

CLAIMS

1. A bifunctional molecule comprising an interleukin 7 (IL-7) variant conjugated to a binding moiety, wherein:
- the binding moiety binds to a target specifically expressed on immune cells surface,
 - 5 - the IL-7 variant presents at least 75% identity with a wild type human IL-7 (wth-IL-7) comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 1, wherein the variant comprises at least one amino acid mutation selected from the group consisting of (i) W142H, W142F or W142Y, (ii) C2S-C141S and C47S-C92S, C2S-C141S and C34S-C129S, or C47S-C92S and C34S-C129S, , (iii) D74E, D74Q or D74N, iv) Q11E, Y12F, M17L, Q22E and/or K81R; or any combination thereof, the amino acid numbering
 - 10 being as shown in SEQ ID NO: 1, which i) reduces affinity of the IL-7 variant for IL-7 receptor (IL-7R) in comparison to the affinity of wth-IL-7 for IL-7R, and ii) improves pharmacokinetics of the bifunctional molecule comprising the IL-7 variant in comparison with a bifunctional molecule comprising wth-IL-7.
2. The molecule according to claim 1, wherein the IL-7 variant comprises an amino acid substitution selected from the group consisting of W142H, W142F and W142Y, the amino acid numbering being as
- 15 shown in SEQ ID NO: 1.
3. The molecule according to claim 1 or 2, wherein the IL-7 variant comprises a group of amino acid substitutions selected from the group consisting of C2S-C141S and C47S-C92S, C2S-C141S and C34S-C129S, and C47S-C92S and C34S-C129S, the amino acid numbering being as shown in SEQ ID NO: 1.
4. The molecule according to any one of claims 1-3, wherein the IL-7 variant comprises in the amino acid
- 20 substitution selected from the group consisting of D74E, D74Q and D74N, the amino acid numbering being as shown in SEQ ID NO: 1.
5. The molecule according to any one of claims 1-4, wherein the IL-7 variant comprises or consists of the amino acid sequence set forth in SEQ ID NO: 2-15.
6. The molecule according to any one of claims 1-5, wherein the binding moiety comprises a heavy chain
- 25 constant domain, preferably a Fc domain, of a human IgG1, optionally with a substitution or a combination of substitutions selected from the group consisting of T250Q/M428L; M252Y/S254T/T256E + H433K/N434F; E233P/L234V/L235A/G236A + A327G/A330S/P331S; E333A; S239D/A330L/I332E; P257I/Q311; K326W/E333S; S239D/I332E/G236A; N297A; L234A/L235A; N297A + M252Y/S254T/T256E; K322A and K444A, preferably selected from the group consisting of N297A optionally in combination with
- 30 M252Y/S254T/T256E, and L234A/L235A.
7. The molecule according to any one of claims 1-5, wherein the binding moiety comprises a heavy chain constant domain, preferably a Fc domain, of a human IgG4, optionally with a substitution or a combination

of substitutions selected from the group consisting of S228P; L234A/L235A, S228P + M252Y/S254T/T256E.17 and K444A.

8. The molecule according to any one of claims 1-7, wherein the immune cell is a T cell, preferably an exhausted T cell.
- 5 9. The molecule according to claim 8, wherein the target is expressed by T cells and the binding moiety binds to a target selected from the group consisting of PD-1, CD28, CD80, CTLA-4, BTLA, TIGIT, CD160, CD40L, ICOS, CD27, OX40, 4-1BB, GITR, HVEM, Tim-1, LFA-1, TIM3, CD39, CD30, NKG2D, LAG3, B7-1, 2B4, DR3, CD101, CD44, SIRPG, CD28H, CD38, CXCR5, CD3, PDL2, CD4 and CD8.
- 10 10. The molecule according to claim 8, wherein the target is expressed by T exhausted cells and the binding moiety binds to a target preferably selected from the group consisting of PD-1, CTLA-4, BTLA, TIGIT, LAG3 and TIM3.
11. The molecule according to any one of claims 1-10, wherein the binding moiety is an antibody or an antigen fragment thereof, and the N-terminal of the IL-7 variant is fused to the C-terminal of a heavy or light chain constant domain of the antibody or antibody fragment thereof, preferably to the C-terminal of the heavy chain constant domain, optionally via a peptide linker.
- 15 12. The molecule according to claim 11, wherein the IL-7 variant is fused to the binding moiety by a peptide linker selected from the group consisting of GGGGS (SEQ ID NO: 68), GGGGSGGGGS (SEQ ID NO: 67), GGGGSGGGGS (SEQ ID NO: 69) and GGGGSGGGGSGGGGS (SEQ ID NO: 70), preferably is (GGGGGS)₃.
- 20 13. The molecule according to any one of claims 1-12, wherein the molecule comprises a first monomer comprising an antigen-binding domain covalently linked via C-terminal end to N-terminal end of a first heterodimeric Fc chain optionally via a peptide linker, said first heterodimeric Fc chain being covalently linked by the C-terminal end to the N-terminal end of the IL-7 variant, optionally via a peptide linker, and a second monomer comprising a complementary second heterodimeric Fc chain devoid of antigen-binding domain.
- 25 14. The molecule according to claim 13, wherein, in the second monomer, the complementary second heterodimeric Fc chain covalently linked to the IL-7 variant, optionally via a peptide linker, preferably covalently linked by C-terminal end to N-terminal of the IL-7 variant, optionally via a peptide linker.
- 30 15. The molecule according to any one of claims 1-12, wherein the molecule comprises a first monomer comprising an antigen-binding domain covalently linked by C-terminal end to N-terminal end of a first heterodimeric Fc chain, optionally via a peptide linker, said first heterodimeric Fc chain being devoid of IL-7 variant, and a second monomer comprising a complementary second heterodimeric Fc chain devoid of antigen-binding domain, said second heterodimeric Fc chain being covalently to the IL-7 variant,

optionally via a peptide linker, preferably linked by C-terminal end to N-terminal of the IL-7 variant, optionally via a peptide linker.

16. The molecule according to any one of claims 1-12, wherein the molecule comprises a first monomer comprising an antigen-binding domain covalently linked via C-terminal end to N-terminal end of a first heterodimeric Fc chain optionally via a peptide linker, and a second monomer comprising an antigen-binding domain covalently linked via C-terminal end to N-terminal end of a complementary second heterodimeric Fc chain optionally via a peptide linker, wherein only one of heterodimeric Fc chains, preferably the first one, is covalently linked by the C-terminal end to the N-terminal end of the IL-7 variant.

17. The molecule according to any one of claims 13-16, wherein the antigen-binding domain is a Fab domain, a Fab', a single-chain variable fragment (scFV) or a single domain antibody (sdAb).

18. The molecule according to any one of claims 13-17, wherein the antigen-binding domain comprises or consists essentially of: (i) a heavy chain comprising a CDR1 of SEQ ID NO: 51, a CDR2 of SEQ ID NO: 53 and a CDR3 of SEQ ID NO: 55, 56, 57, 58, 59, 60, 61 or 62; and (ii) a light chain comprising a CDR1 of SEQ ID NO: 64 or SEQ ID NO: 65, a CDR2 of SEQ ID NO: 66 and a CDR3 of SEQ ID NO: 16.

19. The molecule according to any one of claims 13-17, wherein the antigen-binding domain comprises or consists essentially of:

(a) a heavy chain variable region (VH) comprising or consisting of an amino acid sequence of SEQ ID NO: 18, 19, 20, 21, 22, 23, 24 or 25;

(b) a light chain variable region (VL) comprising or consisting of an amino acid sequence of SEQ ID NO: 27 or SEQ ID NO: 28.

20. The molecule according to any one of claims 13-19, wherein the antigen-binding domain comprises or consists essentially of a heavy chain variable region (VH) of SEQ ID NO: 24 and a light chain variable region (VL) of SEQ ID NO: 28.

21. An isolated nucleic acid sequence or a group of isolated nucleic acid molecules encoding the bifunctional molecule according to any one of claims 1-20.

22. A host cell comprising the isolated nucleic acid according to claim 21.

23. A pharmaceutical composition comprising the bifunctional molecule according to any one of claims 1-20, the nucleic acid according to claim 21 or the host cell according to claim 22, optionally with a pharmaceutically acceptable carrier.

24. The molecule according to any one of claims 1-20, the nucleic acid according to claim 21 or the host cell according to claim 22, or the pharmaceutical composition according to claim 23, for use as a medicament, especially for use in the treatment of a cancer or an infectious disease.