IL-12 Fc Fusion Proteins

[0001] **Field of the invention**

[0002] This invention relates to IL-12 Fc fusion proteins and their use in medicine, pharmaceutical compositions comprising the same, and methods of using the same as agents for treatment and/or prevention of cancer.

[0003] **Background of the invention**

[0004] Interleukin-12 (IL-12) is a cytokine with proven anti-tumor potential showing promising preclinical efficacy in mouse tumor models. However, drug releated toxicities were observed in clinical trials, resulting in suboptimal IL-12 dosing regimen and lack of efficacy in patients.

[0005] To overcome drug related toxicities masking of IL-12 was proposed to prevent systemic activity & toxicity to create a therapeutic window. Masking of the IL-12 activity can be done by e.g. fusing a domain of the IL-12 receptor to IL-12 via a protease-cleavable linker and subsequent local activation by protease-mediated removal of the IL-12 receptor at the tumor site in cancer patients.

[0006] Currently, there are no approved therapies based on IL-12. Hence, there is still a high unmet need for providing new therapeutic IL-12 based biological molecules which may be used for the treatment of cancer.

[0007] **Summary**

[0008] In one aspect, the present disclosure relates to an Interleukin-12 (IL-12) Fc fusion protein comprising a first polypeptide chain and a second polypeptide chain, wherein a) the first polypeptide chain comprises a first Fc domain and an IL-12p35 subunit and an IL-12p40 subunit of IL-12, and b) the second polypeptide chain comprises a second Fc domain and a masking moiety that binds to the IL-12p35 and/or IL-12p40 subunit in the first polypeptide chain; wherein the first and second polypeptide chain are linked via the first Fc domain and the second Fc domain, wherein the IL-12p35 subunit or the IL-12p40 subunit is linked to the C-terminus of the first Fc domain via a first peptide linker, which first peptide linker is protease-cleavable, wherein the masking moiety is linked to the C-terminus of the second Fc domain via a second linker, preferably a peptide linker, and wherein the first or the

second polypeptide chain further comprises a binding moiety selected from the group consisting of: a collagen binding moiety, a heparin binding moiety, and a fibronectin binding moiety.

[0009] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the binding moiety is linked to the C-terminus of the IL-12p35 subunit or to the C-terminus of the IL-12p40 subunit, or the binding moiety is linked to the C-terminus of the masking moiety, and in each case optionally via a third polypeptide linker.

[0010] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the binding moiety is located between the IL-12p35 subunit and the IL-12p40 subunit, or the binding moiety is located between the C-terminus of the first Fc domain and the N-terminus of the IL-12p35subunit or the N-terminus of the IL-12p40 subunit, and in either case the binding moiety may be optionally flanked on one or both sides by a linker or linkers, preferably a peptide linker.

[0011] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the binding moiety is a collagen binding moiety.

[0012] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the collagen binding moiety binds to collagen I.

[0013] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the collagen binding moiety binds to collagen I and has the sequence LxxLxLxxN (SEQ ID NO:41), wherein L is Leucine and N is Asparagine and x is any amino acid.

[0014] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the collagen binding moiety has a length of 20 amino acids (aa), 19aa, 18aa, 17aa, 16aa, 15aa, 14aa, 13aa, 12aa, 11aa, 10a, or 9aa.

[0015] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the collagen binding moiety comprises or consists of any one of the amino acid sequences of SEQ ID NOs:40-47.

[0016] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the binding moiety is a heparin binding moiety.

[0017] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the heparin binding moiety has the sequence VRIQRKKEKMKET (SEQ ID NO:50).

[0018] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the collagen binding moiety binds to collagen IV.

[0019] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the collagen binding moiety has the sequence KLWVLPK (SEQ ID NO:40).

[0020] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the binding moiety is a fibronectin binding moiety.

[0021] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the fibronectin binding moiety has the sequence GGWSHW (SEQ ID NO:49).

[0022] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the IL-12p35 subunit and the IL-12p40 subunit are human.

[0023] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the IL-12p35 subunit comprises a polypeptide having at least 95% identity to SEQ ID NO:1 and the IL-12p40 subunit comprises a polypeptide having at least 95% identity to SEQ ID NO:2, preferably the IL-12p35 subunit comprises the polypeptide of SEQ ID NO:1 and the IL-12p40 subunit comprises the polypeptide of SEQ ID NO:2.

[0024] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the IL-12p40 subunit and the IL-12p35 subunit are linked in a single-chain having the configuration (written from N-terminus to C-terminus) IL-12p40—IL-12p35 or IL-12p35—IL-12p40.

[0025] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the single-chain IL-12p40—IL-12p35 is linked via its IL-12p40 subunit to the C-terminus of the first Fc domain, or the single-chain IL-12p35—IL-12p40 is linked via its IL-12p35 subunit to the first Fc domain, and in both cases via the first peptide linker, which first peptide linker is protease-cleavable.

[0026] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the IL-12p40 subunit and the IL-12p35 subunit are linked to each other via a linker that is rich in amino acid residues glycine and serine, preferably having a length of 5 to 20 amino acids and only including the amino acids glycine and serine, more preferably a glycine and serine linker having the amino acid sequence of SEQ ID NO:22.

[0027] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the single-chain IL-12p40—IL-12p35 comprises a polypeptide having at least 95% identity to SEQ ID NO:8, or the single-chain IL-12p35—IL-12p40 comprises a polypeptide having at least 95% identity to SEQ ID NO:9.

[0028] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the second peptide linker is not protease-cleavable.

[0029] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the masking moiety binds to the IL-12p40 subunit and is selected from the group consisting of: an IL-12 receptor or an IL-12p40 binding fragment thereof, an scFv, or an immunoglobulin single variable domain, preferably a VHH.

[0030] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the first and the second Fc domain each comprise one or more mutations that promote heterodimerization of the Fc domains.

[0031] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the (a) first Fc domain is a human IgG1 Fc domain comprising the mutation T366W and the second Fc domain

is a human IgG1 Fc domain comprising the mutations T366S, L368A and Y407V, or the (b) first Fc domain is a human IgG1 Fc domain comprising the mutations T366S, L368A and Y407V and the second Fc domain is a human IgG1 Fc domain comprising the mutation T366W.

[0032] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the first and the second Fc domain are human IgG1 Fc domains and one of the first or the second Fc domain comprises the mutations H435R and Y436F.

[0033] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the first and the second Fc domain are human IgG1 Fc domains and either the first Fc domain, or the second Fc domain, or both Fc domains comprise the mutations L234A and L235A.

[0034] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the first Fc domain comprises the amino acid sequence of SEQ ID NO:15 and the second Fc domain comprises the amino acid sequence of SEQ ID NO:16, OR the first Fc domain comprises the amino acid sequence of SEQ ID NO:17 and the second Fc domain comprises the amino acid sequence of SEQ ID NO:18, OR the first Fc domain comprises the amino acid sequence of SEQ ID NO:16 and the second Fc domain comprises the amino acid sequence of SEQ ID NO:15, OR the first Fc domain comprises the amino acid sequence of SEQ ID NO:18 and the second Fc domain comprises the amino acid sequence of SEQ ID NO:17.

[0035] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the protease-cleavable linker is cleavable by a matrix metalloproteinase (MMP), preferably an MMP-2, MMP-9, or MMP-13.

[0036] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the protease-cleavable linker comprises or consists of any one of the amino acid sequences of SEQ ID NOs:232-241.

[0037] In another aspect the disclosure relates to an IL-12 Fc fusion protein comprising a first polypeptide chain and a second polypeptide chain, wherein a) the

[0038] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the masking moiety comprises an IL-12 binding immunoglobulin single variable domain comprising the three CDRs contained within any one of the sequences of SEQ ID NOs:61-109.

[0039] In a further example relating to the IL-12 Fc fusion protein as described herein or any of its examples the masking moiety comprises an IL-12 binding immunoglobulin single variable domain comprising any one of the amino acid sequences of SEQ ID NOs:61-109.

[0040] In another aspect the disclosure relates to a cleavage product capable of binding to a human IL-12 receptor comprising the IL-12 cytokine after proteolytic cleavage of the cleavable linker as defined in any one of the IL-12 Fc fusion proteins as described herein and the examples relating thereto.

[0041] In a further example relating to the cleavage product as described herein or any of its examples the cleavage product comprises the IL-12 cytokine and the binding moiety.

[0042] In a further example relating to the cleavage product as described herein or any of its examples the cleavage product comprises or consists of the amino acid sequence of any one of SEQ ID NOs:208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230 or 242 after proteolytic cleavage of the cleavable linker.

[0043] In another aspect the disclosure relates to an IL-12 binding immunoglobulin single variable domain comprising the three CDRs contained within any one of the sequences of SEQ ID NOs:61-109.

[0044] In a further example relating to the IL-12 binding immunoglobulin single variable domain as described herein or any of its examples said immunoglobulin single variable domain is a VHH.

[0045] In a further example relating to the IL-12 binding immunoglobulin single variable domain as described herein or any of its examples said immunoglobulin single variable domain comprises the amino acid sequence of any one of SEQ ID NOs:61-109.

[0046] In another aspect the disclosure relates to a nucleic acid encoding at least one polypeptide of the IL-12 Fc fusion proteins as described herein or any

examples related thereto, or a nucleic acid encoding one of the polypeptide chains of an IL-12 Fc fusion protein as described herein or any examples related thereto, or a nucleic acid encoding an IL-12 binding immunoglobulin single variable domain as described herein or any examples related thereto.

[0047] In another aspect the disclosure relates to a vector comprising the nucleic acid as described herein, optionally wherein the vector comprises nucleic acids encoding both chains of the IL-12 Fc fusion protein.

[0048] In another aspect the disclosure relates to a host cell comprising the nucleic acid as described herein or the vector as described herein, optionally wherein the cell comprises one or more nucleic acids encoding both chains of the IL-12 Fc fusion protein.

[0049] In another aspect the disclosure relates to a method of producing an IL-12 Fc fusion protein comprising culturing the host cell as described herein under a condition that produces the fusion protein and optionally purifying said IL-12 Fc fusion protein.

[0050] In another aspect the disclosure relates to a composition comprising the IL-12 Fc fusion protein as described herein or the examples relating thereto.

[0051] In another aspect the disclosure relates to a pharmaceutical composition comprising the IL-12 Fc fusion protein as described herein or the examples relating thereto and a pharmaceutically acceptable carrier.

[0052] In another aspect the disclosure relates to a kit comprising the IL-12 Fc fusion protein as described herein or the examples relating thereto, or the composition as described herein, or the pharmaceutical composition as described herein.

[0053] In another aspect the disclosure relates to an IL-12 Fc fusion protein as described herein or the examples relating thereto for use in medicine.

[0054] In another aspect the disclosure relates to a cleavage product as described herein or any example relating thereto for use in medicine.

[0055] The disclosure relates to a method of treating or reducing the incidence of cancer in a subject, the method comprising administering to the subject an effective amount of an IL-12 Fc fusion protein as described herein or the examples relating thereto.

[0056] In another aspect the disclosure relates to an IL-12 Fc fusion protein as described herein or the examples relating thereto for use in treating or preventing cancer.

[0057] In another aspect the disclosure relates to the use of an IL-12 Fc fusion protein as described herein or the examples relating thereto in the manufacture of a medicament.

[0058] In another aspect the disclosure relates to the use of an IL-12 Fc fusion protein as described herein or the examples relating thereto in the manufacture of a medicament for reduction of the incidence of or treatment of cancer.

[0059] Where mentioned above, the IL-12 Fc fusion protein, the cleavage product, the IL-12 binding immunoglobulin single variable domain, the nucleic acid, the vector or the host cell may be isolated, i.e. an isolated IL-12 Fc fusion protein, an isolated cleavage product, an isolated IL-12 binding immunoglobulin single variable domain, an isolated nucleic acid, an isolated vector or an isolated host cell.

[0060] **Brief description of the figures**

[0061] **Figures 1A-1H:** Exemplary formats scouted for assembly of IL-12 Fc fusion proteins, with a cleavable IL-12 (p35 as kidney shape, p40 as three individual spheres), an antibody fragment masking domain, cleavable linker (light grey with star) connecting the IL-12 to the Fc, and either Knob-in-holes or wild type Fc.

[0062] **Figure 2:** Functional characterization of 47 VHH-Fc that were discovered via llama immunizations, followed by subsequent phage panning. Only one of the constructs shows inhibition of IL12 >90% with respect to the control molecule in the Promega Bioassay.