members. One ligand for PD-1, PD-Ll is abundant in a variety of human cancers (Dong et al (2002) Nat. Med 8:787-9). The interaction between PD-1 and PD-Ll results in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation allowing immune evasion by the cancerous cells (Dong et al. (2003) J. MoI. Med. 81:281-7; Blank et al. (2005) Cancer Immunol. Immunother. 54:307-314; Konishi et al. (2004) Clin. Cancer Res. 10:5094-100). Immune suppression can be reversed by inhibiting the local interaction of PD-1 with PD-Ll, and the effect is additive when the interaction of PD-1 with PD-L2 is blocked as well (Iwai et al. (2002) Proc. Nat 7. Acad. ScL USA 99: 12293-7; Brown et al. (2003) J. Immunol. 170:1257-66).

Antibodies that bind to PD-1 are described e.g. in WO 2017/055443 A1.

Summary ~~of the invention~~

The present ~~invention~~disclosure provides a novel approach of targeting a mutant form of IL-7 with advantageous properties for immunotherapy directly to immune effector cells, such as cytotoxic T lymphocytes, rather than tumor cells, through conjugation of the mutant IL-7 polypeptide to an antibody that binds to PD-1. This results in cis-delivery of the IL-7 mutant to PD-1 expressing immune subsets, especially tumor reactive T cells e.g. CD8+ PD1+ TCF+ T cell subsets and their progeny.

The IL-7 mutants used in the present ~~invention~~disclosure have been designed to overcome the problems associated with cytokine immunotherapy, in particular toxicity caused by the induction of VLS, tumor tolerance caused by the induction of AICD, and immunosuppression caused by activation of T_{reg} cells. In addition to circumventing escape of tumors from tumor-targeting as mentioned above, targeting of the IL-7 mutant to immune effector cells may further increase the preferential activation of tumor specific CTLs over immunosuppressive T_{reg} cells due to lower PD-1 and IL-7Rα expressing levels on Tregs than CTLs. By using an antibody that binds to PD-1, the suppression of T-cell activity induced by the interaction of PD-1 with its ligand PD-L1 may additionally be reversed, thus further enhancing the immune response.

In ~~a general~~one aspect, the ~~invention~~disclosure provides a mutant interleukin-7 (IL-7) polypeptide comprising at least one amino acid substitution in a position selected from the group of E13, V15, V18, D21, Q22, D25, T72, L77, K81, E84, G85, I88, Q136, K139, N143 and M147 of human IL-7

according to SEQ ID NO:52.~~; i.e. the~~ The numbering above is relative to the human IL-7 sequence SEQ ID NO:52. In some ~~embodiments~~examples of the ~~invention~~disclosure, the mutant interleukin-7 polypeptide comprises at least one amino acid selected from the group of E13A, E13K, V15A, V15K, V18A, V18K, D21A, D21K, Q22A, Q22K, D25A, D25K, T72A, D74K, L77A, L77K, K81A, K81E, E84A, G85K, G85E, I88K, Q136A, Q136K, K139A, K139E, N143K and M147A. In some ~~embodiments~~examples of the ~~invention~~disclosure the mutant interleukin-7 polypetide comprises at least one amino acid substitution selected from the group of V15A, V15K, V18A, V18K, L77A, L77K, K81E, G85K, G85E, I88K and N143K. In some ~~embodimenets~~ examples of the ~~invention~~disclosure the mutant interleukin-7 polypeptide comprises an amino acid sequence selected from the group of SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 135 and SEQ ID NO: 136.

In some ~~embodiments~~examples of the ~~invention~~disclosure the mutant interleukin-7 polypetide comprises an amino acid sequence selected from the group of SEQ ID NO: 55, SEQ IN NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 79, SEQ ID NO: 135 and SEQ ID NO: 136.

In another ~~aspect~~example, the ~~invention~~disclosure provides a mutant interleukin-7 polypeptide comprising an amino acid substitution, which eliminates the N-Glycosylation site of IL-7 at a position selected from the group of position 72, 93 and 118. The substitution may be selected from the group of T72A, T93A and S118A. In another ~~aspect~~example, the ~~invention~~disclosure provide a mutant interleukin-7 polypeptide comprising the amino acid subsitutions T72A, T93A and S118A. In some ~~embodiments~~examples of the ~~invention~~disclosure the mutant interleukin 7 polypeptide comprises an amino acid sequence selected from the group of SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83 and SEQ ID NO: 84.

In another ~~aspect~~example, the ~~invention~~disclosure provides for a mutant interleukin-7 polypeptide comprising at least the amino acid substitutions K81E and G85K or K81E and G85E. In some ~~embodimenets~~ examples of the ~~invention~~disclosure, the mutant interleukin-7 polypeptide comprises an amino acid sequence of SEQ ID NO: 135 or SEQ ID NO: 136.

In a further ~~aspect~~example, the ~~invention~~disclosure provides for a mutant interleukin-7 polypeptide as disclosed herein, wherein said mutant IL-7 polypeptide is linked to a non-IL-7 moiety. The mutant interleukin-7 polypeptide may be linked to a first and a second non-IL-7 moiety. The mutant IL-7 polypeptide may share a carboxy-terminal peptide bond with said first non-IL-7 moiety and an aminoterminal peptide bond with said second non-IL-7 moiety. The non-IL-7 moiety may be an antigen binding moiety or an immune effector cell binding moiety, preferably a PD-1 binding moiety.

In another aspect, the ~~invention~~disclosure provides an immunoconjugate comprising (i) a mutant IL-7 polypeptide as disclosed herein and (ii) an antibody that binds to PD-1. In some ~~embodiments~~examples of the immunoconjugate according to the ~~invention~~disclosure, the antibody comprises (a) a heavy chain variable region (VH) comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3, and a FR-H3 comprising the amino acid sequence of SEQ ID NO:7 at positions 71-73 according to Kabat numbering, and (b) a light chain variable region (VL) comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4, a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

In some ~~embodiments~~examples of the immunoconjugate according to the ~~invention~~disclosure, the antibody comprises (a) a heavy chain variable region (VH) comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO:8, a HVR-H2 comprising the amino acid sequence of SEQ ID NO:9, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:10, and (b) a light chain variable region (VL) comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO:11, a HVR-L2 comprising the amino acid sequence of SEQ ID NO:12, and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:13.

In some ~~embodiments~~examples of the immunoconjugate according to the ~~invention~~disclosure, the antibody comprises (a) a heavy chain variable region (VH) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:14, and (b) a light chain variable region (VL) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO: 17, and SEQ ID NO:18.

In preferred ~~embodiments~~examples of the immunoconjugate of the ~~invention~~disclosure, the mutant IL-7 polypeptide comprises a sequence selected from the group of SEQ ID NO: 55, SEQ IN NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74 , SEQ ID NO: 79, SEQ ID NO: 135 and SEQ ID NO: 136.

In some ~~embodiments~~examples, the immunoconjugate comprises not more than one mutant IL-7 polypeptide. In some ~~embodiments~~examples, the antibody comprises an Fc domain composed of a first and a second subunit. In some such ~~embodiments~~examples, the Fc domain is an IgG class, particularly an IgG1 subclass, Fc domain, and/or the Fc domain is a human Fc domain. In some ~~embodiments~~examples, the antibody is an IgG class, particularly an IgG1 subclass immunoglobulin.

In some ~~embodiments~~examples wherein the immunoconjugate comprises an Fc domain, the Fc domain comprises a modification promoting the association of the first and the second subunit of the Fc domain.

In some ~~embodiments~~examples, in the CH3 domain of the first subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable. In some ~~embodiments~~examples, in the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V) and optionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numberings according to Kabat EU index). In some such ~~embodiments~~examples, in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numberings according to Kabat EU index). In some ~~embodiments~~examples, the mutant IL-7 polypeptide is fused at its amino-terminal amino acid to the carboxy-terminal amino acid of one of the subunits of the Fc domain, particularly the first subunit of the Fc domain, optionally

through a linker peptide. In some such ~~embodiments~~examples, the linker peptide has the amino acid sequence of SEQ ID NO:21.

In some ~~embodiments~~examples wherein the immunoconjugate comprises an Fc domain, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, particularly an Fcγ receptor, and/or effector function, particularly antibody-dependent cell-mediated cytotoxicity (ADCC). In some such ~~embodiments~~examples, said one or more amino acid substitution is at one or more position selected from the group of L234, L235, and P329 (Kabat EU index numbering). In some ~~embodiments~~examples, each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (Kabat EU index numbering).

In some ~~embodiments~~examples, the immunoconjugate according to the ~~invention~~disclosure comprises a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 85, a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 86, and a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 114, SEQ ID NO137 and SEQ ID NO: 138. In some ~~embodiments~~examples, the immunoconjugate essentially consists of a mutant IL-7 polypeptide and an IgG1 immunoglobulin molecule, joined by a linker sequence.

In another aspect, the disclosure provides one or more isolated polynucleotide encoding the mutant IL-7 polypeptide as described herein or the immunoconjugate as described herein.

In another aspect, the disclosure provides one or more vector, particularly expression vector, comprising the polynucleotide(s) as described herein.

In another aspect, the disclosure provides a host cell comprising the polynucleotide(s) as described herein or the vector(s) as described herein.

~~Also~~ In another aspect, the disclosure provide~~sd~~ ~~is~~ a method of producing a mutant IL-7 polypeptide or an immunoconjugate comprising a mutant IL-7 polypeptide and an antibody that binds to PD-1, comprising (a) culturing the host cell of the ~~invention~~disclosure under conditions suitable for the

Expression of the mutant IL-7 polypetide or the immunoconjugate, and optionally (b) recovering the mutant IL-7 polypetide or the immunoconjugate. ~~Also~~ In another aspect, ~~provided by~~ the ~~invention~~disclosure ~~is~~ provides a mutant IL-7 polypetide or an immunoconjugate comprising a mutant IL-7 polypeptide and an antibody that binds to PD-1, produced by ~~said method~~the method as described herein.

The ~~invention~~disclosure further provides a pharmaceutical composition comprising the mutant IL-7 polypetide of the ~~inivention~~ disclosure or the immunoconjugate of the ~~invention~~disclosure and a pharmaceutically acceptable carrier, and methods of using a mutant IL-7 polypeptide or an immunoconjugate of the ~~invention~~disclosure. In another aspect, the disclosure provides a pharmaceutical composition comprising the mutant IL-7 polypeptide as described herein or the immunoconjugate as described herein and a pharmaceutically acceptable carrier.

In particular, the ~~invention~~disclosure encompasses a mutant IL-7 polypeptide according to the ~~invention~~disclosure or an immunoconjugate according to the ~~invention~~disclosure ~~fur~~ for use as a medicmanet, and for use in the treatment of a disease. In a particular ~~embodiment~~example, said disease is cancer. In another aspect, the disclosure provides the mutant IL-7 polypeptide as described herein or the immunoconjugate as described herein for use as a medicament. In another aspect, the disclosure provides the mutant IL-7 polypeptide as described herein or the immunoconjugate as described herein for use in the treatment of a disease.

~~Also encompasses~~In another aspect, ~~by~~ the ~~invention~~disclosure ~~is~~ provides the use~~d~~ of ~~a~~ the mutant IL-7 polypeptide according to the ~~invention~~disclosure or ~~an~~ the immunoconjugate according to the ~~invention~~disclosure in the manufacture of a medicament for the treatment of a disease. In a particular ~~embodiment~~example, said disease is cancer.

~~Further~~ In another aspect, the disclosure provide~~sd~~ ~~is~~ a method of treating a disease in an individual comprising administering to said individual a therapeutically effective amount of a composition comprising the mutant IL-7 polypeptide according to the ~~invention~~disclosure or the immunoconjugate according to the ~~invention~~disclosure in a pharmaceutically acceptable form. In a particular ~~embodiment~~example, said disease is cancer.

~~Also~~ In another aspect, the disclosure provide~~sd~~ ~~is~~ a method of stimulating the immune system of an individual comprising administering to said individual an effective amount of a composition comprising the mutant IL-7 polypetide according to the ~~invention~~disclosure or the immunoconjugate according to the ~~invention~~disclosure in a pharmaceutically acceptable form.

Brief Description of the Drawings

Figure 1A: Schematic representation of an IgG-IL-7 immunoconjugate format, comprising two Fab domains (variable domain, constant domain), a heterodimeric Fc domain and a mutant IL-7 polypeptide fused to a C-terminus of the Fc domain.

Figure 1B: Schematic representation of another IgG-IL-7 immunoconjugate format, comprising two Fab domains (variable domain, constant domain), a homoodimeric Fc domain and two mutant IL-7 polypeptides fused to the C-termini of the Fc domain.

Figure 1C: Schematic representation of another IgG-IL-7 immunoconjugate format, comprising one Fab domain (variable domain, constant domain), a heterodimeric Fc domain and one mutant IL-7 polypeptide fused to a C-terminus of the Fc domain.

Figure 1D: Schematic representation of another IgG-IL-7 immunoconjugate format, comprising two Fab domain (variable domain, constant domain), a heterodimeric Fc domain and one mutant IL-7 polypeptide fused to an N-terminus of one of the Fab domains.

Figure 1E: Schematic representation of another IgG-IL-7 immunoconjugate format, comprising two Fab domain (variable domain, constant domain), a homodimeric Fc domain and two mutant IL-7 polypeptide fused to the N-termini of the Fab domains.

Figures 2A-H: IL-7R signaling by STAT5-phosphrylation upon treatment of PD-1+ CD4 Tcells with increasing doses of PD1-IL7 variants. The IL-7 moiety of the PD1-IL7 variants contain a