

Surprisingly, it was found that in addition to lower immunogenicity these humanised variants may possess other advantageous properties as compared to murine Clone 47 and / or other humanised anti -IL13R α 2 antibodies, for example improved affinity for human IL13R α 2, as outlined elsewhere herein.

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Accordingly, in a first aspectexample, the present disclosure provides a humanised antibody that binds to Interleukin-13 receptor subunit alpha-2 (IL13R α 2), wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 3; SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; and SEQ ID NO: 8.

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In some embodimentexamples, the humanised antibody further comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 4; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11. In some embodimentexamples, the antibody further comprises a constant region derived from one or more human antibodies.

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In some embodimentexamples, the antibody comprises a heavy chain variable region having the amino acid sequence of: SEQ ID NO: 3, and:

- (i) a light chain variable region having the amino acid sequence of SEQ ID NO: 4;
- (ii) a light chain variable region having the amino acid sequence of SEQ ID NO: 9;
- 20 (iii) a light chain variable region having the amino acid sequence of SEQ ID NO: 10; or
- (iv) a light chain variable region having the amino acid sequence SEQ ID NO: 11.

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In some embodimentexamples, the antibody comprises a heavy chain variable region having the amino acid sequence of: SEQ ID NO: 5, and:

- (i) a light chain variable region having the amino acid sequence of SEQ ID NO: 4;
- (ii) a light chain variable region having the amino acid sequence of SEQ ID NO: 9;
- (iii) a light chain variable region having the amino acid sequence of SEQ ID NO: 10; or
- (iv) a light chain variable region having the amino acid sequence SEQ ID NO: 11.

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In some embodimentexamples, the antibody comprises a heavy chain variable region having the amino acid sequence of: SEQ ID NO: 6, and:

- (i) a light chain variable region having the amino acid sequence of SEQ ID NO: 4;
- (ii) a light chain variable region having the amino acid sequence of SEQ ID NO: 9;
- (iii) a light chain variable region having the amino acid sequence of SEQ ID NO: 10; or
- 35 (iv) a light chain variable region having the amino acid sequence SEQ ID NO: 11.

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In some embodimentexamples, the antibody comprises a heavy chain variable region having the amino acid sequence of: SEQ ID NO: 7, and:

- (i) a light chain variable region having the amino acid sequence of SEQ ID NO: 4;
- (ii) a light chain variable region having the amino acid sequence of SEQ ID NO: 9;

- (iii) a light chain variable region having the amino acid sequence of SEQ ID NO: 10; or
- (iv) a light chain variable region having the amino acid sequence SEQ ID NO: 11.

In some embodimentexamples, the antibody comprises a heavy chain variable region having the amino acid sequence of: SEQ ID NO: 8, and:

- (i) a light chain variable region having the amino acid sequence of SEQ ID NO: 4;
- (ii) a light chain variable region having the amino acid sequence of SEQ ID NO: 9;
- (iii) a light chain variable region having the amino acid sequence of SEQ ID NO: 10; or
- (iv) a light chain variable region having the amino acid sequence SEQ ID NO: 11.

In some preferred embodimentexamples, the antibody comprises:

- (i) a heavy chain variable region having the amino acid sequence of SEQ ID NO: 3, and a light chain variable region having the amino acid sequence of SEQ ID NO: 4;
- (ii) a heavy chain variable region having the amino acid sequence of SEQ ID NO: 3, and a light chain variable region having the amino acid sequence of SEQ ID NO: 10;
- (iii) a heavy chain variable region having the amino acid sequence of SEQ ID NO: 3, and a light chain variable region having the amino acid sequence of SEQ ID NO: 11;
- (iv) a heavy chain variable region having the amino acid sequence of SEQ ID NO: 5, and a light chain variable region having the amino acid sequence of SEQ ID NO: 4;
- (v) a heavy chain variable region having the amino acid sequence of SEQ ID NO: 5, and a light chain variable region having the amino acid sequence of SEQ ID NO: 10;
- (vi) a heavy chain variable region having the amino acid sequence of SEQ ID NO: 5, and a light chain variable region having the amino acid sequence of SEQ ID NO: 11; or
- (vii) a heavy chain variable region having the amino acid sequence of SEQ ID NO: 8, and a light chain variable region having the amino acid sequence of SEQ ID NO: 11.

In some more preferred embodimentexamples, the antibody comprises:

- (i) a heavy chain variable region having the amino acid sequence of SEQ ID NO: 3, and a light chain variable region having the amino acid sequence of SEQ ID NO: 4;
- (ii) a heavy chain variable region having the amino acid sequence of SEQ ID NO: 5 and a light chain variable region having the amino acid sequence of SEQ ID NO: 4; and
- (iii) a heavy chain variable region having the amino acid sequence of SEQ ID NO: 8, and a light chain variable region having the amino acid sequence of SEQ ID NO: 11.

In some particularly preferred embodimentexamples, the antibody comprises a heavy chain variable region having the amino acid sequence of: SEQ ID NO: 3, and a light chain variable region having the amino acid sequence of SEQ ID NO: 4.

In some embodimentexamples, the humanised antibody binds IL13R α 2 with an affinity (K_D) no greater than 5×10^{-10} M, such as no greater than 2.5×10^{-10} M or no greater than 1.5×10^{-10} M. In some embodimentexamples, the humanised antibody binds IL13R α 2 with higher affinity (K_D) than an antibody

comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO: 1 and a light chain variable region having the amino acid sequence of SEQ ID NO: 2, and optionally, an antibody constant region. In some embodimentexamples, affinity (K_D) is as determined by a protocol as described in the Examples, for example by Biacore analysis.

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In some embodimentexamples, the humanised antibody competitively inhibits the binding to IL13R α 2 of an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO: 1 and a light chain variable region having the amino acid sequence of SEQ ID NO: 2, and optionally, an antibody constant region.

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In some embodimentexamples, the IL13R α 2 is human IL13R α 2.

The sequences of the antibody heavy chain variable regions and/or the light chain variable regions disclosed herein may be modified by, for example, insertions, substitutions and/or deletions to the extent that the humanised antibody maintains the ability to bind to IL13R α 2. The skilled person can ascertain the maintenance of this activity by performing the functional assays described herein, or known in the art.

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Accordingly, in some embodimentexamples the heavy chain variable region comprises no more than 20 insertions, substitutions and/or deletions, such as no more than 15, no more than 10, no more than 9, no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 insertion, substitution and/or deletion. In some embodimentexamples the light chain variable region comprises no more than 20 insertions, substitutions and/or deletions, such as no more than 15, no more than 10, no more than 9, no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 insertion, substitution and/or deletion.

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In some embodimentexamples the humanised antibodies of the disclosure include antibodies comprising VH and VL domains with amino acid sequences that are identical to the sequences described herein.

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In a second aspectexample the present disclosure provides an antibody drug conjugate comprising a humanised antibody according to the first aspectexample of the disclosure. The conjugates of the disclosure include an antibody conjugated, i.e. covalently attached by a linker, to a drug moiety.

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In a third aspectexample, the present disclosure provides a pharmaceutical composition comprising an antibody according to the first aspectexample or an antibody drug conjugate according to the second aspectexample, and a pharmaceutically acceptable diluent, carrier or excipient.

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The present disclosure also provides such antibodies, conjugates, and pharmaceutical compositions, for use in a method of treatment, and the use of such antibodies, conjugates, and pharmaceutical compositions in the manufacture of a medicament for use in a method of treatment.

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The present disclosure also provides polynucleotides encoding the antibodies of the disclosure, vectors comprising such polynucleotides, and host cells comprising such vectors, as well as methods of producing antibodies of the disclosure comprising culturing such host cells under conditions suitable for the expression of the antibodies of the disclosure.

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In one aspect, the present disclosure relates to an antibody that binds to Interleukin-13 receptor subunit alpha-2 (IL13R α 2), wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO: 3 and a light chain variable region comprising the amino acid sequence shown in SEQ ID NO: 4.

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In another aspect, the present disclosure relates to an antibody drug conjugate comprising the antibody as disclosed herein conjugated to a drug moiety, optionally wherein the drug moiety is a cytotoxic drug moiety.

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In another aspect, the present disclosure relates to a pharmaceutical composition comprising the antibody or antibody drug conjugate as disclosed herein and a pharmaceutically acceptable diluent, carrier or excipient.

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In another aspect, the present disclosure relates to use of an antibody, antibody drug conjugate, or pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating cancer.

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In another aspect, the present disclosure relates to a polynucleotide encoding an antibody as disclosed herein. In yet another aspect, the present disclosure relates to a vector comprising a polynucleotide as disclosed herein. In yet another aspect, the present disclosure relates to a host cell comprising a vector as disclosed herein.

SUMMARY OF THE FIGURES

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Figure 1

Sequence comparison of murine "Clone 47" and "HuCL47" heavy (VH) and light (VK) chain variable domains. CDRs are underlined.

Figure 2

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Comparison of murine Clone47, humanised variant HuCL47, and isotype control B12 antibodies binding to NCI-H1299 Cells.

Figure 3

ELISA binding comparison of murine Clone47, humanised variant HuCL47, and isotype control B12 antibodies.

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DETAILED DESCRIPTION

Aspects and embodiments of the present disclosure will now be discussed with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

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Humanised antibodies

The present disclosure provides humanised anti-IL13R α 2 antibodies.

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“Clone 47” is a murine anti-IL13R α 2 antibody described in Balyasnikova et al (J Biol Chem. 2012 Aug 31; 287(36): 30215–30227). Beginning from the heavy and light chain sequences of murine Clone 47 (SEQ ID NO: 1 and SEQ ID NO: 2 respectively), the present authors have generated a number of humanised heavy and light chain sequences with a view to creating antibodies that have lower immunogenicity in a human individual than chimeric antibodies comprising the heavy and light chain sequences of the murine antibody.

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Surprisingly, it was found that certain humanised variants so produced show improved affinity for human IL13R α 2 as compared to the chimeric Clone 47. The humanised antibodies of the disclosure

CLAIMS:

1. An antibody that binds to Interleukin-13 receptor subunit alpha-2 (IL13R α 2), wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO: 3; and

~~2. The antibody according to claim 1, wherein the antibody further comprises a light chain variable region comprising the amino acid sequence shown in SEQ ID NO: 4.~~

~~32.~~ The antibody according to claim 1 ~~or claim 2~~, wherein the antibody binds IL13R α 2 with an affinity (K_D) no greater than 5×10^{-10} M, such as no greater than 2.5×10^{-10} M or no greater than 1.5×10^{-10} M.

~~43.~~ The antibody according to any one of the preceding claims, wherein the antibody competitively inhibits the binding to IL13R α 2 of an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO: 1 and a light chain variable region having the amino acid sequence of SEQ ID NO: 2, and optionally, an antibody constant region.

~~54.~~ The antibody according to any one of the preceding claims, wherein the antibody binds IL13R α 2 with higher affinity (K_D) than an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO: 1 and a light chain variable region having the amino acid sequence of SEQ ID NO: 2, and optionally, an antibody constant region.

~~65.~~ The antibody according to any one of the preceding claims, wherein the antibody has immunogenicity in a human subject that is lower than that of an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO: 1 and a light chain variable region having the amino acid sequence of SEQ ID NO: 2, and optionally, an antibody constant region.

~~76.~~ The antibody according to any one of the preceding claims, wherein the antibody has improved stability properties as compared to an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO: 1 and a light chain variable region having the amino acid sequence of SEQ ID NO: 2, and optionally, an antibody constant region.

~~87.~~ The antibody according to any one of the preceding claims, wherein the antibody comprises a constant region of either isotype IgG1, IgG2, IgG3 or IgG4, or a mutated IgG constant region, and optionally a light chain constant region of isotype kappa or lambda.

~~98.~~ The antibody of claim ~~8-7~~ wherein the constant region is human.

~~109.~~ The antibody according to any one of claims 1 ~~to -7-6~~ wherein the antibody is a Fab, a Fab', a F(ab')₂, or an scFv.

~~41~~10. An antibody drug conjugate comprising the antibody according to any preceding claim conjugated to a drug moiety, optionally wherein the drug moiety is a cytotoxic drug moiety.

~~42~~11. The antibody drug conjugate of claim ~~41~~10 wherein the drug moiety comprises a pyrolobenzodiazepine dimer.

~~43~~12. A pharmaceutical composition comprising the antibody or antibody drug conjugate according to any preceding claim and a pharmaceutically acceptable diluent, carrier or excipient.

~~44. The antibody, antibody drug conjugate, or pharmaceutical composition according to any preceding claim, for use in a method of treatment, optionally wherein the method of treatment is a method of treating cancer.~~

~~45~~13 Use of an antibody, antibody drug conjugate, or pharmaceutical composition according to any one of claims ~~1- to~~ 13~~12~~, in the manufacture of a medicament for ~~use in a method of treatment, optionally wherein the method of treatment is a method of treating cancer.~~

~~46. A method of treating a proliferative disease comprising administering to a subject in need thereof an antibody, antibody drug conjugate, or pharmaceutical composition according to any one of claims 1-13, optionally wherein the proliferative disease is cancer.~~

~~47~~14. A polynucleotide encoding an antibody according to any one of claims ~~1-40~~9.

~~48~~15. A vector comprising the polynucleotide of claim ~~14, 17,~~

~~16. The vector according to claim 15, optionally wherein the vector is an expression vector.~~

~~49~~17. A host cell comprising a vector according to claim ~~15 or 16,~~18

~~18. The host cell according to claim 17, optionally wherein the host cell is prokaryotic, eukaryotic, or mammalian.~~