oligosaccharide and polypeptide both directly contribute to the interaction site or that the oligosaccharide is required to maintain an active CH2 polypeptide conformation. Modification of the oligosaccharide structure can therefore be explored as a means to increase the affinity of the interaction between $IgG_1$ and FcγR, and to increase ADCC activity of $IgG_1$ antibodies. Umaña et al. (Nat Biotechnol 17, 176-180 (1999) and U.S. Patent No. 6,602,684 (WO 99/54342), the contents of which are hereby incorporated by reference in their entirety) showed that overexpression of β(1,4)-N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing the formation of bisected oligosaccharides, in Chinese hamster ovary (CHO) cells significantly increases the *in vitro* ADCC activity of antibodies produced in those cells. Overexpression of GnTIII in production cell lines leads to antibodies enriched in bisected oligosaccharides, which are generally also non-fucosylated and of the hybrid type. If in addition to GnTIII, mannosidase II (ManII) is overexpressed in production cell lines, antibodies enriched in bisected, non-fucosylated oligosaccharides of the complex type are obtained (Ferrara et al., Biotechn Bioeng 93, 851-861 (2006)). Both types of antibodies show strongly increased ADCC, as compared to antibodies with unmodified glycans, but only antibodies in which the majority of the N-glycans are of the complex type are able to induce significant complement-dependent cytotoxicity (Ferrara et al., Biotechn Bioeng 93, 851-861 (2006)). The critical factor for the increase of ADCC activity appears to be the elimination of fucose from the innermost N-acetylglucosamine residue of the oligosaccharide core, which improves binding of the IgG Fc domain to FcγRIIIa (Shinkawa et al., J Biol Chem 278, 3466-3473 (2003)). Further methods for producing antibodies with reduced fucosylation include, e.g. expression in α(1,6)-fucosyltransferase deficient host cells (Yamane-Ohnuki et al., Biotech Bioeng 87, 614-622 (2004); Niwa et al., J Immunol Methods 306, 151-160 (2006)).

Despite the successes achieved in anti-cancer immunotherapy by the use of free cytokines, immunoconjugates or engineered antibodies, there is a continuous need for novel efficacious and safe treatment options in cancer therapy.

## Summary ~~of the Invention~~

The present inventors have found that the combination of these two strategies for local immune cell activation, i.e. simultaneous stimulation of effector cells by cytokine immunoconjugates and by antibodies engineered to have increased effector functions, greatly improves the efficacy of anti-cancer immunotherapy.

Accordingly, the present ~~invention~~ 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. 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, particularly a full-length IgG class antibody, 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,

by reference in its entirety. In yet another particular embodiment the second antibody is an anti-CEA antibody engineered to have an increased proportion of non-fucosylated oligosaccharides in the Fc region as compared to a non-engineered antibody. Suitable anti-CEA antibodies are described in PCT publication number WO 2011/023787, which is incorporated herein by reference in its entirety. In yet another particular embodiment the second antibody is an anti-HER3 antibody engineered to have an increased proportion of non-fucosylated oligosaccharides in the Fc region as compared to a non-engineered antibody. Suitable anti-HER3 antibodies are described in PCT publication number WO 2011/076683, which is incorporated herein by reference in its entirety. In yet another particular embodiment the second antibody is an anti-CDCP1 antibody engineered to have an increased proportion of non-fucosylated oligosaccharides in the Fc region as compared to a non-engineered antibody. Suitable anti-CDCP1 antibodies are described in PCT publication number WO 2011/023389, which is incorporated herein by reference in its entirety. In one embodiment the second antibody is engineered to have modified glycosylation in the Fc region, as compared to a non-engineered antibody, by producing the antibody in a host cell having altered activity of one or more glycosyltransferase.

In one embodiment 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, by producing the antibody in a host cell having increased β(1,4)-N-acetylglucosaminyltransferase III (GnTIII) activity. In a particular embodiment the host cell additionally has increased α-mannosidase II (ManII) activity. In another embodiment 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, by producing the antibody in a host cell having decreased α(1,6)-fucosyltransferase activity.

In one embodiment the disease is a disorder treatable by stimulation of effector cell function. In one embodiment the disease is a cell proliferation disorder. In a particular embodiment the disease is cancer. In a specific embodiment the cancer is selected from the group of lung cancer, colorectal cancer, renal cancer, prostate cancer, breast cancer, head and neck cancer, ovarian cancer, brain cancer, lymphoma, leukemia, and skin cancer. In one embodiment the individual is a mammal. In a particular embodiment the individual is a human.

In a particular embodiment, the disclosure ~~invention~~ provides a combination of

(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 ~~invention~~ 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.

The ~~invention~~ 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 in an individual.

The ~~invention~~ 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 in an individual in combination with (b) a second antibody engineered to have increased effector function.

Also provided by the ~~invention~~ 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 ~~invention~~ 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.

It is understood that the immunoconjugate and the second antibody used in the pharmaceutical composition, use, methods and kit according to the ~~invention~~ disclosure may incorporate any of the features, singly or in combination, described in the preceding paragraphs in relation to the second antibodies and immunoconjugates useful for the disclosure~~invention~~.

**Short Description of the Drawings**

FIGURE 1. The FAP-targeted 28H1 IgG-IL-2 immunoconjugate (A) or the untargeted DP47GS IgG-IL-2 immunoconjugate (B), comprising the IL-2 quadruple mutant (qm) that lacks binding to CD25, and the anti-EGFR GlycoMab were tested in the human head and neck carcinoma cell line FaDu, intralingually injected into SCID mice. The data show that the combination of the 28H1 IgG-IL2 qm immunoconjugate, but not the DP47GS IgG-IL2 qm immunoconjugate, and the anti-EGFR GlycoMab mediates superior efficacy in terms of enhanced median survival compared to the respective immunoconjugate or the anti-EGFR GlycoMab alone (see Example 1).

FIGURE 2. Overall A549 tumor cell killing by PBMCs (E:T = 10:1, 4 hours), pre-treated or not with 0.57 nM (A) or 5.7 nM (B) FAP-targeted 28H1 IgG-IL2 qm immunoconjugate or IL-2 (Proleukin), in the presence of different concentrations of anti-EGFR GlycoMab (see Example 2).

FIGURE 3. The CEA-targeted CH1A1A IgG-IL-2 immunoconjugate comprising the IL-2 quadruple mutant (qm) that lacks binding to CD25, and the anti-EGFR GlycoMab (A) or cetuximab (B) were tested in the human colorectal carcinoma cell line LS174T, intrasplenically injected into SCID FcγRIII transgenic mice. The data show that the combination of the CH1A1A IgG-IL2 qm immunoconjugate and the anti-EGFR GlycoMab mediates superior efficacy in terms of enhanced median and overall survival compared to the respective immunoconjugate, the anti-EGFR GlycoMab or cetuximab alone, as well as the combination of the CH1A1A IgG-IL2 qm immunoconjugate and cetuximab (see Example 3).

FIGURE 4. The CEA-targeted CH1A1A IgG-IL-2 immunoconjugate comprising the IL-2 quadruple mutant (qm) that lacks binding to CD25, and the anti-EGFR GlycoMab (A) or cetuximab (B) were tested in the human lung carcinoma cell line A549, intravenously injected into SCID FcγRIII transgenic mice. The data show that the combination of the CH1A1A IgG-IL2 qm immunoconjugate and the anti-EGFR GlycoMab mediates superior efficacy in terms of enhanced median and overall survival compared to the respective immunoconjugate or the anti-

**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.

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

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

~~6~~9. The combination of any one of claims 1 to ~~5~~8, wherein the first antibody is a full-length antibody, particularly an IgG class antibody, more particularly and $IgG_1$ sub-class antibody.

~~7~~10. The combination of any one of claims 1 to ~~6~~9, wherein the effector moiety shares an amino- or carboxy-terminal peptide bond with the first antibody.

~~8~~11. The combination of any one of claims 1 to ~~7~~10, wherein the first antibody is engineered to have reduced binding to an activating Fc receptor, particularly reduced binding to human FcγRIIIa.

~~9~~12. The combination of any one of claims 1 to ~~8~~11, wherein the first antibody comprises an amino acid substitution at position P329 of the immunoglobulin heavy chains (Kabat numbering).

~~10~~13. The combination of any one of claims 1 to ~~9~~12, wherein the first antibody comprises the amino acid substitutions L234A, L235A and P329G in the immunoglobulin heavy chains.

~~11~~14. The combination of any one of claims 1 to ~~10~~13, 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.

~~12~~15. The combination of any one of claims 1 to ~~11~~14, wherein the first antibody is directed to an antigen presented on a tumor cell or in a tumor cell environment.

16. The combination of any one of claims 1 to 15, 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. The combination of any one of claims 1 to 16, 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. The combination of any one of claims 1 to 16, 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.

~~13~~19. The combination of any one of claims 1 to ~~12~~18, wherein the second antibody is a full-length IgG class antibody, particularly an $IgG_1$ subclass antibody.

~~14~~20. The combination of any one of claims 1 to ~~13~~19, wherein the effector function is selected from the group of binding to an activating Fc receptor, ADCC, ADCP, CDC, and cytokine secretion.

~~15~~21. The combination of any one of claims 1 to ~~14~~20, wherein the effector function is increased binding to an activating Fc receptor and/or increased ADCC.

~~16~~22. The combination of any one of claims 1 to ~~15~~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.

~~17~~23. The combination of any one of claims 1 to ~~16~~22, 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.

~~18~~24. The combination of any one of claims 1 to ~~17~~23, wherein the second antibody is directed to an antigen presented on a tumor cell.

25. The combination of any one of claims 1 to 24, 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.

~~19~~28. The combination of any one of claims 1 to ~~18~~27, wherein the disease is a disorder treatable by stimulation of effector cell function, particularly cancer.

~~20~~29. The combination of any one of claims 1 to ~~19~~28, wherein the individual is a mammal, particularly a human.

~~21~~30. 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.

~~22~~31. 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 in an individual.

~~23~~32. ~~A method of treating a disease in an individual, comprising administering to the individual a combination~~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 in an individual in combination with~~, and~~ (b) a second antibody engineered to have increased effector function~~, in a therapeutically effective amount~~.

~~24~~33. ~~A method of stimulating effector cell function in an individual, comprising administering to the individual a combination~~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~~, and~~ (b) a second antibody engineered to have increased effector function~~, in an amount effective to stimulate effector cell function~~.

~~25~~34. 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.