

Given the role of Treg-derived TGF- β in suppressing the immune response in the tumour microenvironment, there has been interest in targeting this pathway as an alternative approach to cancer immunotherapy. For example, therapeutic agents capable of dampening this pathway may serve as useful tools to improve the efficacy of cancer vaccines or other cancer immunotherapy strategies designed to harness the power of the body's immune system to treat cancer.

Cuende *et al.* (Sci Transl Med. 2015 Apr 22;7(284):284ra56) describes the production and characterisation of two monoclonal antibodies (MHG-8 and LHG10), which bind to the GARP-TGF- β complex on Tregs and inhibit TGF- β production. These two antibodies are also described and characterised in International patent applications WO2015/015003 and WO2016/125017. These antibodies were shown to be capable of inhibiting the immunosuppressive activity of human Treg in a xenogeneic graft-versus-host disease mouse model. This work serves to validate the GARP-TGF- β complex as a therapeutic target of interest for the purposes of modulating Treg function and consequently treating diseases such as cancer and autoimmune disease where the level of Treg activity plays an important role. There remains a need however, for improved GARP-TGF- β antibodies capable of inhibiting TGF- β release and thereby modifying Treg activity. The present invention addresses this problem as described herein.

SUMMARY

The present disclosure improves upon the state of the art by providing new antibodies and antigen binding fragments thereof, which bind to the human GARP-TGF- β 1 complex. The antibodies and antigen binding fragments of the present disclosure are derived from the GARP-TGF- β 1 antibody "LHG-10", described in International patent applications WO2015/015003 and WO2016/125017. The heavy chain and light chain variable domain sequences of LHG-10 are shown in SEQ ID NOs: 1 and 2, respectively, and the light chain variable domain of a chain-shuffled variant, LHG-10.6 (also described in WO2015/015003 and WO2016/125017), is shown in SEQ ID NO: 3. The antibodies of the present disclosure differ particularly with respect to certain CDR sequences as compared with LHG-10 and LHG-10.6, specifically with respect to the CDR2 and CDR3 sequences of the heavy chain variable domain. The LHG-10 and LHG-

10.6 GARP-TGF- β antibodies possess the heavy chain CDR2 sequence:

RIDPEDGGTKYAQKFQG (SEQ ID NO: 5); and the heavy chain CDR3 sequence:

NEWETVVVGDLMEYEY (SEQ ID NO: 6), whereas the antibodies of the present disclosure comprise the heavy chain CDR2 sequence: RIDPEDAGTKYAQKFQG (SEQ ID NO: 12); and

5 the heavy chain CDR3 sequence: YEWETVVVGDLMEYEY (SEQ ID NO: 13).

The differences in the heavy chain CDR2 and CDR3 sequences reported herein result in antibodies that are improved as compared with the prior art antibodies by virtue of their improved stability. More specifically, the antibodies of the present disclosure are relatively
10 resistant to deamidation, isomerization and oxidation, such that they exhibit enhanced stability. Surprisingly, these specific substitutions in the heavy chain CDR2 and CDR3 regions that lead to improved stability do not significantly decrease the binding affinity of the antibodies for the GARP-TGF- β 1 complex. The improved stability combined with high affinity target binding renders the antibodies of the present disclosure particularly suitable for clinical development as
15 therapeutic agents, for example as cancer therapeutic agents.

In one aspect, the present disclosure provides a recombinant antibody or an antigen binding fragment thereof, which binds to a complex of human glycoprotein A repetitions predominant (GARP) and TGF- β 1, wherein the antibody or antigen binding fragment thereof comprises
20 a heavy chain variable domain (VH), wherein: the VH CDR3 comprises the amino acid sequence YEWETVVVGDLMEYEY (SEQ ID NO: 13), the VH CDR2 comprises the amino acid sequence RIDPEDAGTKYAQKFQG (SEQ ID NO: 12), and the VH CDR1 comprises the amino acid sequence SYYID (SEQ ID NO: 4); and a light chain variable domain (VL), wherein:
the VL CDR3 comprises the amino acid sequence QQYASVPVT (SEQ ID NO: 11),
25 the VL CDR2 comprises the amino acid sequence GASRLKT (SEQ ID NO: 10), and
the VL CDR1 comprises the amino acid sequence QASQSISSYLA (SEQ ID NO: 9).

In another aspect, the present disclosure provides an isolated polynucleotide which encodes the heavy chain variable domain of the antibody or antigen binding fragment as described herein. In
30 yet another aspect, the present disclosure provides an isolated polynucleotide which encodes the light chain variable domain of an antibody or antigen binding fragment as described herein. In yet another aspect, the present disclosure provides an isolated polynucleotide which encodes the antibody or antigen binding fragment as described herein. In yet another aspect, the present disclosure provides an expression vector comprising the polynucleotide as described herein

operably linked to regulatory sequences which permit expression of the antibody, antigen binding fragment, heavy chain variable domain, or light chain variable domain in a host cell or cell-free expression system.

5 In a further aspect, the present disclosure provides a host cell or cell-free expression system containing the expression vector as described herein. In a further aspect, the present disclosure provides a method of producing a recombinant antibody or antigen binding fragment thereof, comprising culturing the host cell or cell-free expression system as described herein under conditions which permit expression of the antibody or antigen binding fragment; and recovering
10 the expressed antibody or antigen binding fragment. In a further aspect, the present disclosure provides a pharmaceutical composition comprising an antibody or antigen binding fragment as described herein and at least one pharmaceutically acceptable carrier or excipient. In a further aspect, the present disclosure provides a recombinant antibody, which binds to a complex of human glycoprotein A repetitions predominant (GARP) and TGF- β 1, wherein the antibody
15 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 16, and a light chain comprising the amino acid sequence of SEQ ID NO: 17.

In a first example, the present disclosure provides an antibody or antigen binding fragment thereof, which binds to a complex of human GARP-TGF- β 1, wherein the antibody or antigen
20 binding fragment thereof comprises a heavy chain variable domain (VH) wherein:
the VH CDR3 comprises the amino acid sequence YEWETVVVGDLMEYHEY (SEQ ID NO: 13),
the VH CDR2 comprises the amino acid sequence RIDPEDAGTKYAQKFQG (SEQ ID NO: 12),
and
the VH CDR1 comprises the amino acid sequence SYYID (SEQ ID NO: 4).

25 In certain examples, the present disclosure provides an antibody or antigen binding fragment thereof, which binds to a complex of human GARP-TGF- β 1, wherein the antibody or antigen binding fragment thereof comprises a heavy chain variable domain (VH) wherein:
the VH CDR3 consists of the amino acid sequence YEWETVVVGDLMEYHEY (SEQ ID NO:
30 13),
the VH CDR2 consists of the amino acid sequence RIDPEDAGTKYAQKFQG (SEQ ID NO: 12),
and
the VH CDR1 consists of the amino acid sequence SYYID (SEQ ID NO: 4).

The antibody or antigen binding fragment may additionally comprise a light chain variable domain (VL) wherein:

the VL CDR3 comprises the amino acid sequence QQYASVPVT (SEQ ID NO: 11),
the VL CDR2 comprises the amino acid sequence GASRLKT (SEQ ID NO: 10), and
5 the VL CDR1 comprises the amino acid sequence QASQSISSYLA (SEQ ID NO: 9).

In certain examples, the antibody or antigen binding fragment may additionally comprise a light chain variable domain (VL) wherein:

the VL CDR3 consists of the amino acid sequence QQYASVPVT (SEQ ID NO: 11),
10 the VL CDR2 consists of the amino acid sequence GASRLKT (SEQ ID NO: 10), and
the VL CDR1 consists of the amino acid sequence QASQSISSYLA (SEQ ID NO: 9).

In certain examples, the antibody or antigen binding fragment thereof comprises
a heavy chain variable domain (VH), wherein:

15 the VH CDR3 comprises the amino acid sequence YEWETVVVGDLMEYEEY (SEQ ID NO: 13),
the VH CDR2 comprises the amino acid sequence RIDPEDAGTKYAQKFQG (SEQ ID NO: 12),
and
the VH CDR1 comprises the amino acid sequence SYYID (SEQ ID NO: 4); and

a light chain variable domain (VL), wherein:

20 the VL CDR3 comprises the amino acid sequence QQYASVPVT (SEQ ID NO: 11),
the VL CDR2 comprises the amino acid sequence GASRLKT (SEQ ID NO: 10), and
the VL CDR1 comprises the amino acid sequence QASQSISSYLA (SEQ ID NO: 9).

In certain examples, the antibody or antigen binding fragment thereof comprises

25 a heavy chain variable domain (VH), wherein:

the VH CDR3 consists of the amino acid sequence YEWETVVVGDLMEYEEY (SEQ ID NO:
13),

the VH CDR2 consists of the amino acid sequence RIDPEDAGTKYAQKFQG (SEQ ID NO: 12),
and

30 the VH CDR1 consists of the amino acid sequence SYYID (SEQ ID NO: 4); and

a light chain variable domain (VL), wherein:

the VL CDR3 consists of the amino acid sequence QQYASVPVT (SEQ ID NO: 11),
the VL CDR2 consists of the amino acid sequence GASRLKT (SEQ ID NO: 10), and
the VL CDR1 consists of the amino acid sequence QASQSISSYLA (SEQ ID NO: 9).

In certain examples, the antibodies or antigen binding fragments include at least one heavy chain variable domain (VH) and/or at least one light chain variable domain (VL) that is a humanised, germlined or affinity variant of a camelid-derived VH or VL domain.

5

In certain examples, provided herein are antibodies or antigen binding fragments thereof, which bind to the complex of human GARP and human TGF- β 1, wherein the antibodies or antigen binding fragments comprise a heavy chain variable domain selected from the following:

- (i) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 14; or
- 10 (ii) a VH comprising or consisting of an amino acid sequence having at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO:14.

Alternatively or in addition, the antibodies or antigen binding fragments may comprise a light chain variable domain (VL) selected from the following:

- 15 (i) a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 15; or
- (ii) a VL comprising or consisting of an amino acid sequence having at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15.

For examples wherein the domains of the antibodies or antigen binding fragments are defined
20 by a particular percentage sequence identity to a reference sequence, the VH and/or VL domains may retain identical CDR sequences to those present in the reference sequence such that the variation is present only within the framework regions.

In a particular example, provided herein are antibodies or antigen binding fragments thereof,
25 wherein the heavy chain variable domain (VH) comprises or consists of the amino acid sequence of SEQ ID NO: 14 and the light chain variable domain (VL) comprises or consists of the amino acid sequence of SEQ ID NO: 15.

In certain examples, the antibodies of the disclosure include the CH1 domain, hinge region,
30 CH2 domain and CH3 domain of a human antibody, in particular human IgG1, IgG2, IgG3 or IgG4. In certain examples, the antibody includes the CH3 region of a human IgG4 and includes the substitution S228P in the CH3 domain.

The antibodies which bind the GARP-TGF- β 1 complex may comprise at least one full-length immunoglobulin heavy chain and/or at least one full-length lambda or kappa light chain. In certain examples, the antibodies comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 16 and a light chain comprising the amino acid sequence of SEQ ID NO: 17. In certain examples, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 16. In certain examples, provided herein are monoclonal antibodies comprising a light chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 17. In certain examples, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 16, and a light chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 17.

For examples wherein the heavy and/or light chains of the antibodies are defined by a particular percentage sequence identity to a reference sequence, the heavy chain and/or light chain may retain identical CDR sequences to those present in the reference sequence such that the variation is present only outside the CDR regions.

Unless otherwise stated in the present application, % sequence identity between two amino acid sequences may be determined by comparing these two sequences aligned in an optimum manner and in which the amino acid sequence to be compared can comprise additions or deletions with respect to the reference sequence for an optimum alignment between these two sequences. The percentage of identity is calculated by determining the number of identical positions for which the amino acid residue is identical between the two sequences, by dividing this number of identical positions by the total number of positions in the comparison window and by multiplying the result obtained by 100 in order to obtain the percentage of identity between these two sequences. For example, it is possible to use the BLAST program, "BLAST 2 sequences" (Tatusova et al, "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250), the parameters used being those given by default (in particular for the parameters "open gap penalty": 5, and "extension gap penalty": 2; the matrix chosen being, for example, the matrix "BLOSUM 62" proposed by the

program), the percentage of identity between the two sequences to be compared being calculated directly by the program.

The GARP-TGF- β 1 antibodies or antigen binding fragments thereof provided herein may each exhibit one or more of the following properties/features:

- the antibody or antigen binding fragment may cross-react with the GARP-TGF- β complex of Cynomolgus origin;
- the antibody or antigen binding fragment may bind to human GARP-TGF- β 1 with high affinity;
- the antibody or antigen binding fragment may include a VH domain and VL domain that when tested as a Fab fragment exhibit an off-rate (K_{off}) for the complex of human GARP and TGF- β 1 of less than $5 \times 10^{-4} \text{ s}^{-1}$;
- the antibody or antigen binding fragment may include a VH domain and VL domain that when tested as a Fab fragment exhibit an off-rate (K_{off}) for the complex of human GARP and TGF- β 1 in the range $1 \times 10^{-6} \text{ s}^{-1}$ to $5 \times 10^{-4} \text{ s}^{-1}$;
- the antibody or antigen binding fragment may include a VH domain and VL domain that when tested as a mAb exhibit a K_D of less than $1.7 \times 10^{-9} \text{ M}$;
- the antibody or antigen binding fragment may block or inhibit release of active TGF- β 1 from regulatory T cells.

In further examples, the disclosure also provides polynucleotide molecules which encode the above-listed antibodies and antigen binding fragments, in addition to expression vectors comprising the polynucleotides, host cells containing the vectors, and methods of recombinant expression/production of the antibodies described herein.

In a still further example, the disclosure provides a pharmaceutical composition comprising any one of the GARP-TGF- β 1 antibodies or antigen binding fragments thereof described herein, and a pharmaceutically acceptable carrier or excipient.

A still further example of the disclosure concerns methods of medical treatment using the above-listed GARP-TGF- β 1 antibodies or antigen binding fragments thereof, particularly in the prophylaxis and/or treatment of TGF- β -related disorders. In certain examples, the disclosure relates to methods of treatment using the GARP-TGF- β 1 antibodies or antigen binding fragments thereof, wherein the disease or condition to be treated is selected from the group

consisting of inflammatory diseases, chronic infection, cancer, fibrosis, cardiovascular disease, cerebrovascular disease and neurodegenerative disease. In certain examples, the GARP-TGF- β 1 antibodies or antigen binding fragments thereof are administered in combination with another treatment as part of a combination therapy. For example, the GARP-TGF- β 1 antibodies or
5 antigen binding fragments thereof may be administered in combination with an immunotherapeutic agent, optionally an immunostimulatory antibody or a tumour vaccine.

These and other examples of the disclosure will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It
10 should be understood, however, that the following description, while indicating various examples of the disclosure and numerous specific details thereof, is given by way of illustration and not of limitation. Many substitutions, modifications, additions and/or rearrangements may be made within the scope of the disclosure without departing from the spirit thereof, and the disclosure includes all such substitutions, modifications, additions and/or rearrangements.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic showing the binding of latent TGF- β to GARP on the surface of
20 regulatory T cells. TGF- β is produced as a precursor, “pro-TGF- β ” and undergoes cleavage to produce “latent-TGF- β ”, a form in which the mature TGF- β dimer remains non-covalently associated with the latency associated peptide (LAP) region of each polypeptide. It is this latent form that binds to GARP on the surface of Treg cells. Integrins $\alpha\beta$ 6 and $\alpha\beta$ 8 are thought to be responsible for mediating the release of mature or “active TGF- β ” from the cell surface. This
25 active form can act in a paracrine fashion to bring about effects in a variety of target cells, or can act as an autocrine mediator by binding to the TGF- β receptor on Treg cells.

Figure 2 shows target binding activity as measured by Biacore™ or surface plasmon resonance (SPR) for antibodies 39B6 IgG1^{N297Q} and 39B6 IgG4^{S228P} over a 56-day period for samples
30 stored at -20 °C, 5 °C and 37 °C in PBS or PBSTween (PBSTw). The reference sample (-20 °C) was set as 100% binding activity at each time point.

Figure 3 shows the results of testing the 39B6-A antibody variants in an assay designed to monitor SMAD2 phosphorylation downstream of TGF- β receptor activation. SMAD2

CLAIMS

1. A recombinant antibody or an antigen binding fragment thereof, which binds to a complex of human glycoprotein A repetitions predominant (GARP) and TGF- β 1, wherein the antibody
5 or antigen binding fragment thereof comprises
a heavy chain variable domain (VH), wherein:
the VH CDR3 comprises the amino acid sequence YEWETVVVGDLMEYEY (SEQ ID NO:
13),
the VH CDR2 comprises the amino acid sequence RIDPEDAGTKYAQKFQG (SEQ ID NO:
10 12), and
the VH CDR1 comprises the amino acid sequence SYYID (SEQ ID NO: 4); and
a light chain variable domain (VL), wherein:
the VL CDR3 comprises the amino acid sequence QQYASVPVT (SEQ ID NO: 11),
the VL CDR2 comprises the amino acid sequence GASRLKT (SEQ ID NO: 10), and
15 the VL CDR1 comprises the amino acid sequence QASQSISSYLA (SEQ ID NO: 9).
2. The antibody of claim 1, wherein the heavy chain variable domain (VH) comprises the amino acid sequence of SEQ ID NO: 14 and the light chain variable domain (VL) comprises the amino acid sequence of SEQ ID NO: 15.
- 20 3. The antibody of claim 1 or 2, which comprises the CH1 domain, hinge region, CH2 domain and/or CH3 domain of a human IgG.
4. The antibody of claim 3, wherein the human IgG is IgG1.
- 25 5. The antibody of claim 3, wherein the human IgG is IgG4.
6. The antibody of claim 5, wherein the human IgG4 has the substitution S228P in the CH3 domain.
- 30 7. An isolated polynucleotide which encodes the heavy chain variable domain of the antibody or antigen binding fragment of any of claims 1-6.
8. The isolated polynucleotide of claim 7, which comprises the sequence of SEQ ID NO:18.
- 35

9. An isolated polynucleotide which encodes the light chain variable domain of an antibody or antigen binding fragment of any of claims 1-6.

10. The isolated polynucleotide of claim 9, which comprises the sequence of SEQ ID NO:19.

5

11. An isolated polynucleotide which encodes the antibody or antigen binding fragment of any of claims 1-6.

12. An expression vector comprising the polynucleotide of any of claims 7-11 operably linked to regulatory sequences which permit expression of the antibody, antigen binding fragment, heavy chain variable domain, or light chain variable domain in a host cell or cell-free expression system.

13. A host cell or cell-free expression system containing the expression vector of claim 12.

15

14. A method of producing a recombinant antibody or antigen binding fragment thereof, comprising

culturing the host cell or cell-free expression system of claim 13 under conditions which permit expression of the antibody or antigen binding fragment; and

20

recovering the expressed antibody or antigen binding fragment.

15. A pharmaceutical composition comprising an antibody or antigen binding fragment according to any of claims 1 to 6 and at least one pharmaceutically acceptable carrier or excipient.

25

16. A recombinant antibody, which binds to a complex of human glycoprotein A repetitions predominant (GARP) and TGF- β 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 16, and a light chain comprising the amino acid sequence of SEQ ID NO: 17.