

cancer pathobiology, including invasion and metastasis, genetic instability, anchorage-independent growth, tumorigenesis, chemoresistance and resistance to apoptosis, angiogenesis, and evasion from the immune system (Viklund J et al., Curr Med Chem. 2017;24(26):2827-2845; Kolosenko et al., Semin Cancer Biol. 2017 Apr;43:119-133; Corbet et al., Nat Rev Cancer 17, 5 577–593 (2017)).

Some external pH conditional cancer immunotherapy approaches support that tumor acidosis can be utilized for conditional drug activity. Amongst other are CCT-301-59, a pH dependent anti-ROR2 conditionally active biologic – CarT cell therapy, and a pH dependent CTLA-4 check point inhibitor, both developed by BioAtla (<https://www.bioatla.com/technology/cab/>) and pH-sensitive liposomes delivering cancer antigens for vaccination (Yuba et al., Biomaterials. 2013 10 Apr;34(12):3042-52).

Tumor acidosis is discussed in Corbet, C., Feron, O. Tumour acidosis: from the passenger to the driver's seat. Nat Rev Cancer 17, 577–593 (2017) or Damgaci et al., Immunology. 2018 Jul;154(3):354-362.

15 Thus there remains a need in the art to further enhance the therapeutic usefulness of IL-2 proteins.

Summary of the invention

The present ~~invention~~disclosure is based, in part, on the recognition (i) that tumor microenvironments (TME) exhibit decreased pH levels compared to the neutral pH in the periphery, i.e. neutral systemic pH, and (ii) that pH-dependent mutant interleukin-2 polypeptides 20 have a reduced or abolished systemic activity at neutral pH and full activity in the tumor microenvironment at acidic pH.

Accordingly, in ~~a first~~one aspect the ~~invention~~disclosure provides a mutant interleukin-2 (IL-2) polypeptide comprising one or more amino acid substitutions, each compared to a wild-type IL-2, preferably human IL-2 according to SEQ ID NO: 144, wherein the one or more amino acid 25 substitutions abolishes or reduces binding to the IL-2 receptor, preferably to the intermediate-affinity IL-2 receptor (IL2R $\beta\gamma$), at neutral pH and facilitate binding to the IL-2 receptor, preferably to the intermediate-affinity IL-2 receptor (IL2R $\beta\gamma$), at decreased pH. In one ~~embodiment~~example, the mutant

IL-2 polypeptide exhibits reduced or abolished IL-2 receptor binding, preferably intermediate-affinity IL-2 receptor binding, at pH 7.4 and/or pH 7.0 and retained IL-2 receptor binding, preferably intermediate-affinity IL-2 receptor binding, at a pH 6 and/or pH 6.5. In one [embodimentexample](#) said one or more amino acid substitutions is at a position selected from the group of positions corresponding to residue 6, 8, 11, 12, 13, 15, 16, 19, 20, 22, 23, 81, 84, 87, 91, 95, 120, 123, 126, 130, 133 of human IL-2 according to SEQ ID NO: 144. In one [embodimentexample](#) said one or more amino acid substitutions is selected from the group of S6Y, K8E, Q11E, Q11T, L12D, L12E, L12Q, L12S, L12T, Q13H, Q13R, E15Q, H16D, H16E, H16N, H16Q, L19D, L19Q, D20E, D20Q, Q22D, Q22E, Q22H, M23E, M23N, M23Q, R81D, R81E, R81H, R81N, R81Q, D84E, D84Q, S87D, S87E, S87N, S87Q, V91D, V91E, V91N, E95D, E95Q, R120E, R120H, T123E, T123Q, Q126E, Q126H, S130E, T133D, T133E, T133N, T133Q. In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitutions (i) L12E, D20E, M23N, R81N, D84E, S87E, R120E, T123E, S130E, T133N; (ii) Q11E, D20Q, M23E, R81D, D84E, S87E, S130E, T133N; (iii) L12E, L19D, R81D, R120E, T123E, S130E, T133E; (iv) R81Q, S87E, V91D, T123E, S130E, T133D; (v) Q11E, L12E, M23Q, R81D, S87E, V91D, S130E, T133Q; (vi) Q11E, L19D, R81D, D84E, S130E, T133D; (vii) R81D, D84Q, S87D, V91N, T123Q, S130E, T133D; (viii) L19D, R81E, D84E, S87Q, R120H, S130E, T133E; (ix) L19D, M23N, R81D, T133E; (x) Q11E, L12E, M23Q, R81Q, S87D, V91N, E95Q, R120H, T123E, S130E, T133E; (xi) L19D, R81E, S130E, T133D; (xii) R81Q, S87E, V91D, R120E, S130E, T133D; (xiii) L12Q, L19Q, R81H, V91E, T123E, S130E, T133E; (xiv) K8E, D20E, M23N, R81H, D84Q, S87E, R120H, S130E, T133D; (xv) L12E, L19Q, R81H, R120E, T133D; (xvi) H16E, L19D, Q22E, M23Q, R81D, D84E, S87D, R120H, S130E, T133E; (xvii) Q11E, L12E, H16Q, L19D, Q22E, M23N, R81E, D84E, S87E, R120H, S130E, T133E (SEQ ID NO: 21); (xviii) Q11E, H16E, L19D, M23E, R81D, S87E, R120H, Q126E, T133D; (xix) Q11E, L12S, E15Q, H16N, L19D, M23E, R81E, D84E, S87D, R120H, S130E, T133E; (xx) Q11E, H16E, M23E, R81N, D84E, S87E, R120H, Q126E, S130E, T133E; (xxi) Q11E, E15Q, H16E, Q22E, M23E, R81H, D84E, S87E, R120H, S130E, T133D; (xxii) Q11E, L12D, Q13H, E15Q, H16E, Q22E, M23E, R81N, D84E, S87E, R120H, S130E, T133E; (xxiii) Q11E, E15Q, H16E, L19D, R81E, S87E, R120H, S130E, T133E; (xxiv) H16E, L19D, M23Q, R81N, D84E, S87D, R120H, S130E, T133D; (xxv) Q11E, L12T, E15Q, H16E, L19D, Q22H, R81D, D84E, S87E, R120H, S130E, T133E; (xxvi) Q11E, L12T, E15Q, H16E, L19D, Q22H, M23E, R81E, D84E, S87E, R120H, S130E, T133E; (xxvii) Q11E, E15Q, H16E, L19D, R81E, D84E, S87E, R120H, S130E, T133E; (xxviii) H16E, Q22E, M23Q, S87N, R120H, S130E, T133E; (xxix) H16E, L19D, Q22D,

M23Q, R81D, D84E, S87E, R120H, S130E, T133E; (xxiix) Q11E, L12E, Q13H, E15Q, H16N, L19D, Q22E, M23Q, R81E, D84E, S87D, E95D, R120H, T133E; (xxix) Q11E, L12T, H16E, L19D, Q22E, R81D, D84E, S87E, R120H, S130E, T133D; (xxx) Q11T, L12E, E15Q, H16E, L19D, R81D, D84E, S87E, R120E, S130E, T133D; (xxxi) Q11E, E15Q, H16E, L19D, R81D, D84E, S87E, R120H, S130E, T133E; (xxxii) Q11E, E15Q, H16E, L19D, R81Q, D84E, S87E, R120H, S130E, T133E; (xxxiii) Q11E, L12S, H16E, L19D, M23Q, R81D, D84E, S87E, R120H, S130E, T133E; (xxxiv) S6Y, L12E, Q13R, H16Q, Q22E, M23Q, R81N, D84E, S87E, R120H, S130E, T133D; (xxxv) H16D, M23N, R81D, D84E, R120H, S130E, T133E; (xxxvi) Q11E, L12TE, H16Q, L19D, M23E, R81D, D84E, S87E, R120H, S130E, T133E; (xxxvii) Q11E, L12E, H16NE, M23N, R81E, D84E, R120H, S130E, T133E; (xxxviii) E15Q, H16E, L19D, R81D, D84E, S87E; (xxxix) Q11E, R120H, S130E, T133D; or (xl) Q11E, R81D, D84E, S87E, R120H, S130E, T133D.

In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution Q11E (as in SEQ ID NO: 44). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution E15Q (as in SEQ ID NO: 45). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution H16E (as in SEQ ID NO: 46). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution L19D (as in SEQ ID NO: 47). In one [embodimentexample](#) mutant IL-2 polypeptide comprises the amino acid substitution Q22E (as in SEQ ID NO: 48). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution M23Q (as in SEQ ID NO: 49). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution R81D (as in SEQ ID NO: 50). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution D84E (as in SEQ ID NO: 51). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution S87E (as in SEQ ID NO: 52). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution R120H (as in SEQ ID NO: 53). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution Q126E (as in SEQ ID NO: 54). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution Q126H (as in SEQ ID NO: 55). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution S130E (as in SEQ ID NO: 56). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution T133E (as in SEQ ID NO: 57). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitution T133D (as in SEQ ID NO: 58).

In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitutions E15Q, H16E, L19D, R81D, D84E and S87E (as in SEQ ID NO: 59). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitutions Q11E, R120H, S130E and T133D (as in SEQ ID NO: 60). In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitutions Q11E, R81D, D84E, S87E, R120H, S130E and T133D (as in SEQ ID NO: 61).

In one [embodimentexample](#) the mutant IL-2 polypeptide comprises a first amino acid mutation that abolishes or reduces affinity of the mutant IL-2 polypeptide to the high-affinity IL-2 receptor and preserves affinity of the mutant IL-2 polypeptide to the intermediate-affinity IL-2 receptor, each compared to a wild-type IL-2 polypeptide. In one [embodimentexample](#) said first amino acid mutation is at a position corresponding to residue 72 of human IL-2. In one [embodimentexample](#) said amino acid mutation is an amino acid substitution, selected from the group of L72G, L72A, L72S, L72T, L72Q, L72E, L72N, L72D, L72R, and L72K. In a more specific [embodimentexample](#) said first amino acid mutation is the amino acid substitution L72G.

In certain [embodimentexamples](#) the mutant IL-2 polypeptide comprises a second amino acid mutation that abolishes or reduces affinity of the mutant IL-2 polypeptide to the high-affinity IL-2 receptor and preserves affinity of the mutant IL-2 polypeptide to the intermediate-affinity IL-2 receptor, each compared to a wild-type IL-2 polypeptide. In one [embodimentexample](#) said second amino acid mutation is at a position selected from the positions corresponding to residue 35, 38, 42, 43, and 45 of human IL-2. In a specific [embodimentexample](#) said second amino acid mutation is at a position corresponding to residue 42 of human IL-2. In a more specific [embodimentexample](#) said second amino acid mutation is an amino acid substitution, selected from the group of F42A, F42G, F42S, F42T, F42Q, F42E, F42N, F42D, F42R, and F42K. In an even more specific [embodimentexample](#) said second amino acid mutation is the amino acid substitution F42A.

In certain [embodimentexamples](#) the mutant interleukin-2 polypeptide comprises a third amino acid mutation that abolishes or reduces affinity of the mutant IL-2 polypeptide to the high-affinity IL-2 receptor and preserves affinity of the mutant IL-2 polypeptide to the intermediate-affinity IL-2 receptor, each compared to a wild-type IL-2 polypeptide. In a particular [embodimentexample](#), the mutant interleukin-2 polypeptide comprises three amino acid mutations that abolish or reduce affinity of the mutant IL-2 polypeptide to the high-affinity IL-2 receptor and preserve affinity of the mutant IL-2 polypeptide to the intermediate-affinity IL-2 receptor, each compared to a wild-type IL-2 polypeptide, wherein said three amino acid mutations are at positions corresponding to residue 42, 45, and 72 of human IL-2. In one [embodimentexample](#) said three amino acid mutations are amino acid substitutions selected from the group of F42A, F42G, F42S, F42T, F42Q, F42E, F42N, F42D, F42R, F42K, Y45A, Y45G,

Y45S, Y45T, Y45Q, Y45E, Y45N, Y45D, Y45R, Y45K, L72G, L72A, L72S, L72T, L72Q, L72E, L72N, L72D, L72R, and L72K. In a specific [embodimentexample](#) said three amino acid mutations are the amino acid substitutions F42A, Y45A and L72G. In certain [embodimentexamples](#) the mutant interleukin-2 polypeptide further comprises an amino acid
5 mutation which eliminates the O-glycosylation site of IL-2 at a position corresponding to residue 3 of human IL-2. In one [embodimentexample](#) said amino acid mutation which eliminates the O-glycosylation site of IL-2 at a position corresponding to residue 3 of human IL-2 is an amino acid substitution selected from the group of T3A, T3G, T3Q, T3E, T3N, T3D, T3R, T3K, and T3P. In a specific [embodimentexample](#) the amino acid mutation which eliminates the O-
10 glycosylation site of IL-2 at a position corresponding to residue 3 of human IL-2 is T3A. In certain [embodimentexamples](#) the mutant IL-2 polypeptide further comprises an amino acid at a position corresponding to residue 125 of human IL-2. In a specific [embodimentexample](#) the amino acid at a position corresponding to residue 125 of human IL-2 is C125A. In certain [embodimentexamples](#) the mutant IL-2 polypeptide is essentially a full-length IL-2 molecule,
15 particularly a human full-length IL-2 molecule. In one [embodimentexample](#) the mutant IL-2 polypeptide comprises an amino acid substitution selected from the group T3A, F42A, Y45A, L72G, C125A. In one [embodimentexample](#) the mutant IL-2 polypeptide comprises the amino acid substitutions T3A, F42A, Y45A, L72G and C125A.

In one [embodimentexample](#) the mutant IL-2 polypeptide is linked to a non-IL-2 moiety. In one
20 [embodimentexample](#) the mutant IL-2 polypeptide is linked to a first and a second non-IL-2 moiety. In one [embodimentexample](#) the mutant IL-2 polypeptide is linked to a first and a second non-IL-2 moiety. In one [embodimentexample](#) the mutant IL-2 polypeptide shares a carboxy-terminal peptide bond with said first non-IL-2 moiety and an amino-terminal peptide bond with said second non-IL-2 moiety. In certain [embodimentexample](#) the non-IL-2 moiety is a targeting
25 moiety. In certain [embodimentexamples](#) said non-IL-2 moiety is an antigen binding moiety or an effector cell binding moiety. In one [embodimentexample](#) said antigen binding moiety is an antibody. In another [embodimentexample](#) said antigen binding moiety or effector cell binding moiety is an antibody fragment. In a more specific [embodimentexample](#) said antigen binding moiety effector cell binding moiety is selected from a Fab molecule and a scFv molecule. In a
30 particular [embodimentexample](#) said antigen binding moiety or effector cell binding moiety is a Fab molecule. In another [embodimentexample](#) said antigen binding moiety or effector cell binding moiety is a scFv molecule. In one [embodimentexample](#) said antigen binding moiety or effector cell binding moiety is an immunoglobulin molecule. In a more specific [embodimentexample](#) said antigen binding moiety or effector cell binding moiety is an IgG class,
35 particularly an IgG₁ subclass, immunoglobulin molecule. In certain [embodimentexamples](#) said antigen

binding moiety is directed to an antigen presented on a tumor cell or in a tumor cell environment, particularly an antigen selected from the group of Fibroblast Activation Protein (FAP), the A1 domain of Tenascin-C (TNC A1), the A2 domain of Tenascin-C (TNC A2), the Extra Domain B of Fibronectin (EDB), Carcinoembryonic Antigen (CEA) and the Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP). In certain embodimentexamples said effector cell binding moiety is directed to an effector cell present in the tumor cell environment in order to achieve cis-targeting, particularly a target selected from the group of CD8, PD-1, LAG3, TIM3, TIGIT, CD28, 4-1BB, OX40. GITR. ICOS, CXCR3, CXCR5.

In a further ~~the~~ aspect the inventiondisclosure provides an immunoconjugate comprising a mutant IL-2 polypeptide as described herein, and an antigen binding moiety and/or an effector cell binding moiety. In a further aspect, the present disclosure provides an immunoconjugate comprising a mutant IL-2 polypeptide as described herein and an antigen binding moiety or an effector cell binding moiety. In one embodimentexample of the immunoconjugate according to the inventiondisclosure the mutant IL-2 polypeptide shares an amino- or carboxy-terminal peptide bond with said antigen binding moiety or said effector cell binding moiety. In particular embodimentexamples the immunoconjugate comprises as first and a second antigen binding moiety or a first and a second effector cell antigen binding moiety or an antigen binding moiety and an effector cell binding moiety. In one such embodimentexample the mutant IL-2 polypeptide comprised in the immunoconjugate according to the inventiondisclosure shares an amino- or carboxy-terminal peptide bond with said first antigen binding moiety and said second antigen binding moiety shares an amino- or carboxy-terminal peptide bond with either a) said mutant IL-2 polypeptide or b) said first antigen binding moiety. In another embodimentexample the mutant IL-2 polypeptide comprised in the immunoconjugate according to the inventiondisclosure shares an amino- or carboxy-terminal peptide bond with said first effector cell binding moiety and said second effector cell binding moiety shares an amino- or carboxy-terminal peptide bond with either a) said mutant IL-2 polypeptide or b) said first effector cell binding moiety. In another embodimentexample the mutant IL-2 polypeptide comprised in the immunoconjugate according to the inventiondisclosure shares an amino- or carboxy-terminal peptide bond with the antigen binding moiety and the effector cell binding moiety shares an amino- or carboxy-terminal peptide bond with either a) said mutant IL-2 polypeptide or b) said antigen binding moiety. In another embodimentexample the mutant IL-2 polypeptide comprised in the immunoconjugate according to the inventiondisclosure shares an amino- or carboxy-terminal peptide bond with the effector cell binding moiety and the antigen binding moiety shares an amino- or carboxy-terminal peptide bond with either a) said mutant IL-2 polypeptide or b) said effector cell binding moiety.

In one embodimentexample the antigen binding moiety and/or the effector cell binding moiety comprised in the immunoconjugate according to the inventiondisclosure is an antibody. In another embodimentexample said antigen binding moiety and/or the effector cell binding moiety is an antibody fragment. In a specific embodimentexample said antigen binding moiety and/or the effector cell binding moiety is selected from a Fab molecule and a scFv molecule. In a particular embodimentexample said antigen binding moiety and/or the effector cell binding moiety is a Fab molecule. In another particular embodimentexample said antigen binding moiety and/or the effector cell binding moiety is an immunoglobulin molecule. In a more specific embodimentexample said antigen binding moiety and/or the effector cell binding moiety is an IgG class, particularly an IgG₁ subclass, immunoglobulin molecule. In certain embodimentexamples said antigen binding moiety is directed to an antigen presented on a tumor cell or in a tumor cell environment, particularly an antigen selected from the group of Fibroblast Activation Protein (FAP), the A1 domain of Tenascin-C (TNC A1), the A2 domain of Tenascin-C (TNC A2), the Extra Domain B of Fibronectin (EDB), Carcinoembryonic Antigen (CEA) and the Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP). In certain embodimentexamples the effector cell binding moiety is directed to an effector cell present in a tumor cell environment in order to achieve cis-targeting, particularly a target selected from the group of CD8, PD-1, LAG3, TIM3, TIGIT, CD28, 4-1BB, OX40, GITR, ICOS, CXCR3, CXCR5.

The inventiondisclosure further provides isolated polynucleotides encoding a mutant IL-2 polypeptide or an immunoconjugate as described herein, expression vectors comprising said polynucleotides, and host cells comprising the polynucleotides or the expression vectors. In a further aspect, the present disclosure provides an isolated polynucleotide encoding the mutant IL-2 polypeptide or immunoconjugate as described herein. In a further aspect, the present disclosure provides an expression vector comprising the polynucleotide as described herein. In a further aspect, the present disclosure provides a host cell comprising the polynucleotide as described herein or the expression vector as described herein.

Also provided is a method of producing a mutant IL-2 polypeptide or an immunoconjugate as described herein, a pharmaceutical composition comprising a mutant IL-2 polypeptide or an immunoconjugate as described herein and a pharmaceutically acceptable carrier, and methods of using a mutant IL-2 polypeptide or an immunoconjugate as described herein. In a further aspect,

5 the present disclosure provides a method of producing a mutant IL-2 polypeptide or an immunoconjugate thereof, comprising culturing the host cell as described herein under conditions suitable for the expression of the mutant IL-2 polypeptide or the immunoconjugate. In a further aspect, the present disclosure provides a mutant IL-2 polypeptide or immunoconjugate produced by the method as described herein. In a further aspect, the present disclosure provides a
10 pharmaceutical composition comprising the mutant IL-2 polypeptide or immunoconjugate as described herein and a pharmaceutically acceptable carrier.

In ~~particular~~ a further aspect, the ~~invention~~ disclosure encompasses ~~a~~ the mutant IL-2 polypeptide or ~~an~~ immunoconjugate as described herein for use in the treatment of a disease in an individual in need thereof. In a particular ~~embodiment~~ example said disease is cancer. In a particular
15 ~~embodiment~~ example the individual is a human.

~~Also~~ In a further aspect, also ~~encompassed~~ encompassed by the ~~invention~~ disclosure is the use of the mutant IL-2 polypeptide or immunoconjugate as described herein ~~for~~ in the manufacture of a medicament for treating a disease in an individual in need thereof.

Further provided is a method of treating disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising a mutant IL-2 polypeptide or an immunoconjugate as described herein. Said disease preferably is cancer.

Also provided 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-2 polypeptide or immunoconjugate described herein in a pharmaceutically acceptable form. In a further aspect, the present disclosure provides a composition comprising the mutant IL-2 polypeptide or immunoconjugate as described herein in a pharmaceutically acceptable form for use in stimulating the immune system of an individual.

Brief Description of the Drawings

Figure 1. A schematic representation of the constructs comprising the extracellular domains (ECD) of the IL2R beta and gamma (IL2R $\beta\gamma$) subunits.

Figure 2. Analysis of IL2v binding to the receptor IL2R $\beta\gamma$ at pH 6 and pH 7.4 by ELISA. Bacterial supernatant containing IL2v was incubated with previously coated IL2R $\beta\gamma$. Prior to the detection by an anti-Flag/HRP secondary antibody, extensive washing for 30 minutes was performed at pH 6 or pH 7.4.

Figure 3. Binding analysis for the first set of selected pH-dependent IL2v variants to IL2R $\beta\gamma$ at pH 6 and pH 7.4 by ELISA. Bacterial supernatants containing clonal pH-dependent IL2v variants were incubated with previously coated IL2R $\beta\gamma$. Prior to the detection by an anti-Flag/HRP secondary antibody, extensive washing for 30 minutes was performed at pH 6 or pH 7.4. The absorption at pH 6 was normalized to a value of 1 and the ratio of absorption levels was compared.

Figure 4. Binding analysis for the second set of selected pH-dependent IL2v variants to IL2R $\beta\gamma$ at pH 6, pH 6.5, pH 7 and pH 7.4 by ELISA. Bacterial supernatants containing clonal pH-dependent IL2v variants were incubated with previously coated IL2R $\beta\gamma$. Prior to the detection by an anti-Flag/HRP secondary antibody, extensive washing for 30 minutes was performed at pH 6 or pH 7.4. The absorption at pH 6 was normalized to a value of 1 and the ratio of absorption levels was compared.

CLAIMS

1. A mutant interleukin-2 (IL-2) polypeptide comprising one or more amino acid substitutions, each compared to a wild-type IL-2, preferably human IL-2 according to SEQ ID NO: 144,
5 wherein the one or more amino acid substitutions abolishes or reduces binding to the IL-2 receptor, preferably to the intermediate-affinity IL-2 receptor (IL2R $\beta\gamma$), at neutral pH and facilitate binding to the IL-2 receptor, preferably to the intermediate-affinity IL-2 receptor (IL2R $\beta\gamma$), at decreased pH.
2. The mutant IL-2 polypeptide of claim 1, wherein said one or more amino acid substitutions is
10 at a position selected from the group of positions corresponding to residue 6, 8, 11, 12, 13, 15, 16, 19, 20, 22, 23, 81, 84, 87, 91, 95, 120, 123, 126, 130, 133 of human IL-2 according to SEQ ID NO: 144.
3. The mutant IL-2 polypeptide of claim 1 or 2, wherein said one or more amino acid substitutions is selected from the group of S6Y, K8E, Q11E, Q11T, L12D, L12E, L12Q, L12S,
15 L12T, Q13H, Q13R, E15Q, H16D, H16E, H16N, H16Q, L19D, L19Q, D20E, D20Q, Q22D, Q22E, Q22H, M23E, M23N, M23Q, R81D, R81E, R81H, R81N, R81Q, D84E, D84Q, S87D, S87E, S87N, S87Q, V91D, V91E, V91N, E95D, E95Q, R120E, R120H, T123E, T123Q, Q126E, Q126H, S130E, T133D, T133E, T133N, T133Q.
4. The mutant IL-2 polypeptide of any of claims 1 to 3, wherein the mutant IL-2 polypeptide
20 comprises the amino acid substitutions
 - (i) L12E, D20E, M23N, R81N, D84E, S87E, R120E, T123E, S130E, T133N;
 - (ii) Q11E, D20Q, M23E, R81D, D84E, S87E, S130E, T133N;
 - (iii) L12E, L19D, R81D, R120E, T123E, S130E, T133E;
 - (iv) R81Q, S87E, V91D, T123E, S130E, T133D;
 - 25 (v) Q11E, L12E, M23Q, R81D, S87E, V91D, S130E, T133Q;
 - (vi) Q11E, L19D, R81D, D84E, S130E, T133D;

(vii) R81D, D84Q, S87D, V91N, T123Q, S130E, T133D;

(viii) L19D, R81E, D84E, S87Q, R120H, S130E, T133E;

(iix) L19D, M23N, R81D, T133E;

(ix) Q11E, L12E, M23Q, R81Q, S87D, V91N, E95Q, R120H, T123E, S130E, T133E;

5 (x) L19D, R81E, S130E, T133D;

(xi) R81Q, S87E, V91D, R120E, S130E, T133D;

(xii) L12Q, L19Q, R81H, V91E, T123E, S130E, T133E;

(xiii) K8E, D20E, M23N, R81H, D84Q, S87E, R120H, S130E, T133D;

(xiv) L12E, L19Q, R81H, R120E, T133D;

10 (xv) H16E, L19D, Q22E, M23Q, R81D, D84E, S87D, R120H, S130E, T133E;

(xvi) Q11E, L12E, H16Q, L19D, Q22E, M23N, R81E, D84E, S87E, R120H, S130E, T133E;

(xvii) Q11E, H16E, L19D, M23E, R81D, S87E, R120H, Q126E, T133D;

(xviii) Q11E, L12S, E15Q, H16N, L19D, M23E, R81E, D84E, S87D, R120H, S130E, T133E;

(xix) Q11E, H16E, M23E, R81N, D84E, S87E, R120H, Q126E, S130E, T133E;

15 (xx) Q11E, E15Q, H16E, Q22E, M23E, R81H, D84E, S87E, R120H, S130E, T133D;

(xxi) Q11E, L12D, Q13H, E15Q, H16E, Q22E, M23E, R81N, D84E, S87E, R120H, S130E, T133E;

(xxii) Q11E, E15Q, H16E, L19D, R81E, S87E, R120H, S130E, T133E;

(xxiii) H16E, L19D, M23Q, R81N, D84E, S87D, R120H, S130E, T133D;

20 (xxiv) Q11E, L12T, E15Q, H16E, L19D, Q22H, R81D, D84E, S87E, R120H, S130E, T133E;

(xxv) Q11E, L12T, E15Q, H16E, L19D, Q22H, M23E, R81E, D84E, S87E, R120H, S130E, T133E;

(xxvi) Q11E, E15Q, H16E, L19D, R81E, D84E, S87E, R120H, S130E, T133E;

(xxvii) H16E, Q22E, M23Q, S87N, R120H, S130E, T133E;

(xxviii) H16E, L19D, Q22D, M23Q, R81D, D84E, S87E, R120H, S130E, T133E;

(xxix) Q11E, L12E, Q13H, E15Q, H16N, L19D, Q22E, M23Q, R81E, D84E, S87D, E95D,
5 R120H, T133E;

(xxix) Q11E, L12T, H16E, L19D, Q22E, R81D, D84E, S87E, R120H, S130E, T133D;

(xxx) Q11T, L12E, E15Q, H16E, L19D, R81D, D84E, S87E, R120E, S130E, T133D;

(xxxi) Q11E, E15Q, H16E, L19D, R81D, D84E, S87E, R120H, S130E, T133E;

(xxxii) Q11E, E15Q, H16E, L19D, R81Q, D84E, S87E, R120H, S130E, T133E;

10 (xxxiii) Q11E, L12S, H16E, L19D, M23Q, R81D, D84E, S87E, R120H, S130E, T133E;

(xxxiv) S6Y, L12E, Q13R, H16Q, Q22E, M23Q, R81N, D84E, S87E, R120H, S130E, T133D;

(xxxv) H16D, M23N, R81D, D84E, R120H, S130E, T133E;

(xxxvi) Q11E, L12T, H16Q, L19D, M23E, R81D, D84E, S87E, R120H, S130E, T133E;

(xxxvii) Q11E, L12E, H16N, M23N, R81E, D84E, R120H, S130E, T133E;

15 (xxxviii) E15Q, H16E, L19D, R81D, D84E, S87E;

(xxxix) Q11E, R120H, S130E, T133D; or

(xl) Q11E, R81D, D84E, S87E, R120H, S130E, T133D.

5. The mutant IL-2 polypeptide of any of claims 1 to 4, wherein the mutant IL-2 polypeptide
comprises any amino acid substitution selected from the group T3A, F42A, Y45A, L72G,
20 C125A.

6. The mutant IL-2 polypeptide of any of claims 1 to 5, wherein the mutant IL-2 polypeptide
comprises the amino acid substitutions F42A, Y45A and L72G.

7. The mutant IL-2 polypeptide of any of claims 1 to 6, wherein the mutant IL-2 polypeptide comprises the amino acid substitutions T3A, F42A, Y45A, L72G and C125A.

8. The mutant IL-2 polypeptide of any one of claims 1 to 7, wherein said mutant IL-2 polypeptide is linked to a non-IL-2 moiety.

5 9. The mutant IL-2 polypeptide of any one of claims 1 to 8, wherein said mutant IL-2 polypeptide is linked to a first and a second non-IL-2 moiety.

10. The mutant IL-2 polypeptide of claim 9, wherein said mutant IL-2 polypeptide shares a carboxy-terminal peptide bond with said first non-IL-2 moiety and an amino-terminal peptide bond with said second non-IL-2 moiety.

10 11. The mutant IL-2 polypeptide of any one of claims 8 to 10, wherein said non-IL-2 moiety is an antigen binding moiety or an effector cell binding moiety.

12. An immunoconjugate comprising a mutant IL-2 polypeptide of any one of claims 1 to 7 and an antigen binding moiety or an effector cell binding moiety.

15 13. The immunoconjugate of claim 12, wherein said mutant IL-2 polypeptide shares an amino- or carboxy-terminal peptide bond with said antigen binding moiety or the effector cell binding moiety.

14. The immunoconjugate of claims 12 or 13, wherein said immunoconjugate comprises a first and a second antigen binding moiety or a first and a second effector cell antigen binding moiety or an antigen binding moiety and an effector cell binding moiety.

20 15. The immunoconjugate of claim 14,

(i) wherein said mutant IL-2 polypeptide shares an amino- or carboxy-terminal peptide bond with said first antigen binding moiety and said second antigen binding moiety shares an amino- or carboxy-terminal peptide bond with either a) said mutant IL-2 polypeptide or b) said first antigen binding moiety;

25 (ii) wherein said mutant IL-2 polypeptide shares an amino- or carboxy-terminal peptide bond with said first effector cell binding moiety and said second effector cell binding moiety shares an amino- or carboxy-terminal peptide bond with either a) said mutant IL-2 polypeptide or b) said first effector cell binding moiety;

(iii) wherein said mutant IL-2 polypeptide shares an amino- or carboxy-terminal peptide bond with the antigen binding moiety and the effector cell binding moiety shares an amino- or carboxy-terminal peptide bond with either a) said mutant IL-2 polypeptide or b) said antigen binding moiety; or

5 (iv) wherein said mutant IL-2 polypeptide shares an amino- or carboxy-terminal peptide bond with the effector cell binding moiety and the antigen binding moiety shares an amino- or carboxy-terminal peptide bond with either a) said mutant IL-2 polypeptide or b) said effector cell binding moiety.

16. The mutant IL-2 polypeptide of claim 11 or the immunoconjugate of any one of claims 12 to
10 15, wherein said antigen binding moiety is an antibody or an antibody fragment.

17. The mutant IL-2 polypeptide of claim 11 or the immunoconjugate of any one of claims 12 to 16, wherein said antigen binding moiety and/or said effector cell binding moiety is selected from a Fab molecule and a scFv molecule.

18. The mutant IL-2 polypeptide of claim 11 or the immunoconjugate of any one of claims 12 to
15 17, wherein said antigen binding moiety and/or said effector cell binding moiety is an immunoglobulin molecule, particularly an IgG molecule.

19. The mutant IL-2 polypeptide of claim 11 or the immunoconjugate of any one of claims 12 to 18, wherein said antigen binding moiety is directed to an antigen presented on a tumor cell or in a tumor cell environment and/or wherein said effector cell binding moiety is directed to an
20 effector cell present in a tumor cell environment in order to achieve cis-targeting.

20. An isolated polynucleotide encoding the mutant IL-2 polypeptide or immunoconjugate of any one of claims 1 to 19.

21. An expression vector comprising the polynucleotide of claim 20.

22. A host cell comprising the polynucleotide of claim 20 or the expression vector of claim 21.

25 23. A method of producing a mutant IL-2 polypeptide or an immunoconjugate thereof, comprising culturing the host cell of claim 22 under conditions suitable for the expression of the mutant IL-2 polypeptide or the immunoconjugate.

24. A mutant IL-2 polypeptide or immunoconjugate produced by the method of claim 23.

25. A pharmaceutical composition comprising the mutant IL-2 polypeptide or immunoconjugate of any one of claims 1 to 19 or 24 and a pharmaceutically acceptable carrier.

26. The mutant IL-2 polypeptide or immunoconjugate of any one of claims 1 to 19 or 24 for use in the treatment of a disease in an individual in need thereof.

5 27. The mutant IL-2 polypeptide or immunoconjugate for use of claim 26, wherein said disease is cancer.

28. Use of the mutant IL-2 polypeptide or immunoconjugate of any one of claims 1 to 19 or 24 ~~for~~ in the manufacture of a medicament for treating a disease in an individual in need thereof.

10 ~~29. A method of treating disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the mutant IL-2 polypeptide or immunoconjugate of any one of claims 1 to 19 or 24 in a pharmaceutically acceptable form.~~

~~30~~ 29. The ~~method~~ use of claim ~~29~~ 28, wherein said disease is cancer.

15 ~~31~~ 30. A ~~method of stimulating the immune system of an individual, comprising administering to said individual a effective amount of a~~ composition comprising the mutant IL-2 polypeptide or immunoconjugate of any one of claims 1 to 19 or 24 in a pharmaceutically acceptable form for use in stimulating the immune system of an individual.