

Urech et al. 2010 Ann Rheum Dis 69: 443), shows a better binding to TNF- $\alpha$  than scFv1 (see Fig. 1 of WO 2015/144852 A1). Therefore, WO 2015/144852 A1 does not disclose anti-TNF- $\alpha$  antibodies having high affinity for human TNF $\alpha$  ( $K_D < 125$  pM).

- WO 2015/065987 A1 describes anti-TNF- $\alpha$  antibodies, anti-IL-6 antibodies, and bispecific antibodies binding to both antigens. Certain anti-TNF $\alpha$  antibodies showed some cross-reactivity with TNF $\alpha$  from *Cynomolgus* (Fig. 17). The anti-TNF $\alpha$  antibodies, however, exhibited a significantly lower potency than infliximab in an L929 neutralization assay ([0152]; Fig. 5). Therefore, WO 2015/065987 A1 does not disclose anti-TNF- $\alpha$  antibodies having a potency to inhibit TNF $\alpha$ -induced apoptosis in L929 cells greater than that of infliximab.
- Drugs in R&D, Vol. 4 No. 3, 2003, pages 174-178 describes the humanized antibody "Humicade" (CDP 571; BAY 103356), a monoclonal anti-TNF $\alpha$  antibody with high affinity. The potency of Humicade to inhibit TNF $\alpha$ -induced apoptosis in L929 cells, however, appears to be limited (see, e.g., US 2003/0199679 A1 at [0189]). The reference therefore does not disclose anti-TNF- $\alpha$  antibodies having a potency to inhibit TNF $\alpha$ -induced apoptosis in L929 cells greater than that of infliximab.

Saldanha J W et al: "Molecular Engineering I: Humanization", Handbook of Therapeutic Antibodies, Chapter 6, 2007-01-01, WILEY-VCH, Weinheim, p.119-144 discloses different strategies for humanization of monoclonal antibodies including CDR Grafting, Resurfacing/Veneering, SDR transfer and DeImmunization Technology.

- There is a need for improved antibody molecules to treat chronic inflammatory diseases such as inflammatory bowel disorders. The antibody molecules should at least have (i) high affinity for human TNF $\alpha$  (i.e. a  $K_D < 1$  nM, particularly  $< 100$  pM), (ii) a high potency to inhibit TNF $\alpha$ -induced apoptosis in L929 cells, (iii) a high potency to inhibit LPS-induced cytokine secretion, (iv) substantial affinity to TNF $\alpha$  from *Cynomolgus* and *Rhesus* (e.g. a  $K_D < 1$  nM), and (v) a high melting temperature of the variable domain as determined in a thermal unfolding experiment (e.g. a  $T_m$  at least  $60^\circ\text{C}$ , particularly at least  $63^\circ\text{C}$ , more particularly at least  $66^\circ\text{C}$ ).

## SUMMARY

- The inventors of the present application found that certain anti-TNF $\alpha$  antibodies and functional fragments thereof exhibit a combination of two or more favorable properties, including high affinity for human TNF $\alpha$  ( $K_D < 1$  nM, particularly  $< 100$  pM), a potency to inhibit

TNF $\alpha$ -induced apoptosis in L929 cells similar to, and particularly greater than, that of infliximab, a potency to inhibit LPS-induced cytokine secretion greater than that of adalimumab, and/or substantial affinity ( $K_D < 1$  nM) to TNF $\alpha$  from animals such as Cynomolgus monkey (*Macaca fascicularis*) and/or Rhesus macaques (*Macaca mulatta*). In addition, the antibodies and functional fragments thereof were specific for TNF $\alpha$  in that they did not significantly bind to TNF $\beta$ , and exhibit a significant stability, as determined in a thermal unfolding assay of the variable domain.

The disclosure describes antibody molecules and functional fragments thereof. In one aspect, the present disclosure refers to the use of the antibody or functional fragment thereof as defined herein in the manufacture of a medicament for treating a TNF $\alpha$ -related disorder or disease selected from the list of: arthritis in general, rheumatoid arthritis, osteoarthritis, reactive arthritis, juvenile arthritis; psoriasis, including psoriatic arthritis; inflammatory bowel disease, including Crohn's disease, ulcerative colitis, including proctitis, sigmoiditis, left-sided colitis, extensive colitis and pancolitis, undetermined colitis, microscopic colitis, including collagenous and lymphocytic colitis, colitis in connective tissue disease, diversion colitis, colitis in diverticular disease, eosinophilic colitis and pouchitis.

The present disclosure therefore describes to the subject matter defined in the following items (1) to (47):

(1) An antibody or a functional fragment thereof capable of binding to human tumor necrosis factor alpha (TNF $\alpha$ ), wherein said antibody or functional fragment thereof comprises (i) a  $V_L$  domain comprising a CDR1 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:1, a CDR2 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:2, and a CDR3 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:3, and (ii) a  $V_H$  domain comprising a CDR1 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:4, a CDR2 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:5, and a CDR3 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:6.

(2) The antibody or functional fragment of item (1), wherein said antibody or functional fragment comprises (i) a  $V_L$  domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:7, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:2, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:8, and (ii) a  $V_H$  domain comprising a CDR1 region having the amino acid

sequence as shown in SEQ ID NO:9, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:10, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:11.

- 5 (3) The antibody or functional fragment of any one of the preceding items, wherein said antibody or functional fragment comprises a V<sub>H</sub> domain having an amino acid sequence selected from the amino acid sequences as shown in SEQ ID NOs:12 to 14.

## Claims

1. An antibody or a functional fragment thereof capable of binding to human tumor necrosis factor alpha (TNF $\alpha$ ), wherein said antibody or functional fragment comprises (i) a V<sub>L</sub> domain comprising a CDR1 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:1, a CDR2 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:2, and a CDR3 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:3, and (ii) a V<sub>H</sub> domain comprising a CDR1 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:4, a CDR2 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:5, and a CDR3 region having an amino acid sequence in accordance with the amino acid sequence as shown in SEQ ID NO:6.
2. The antibody or functional fragment thereof of claim 1, wherein said antibody or functional fragment comprises (i) a V<sub>L</sub> domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:7, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:2, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:8, and (ii) a V<sub>H</sub> domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:9, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:10, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:11.
3. The antibody or functional fragment thereof of claim 1 or 2, wherein said antibody or functional fragment
  - (i) binds to human TNF $\alpha$  with a dissociation constant (K<sub>D</sub>) of less than 1 nM, particularly less than 750 pM, more particularly less than 100 pM;
  - (ii) is cross-reactive with *Macaca mulatta* (Rhesus) TNF $\alpha$  and with *Macaca fascicularis* (Cynomolgus) TNF $\alpha$ ;
  - (iii) has a potency to inhibit TNF $\alpha$ -induced apoptosis that is greater than the potency of infliximab;
  - (iv) comprises a variable domain having a melting temperature, determined by differential scanning fluorimetry, of at least 60°C, particularly at least 63°C, more particularly at least 66°C; and/or
  - (v) is capable of binding to human TNF $\alpha$ <sub>Trimer</sub> in a stoichiometry (antibody : TNF $\alpha$ <sub>Trimer</sub>) of at least 2.

4. The antibody or functional fragment thereof of any one of the preceding claims, which binds to human TNF $\alpha$  with a  $K_D$  of less than 75 pM.
5. The antibody or functional fragment thereof of any one of the preceding claims, wherein said antibody or functional fragment comprises a  $V_H$  domain having the amino acid sequence as shown in SEQ ID NO:14.
6. The antibody or functional fragment thereof of any one of the preceding claims, wherein said antibody or functional fragment comprises a  $V_L$  domain having the amino acid sequence as shown in SEQ ID NO:17.
7. The antibody or functional fragment thereof of any one of the preceding claims, which is a single-chain variable fragment (scFv).
8. The antibody or functional fragment thereof of claim 7, wherein said scFv has the amino acid sequence as shown in SEQ ID NO:20.
9. The antibody or functional fragment thereof of any one of claims 1 to 6, which is an immunoglobulin G (IgG).
10. A nucleic acid encoding the antibody or functional fragment thereof of any one of the preceding claims.
11. A vector or plasmid comprising the nucleic acid of claim 10.
12. A cell comprising the nucleic acid of claim 10 or the vector or plasmid of claim 11.
13. A method of preparing the antibody or functional fragment thereof of any one of claims 1 to 9, comprising culturing the cell of claim 12 in a medium under conditions that allow expression of the nucleic acid encoding the antibody or functional fragment thereof, and recovering the antibody or functional fragment thereof from the cells or from the medium.
14. A pharmaceutical composition comprising the antibody or functional fragment thereof of any one of claims 1 to 9, and optionally a pharmaceutically acceptable carrier and/or excipient.
15. Use of the antibody or functional fragment thereof as defined in any one of claims 1 to 9 in the manufacture of a medicament for treating a TNF $\alpha$ -related disorder or disease selected from the list of: arthritis in general, rheumatoid arthritis, osteoarthritis, reactive arthritis, juvenile arthritis; psoriasis, including psoriatic arthritis; inflammatory bowel

disease, including Crohn's disease, ulcerative colitis, including proctitis, sigmoiditis, left-sided colitis, extensive colitis and pancolitis, undetermined colitis, microscopic colitis, including collagenous and lymphocytic colitis, colitis in connective tissue disease, diversion colitis, colitis in diverticular disease, eosinophilic colitis and pouchitis.