

Claims

1. An interleukin-15 (IL-15) variant comprising amino acid substitutions at position G78 and at position N79 of a mature human IL-15.
2. The IL-15 variant of claim 1, wherein the IL-15 variant comprises the amino acid substitutions G78A, G78V, G78L or G78I, and N79Q, N79H or N79M, preferably G78A and N79Q.
3. The IL-15 variant of claim 1 or claim 2, wherein the IL-15 variant has been expressed in a mammalian cell line, preferably the mammalian cell line is selected from CHO cells, HEK293 cells, COS cells, PER.C6 cells, SP20 cells, NSO cells or any cells derived therefrom, more preferably CHO cells.
4. The IL-15 variant of any of claims 1 to 3, wherein the amino acid substitutions
 - (a) reduce deamidation at N77 and glycosylation at N79 of the IL-15 variant compared to mature human IL-15,
 - (b) result in less than 30% of glycosylated IL-15 variant, preferably less than 25% of glycosylated IL-15 variant, and/or,
 - (c) increase glycosylation at N71 of the IL-15 variant compared to mature human IL-15.
5. The IL-15 variant of any of claims 1 to 4, wherein the amino acid substitutions do not substantially reduce the IL-15 activity of the IL-15 variant on the proliferation induction of Kit225 cells, 32Db cells, human PBMC or in the Promega IL-15-bioassay.
6. The IL-15 variant of any of claims 1 to 5, wherein the IL-15 variant does not have a substitution at position N71 and/or at position N77.
7. The IL-15 variant of any of the claims 1 to 6, wherein the IL-15 variant comprises at least one further substitution that reduces the binding to the IL-2/IL-15R β and/or to the γ_c receptor and/or the IL-15R α ,
 optionally wherein
 - (a) the site for the further substitution reducing binding to the IL-2/IL-15R β and/or to the γ_c receptor is selected from the list consisting of N1, N4, S7, D8, K10, K11, D30, D61, E64, N65, L69, N72, E92, Q101, Q108, and I111, preferably from the list consisting of D61, N65 and Q101, most preferably N65;

- (b) the further substitution reducing binding to the IL-2/IL-15R β and/or to the γ c receptor is selected the list consisting of N1D, N1A, N1G, N4D, S7Y, S7A, D8A, D8N, K10A, K11A, D30N, D61A, D61N, E64Q, N65D, N65A, N65E, N65R, N65K, L69R, N72R, Q101D, Q101E, Q108D, Q108A, Q108E and Q108R,
 5 preferably from the list consisting of D8A, D8N, D61A, D61N, N65A, N65D, N72R, Q101D, Q101E and Q108A,
 more preferably selected from the list consisting of D61A, N65A and Q101, most preferably N65A; or
- (c) the further substitution reducing binding to the IL-2/IL-15R β and/or to the γ c receptor is a
 10 combined substitution and is selected form the list consisting of D8N/N65A, D61A/N65A and D61A/N65A/Q101D, and/or

optionally wherein

- (a) the site for the further substitution reducing binding to the IL-15R α is selected from the list consisting of L44, L45, E46, L47, V49, I50, S51, E64, L66, I67, I68 and L69,
 15 (b) the further substitution reducing binding to the IL-15R α is selected from the list consisting of L44D, E46K, E46G, L47D, V49D, V49R, I50D, L66D, L66E, I67D, and I67E, or
 (c) the further substitution reducing binding to the IL-15R α is a combined substitution selected form the list consisting of E46G/V49R, N1A/D30N/E46G/V49R, N1G/D30N/E46G/V49R/E64Q, V49R/E46G/N1A/D30N and
 20 V49R/E46G/N1G/E64Q/D30N.

8. A conjugate comprising an IL-15 variant of any of the claims 1 to 7, optionally wherein the conjugate further comprises the sushi domain of an IL-15R α or a derivative thereof.
9. A fusion protein comprising an IL-15 variant of any of the claims 1 to 7, optionally wherein in the fusion protein further comprises the sushi domain of an IL-15R α or a derivative thereof, a
 25 targeting moiety, and/or a half-life extending moiety, and optionally one or more linker(s).
10. The fusion protein of claim 9, wherein the fusion protein comprises, preferably in N- to C-terminal order, the human IL-15R α sushi domain, a linker and the IL-15 variant of any of the claims 1 to 7,
 preferably wherein the human IL-15R α sushi domain comprises the sequence of SEQ ID NO:
 30 5,
 the linker has a length of 18 to 22 amino acids and is composed of serines and glycines, and more preferably wherein the fusion protein is SEQ ID NO: 9 or SEQ ID NO: 10.

11. The fusion protein of any of the claims 9 or 10, wherein the targeting moiety is an antibody or functional variant thereof that preferably binds to a tumor antigen, a tumor extracellular matrix antigen, or a tumor neovascularization antigen, or is an immunomodulatory antibody, optionally wherein the fusion protein is fused to the C-terminus of at least one heavy chain of the antibody or to the C-terminus of both light chains of the antibody.
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12. A nucleic acid encoding the IL-15 variant of any of the claims 1 to 7, the conjugate of claim 8, or the fusion protein of any of the claims 9 to 11.
13. A vector comprising the nucleic acid of claim 12.
14. A host cell comprising the nucleic acid of claim 12 or the vector of claim 13.
- 10 15. The IL-15 variant of any of the claims 1 to 7, the conjugate of claim 8, or the fusion protein of any of the claims 9 to 11, the nucleic acid of claim 12 or the vector of claim 13 for use in treatment.
16. A pharmaceutical composition comprising the IL-15 variant of any of the claims 1 to 7, the conjugate of claim 8, or the fusion protein of any of the claims 9 to 11, the nucleic acid of claim 12 or the vector of claim 13 and a pharmaceutically acceptable carrier.
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17. Use of the IL-15 variant of any of the claims 1 to 7, the conjugate of claim 8, or the fusion protein of any of the claims 9 to 11, the nucleic acid of claim 12 or the vector of claim 13 in the manufacture of a medicament for the treatment of a subject suffering from, at risk of developing and/or being diagnosed for a neoplastic disease or an infectious disease.