Accordingly, the present disclosure, in one aspect, provides a combination of (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety, and (b) a second antibody engineered to have increased effector function, for use in treating a disease in an individual in need thereof, wherein the first antibody comprises in its Fc region one or more amino acid mutation that reduces the binding affinity of the immunoconjugate to an activating Fc receptor, and wherein the second antibody is engineered by introduction of one or more amino acid mutations in the Fc region or by modification of the glycosylation in the Fc region. In one embodiment the effector moiety is a cytokine. In one embodiment the cytokine is selected from the group consisting of IL-2, GM-CSF, IFN-α, and IL-12. In a particular embodiment the effector moiety is IL-2. In another embodiment the effector moiety is IL-12. In another particular embodiment the IL-2 effector moiety is a mutant IL-2 effector moiety comprising at least one amino acid mutation, particularly an amino acid substitution, that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the α-subunit of the IL-2 receptor but preserves the affinity of the mutant IL-2 effector moiety to the intermediate-affinity IL-2 receptor, compared to the non-mutated IL-2 effector moiety. In a specific embodiment, the mutant IL-2 effector moiety comprises one, two or three amino acid substitutions at one, two or three position(s) selected from the positions corresponding to residue 42, 45, and 72 of human IL-2 (SEQ ID NO: 1). In a more specific embodiment, the mutant IL-2 effector moiety comprises three amino acid substitutions at the positions corresponding to residue 42, 45 and 72 of human IL-2. In an even more specific embodiment, the mutant IL-2 effector moiety is human IL-2 comprising the amino acid substitutions F42A, Y45A and L72G. In certain embodiments the mutant IL-2 effector moiety additionally comprises an amino acid mutation at a position corresponding to position 3 of human IL-2, which eliminates the O-glycosylation site of IL-2. In a specific embodiment the mutant IL-2 effector moiety comprises the amino acid sequence of SEQ ID NO: 2. In one embodiment the effector moiety is a single-chain effector moiety.

In one embodiment the first antibody is a full-length IgG class antibody, particularly a full-length IgG_1 sub-class antibody. In one embodiment the effector moiety shares an amino- or carboxy-terminal peptide bond with the first antibody. In one embodiment the effector moiety shares an amino-terminal peptide bond with the first antibody. In one embodiment, the effector moiety is fused at its N-terminus to the C-terminus of one of the heavy chains of the first antibody. In a particular embodiment, the immunoconjugate comprises not more than one effector moiety. In one embodiment the immunoconjugate essentially consists of an effector moiety and a first antibody joined by one or more peptide linkers. In a specific embodiment the immunoconjugate comprises an effector moiety, particularly a single chain effector moiety, and a first antibody,

(a) an immunoconjugate comprising a first full-length IgG class antibody engineered to have reduced effector function by introduction of one or more amino acid mutation in the Fc region and a cytokine, wherein the effector moiety is fused at its amino-terminal amino acid to the carboxy-terminus of one of the heavy chains of the first antibody, optionally through a peptide linker, and

(b) a second full-length IgG class antibody engineered to have increased effector function by modification of the glycosylation in the Fc region, for use in treating a disease in an individual in need thereof. In another aspect the disclosure provides a pharmaceutical composition comprising (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety, and (b) a second antibody engineered to have increased effector function, in a pharmaceutically acceptable carrier, wherein the effector moiety is a mutant IL-2 effector moiety comprising at least one amino acid mutation, particularly an amino acid substitution, that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the α-subunit of the IL-2 receptor but preserves the affinity of the mutant IL-2 effector moiety to the intermediate-affinity IL-2 receptor, compared to the non-mutated IL-2 effector moiety, wherein the first antibody comprises in its Fc region one or more amino acid mutation that reduces the binding affinity of the immunoconjugate to an activating Fc receptor, and wherein the second antibody is engineered by introduction of one or more amino acid mutations in the Fc region or by modification of the glycosylation in the Fc region.

The disclosure, in another aspect, also encompasses the use of (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety, and (b) a second antibody engineered to have increased effector function, for the manufacture of a medicament for the treatment of ~~a disease~~ cancer in an individual, wherein the effector moiety is a mutant IL-2 effector moiety comprising at least one amino acid mutation, particularly an amino acid substitution, that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the α-subunit of the IL-2 receptor but preserves the affinity of the mutant IL-2 effector moiety to the intermediate-affinity IL-2 receptor, compared to the non-mutated IL-2 effector moiety, wherein the first antibody comprises in its Fc region one or more amino acid mutation that reduces the binding affinity of the immunoconjugate to an activating Fc receptor, and wherein the second antibody is engineered by introduction of one or more amino acid mutations in the Fc region or by modification of the glycosylation in the Fc region.

The disclosure further provides a method of treating a disease in an individual, comprising administering to the individual a combination of (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety, and (b) a second antibody engineered to have increased effector function, in a therapeutically effective amount.

In another aspect, the disclosure provides use of (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety for the manufacture of a medicament for treatment of ~~a disease~~ cancer in an individual in combination with (b) a second antibody engineered to have increased effector function, wherein the effector moiety is a mutant IL-2 effector moiety comprising at least one amino acid mutation, particularly an amino acid substitution, that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the α-subunit of the IL-2 receptor but preserves the affinity of the mutant IL-2 effector moiety to the intermediate-affinity IL-2 receptor, compared to the non-mutated IL-2 effector moiety, wherein the first antibody comprises in its Fc region one or more amino acid mutation that reduces the binding affinity of the immunoconjugate to an activating Fc receptor, and wherein the second antibody is engineered by introduction of one or more amino acid mutations in the Fc region or by modification of the glycosylation in the Fc region.

Also provided by the disclosure is a method of stimulating effector cell function in an individual, comprising administering to the individual a combination of (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety, and (b) a second antibody engineered to have increased effector function, in an amount effective to stimulate effector cell function.

~~In another aspect, the present disclosure provides use of (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety for the manufacture of a medicament for stimulation of effector cell function in an individual in combination with (b) a second antibody engineered to have increased effector function.~~

In a further aspect, the disclosure provides a kit intended for the treatment of a disease, comprising in the same or in separate containers (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety, (b) a second antibody engineered to have increased effector function, and (c) optionally a package insert comprising printed instructions directing the use of the combined treatment as a method for treating the disease, wherein the effector moiety is a mutant IL-2 effector moiety comprising at least one amino acid mutation, particularly an amino acid substitution, that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the α-subunit of the IL-2 receptor but preserves the affinity of the mutant IL-2 effector moiety to the intermediate-affinity IL-2 receptor, compared to the non-mutated IL-2 effector moiety, wherein the first antibody comprises in its Fc region one or more amino acid mutation that reduces the binding affinity of the immunoconjugate to an activating Fc receptor, and wherein the second antibody is engineered by introduction of one or more amino acid mutations in the Fc region or by modification of the glycosylation in the Fc region.

Claims

1. A combination of (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety, and (b) a second antibody engineered to have increased effector function, for use in treating a disease in an individual in need thereof~~.~~,

~~2. The combination of claim 1, wherein the effector moiety is a cytokine.~~

~~3. The combination of claim 1 or 2, wherein the effector moiety is a cytokine selected from the group consisting of IL-2, GM-CSF, IFN-α, and IL-12.~~

~~4. The combination of any one of claims 1 to 3, wherein the effector moiety is IL-2.~~

~~5. The combination of claim 4,~~ wherein the ~~IL-2~~ effector moiety is a mutant IL-2 effector moiety comprising at least one amino acid mutation, particularly an amino acid substitution, that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the α-subunit of the IL-2 receptor but preserves the affinity of the mutant IL-2 effector moiety to the intermediate-affinity IL-2 receptor, compared to the non-mutated IL-2 effector moiety~~.~~,

wherein the first antibody comprises in its Fc region one or more amino acid mutation that reduces the binding affinity of the immunoconjugate to an activating Fc receptor, and

wherein the second antibody is engineered by introduction of one or more amino acid mutations in the Fc region or by modification of the glycosylation in the Fc region.

~~6~~2. The combination of claim ~~5~~1, wherein the mutant IL-2 effector moiety comprises three amino acid substitutions at the positions corresponding to residue 42, 45 and 72 of human IL-2 (SEQ ID NO: 1).

~~7~~3. The combination of claim ~~5~~1 or ~~6~~2, wherein the mutant IL-2 effector moiety is human IL-2 comprising the amino acid substitutions F42A, Y45A and L72G.

~~8~~4. The combination of any one of claims ~~5~~1 to ~~7~~3, wherein the mutant IL-2 effector moiety comprises the amino acid sequence of SEQ ID NO: 2.

~~9~~5. The combination of any one of claims 1 to ~~8~~4, wherein the first antibody is a full-length antibody, particularly an IgG class antibody, more particularly and IgG_1 sub-class antibody.

~~10~~6. The combination of any one of claims 1 to ~~9~~5, wherein the effector moiety shares an amino- or carboxy-terminal peptide bond with the first antibody.

~~11~~7. The combination of any one of claims 1 to ~~10~~6, wherein the ~~first antibody is engineered to have reduced binding to an~~ activating Fc receptor is an FcγRIIIa, FcγRI or FcγRIIa receptor, particularly ~~reduced binding to~~ human FcγRIIIa.

~~12~~8. The combination of any one of claims 1 to ~~11~~7, wherein the first antibody comprises an amino acid substitution at position P329 of the immunoglobulin heavy chains (~~Kabat~~ EU numbering as described in Kabat).

~~13~~9. The combination of any one of claims 1 to ~~12~~8, wherein the first antibody comprises the amino acid substitutions L234A, L235A and P329G (EU numbering as described in Kabat) in the immunoglobulin heavy chains.

~~14~~10. The combination of any one of claims 1 to ~~13~~9, wherein the immunoconjugate essentially consists of an effector moiety~~, particularly a single chain effector moiety,~~ and a first antibody engineered to have reduced effector function, wherein the effector moiety is fused at its amino-terminal amino acid to the carboxy-terminus of one of the heavy chains of the first antibody, optionally through a peptide linker.

~~15~~11. The combination of any one of claims 1 to ~~14~~10, wherein the first antibody is directed to an antigen presented on a tumor cell or in a tumor cell environment.

~~16~~12. The combination of any one of claims 1 to ~~15~~11, wherein the first antibody is directed to 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 Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP).

~~17~~13. The combination of any one of claims 1 to ~~16~~12, wherein the first antibody is directed to Fibroblast Activated Protein (FAP) and comprises a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67 and SEQ ID NO: 69, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66 and SEQ ID NO: 68.

~~18~~14. The combination of any one of claims 1 to ~~16~~13, wherein the first antibody is directed to Carcinoembryonic Antigen (CEA) and comprises a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 114, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 115.

~~19~~15. The combination of any one of claims 1 to ~~18~~14, wherein the second antibody is a full-length IgG class antibody, particularly an IgG_1 subclass antibody.

~~20~~16. The combination of any one of claims 1 to ~~19~~15, wherein the effector function is selected from the group of binding to an activating Fc receptor, ADCC, ADCP, CDC, and cytokine secretion.

~~21~~17. The combination of any one of claims 1 to ~~20~~16, wherein the effector function is increased binding to an activating Fc receptor and/or increased ADCC.

~~22. The combination of any one of claims 1 to 21, wherein the second antibody is engineered by introduction of one or more amino acid mutations in the Fc region or by modification of the glycosylation in the Fc region.~~

~~23~~18. The combination of any one of claims 1 to ~~22~~17, wherein the second antibody is engineered to have an increased proportion of non-fucosylated oligosaccharides in the Fc region as compared to a non-engineered antibody.

~~24~~19. The combination of any one of claims 1 to ~~23~~18, wherein the second antibody is directed to an antigen presented on a tumor cell.

~~25~~20. The combination of any one of claims 1 to ~~24~~19, wherein the second antibody is directed to an antigen selected from the group of CD20, Epidermal Growth Factor Receptor (EGFR), HER2, HER3, Insulin-like Growth Factor 1 Receptor (IGF-1R), Carcinoembryonic Antigen (CEA), c-Met, CUB domain-containing protein-1 (CDCP1), and Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP).

~~26. The combination of any one of claims 1 to 25, wherein the second antibody is directed to EGFR and is selected from the group of (i) an IgG class antibody comprising the heavy chain variable domain of SEQ ID NO: 102 and the light chain variable domain of SEQ ID NO: 103, and (ii) cetuximab.~~

~~27. The combination of any one of claims 1 to 25, wherein the second antibody is directed to Her3 and comprises the heavy chain variable domain of SEQ ID NO: 142 and the light chain variable domain of SEQ ID NO: 146.~~

~~28~~21. The combination of any one of claims 1 to ~~27~~20, wherein the disease is a disorder treatable by stimulation of effector cell function, particularly cancer.

~~29~~22. The combination of any one of claims 1 to ~~28~~21, wherein the individual is a mammal, particularly a human.

~~30~~23. A pharmaceutical composition comprising (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety, and (b) a second antibody engineered to have increased effector function, in a pharmaceutically acceptable carrier~~.~~, wherein the effector moiety is a mutant IL-2 effector moiety comprising at least one amino acid mutation, particularly an amino acid substitution, that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the α-subunit of the IL-2 receptor but preserves the affinity of the mutant IL-2 effector moiety to the intermediate-affinity IL-2 receptor, compared to the non-mutated IL-2 effector moiety,

wherein the first antibody comprises in its Fc region one or more amino acid mutation that reduces the binding affinity of the immunoconjugate to an activating Fc receptor, and

wherein the second antibody is engineered by introduction of one or more amino acid mutations in the Fc region or by modification of the glycosylation in the Fc region.

~~31~~24. Use of (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety, and (b) a second antibody engineered to have increased effector function, for the manufacture of a medicament for the treatment of ~~a disease~~ cancer in an individual~~.~~,

wherein the effector moiety is a mutant IL-2 effector moiety comprising at least one amino acid mutation, particularly an amino acid substitution, that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the α-subunit of the IL-2 receptor but preserves the affinity of the mutant IL-2 effector moiety to the intermediate-affinity IL-2 receptor, compared to the non-mutated IL-2 effector moiety,

wherein the first antibody comprises in its Fc region one or more amino acid mutation that reduces the binding affinity of the immunoconjugate to an activating Fc receptor, and

wherein the second antibody is engineered by introduction of one or more amino acid mutations in the Fc region or by modification of the glycosylation in the Fc region.

~~32~~25. Use of (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety for the manufacture of a medicament for treatment of ~~a disease~~cancer in an individual in combination with (b) a second antibody engineered to have increased effector function~~.~~,

wherein the effector moiety is a mutant IL-2 effector moiety comprising at least one amino acid mutation, particularly an amino acid substitution, that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the α-subunit of the IL-2 receptor but preserves the affinity of the mutant IL-2 effector moiety to the intermediate-affinity IL-2 receptor, compared to the non-mutated IL-2 effector moiety,

wherein the first antibody comprises in its Fc region one or more amino acid mutation that reduces the binding affinity of the immunoconjugate to an activating Fc receptor, and

wherein the second antibody is engineered by introduction of one or more amino acid mutations in the Fc region or by modification of the glycosylation in the Fc region.

~~33. Use of (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety for the manufacture of a medicament for stimulation of effector cell function in an individual in combination with (b) a second antibody engineered to have increased effector function~~.

~~34~~26. A kit intended for the treatment of a disease, comprising in the same or in separate containers (a) an immunoconjugate comprising a first antibody engineered to have reduced effector function and an effector moiety, (b) a second antibody engineered to have increased effector function, and (c) optionally a package insert comprising printed instructions directing the use of the combined treatment as a method for treating the disease~~.~~,

wherein the effector moiety is a mutant IL-2 effector moiety comprising at least one amino acid mutation, particularly an amino acid substitution, that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the α-subunit of the IL-2 receptor but preserves the affinity of the mutant IL-2 effector moiety to the intermediate-affinity IL-2 receptor, compared to the non-mutated IL-2 effector moiety,

wherein the first antibody comprises in its Fc region one or more amino acid mutation that reduces the binding affinity of the immunoconjugate to an activating Fc receptor, and

wherein the second antibody is engineered by introduction of one or more amino acid mutations in the Fc region or by modification of the glycosylation in the Fc region.