypeptide of any of claims ~~7~~1 to 5, wherein:

(i) the TPA is an extended TPA leader sequence with an amino acid sequence according to SEQ ID NO: 42, or the TPA is an extended TPA leader sequence with an amino acid sequence according to SEQ ID NO: 42 and is ~~preferably~~ present at the N-terminus of the polypeptide; and/or

(ii) the invariant chain is selected from the group consisting of:

(a) human invariant chain according to SEQ ID NO: 36, mouse invariant chain according to SEQ ID NO: 37, and Mandarin fish invariant chain according to SEQ ID NO: 38;

(b) an immune stimulatory fragment of an invariant chain according to (a); and/or

(c) an immune stimulatory variant of (a) or (b), wherein the variant has at least ~~70~~95% sequence identity to the invariant chain according to (a) or a fragment thereof according to (b).

~~9~~7. A nucleic acid encoding the polypeptide of any of claims 1 to ~~8~~6.

~~10~~8. A vector comprising the nucleic acid of claim ~~9~~ 7 operatively linked to an expression control sequence.

~~11~~9. A collection of one or more expression vectors each comprising a nucleic acid according to claim ~~9~~7, wherein each expression vector is selected from the group consisting of a plasmid; a cosmid; an RNA; an RNA-formulated with an adjuvant; an RNA formulated in liposomal particles; a self-amplifying RNA (SAM); a SAM formulated with an adjuvant; a SAM formulated in liposomal particles; a viral vector; ~~preferably~~ or an alphavirus vector, a venezuelan equine encephalitis (VEE) virus vector, a sindbis (SIN) virus vector, a semliki forest virus (SFV) virus vector, a simian or human cytomegalovirus (CMV) vector, a Lymphocyte choriomeningitis virus (LCMV) vector, a retroviral or lentiviral vector~~. P,~~ ~~preferably~~ or a replication competent or incompetent Great Apes derived adenoviral vector ~~preferably~~ derived from chimpanzee or bonobo or gorilla, a poxvirus vector, a vaccinia virus vector or a modified vaccinia ankara (MVA) vector.

~~12~~10. ~~A~~ Use of the composition comprising a vaccine comprising the polypeptide of any of claims 1 to ~~8~~6, the nucleic acid of claim ~~9~~7, the vector of claim ~~10~~ 8 or a collection of vectors according to claim ~~11~~ 9 and at least one modulator of a checkpoint molecule or immunomodulator or a nucleic acid encoding the modulator or immunomodulator or a vector comprising the nucleic acid encoding the modulator or immunomodulator ~~for use~~ in the manufacture of a medicament for preventing or treating a proliferative disease in a subject.

~~13~~11. The ~~composition~~ use of claim ~~12~~10, wherein the modulator of a checkpoint molecule is selected from the group consisting of:

(a) an agonist of a tumor necrosis factor (TNF) receptor superfamily member, ~~preferably~~ or of CD27, CD40 (e.g. CP-870,893), OX40, GITR or CD137; and/or

(b) an antagonist of PD-1, PD-L1, CD274, A2AR, B7-H3 (e.g. MGA271), B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, TIM-3, or VISTA or an antagonist of a B7-CD28 superfamily member, ~~preferably~~ or of CD28 or ICOS or an antagonist of a ligand thereof; and/or

(c) the other immunomodulator is a T cell growth factor like IL-2, IL-12, IL-15.

~~14~~12. The ~~composition~~ use of claim ~~12~~ 10 or ~~13~~11, wherein administration of the modulator of a checkpoint molecule is to be initiated before initiation of administration of the vaccine, or wherein administration of the checkpoint inhibitor is to be initiated after initiation of administration of the vaccine, or wherein administration of the checkpoint inhibitor is to be initiated simultaneously with the initiation of administration of the vaccine, or wherein administration of the checkpoint inhibitor is to be initiated simultaneously with the initiation of administration of the vaccine and~~preferably~~ the vaccination regimen is a heterologous prime boost with two different viral vectors.

~~15~~13. The ~~composition~~ use of any one of claims ~~12~~ 10 to ~~14~~12, wherein the subject is suffering from or is at risk of suffering from:

(a) Malignant neoplasms of lip, oral cavity and pharynx; and/or

(b) Malignant neoplasms of digestive organs; and/or

(c) Malignant neoplasms of respiratory and intrathoracic organs; and/or

(d) Malignant neoplasms of bone and articular cartilage; and/or

(e) Melanoma and other malignant neoplasms of skin; and/or

(f) Malignant neoplasms of mesothelial and soft tissue; and/or

(g) Malignant neoplasm of breast; and/or

(h) Malignant neoplasms of female genital organs; and/or

(i) Malignant neoplasms of male genital organs; and/or

(j) Malignant neoplasms of urinary tract; and/or

(k) Malignant neoplasms of eye, brain and other parts of central nervous system; and/or

(l) Malignant neoplasms of thyroid and other endocrine glands; and/or

(m) Malignant neoplasms of lymphoid, haematopoietic and related tissue.

~~16~~14. The ~~composition~~ use of any one claims ~~12~~ 10 to ~~14~~12, wherein the subject has a tumor according to the TNM classification of at least stage T1 with any stage of N and M, and/or a tumor that is characterized by a lesion of at least about 3 mm in diameter.

~~17~~15. A vaccination kit comprising in separate packaging:

(i) a vaccine comprising the polypeptide of any of claims 1 to ~~8~~6, the nucleic acid of claim ~~9~~7, the vector of claim ~~10~~ 8 or a collection of vectors according to claim ~~11~~9; and

(ii) at least one modulator of a checkpoint molecule or a nucleic acid encoding the modulator or a vector comprising the nucleic acid encoding the modulator.