Immunol 177, 306-14 (2006); Boyman et al., Science 311, 1924-27 (2006)). In an alternative approach, IL-2 has been mutated in various ways to reduce its toxicity and/or increase its efficacy. Hu et al. (Blood 101, 4853-4861 (2003), US Pat. Publ. No. 2003/0124678) have substituted the arginine residue in position 38 of IL-2 by tryptophan to eliminate IL-2's vasopermeability activity. Shanafelt et al. (Nature Biotechnol 18, 1197-1202 (2000)) have mutated asparagine 88 to arginine to enhance selectivity for T cells over NK cells. Heaton et al. (Cancer Res 53, 2597-602 (1993); US Pat. No. 5,229,109) have introduced two mutations, Arg38Ala and Phe42Lys, to reduce the secretion of proinflammatory cytokines from NK cells. Gillies et al. (US Pat. Publ. No. 2007/0036752) have substituted three residues of IL-2 (Asp20Thr, Asn88Arg, and Gln126Asp) that contribute to affinity for the intermediate-affinity IL-2 receptor to reduce VLS. Gillies et al. (WO 2008/0034473) have also mutated the interface of IL-2 with CD25 by amino acid substitution Arg38Trp and Phe42Lys to reduce interaction with CD25 and activation of T_{reg} cells for enhancing efficacy. To the same aim, Wittrup et al. (WO 2009/061853) have produced IL-2 mutants that have enhanced affinity to CD25, but do not activate the receptor, thus act as antagonists. The mutations introduced were aimed at disrupting the interaction with the β- and/or γ-subunit of the receptor.

However, none of the known IL-2 mutants was shown to overcome all of the above-mentioned problems associated with IL-2 immunotherapy, namely toxicity caused by the induction of VLS, tumor tolerance caused by the induction of AICD, and immunosuppression caused by activation of T_{reg} cells. Thus there remains a need in the art to further enhance the therapeutic usefulness of IL-2 proteins.

Summary

The present disclosure is based, in part, on the recognition that the interaction of IL-2 with the α-subunit of the trimeric, high-affinity IL-2 receptor is responsible for the problems associated with IL-2 immunotherapy.

Accordingly, the disclosure provides a mutant interleukin-2 (IL-2) polypeptide comprising 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

site of IL-2 at a position corresponding to residue 3 of human IL-2 is T3A. In certain embodiments the mutant IL-2 polypeptide is essentially a full-length IL-2 molecule, particularly a human full-length IL-2 molecule.

The disclosure further provides for a mutant interleukin-2 polypeptide linked to a non-IL-2 moiety. In certain embodiments said non-IL-2 moiety is a targeting moiety. In certain embodiments said non-IL-2 moiety is an antigen binding moiety. In one embodiment said antigen binding moiety is an antibody. In another embodiment said antigen binding moiety is an antibody fragment. In a more specific embodiment said antigen binding moiety is selected from a Fab molecule and a scFv molecule. In a particular embodiment said antigen binding moiety is a Fab molecule. In another embodiment said antigen binding moiety is a scFv molecule. In particular embodiments the mutant IL-2 polypeptide is linked to a first and a second non-IL-2 moiety. In one such embodiment the mutant interleukin-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 one embodiment said antigen binding moiety is an immunoglobulin molecule. In a more specific embodiment said antigen binding moiety is an IgG class, particularly an IgG_1 subclass, immunoglobulin molecule. In certain embodiments 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).

Also provided by the disclosure is an immunoconjugate comprising a mutant IL-2 polypeptide as described herein, and an antigen binding moiety. In one embodiment of the immunoconjugate according to the disclosure the mutant IL-2 polypeptide shares an amino- or carboxy-terminal peptide bond with said antigen binding moiety. In particular embodiments the immunoconjugate comprises as first and a second antigen binding moiety. In one such embodiment the mutant IL-2 polypeptide comprised in the immunoconjugate according to the disclosure shares an amino- or carboxy-terminal peptide bond with a first antigen binding moiety and a second antigen binding moiety shares an amino- or carboxy-terminal peptide bond with either i) the mutant IL-2 polypeptide or ii) said first antigen binding moiety. In one embodiment the antigen binding moiety comprised in the immunoconjugate according to the disclosure is an antibody, in another embodiment said antigen binding moiety is an antibody fragment. In a specific embodiment said

antigen binding moiety is selected from a Fab molecule and a scFv molecule. In a particular embodiment said antigen binding moiety is a Fab molecule. In another particular embodiment said antigen binding moiety is an immunoglobulin molecule. In a more specific embodiment said antigen binding moiety is an IgG class, particularly an IgG_1 subclass, immunoglobulin molecule. In certain embodiments 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 one aspect, the disclosure relates to an immunoconjugate comprising a mutant IL-2 polypeptide and an antigen binding moiety, wherein said mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions F42A, Y45Aand L72G (numbering relative to the human IL-2 sequence SEQ ID NO: 1); and wherein said antigen binding moiety is an IgG_1 subclass immunoglobulin molecule specific for FAP, comprising (i) the heavy chain variable region sequence of SEQ ID NO: 41 and the light chain variable region sequence of SEQ ID NO: 39; (ii) the heavy chain variable region sequence of SEQ ID NO: 51 and the light chain variable region sequence of SEQ ID NO: 49; (iii) the heavy chain variable region sequence of SEQ ID NO: 111 and the light chain variable region sequence of SEQ ID NO: 109; (iv) the heavy chain variable region sequence of SEQ ID NO: 143 and the light chain variable region sequence of SEQ ID NO: 141; or (iv) the heavy chain variable region sequence of SEQ ID NO: 151 and the light chain variable region sequence of SEQ ID NO: 149.

The disclosure 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 another aspect, the disclosure relates to an isolated polynucleotide encoding the immunoconjugate as described herein.

In another aspect, the disclosure relates to a host cell comprising the polynucleotide 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 another aspect, the disclosure relates to a method of producing an immunoconjugate comprising a mutant IL-2 polypeptide and an antigen binding moiety, comprising culturing the host cell as described herein under conditions suitable for the expression of the immunoconjugate.

In another aspect, the disclosure relates to an immunoconjugate produced by the method as described herein.

In another aspect, the disclosure relates to a pharmaceutical composition comprising the immunoconjugate as described herein and a pharmaceutically acceptable carrier.

In particular, the disclosure encompasses a 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 another aspect, the disclosure relates to the immunoconjugate as described herein for use in the treatment of a disease in an individual in need thereof. In a particular embodiment said disease is cancer. In a particular embodiment the individual is a human.

Also emcompassed by the disclosure is the use of the mutant IL-2 polypeptide or immunoconjugate as described herein for the manufacture of a medicament for treating a disease in an individual in need thereof. In another aspect, the disclosure relates to use of the immunoconjugate as described herein in the manufacture of a medicament for treating a disease in an individual in need thereof. In another aspect, the disclosure relates to use of the immunoconjugate as described herein in the manufacture of a medicament for stimulating the immune system of an individual.

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.

14. The immunoconjugate of any one of claims 1 to 13, comprising a polypeptide sequence of SEQ ID NO: 301, a polypeptide sequence of SEQ ID NO: 303, and a polypeptide sequence of SEQ ID NO: 231.

15. The immunoconjugate of any one of claims 1 to 14, essentially consisting of a mutant IL-2 polypeptide and an IgG_1 molecule, joined by a linker sequence.

16. An isolated polynucleotide encoding the immunoconjugate of any one of claims 1 to 15.

17. A host cell comprising the polynucleotide of claim 16.

18. A method of producing an immunoconjugate comprising a mutant IL-2 polypeptide and an antigen binding moiety, comprising culturing the host cell of claim 17 under conditions suitable for the expression of the immunoconjugate.

19. An immunoconjugate produced by the method of claim 18.

20. A pharmaceutical composition comprising the immunoconjugate of any one of claims 1 to 15 or 19 and a pharmaceutically acceptable carrier.

21. The immunoconjugate of any one of claims 1 to 15 or 19 for use in the treatment of a disease in an individual in need thereof.

22. The immunoconjugate of claim 21, wherein said disease is cancer.

23. Use of the immunoconjugate of any one of claims 1 to 15 or 19 in the manufacture of a medicament for treating a disease in an individual in need thereof.

24. The use of claim 23, wherein said disease is cancer.

25. Use of the immunoconjugate of any one of claims 1 to 15 or 19 in the manufacture of a medicament for stimulating the immune system of an individual.