

in curing cancer is to induce a diverse population of immune T cells capable of recognizing and eliminating as large a number of cancer cells as possible at once, to decrease the chance that cancer cells can “escape” the T cell response. Therefore, it is desirable that the vaccine encodes quite a large number of cancer specific antigens. This is particular relevant for the recently described personalized vaccine approach based on cancer specific neoantigens. In order to optimize the probability of success as many neoantigens as possible should be targeted by the vaccine, however the maximal insert size of vectors is limited. Full-length INV sequences or large fragments thereof occupy a relatively large portion of the vaccine antigen insert. Therefore, the use of short polypeptide as T cell enhancer is preferable in the context of anti-cancer vaccination especially when using several cancer specific antigens in the vaccine.

Genetic vaccination platforms based on adenovirus, in particular Great Apes derived Adenovirus (GAd) viral vector were shown to be very potent for induction of T cell responses and Great Ape derived Adenoviruses are suitable for encoding large antigens in the format of artificial genes composed of polynucleotides encoding fragments from different proteins linked one after the other (Borthwick, N., et al., Mol Ther, 2014. 22(2): p. 464-75). Unexpectedly, when used in the context of cancer specific neoantigens, no T cell mediated immune response was induced.

The present inventors identified specific INV sequences able to restore immunogenicity, which are also referred to in the present application as “T cell enhancer amino acid sequence”. Such T cell enhancer amino acid sequences were suitable in overcoming the lack of or poor immunogenicity of the cancer specific neoantigens. In particular two short fragments of a non-human *Teleostei* INV were identified both not including the transmembrane domain that acted as potent T cell enhancers.

The use of human INVs or of INVs of phylogenetically closely related species may result in undesired induction of an immune response against this self sequence in the context of vaccination. The autoimmune response would be in this case directed towards normal tissues in which INV is expressed. The present inventors have surprisingly found that INVs of teleosts although quite different from mammalian INVs increase the T cell response against antigens in mammals, that this T cell response enhancing effect is exerted on multiple antigens fused to a teleost invariant chain and that already a short fragment of teleost INV is sufficient to elicit this response. Thus, the present invention provides inter alia: (i) an improved enhancer of T cell response against antigens in mammals, with a decreased likelihood of eliciting unwanted T cell responses against healthy tissue, (ii) an enhancer of T cell response against multiple antigens, and (iii) a short fragment capable of eliciting a T cell that maximizes the ability to fuse a large number of antigens or antigenic fragments thereof.

In a first aspect the present disclosure provides a polypeptide comprising:

- (a) a fragment of an invariant chain (INV) of a *Teleostei*, which has T cell response enhancer activity, comprising or consisting of between 16 to 27 amino acids of the membrane proximal domain (MPD) of an INV of the *Teleostei*, wherein the MPD is preferably characterized by the following amino acid sequence:

$X_1 QKX_2 QIHTLQKX_3 SX_4 RX_5 X_6 X_7 QX_8 TRX_9 SX_{10} AV$

wherein

X_1 is G, D, S or N;

X_2 is E or Q;

10 X_3 is N or S;

X_4 is D or E;

X_5 is M or L;

X_6 is G, N, S or T;

X_7 is K or R;

15 X_8 is L or M;

X_9 is S, T or A; and

X_{10} is Q or H;

and wherein the 16 to 27 amino acids of the MPD are preferably at least 70% identical to SEQ ID NO: 7;

20 and optionally one or more antigens or antigenic fragments thereof;

or

- (b) a full length *Teleostei* INV of SEQ ID NO: 1 or a variant thereof, which has T cell response enhancer activity, wherein the amino acid sequence of the MPD of the variant is at least 80% identical to SEQ ID NO: 7 and one or more antigens or antigenic fragments thereof.

25 In a second aspect the present disclosure relates to a polynucleotide encoding the polypeptide according to the first aspect of the present disclosure, wherein the polynucleotide is preferably DNA or RNA, more preferably a DNA.

In a third aspect the present disclosure relates to a vector comprising the polynucleotide according to the second aspect of the disclosure.

30 In a fourth aspect the present disclosure relates to a collection of two or more different vectors, wherein the different vectors each comprise a polynucleotide according to the second aspect of the present disclosure encoding a different polypeptide according to the first aspect of the present disclosure.

35 In a fifth aspect the present disclosure relates to a pharmaceutical composition comprising the polypeptide of the first aspect of the present disclosure, a polynucleotide of the second aspect of the

present disclosure or a vector of the third aspect of the present disclosure or collection of vectors of the fourth aspect of the disclosure and a pharmaceutically acceptable excipient and optionally one or more adjuvants.

In another aspect, the present disclosure refers to a kit of parts comprising the pharmaceutical composition as described herein and separately packaged at least one immunomodulator, or at least one polynucleotide encoding the immunomodulator, or a vector comprising the polynucleotide encoding the immunomodulator.

In an example the present disclosure relates to a kit of parts comprising the pharmaceutical composition of the fifth aspect of the present disclosure and separately packaged at least one immunomodulator, for example a modulator of a checkpoint molecule (MCM), or at least one polynucleotide encoding the immunomodulator, for example a MCM, or a vector comprising the polynucleotide encoding the immunomodulator, for example a MCM.

In yet another aspect, the present disclosure refers to the use of a polypeptide as described herein, the polynucleotide as described herein, a vector or a collection of vectors as described herein, or a pharmaceutical composition or kits comprising such pharmaceutical compositions as described herein in the manufacture of a medicament for preventing or treating a proliferative disease, preferably cancer, viral disease, fungal disease or bacterial disease.

In an example the present disclosure relates to a polypeptide according to the first aspect of the disclosure, a polynucleotide according to the second aspect of the disclosure, or a vector or a collection of vectors according to third or fourth aspect of the disclosure for use as a medicament.

In another example the present disclosure relates to polypeptide according to the first aspect of the disclosure, a polynucleotide according to the second aspect of the disclosure, or a vector or a collection of vectors according to third or fourth aspect of the disclosure, or a pharmaceutical composition according to the fifth aspect of the disclosure or kits comprising such pharmaceutical compositions according to the sixth aspect of the disclosure for use in preventing or treating a proliferative disease, preferably cancer, viral disease or bacterial disease.

FIGURE LEGENDS

Fig. 1: Immunogenicity of GAd vectors at doses of 5×10^7 vp or 5×10^8 vp. Vectors encode for Mandarin Fish INV full length (MF_INV_FL), Mandarin Fish INV short version (residue 1 to 81, MF_INV_SH), Mandarin Fish INV variants A (residue 62 to 88, MF_INV_A) and B (residue 66 to 81, MF_INV_B) linked to the CT26 pentatope antigen. Penta represents the CT26 pentatope antigen without the Mandarin Fish INV sequence or a fragment thereof and with only an initial

methione. Values reported were obtained by an ELISpot assay on spleen cells of immunized animals. Splenocytes were stimulated ex vivo two weeks post vaccination with a pool of five synthetic peptides corresponding to the sequences of the five cancer specific neoantigens containing the mutation. Responses are expressed as number of T cells producing IFN γ per millions of splenocytes. Shown at the bottom is the number of mice showing a positive response out of a total number of 6 immunized mice for each tested vector construct. The dashed line represents the threshold for a positive response.

Fig. 2: Alignment of invariant chains of various *Teleostei* species. The first box characterizes the 26 amino acid long transmembrane domain (TMD). The membrane proximal domain (MPD) is immediately C-terminally of the TMD and highlighted with a second box. The location and extend of the short 16 amino acid long invariant chain fragment MF_INV_B comprised in the MPD is highlighted by a series of asterisk (*) below the second box. The INV sequences are from top to

CLAIMS

1. A polypeptide comprising:

- (a) a fragment of an invariant chain (INV) of a *Teleostei* comprising or consisting of between 16 to 27 amino acids of the membrane proximal domain (MPD) of an INV of the *Teleostei*, wherein the MPD is characterized by the following amino acid sequence (SEQ ID NO: 31):

X₁ QKX₂ QIHTLQKX₃ SX₄ RX₅ X₆ X₇ QX₈ TRX₉ SX₁₀ AV

wherein

X₁ is G, D, S or N;

X₂ is E or Q;

X₃ is N or S;

X₄ is D or E;

X₅ is M or L;

X₆ is G, N, S or T;

X₇ is K or R;

X₈ is L or M;

X₉ is S, T or A; and

X₁₀ is Q or H;

and wherein the 16 to 27 amino acids of the MPD are preferably at least 70% identical to SEQ ID NO: 7;

and optionally one or more antigens and/or one or more antigenic fragments thereof;

or

- (b) a full length *Teleostei* INV of SEQ ID NO: 1 or a variant thereof, which has T cell response enhancer activity, wherein the amino acid sequence of the MPD of the variant is at least 80% identical to SEQ ID NO: 7 and one or more antigens and/or one or more antigenic fragments thereof.

2. The polypeptide of claim 1, wherein the amino acid sequence of:

- (a) the MPD according to alternative (a) of claim 1 is any of SEQ ID NOs: 7 to 12; or

- (b) the fragment according to alternative (a) of claim 1 comprises or consists of any of SEQ ID NOs: 7 to 18; or

- (c) the *Teleostei* invariant chain according to alternative (b) of claim 1 is any of SEQ ID NOs: 1 to 6.

3. The polypeptide of claim 1 (a) or claim 2 alternative (a) or (b), wherein the fragment consists of at least 16 to 27 N-terminal amino acids of the MPD and:

- (i) 1 to 26 consecutive amino acids of the transmembrane domain (TMD) of an INV of a *Teleostei* immediately N-terminal of the MPD, wherein the TMD of the INV of the *Teleostei* is preferably characterized by the following amino acid sequence (SEQ ID NO: 32):

AY₁KY₂AY₃LT₄TTLY₄CLLY₅Y₆SQVFTAYY₇VF

wherein

Y₁ is L or F;

Y₂ is I or V;

Y₃ is G or A;

Y₄ is T or A;

Y₅ is L or V;

Y₆ is A or S; and

Y₇ is M or T;

and/or

- (ii) 1 to 19 consecutive amino acids of the INV of a *Teleostei* immediately C-terminal of the MPD preferably characterized by the following amino acid sequence (SEQ ID NO: 33):

APZ₁Z₂MZ₃Z₄PMZ₅SLPZ₆Z₇Z₈DZ₉Z₁₀

wherein

Z₁ is M, V or A;

Z₂ is R or K;

Z₃ is H, A or Q;

Z₄ is M or L;

Z₅ is N or S;

Z₆ is M or L;

Z₇ is M, L or V;

Z₈ is M or S;

Z₉ is F or Y; and

Z₁₀ is T or S.

4. The polypeptide according to any of claims 1 to 3, wherein the antigen in each case is independently selected from the group consisting of a cancer-specific antigen, a cancer specific neoantigen or, a viral protein, a bacterial protein, wherein preferably:

(i) the polypeptide comprises at least five different antigens and/or antigenic fragments thereof;

and/or

(ii) the one or more antigens and/or one or more antigenic fragments thereof are C-terminally of the fragment of the INV according to alternative (a) of claim 1 or the full length INV according to alternative (b) of claim 1.

5. A polynucleotide encoding the polypeptide according to any one of claims 1 to 4, wherein the polynucleotide is preferably DNA or RNA, more preferably a DNA.

6. A vector comprising the polynucleotide according to claim 5.

7. A collection of two or more different vectors, wherein the different vectors each comprise a polynucleotide according to claim 5 encoding a different polypeptide according to claims 1 to 4.

8. The vector of claim 9 or the collection of vectors of claim 10, wherein the vector in each case is independently selected from the group consisting of a plasmid; a cosmid; a liposomal particle, a viral vector or a virus like particle; 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 vector, a lentiviral vector, an adenoviral vector, an adeno-associated virus vector a poxvirus vector, a vaccinia virus vector or a modified vaccinia ankara (MVA) vector, wherein the adenoviral vector is preferably a Great Apes adenoviral vector, more preferably a chimpanzee or bonobo or gorilla adenoviral vector.

9. A pharmaceutical composition comprising the polypeptide of any of claims 1 to 4, polynucleotide of claim 5, or a vector(s) of claim 6 to 8, a pharmaceutically acceptable excipient and optionally one or more adjuvants.

10. The pharmaceutical composition of claim 9 comprising at least one immunomodulator, or at least one polynucleotide encoding the immunomodulator, or a vector or liposomal particle comprising the polynucleotide encoding the immunomodulator.

11. A kit of parts comprising the pharmaceutical composition of claim 9 and separately packaged at least one immunomodulator, or at least one polynucleotide encoding the immunomodulator, or a vector comprising the polynucleotide encoding the immunomodulator.
12. The pharmaceutical composition of claim 10 or the kit of parts of claim 11, wherein:
 - (i) the immunomodulator is a MCM and is selected from the group consisting of:
 - (a) an agonist of a tumor necrosis factor (TNF) receptor superfamily member, preferably of CD27, CD40 (e.g. CP-870,893), OX40, GITR or CD137; and/or
 - (b) an antagonist of PD-1, 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 of CD28 or ICOS or an antagonist of a ligand thereof;
 - and/or
 - (ii) the immunomodulator is a T cell growth factor, preferably IL-2, IL-12, or IL-15.
13. Use of a polypeptide according to claims 1 to 4, the polynucleotide according to claim 5, a vector or a collection of vectors according to claim 6 to 8, or a pharmaceutical composition or kits comprising such pharmaceutical compositions according to claims 9 to 12 in the manufacture of a medicament for preventing or treating a proliferative disease, preferably cancer, viral disease, fungal disease or bacterial disease.
14. The use according to claim 13, wherein the cancer is selected from the group consisting of malignant neoplasms of lip, oral cavity, pharynx, a digestive organ, respiratory organ, intrathoracic organ, bone, articular cartilage, skin, mesothelial tissue, soft tissue, breast, female genital organs, male genital organs, urinary tract, brain and other parts of central nervous system, thyroid gland, endocrine glands, lymphoid tissue, and haematopoietic tissue.
15. The use according to claim 13, wherein at least one immunomodulator, or at least one polynucleotide encoding the immunomodulator, or a vector or liposomal particle comprising the polynucleotide encoding the immunomodulator, is to be administered prior to, concomitantly with or subsequently to the administration of said medicament.