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 ~~of the invention~~

The present ~~invention~~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, ~~in a first aspect~~ the ~~invention~~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.

In a first aspect, there is provided a mutant interleukin-2 (IL-2) polypeptide, comprising 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 (SEQ ID NO: 1).

The ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~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 ~~invention~~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).

The ~~invention~~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 a second aspect, there is provided an isolated polynucleotide encoding the mutant IL-2 polypeptide as defined above.

In a third aspect, there is provided an expression vector comprising the polynucleotide as defined above.

In a fourth aspect, there is provided a host cell comprising the polynucleotide as defined above or the expression vector as defined above.

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 fifth aspect, there is provided a method of producing a mutant IL-2 polypeptide, comprising culturing the host cell as defined above under conditions suitable for the expression of the mutant IL-2 polypeptide.

In a sixth aspect, there is provided a pharmaceutical composition comprising the mutant IL-2 polypeptide as defined above and a pharmaceutically acceptable carrier.

In particular, the ~~invention~~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 a particular embodiment said disease is cancer. In a particular embodiment the individual is a human.

In a seventh aspect, there is provided the mutant IL-2 polypeptide as defined above for use in the treatment of a disease in an individual in need thereof.

Also emcompassed by the ~~invention~~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 an eighth aspect, there is provided the use of the mutant IL-2 polypeptide as defined above 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 ninth aspect, there is provided the use of the mutant IL-2 polypeptide as defined above in the manufacture of a medicament for stimulating the immune system of an individual.