

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



(10) International Publication Number

WO 2014/205501 A1

(43) International Publication Date
31 December 2014 (31.12.2014)

WIPO | PCT

(51) International Patent Classification:

C07K 16/24 (2006.01) A61P 37/02 (2006.01)
A61K 39/395 (2006.01) A61P 37/08 (2006.01)
A61P 35/00 (2006.01)

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(21) International Application Number:

PCT/AU2014/000673

(22) International Filing Date:

27 June 2014 (27.06.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

2013902377 27 June 2013 (27.06.2013) AU

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

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WO 2014/205501 A1

(54) Title: IL-21 BINDING PROTEINS AND USES THEREOF

(57) Abstract: The present disclosure provides proteins comprising antigen binding sites of antibodies that bind to interleukin-21 (IL-21) and uses thereof, e.g., in therapy.

IL-21 BINDING PROTEINS AND USES THEREOF**RELATED APPLICATION DATA**

The present application claims priority from Australian provisional Patent Application 5 No. 2013902377 entitled "A super-agonist monoclonal antibody to human IL-21 and its application in immunomodulation" filed 27 June 2013, the entire contents of which are herein incorporated by reference.

FIELD

10 The present disclosure relates to proteins comprising antigen binding sites of antibodies that bind to interleukin-21 (IL-21) and uses thereof, e.g., in therapy.

SEQUENCE LISTING

15 The present application is filed with a sequence listing in electronic form. The entire contents of the sequence listing are herein incorporated by reference and constitute part of the specification description.

BACKGROUND

20 Cytokines generally stimulate proliferation or differentiation of cells of the hematopoietic lineage or participate in the immune and inflammatory response mechanisms of the body. The interleukins are a family of cytokines that mediate immunological responses. Central to an immune response is the T cell, which produces many cytokines and effects adaptive immunity to antigens. Cytokines produced by the T cell have been classified as TH1 and TH2. Type 1 cytokines include IL-2, IFN- γ , LT- α , and are involved in inflammatory responses, viral immunity, 25 intracellular parasite immunity and allograft rejection. Type 2 cytokines include IL-4, IL-5, IL-6, IL-10 and IL-13, and are involved in humoral responses, helminth immunity and allergic response. Shared cytokines between Type 1 and 2 include IL-3, GM-CSF and TNF- α . There is some evidence to suggest that Type 1 and Type 2 producing T cell populations preferentially migrate into different types of inflamed tissue.

30 Natural killer (NK) cells have a common progenitor cell with T cells and B cells, and play a role in immune surveillance. NK cells, which comprise up to 15% of blood lymphocytes, do not express antigen receptors, and are a component of innate immunity. NK cells are involved in the recognition and killing of tumor cells and virally infected cells. *In vivo*, NK cells are believed to require activation, however, *in vitro*, NK cells have been shown to kill some 35 types of tumor cells without activation.

IL-21 has been shown to be a potent modulator of cytotoxic T cells and NK cells. IL-21 has been shown to co-stimulate the expansion of NK cells, and it has been demonstrated to enhance the effector functions of these cells. T cell responses include enhancement of primary antigen response as modulation of memory T cell functions.

5 It has been shown that IL-21R knock-out mice express higher levels of IgE and lower levels of IgG1 than normal mice after antigen exposure. IgE levels decreased after mice were injected with IL-21. This has implications for the role of IL-21 in controlling allergic responses because of the role of IgE in hypersensitivity type 1 responses.

10 IL-21 has been tried as therapy for alleviating allergic responses. It was shown to be successful in decreasing pro-inflammatory cytokines produced by T cells in addition to decreasing IgE levels in a mouse model for rhinitis (nasal passage inflammation). This has strong implications for the pharmacological development of IL-21 for controlling both localized and systemic allergies.

15 A role for IL-21 in modulating the differentiation programming of human T cells was first reported by Li *et al* (*Journal of immunology* 175 (4): 2261–9 (2005)), where it was shown to enrich for a population of central memory-type CTL with a unique CD28+ CD127hi CD45RO+ phenotype with IL-2 producing capacity. Tumor-reactive antigen-specific CTL generated by priming in the presence of IL-21 led to a stable, 'helper-independent' phenotype.

20 IL-21 was approved for Phase 1 clinical trials in metastatic melanoma (MM) and renal cell carcinoma (RCC) patients. It was shown to be safe for administration with flu-like symptoms as side effects. Dose-limiting toxicities included low lymphocyte, neutrophil, and thrombocyte count as well as hepatotoxicity. According to the Response Evaluation Criteria in Solid Tumors (RECIST) response scale, 2 out of 47 MM patients and 4 out of 19 RCC patients showed complete and partial responses, respectively. In addition, there was an increase of 25 perforin, granzyme B, IFN- γ , and CXCR3 mRNA in peripheral NK cells and CD8+ T cells. This suggested that IL-21 enhances the CD8+ effector functions thus leading to anti-tumor response. IL-21 proceeded to Phase 2 clinical trials where it was administered alone or coupled with drugs as sorafenib and rituximab.

30 IL-21 may also be a critical factor in the control of persistent viral infections. IL-21 (or IL-21R) knock-out mice infected with chronic LCMV (lymphocytic choriomeningitis virus) were not able to overcome chronic infection compared to normal mice. Besides, these mice with impaired IL-21 signaling had more dramatic exhaustion of LCMV-specific CD8+ T cells, suggesting that IL-21 produced by CD4+ T cells is required for sustained CD8+ T cell effector activity and then, for maintaining immunity to resolve persistent viral infection. Thus, IL-21 may 35 contribute to the mechanism by which CD4+ T helper cells orchestrate the immune system response to viral infections.

In HIV infected subjects, IL-21 has been reported to critically improve the HIV-specific cytotoxic T cell responses and NK cell functions. It has also been shown that HIV-specific CD4 T cells from "HIV controllers" (rare individuals who don't progress to AIDS by controlling the virus replication without treatment) are able to produce significantly more IL-21 than those of 5 progressors. In addition, IL-21 producing virus specific CD8 T cells were also preferentially found in HIV controllers. These data and the fact that IL-21 stimulated CD8 or NK cells are able to inhibit HIV viral replication *in vitro* show that this cytokine could potentially be useful for anti-HIV therapeutics.

Monoclonal antibodies directed against IL-21 have been developed, however these 10 antibodies have been shown to antagonize or inhibit the function of IL-21. These antagonistic antibodies are useful in suppressing inflammatory disorders. There have been no publications to date of the development of antibodies which agonize or enhance IL-21 activity.

SUMMARY

15 The present inventors have generated an antibody that binds to IL-21 and enhances the activity of IL-21. The present inventors have therefore developed, for the first time, an antibody which functions as an IL-21 agonist.

In one example, the present disclosure provides an IL-21-binding protein comprising an 20 antigen binding domain of an antibody, the antigen binding domain binds to or specifically binds to IL-21 and enhances IL-21 activity.

In one example the IL-21-binding protein enhances IL-21-mediated proliferation of B cells *in vitro* or *in vivo*

In another example, the IL-21-binding protein enhances IL-21-mediated cytotoxicity of CD8⁺ T cells *in vitro* or *in vivo*.

25 In another example, the IL-21 binding protein enhances IL-2-mediated proliferation of NK cells.

In one example, the IL-21 binding protein is an IL-21 agonist.

In another example, the IL-21-binding protein enhances IL-21 half-life *in vivo*. For 30 example, the IL-21-binding protein may enhance IL-21 half-life *in vivo* from hours to days. In one example, the IL-21 binding protein enhances IL-21 half-life *in vivo* for at least 24 hours.

In another example, activity of IL-21 is enhanced at least five-fold compared to IL-21 activity in the absence of the IL-21 binding protein.

The present disclosure additionally or alternatively provides an IL-21-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain 35 binds to or specifically binds to an epitope of IL-21 comprising the sequence STNAGRRQK (SEQ ID NO:23). In another example, the IL-21 binds to or specifically binds with the an epitope

comprising the sequence PPSTNAGRRQKHRLT as set forth in SEQ ID NO:19. In another example, the IL-21-binding protein binds to or specifically binds to an epitope of IL-21 comprising the sequence PPSTNAGRRQKHRLTCPSCDSYE as set forth in SEQ ID NO:20. In another example, the IL-21 binding protein binds to, or specifically binds to an epitope of IL-21 5 comprising the sequence IKKLKRKPPSTNAGRRQKHRLTCPSCDSYE set forth in SEQ ID NO:4.

The present disclosure additionally or alternatively provides an IL-21-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-21 and wherein the protein competitively inhibits binding of 10 antibody 2P2 (comprising a V_H comprising a sequence set forth in SEQ ID NO: 2 and a V_L comprising a sequence set forth in SEQ ID NO: 3) to IL-21.

In one example, the IL-21-binding protein competitively inhibits binding of antibody 2P2 to an epitope sequence set forth in SEQ ID NO: 19, 20 or 4.

In one example, the IL-21-binding protein binds to, or specifically binds to mammalian 15 IL-21 (hIL-21). In another example, the IL-21 binding protein binds to, or specifically binds to human IL-21. In a further example, the IL-21 binding protein binds to, or specifically binds to cat or dog IL-21.

In one example, the IL-21-binding protein does not detectably bind to mouse IL-21.

In one example, the IL-21-binding protein has an affinity constant (K_D) for mammalian 20 IL-21 or hIL-21 of about 9×10^{-9} M or less. For example, the K_D is about 8×10^{-9} M or less or about 7×10^{-9} M or less or about 6×10^{-9} M or less or about 5×10^{-9} M or less. In one example, the K_D is about 4.8×10^{-9} M or less.

In another example, the IL-21-binding protein has an affinity constant (K_D) for mammalian IL-21 or hIL-21 of about 2×10^{-9} M or less. For example, the K_D is about 1×10^{-9} M or 25 less or about 9×10^{-10} M or less or about 8×10^{-10} M or less or about 7×10^{-10} M or less. In one example, the K_D is about 5×10^{-10} M or less. In one example, the K_D is about 4×10^{-10} M or less. In one example, the K_D is about 3×10^{-10} M or less. In one example, the K_D is about 2×10^{-10} M or less. In one example, the K_D is about 1.5×10^{-10} M or less.

In one example, relating to each of the foregoing examples, the K_D can be 0.1×10^{-12} M 30 or more or 1×10^{-12} M or more.

In one example, the K_D is assessed using a biosensor, e.g., by surface plasmon resonance. For example, the IL-21-binding protein is immobilized and the level of binding to hIL-21 or mammalian IL-21 is determined.

The present disclosure additionally or alternatively provides an IL-21-binding protein 35 comprising an antigen binding domain of an antibody, wherein the antigen binding domain

binds to or specifically binds to IL-21, wherein the antigen binding domain comprises at least one of:

- (i) a V_H comprising a complementarity determining region (CDR) 1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at 5 least 99% identical to a sequence set forth in SEQ ID NO:5, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set in SEQ ID NO:6 and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 7;
- 10 (ii) a V_H comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO: 2;
- (iii) a V_L comprising a CDR1 comprising a sequence at least about 80 %, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set 15 forth in SEQ ID NO: 8, a CDR2 comprising a sequence at least about 80 %, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 9 and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 10;
- (iv) a V_L comprising a sequence at least about 95% identical to a sequence set forth in SEQ 20 ID NO: 3;
- (v) a V_H comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 5, a CDR2 comprising a sequence set forth between in SEQ ID NO: 6 and a CDR3 comprising a sequence set forth in SEQ ID NO: 7;
- (vi) a V_H comprising a sequence set forth in SEQ ID NO: 2;
- 25 (vii) a V_L comprising a CDR1 comprising a sequence set SEQ ID NO: 8, a CDR2 comprising a sequence set forth in SEQ ID NO: 9 and a CDR3 comprising a sequence set forth in SEQ ID NO: 10;
- (viii) a V_L comprising a sequence set forth in SEQ ID NO: 3;
- (ix) a V_H comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 5, a CDR2 30 comprising a sequence set forth between in SEQ ID NO: 6 and a CDR3 comprising a sequence set forth in SEQ ID NO: 7; and a V_L comprising a CDR1 comprising a sequence set SEQ ID NO: 8, a CDR2 comprising a sequence set forth in SEQ ID NO: 9 and a CDR3 comprising a sequence set forth in SEQ ID NO: 10; and
- (x) a V_H comprising a sequence set forth in SEQ ID NO: 2 and a V_L comprising a sequence 35 set forth in SEQ ID NO: 3.

In one example, differences between the recited sequence and the IL-21-binding protein are substitutions.

The skilled artisan will be capable of determining sites for substitutions to an IL-21-binding protein of the disclosure, e.g., within a framework region of a variable region containing 5 protein.

The present disclosure also provides an IL-21-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-21 and enhances IL-21 signaling and wherein the antigen binding domain comprises a V_H comprising a sequence set forth in SEQ ID NO: 2 and a V_L comprising a 10 sequence set forth in SEQ ID NO: 3.

In one example, an IL-21-binding protein described herein comprises at least a V_H and a V_L , wherein the V_H and V_L bind to form a Fv comprising an antigen binding domain. The skilled artisan will understand that the antigen binding domain comprises the binding site of the antibody.

15 In one example, the V_H and the V_L are in a single polypeptide chain. For example, the protein is:

- (i) a single chain Fv fragment (scFv);
- (ii) a dimeric scFv (di-scFv);
- (iii) one of (i) or (ii) linked to a constant region of an antibody, Fc or a heavy chain constant 20 domain (C_H) 2 and/or C_H 3; or
- (iv) one of (i) or (ii) linked to a protein that binds to an immune effector cell.

In one example, the V_L and V_H are in separate polypeptide chains.

For example, the protein is:

- (i) a diabody;
- (ii) a triabody;
- (iii) a tetrabody;
- (iv) a Fab;
- (v) a $F(ab');$
- (vi) a Fv;
- 30 (vii) one of (i) to (vi) linked to a constant region of an antibody, Fc or a heavy chain constant domain (C_H) 2 and/or C_H 3;
- (viii) one of (i) to (vi) linked to a protein that binds to an immune effector cell.

The foregoing proteins can also be referred to as antigen binding domains of antibodies.

35 In one example, the protein is an antibody, for example, a monoclonal antibody. In one example, the antibody is a naked antibody.

In one example, a protein (or antibody) is chimeric, de-immunized, humanized, human or primatized.

In one example, the protein or antibody is human.

5 In one example, the complementarity determining region sequences (CDRs) are defined according to the Kabat numbering system.

In another example, the CDRs are defined according to the IMGT numbering system.

Reference herein to a protein or antibody that "binds to" IL-21 provides literal support for a protein or antibody that "binds specifically to" or "specifically binds to" IL-21.

10 The present disclosure also provides antigen binding domains or antigen binding fragments of the foregoing antibodies.

15 In one example, a protein or antibody as described herein comprises a human constant region, e.g., an IgG constant region, such as an IgG1, IgG2, IgG3 or IgG4 constant region or mixtures thereof. In the case of an antibody or protein comprising a V_H and a V_L , the V_H can be linked to a heavy chain constant region and the V_L can be linked to a light chain constant region.

20 The C-terminal lysine of the heavy chain constant region of a whole antibody (or an IL-21-binding protein comprising a constant region or a C_{H3}) of the disclosure may be removed, for example, during production or purification of the antibody or protein, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, whole 25 antibodies (or IL-21-binding proteins) may comprise populations with all C-terminal lysine residues removed, populations with no C-terminal lysine residues removed, and/or populations having a mixture of protein with and without the C-terminal lysine residue. In some examples, the populations may additionally comprise protein in which the C-terminal lysine residue is removed in one of the heavy chain constant regions. Similarly, a composition of whole antibodies may comprise the same or a similar mix of antibody populations with or without the C-terminal lysine residue.

30 In one example, a protein or antibody as described herein comprises a constant region of an IgG4 antibody or a stabilized constant region of an IgG4 antibody. In one example, the protein or antibody comprises an IgG4 constant region with a proline at position 241 (according to the numbering system of Kabat (Kabat *et al.*, Sequences of Proteins of Immunological Interest Washington DC United States Department of Health and Human Services, 1987 and/or 1991)).

35 In one example a protein or antibody as described herein or a composition of a protein or antibody as described herein, comprises a heavy chain constant region, including a stabilized heavy chain constant region, comprising a mixture of sequences fully or partially with or without the C-terminal lysine residue.

In one example, an antibody of the disclosure comprises a V_H disclosed herein linked or fused to an IgG4 constant region or stabilized IgG4 constant region (e.g., as discussed above) and the V_L is linked to or fused to a kappa light chain constant region.

5 The functional characteristics of an IL-21-binding protein of the disclosure will be taken to apply *mutatis mutandis* to an antibody of the disclosure.

In one example, an IL-21-binding protein or antibody as described herein is isolated and/or recombinant.

10 In one example, an IL-21-binding protein or antibody of the disclosure is conjugated to another compound, for example, a detectable label or a compound that extends the half-life of the protein or antibody, such as polyethylene glycol or an albumin binding protein. Other suitable compounds are described herein.

The present disclosure also provides a nucleic acid encoding the IL-21-binding protein or antibody of the present disclosure or a polypeptide thereof.

15 The present disclosure additionally or alternatively provides an IL-21 binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-21, wherein the antigen binding domain is encoded by nucleic acid sequence comprising at least one of:

- (i) a V_H comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO: 17;
- 20 (ii) a V_L comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO: 18;
- (iii) a V_H comprising a sequence set forth in SEQ ID NO: 17;
- (iv) a V_L comprising a sequence set forth in SEQ ID NO: 18; and
- (v) a V_H comprising a sequence set forth in SEQ ID NO: 17 and a V_L comprising a sequence set forth in SEQ ID NO: 18.

25 In one example, such a nucleic acid is included in an expression construct in which the nucleic acid is operably linked to a promoter. Such an expression construct can be in a vector, e.g., a plasmid.

30 In examples of the disclosure directed to single polypeptide chain IL-21-binding proteins, the expression construct may comprise a promoter linked to a nucleic acid encoding that polypeptide chain.

35 In examples directed to multiple polypeptide chains that form an IL-21-binding protein, an expression construct comprises a nucleic acid encoding a polypeptide comprising, e.g., a V_H operably linked to a promoter and a nucleic acid encoding a polypeptide comprising, e.g., a V_L operably linked to a promoter.

In another example, the expression construct is a bicistronic expression construct, e.g., comprising the following operably linked components in 5' to 3' order:

- (i) a promoter
- (ii) a nucleic acid encoding a first polypeptide;
- 5 (iii) an internal ribosome entry site; and
- (iv) a nucleic acid encoding a second polypeptide,

wherein the first polypeptide comprises a V_H and the second polypeptide comprises a V_L , or *vice versa*.

The present disclosure also contemplates separate expression constructs one of which 10 encodes a first polypeptide comprising a V_H and another of which encodes a second polypeptide comprising a V_L . For example, the present disclosure also provides a composition comprising:

- (i) a first expression construct comprising a nucleic acid encoding a polypeptide comprising a V_H operably linked to a promoter; and
- 15 (ii) a second expression construct comprising a nucleic acid encoding a polypeptide comprising a V_L operably linked to a promoter.

The present disclosure also provides an isolated or recombinant cell expressing an IL-21-binding protein of the disclosure.

In one example, the cell comprises the expression construct of the disclosure or:

- 20 (i) a first expression construct comprising a nucleic acid encoding a polypeptide comprising a V_H operably linked to a promoter; and
- (ii) a second expression construct comprising a nucleic acid encoding a polypeptide comprising a V_L operably linked to a promoter,

wherein the first and second polypeptides associate to form an IL-21-binding protein of the 25 present disclosure.

Examples of cells of the present disclosure include bacterial cells, yeast cells, insect cells or mammalian cells.

The present disclosure additionally provides methods for producing an IL-21-binding protein or antibody of the disclosure. For example, such a method involves maintaining the 30 expression construct(s) of the disclosure under conditions sufficient for the IL-21-binding protein or antibody to be produced.

In one example, a method for producing an IL-21-binding protein or antibody of the disclosure comprises culturing the cell of the disclosure under conditions sufficient for the IL-21-binding protein or antibody to be produced and, optionally, secreted.

In one example, the method for producing an IL-21-binding protein or antibody of the disclosure additionally comprises isolating the protein or antibody and, optionally, formulating the IL-21-binding protein or antibody into a pharmaceutical composition.

5 The present disclosure additionally provides a composition comprising an IL-21-binding protein or antibody of the disclosure and a pharmaceutically acceptable carrier.

In some examples, the composition comprises:

- (i) an antibody of the disclosure comprising a C-terminal lysine residue from the heavy chain;
- (ii) an antibody of the disclosure lacking a C-terminal lysine residue from the heavy chain;
- 10 and/or
- (iii) an antibody of the disclosure comprising a C-terminal lysine residue on one heavy chain and lacking a C-terminal lysine residue on another (or the other) heavy chain, and, optionally, a pharmaceutically acceptable carrier.

15 The present disclosure also provides a complex comprising an IL-21 binding protein of the disclosure and IL-21. In one example, the IL-21 is recombinant IL-21. Examples of recombinant IL-21 are known in the art.

20 The present disclosure also provides a method for treating or preventing an infection or infectious disease, an allergic immune response or cancer in a subject, the method comprising administering an IL-21-binding protein or complex of the disclosure. In this regard, an IL-21-binding protein or complex can be used to prevent a relapse of a condition, and this is considered preventing the condition.

In one example, the infection is a chronic infection. In another example, the infection is a viral infection, such as HIV infection.

25 In one example, the infectious disease is hepatitis B or C.

Exemplary cancers include hematologic cancers, cancers of epithelial origin, liver cancer, pancreatic cancer, gastric cancer, osteosarcoma, endometrial cancer and ovarian cancer.

The present disclosure also provides a method for enhancing IL-21 activity in a subject, the method comprising administering the IL-21-binding protein, or complex of the disclosure.

30 In one example, a method described herein comprises administering between about 0.05mg/kg and 30mg/kg of the IL-21-binding protein or complex. For example, the method comprises administering between 0.1mg/kg and 10mg/kg or between 0.2mg/kg and 5mg/kg of the IL-21-binding protein or complex. In one example, the method comprises administering about 0.5-2.0mg/kg of the IL-21-binding protein or complex. The present disclosure also provides for use of an IL-21-binding protein or complex as described herein in any example in medicine.

The present disclosure also provides for use of an IL-21-binding protein or complex as described herein according to any example in the manufacture of a medicament to treat an infection, an allergic immune response or cancer.

The present disclosure also provides a method for localizing and/or detecting and/or 5 diagnosing and/or prognosing an IL-21-mediated condition associated with a cell expressing IL-21, the method comprising detecting *in vivo* an IL-21-binding protein or antibody as described herein bound to the IL-21 expressing cell, if present, wherein the IL-21-binding protein or antibody is conjugated to a detectable tag.

In one example, the method additionally comprises administering the IL-21-binding 10 protein to the subject.

The present disclosure also provides a method of culturing expanding a population of cells comprising immune system precursor cells, the method comprising culturing the population of cells *in vitro* or *ex vivo* in the presence of an IL-21 binding protein or complex of the present disclosure.

15 It will be appreciated that culturing the cells in the presence of a an IL-21 binding protein or complex of the present disclosure will enhance proliferation and differentiation of immune system cells such as NK cells, B cells and T cells.

In one embodiment, the method of culturing further comprises the addition of exogenous IL-21 to the cell population.

20 Cell populations comprising immune system precursor cells may be isolated from a biological sample taken from a mammalian subject. The sample may originate from a number of sources, including, but not limited to peripheral blood, leukapheresis blood product, apheresis blood product, bone marrow, thymus, tissue biopsy, tumor, lymph node tissue, gut associated lymphoid tissue, mucosa associated lymphoid tissue, liver, sites of immunologic 25 lesions (e.g., synovial fluid), pancreas, and cerebrospinal fluid. The donor subject is preferably human, and can be fetal, neonatal, child, adult, and may be normal, diseased, or susceptible to a disease of interest.

In some embodiments, the cell sample comprises peripheral blood mononuclear cells (PBMCs) from a blood sample. By "peripheral blood mononuclear cells" or "PBMCs" is meant 30 lymphocytes (including T-cells, B-cells, NK cells, etc.) and monocytes. In general, PBMCs are isolated from a patient using standard techniques. In some embodiments, only PBMCs are taken, either leaving or returning substantially all of the red blood cells and polymorphonuclear leukocytes to the donor. PBMCs may be isolated using methods known in the art, such as leukapheresis. In general, a 5 to 7 liter leukapheresis step is performed, which essentially 35 removes PBMCs from a patient, returning the remaining blood components. Collection of the sample is preferably performed in the presence of an anticoagulant (e.g., heparin).

It will be appreciated that *in vitro* and *ex vivo* cell culture, as described herein, can be used for the expansion of NK cells, B cells and T cells.

It will be appreciated that the present disclosure also provides a method for treating or preventing an infection or infectious disease, an allergic immune response or cancer in a 5 subject, by administering the cells which have been expanded *in vitro* or *ex vivo*, in accordance with the method described above.

The *in vitro* or *ex vivo* expanded cell population obtained by the method disclosed above, alone or in combination with an IL-21 binding protein or complex of the present disclosure, can be used to prevent a relapse of a condition, and this is considered preventing 10 the condition. Exemplary infections include HIV, hepatitis B or Hepatitis C. Exemplary cancers include hematologic cancers, cancers of epithelial origin, liver cancer, pancreatic cancer, gastric cancer, osteosarcoma, endometrial cancer and ovarian cancer.

The present disclosure also provides a method for detecting IL-21 or a cell expressing same in a sample, the method comprising contacting the sample with a protein or antibody as 15 described herein according to any example such that a complex forms and detecting the complex, wherein detection of the complex is indicative of IL-21 or a cell expressing same in the sample. In one example, the method is performed *ex vivo* or *in vitro*.

The present disclosure also provides a kit (e.g., a package or article of manufacture) comprising an IL-21-binding protein or complex as described herein according to any example, 20 optionally, packaged with instructions for use in a method as described herein.

KEY TO SEQUENCE LISTING

SEQ ID NO 1: amino acid sequence of Homo sapiens IL-21

SEQ ID NO 2: amino acid sequence of VH chain of antibody 2P2

25 SEQ ID NO 3: amino acid sequence of VL chain of antibody 2P2

SEQ ID NO 4: amino acid sequence of 2P2 binding epitope

SEQ ID NO 5: amino acid sequence of CDR1 of VH chain of antibody 2P2 using IMGT numbering scheme

30 SEQ ID NO 6: amino acid sequence of CDR2 of VH chain of antibody 2P2 using IMGT numbering scheme

SEQ ID NO 7: amino acid sequence of CDR3 of VH chain of antibody 2P2 using IMGT numbering scheme

SEQ ID NO 8: amino acid sequence of CDR1 of VL chain of antibody 2P2 using IMGT numbering scheme

35 SEQ ID NO 9: amino acid sequence of CDR2 of VL chain of antibody 2P2 using IMGT numbering scheme

SEQ ID NO 10: amino acid sequence of CDR3 of VL chain of antibody 2P2 using IMGT numbering scheme

SEQ ID NO 11: amino acid sequence of CDR1 of VH chain of antibody 2P2 using Kabat numbering scheme

5 SEQ ID NO 12: amino acid sequence of CDR2 of VH chain of antibody 2P2 using Kabat numbering scheme

SEQ ID NO 13: amino acid sequence of CDR3 of VH chain of antibody 2P2 using Kabat numbering scheme

SEQ ID NO 14: amino acid sequence of CDR1 of VL chain of antibody 2P2 using Kabat

10 numbering scheme

SEQ ID NO 15: amino acid sequence of CDR2 of VL chain of antibody 2P2 using Kabat numbering scheme

SEQ ID NO 16: amino acid sequence of CDR3 of VL chain of antibody 2P2 using Kabat numbering scheme

15 SEQ ID NO 17: nucleotide sequence encoding the VH chain of antibody 2P2

SEQ ID NO 18: nucleotide sequence encoding the VL chain of antibody 2P2

SEQ ID NO 19: amino acid sequence of 2P2 binding epitope

SEQ ID NO 20: amino acid sequence of 2P2 binding epitope

SEQ ID NO 21: amino acid sequence of 2P2 binding epitope

20 SEQ ID NO 22: amino acid sequence of 2P2 binding epitope

SEQ ID NO 23: core amino acid sequence of 2P2 binding epitope

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows that mAB 2P2 in the presence of 2 ng/ml of hIL-21 increased the proliferation of Ba/F3 cells expressing human IL-21 receptor (BaF3-hIL-21R) in a dose dependent manner (n = 3 ± SEM).

Figure 2 is a diagrammatic representation showing the CDRs of the V_H and V_L of antibody 2P2 determined by the IMGT numbering scheme.

Figure 3 shows that mAb 2P2 increased the proliferation of Ba/F3-hIL-21R cells but not Ba/F3-mIL-21R cells (A) and that mAb 2P2 increased hIL-21 bioactivity by more than 10 fold (B) (n = 3 ± SEM).

Figure 4 shows that mAb 2P2 increased hIL-21-mediated proliferation of human B cells; and that 5ng/ml of hIL-21 plus 1ug/ml of mAb 2P2 demonstrated a similar bioactivity to 100ng/ml of hIL-21, indicating about a 20-fold increase of hIL-21biotactivity by mAb 2P2.

Figure 5 shows that mAb 2P2 increased hIL-21-mediated activation of human CD8 T cells to express cytotoxic molecules including Granzyme B and Perforin, which are important for anti-virus and anti-tumour immunity.

5 **Figure 6** shows the results of half-life determination of IL-21/2P2 complexes or IL-21 alone, administered either intravenously (IV) or Subcutaneously (SC) (n = 3 ± SEM).

Figure 7 shows the results of 2P2 enhancement of IL-21 mediated B cell proliferation in the lymph node for follicular B cells (A), marginal zone B cells (B) and transition B cells (C) (n = 3 ± SEM; *P > 0.05, ** P > 0.01 ***P > 0.001).

10 **Figure 8** shows the results of 2P2 enhancement of IL-21 mediated B cell proliferation in the spleen for follicular B cells (A), marginal zone B cells (B) and transition B cells (C) (n = 3 ± SEM; *P > 0.05, ** P > 0.01 ***P > 0.001).

Figure 9 shows residues of human IL-21 (indicated in bold) which according to structural simulation are thought to be involved in binding by 2P2. Residues located in IL-21 Helix regions are underlined.

15 **Figure 10** shows binding between peptide fragments of human IL-21 and 2P2 and control antibody 3A3 determined by ELISA.

Figure 11 shows (A) binding between human IL-21 or mouse IL-21 with 2P2 (B) shows binding between human IL-21 and mouse IL-21 with control antibody 3A3.

20 DETAILED DESCRIPTION

General

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or groups of compositions of matter. Thus, as used herein, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. For example, reference to "a" includes a single as well as two or more; reference to "an" includes a single as well as two or more; reference to "the" includes a single as well as two or more and so forth.

30 Those skilled in the art will appreciate that the present disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present disclosure is not to be limited in scope by the specific examples described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the present disclosure.

Any example of the present disclosure herein shall be taken to apply *mutatis mutandis* 5 to any other example of the disclosure unless specifically stated otherwise.

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (for example, in cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

10 Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, *A Practical Guide to Molecular Cloning*, John Wiley and Sons (1984), J. Sambrook *et al.* *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbour 15 Laboratory Press (1989), T.A. Brown (editor), *Essential Molecular Biology: A Practical Approach*, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), *DNA Cloning: A Practical Approach*, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel *et al.* (editors), *Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) 20 *Antibodies: A Laboratory Manual*, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan *et al.* (editors) *Current Protocols in Immunology*, John Wiley & Sons (including all updates until present).

The description and definitions of variable regions and parts thereof, immunoglobulins, 25 antibodies and fragments thereof herein may be further clarified by the discussion in Kabat *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, Md., 1987 and 1991, Bork *et al.*, *J Mol. Biol.* 242, 309-320, 1994, Chothia and Lesk *J. Mol Biol.* 196:901 -917, 1987, Chothia *et al.* *Nature* 342, 877-883, 1989 and/or or Al-Lazikani *et al.*, *J Mol Biol* 273, 927-948, 1997.

The term "and/or", e.g., "X and/or Y" shall be understood to mean either "X and Y" or "X 30 or Y" and shall be taken to provide explicit support for both meanings or for either meaning.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

35 As used herein the term "derived from" shall be taken to indicate that a specified integer may be obtained from a particular source albeit not necessarily directly from that source.

Reference herein to a range of, e.g., residues, will be understood to be inclusive. For example, reference to "a region comprising amino acids 56 to 65" will be understood in an inclusive manner, i.e., the region comprises a sequence of amino acids as numbered 56, 57, 58, 59, 60, 61, 62, 63, 64 and 65 in a specified sequence.

5

Selected Definitions

For the purposes of nomenclature only and not limitation an exemplary sequence of a human IL-21 is set out in SEQ ID NO 1.

The phrase "enhances IL-21 activity" is understood to mean that the IL-21 binding protein of the present disclosure enhances any one or more activities of naturally occurring IL-21, including but not limited to stimulating proliferation, cytotoxicity or maturation of NK cells, stimulating proliferation or differentiation of B cells and T cells; stimulating antibody production and affinity maturation in B cells; stimulating cytotoxicity of CD8+ T cells; stimulating interferon gamma production in T-cells and NK-cells; inhibiting dendritic cell (DC) activation and maturation; inhibiting release of inflammatory mediators from mast cells, enhancing phagocytosis of macrophages, inhibiting generation or survival of T_{Reg} cells, and stimulating the proliferation of bone marrow progenitor cells.

The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide that by virtue of its origin or source of derivation is not associated with naturally-associated components that accompany it in its native state; is substantially free of other proteins from the same source. A protein may be rendered substantially free of naturally associated components or substantially purified by isolation, using protein purification techniques known in the art. By "substantially purified" is meant the protein is substantially free of contaminating agents, e.g., at least about 70% or 75% or 80% or 85% or 90% or 95% or 96% or 97% or 98% or 99% free of contaminating agents.

The term "recombinant" shall be understood to mean the product of artificial genetic recombination. Accordingly, in the context of a recombinant protein comprising an antibody antigen binding domain, this term does not encompass an antibody naturally-occurring within a subject's body that is the product of natural recombination that occurs during B cell maturation. However, if such an antibody is isolated, it is to be considered an isolated protein comprising an antibody antigen binding domain. Similarly, if nucleic acid encoding the protein is isolated and expressed using recombinant means, the resulting protein is a recombinant protein comprising an antibody antigen binding domain. A recombinant protein also encompasses a protein expressed by artificial recombinant means when it is within a cell, tissue or subject, e.g., in which it is expressed.

The term "protein" shall be taken to include a single polypeptide chain, i.e., a series of contiguous amino acids linked by peptide bonds or a series of polypeptide chains covalently or non-covalently linked to one another (i.e., a polypeptide complex). For example, the series of polypeptide chains can be covalently linked using a suitable chemical or a disulphide bond.

5 Examples of non-covalent bonds include hydrogen bonds, ionic bonds, Van der Waals forces, and hydrophobic interactions.

The term "polypeptide" or "polypeptide chain" will be understood from the foregoing paragraph to mean a series of contiguous amino acids linked by peptide bonds.

As used herein, the term "antigen binding domain" shall be taken to mean a region of 10 an antibody that is capable of specifically binding to an antigen, i.e., a V_H or a V_L or an Fv comprising both a V_H and a V_L . The antigen binding domain need not be in the context of an entire antibody, e.g., it can be in isolation (e.g., a domain antibody) or in another form, e.g., as described herein, such as a scFv.

For the purposes for the present disclosure, the term "antibody" includes a protein 15 capable of specifically binding to one or a few closely related antigens (e.g., IL-21) by virtue of an antigen binding domain contained within a Fv. This term includes four chain antibodies (e.g., two light chains and two heavy chains), recombinant or modified antibodies (e.g., chimeric antibodies, humanized antibodies, human antibodies, CDR-grafted antibodies, primatized antibodies, de-immunized antibodies, synhumanized antibodies, half-antibodies, bispecific 20 antibodies). An antibody generally comprises constant domains, which can be arranged into a constant region or constant fragment or fragment crystallizable (Fc). Exemplary forms of antibodies comprise a four-chain structure as their basic unit. Full-length antibodies comprise two heavy chains (~50 to 70 kD) covalently linked and two light chains (~23 kDa each). A light chain generally comprises a variable region (if present) and a constant domain and in 25 mammals is either a κ light chain or a λ light chain. A heavy chain generally comprises a variable region and one or two constant domain(s) linked by a hinge region to additional constant domain(s). Heavy chains of mammals are of one of the following types α , δ , ϵ , γ , or μ . Each light chain is also covalently linked to one of the heavy chains. For example, the two 30 heavy chains and the heavy and light chains are held together by inter-chain disulfide bonds and by non-covalent interactions. The number of inter-chain disulfide bonds can vary among different types of antibodies. Each chain has an N-terminal variable region (V_H or V_L wherein each are ~110 amino acids in length) and one or more constant domains at the C- terminus. The constant domain of the light chain (C_L which is ~110 amino acids in length) is aligned with 35 and disulfide bonded to the first constant domain of the heavy chain (C_H1 which is 330 to 440 amino acids in length). The light chain variable region is aligned with the variable region of the heavy chain. The antibody heavy chain can comprise 2 or more additional C_H domains (such

as, C_H2, C_H3 and the like) and can comprise a hinge region between the C_H1 and C_H2 constant domains. Antibodies can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂) or subclass. In one example, the antibody is a murine (mouse or rat) antibody or a primate (such as, human) antibody. In one example the antibody 5 heavy chain is missing a C-terminal lysine residue. In one example, the antibody is humanized, synhumanized, chimeric, CDR-grafted or deimmunized.

The terms "full-length antibody," "intact antibody" or "whole antibody" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antigen binding fragment of an antibody. Specifically, whole antibodies include those with heavy and 10 light chains including an Fc region. The constant domains may be wild-type sequence constant domains (e.g., human wild-type sequence constant domains) or amino acid sequence variants thereof.

As used herein, "variable region" refers to the portions of the light and/or heavy chains of an antibody as defined herein that is capable of specifically binding to an antigen and, 15 includes amino acid sequences of complementarity determining regions (CDRs); i.e., CDRI, CDR2, and CDR3, and framework regions (FRs). For example, the variable region comprises three or four FRs (e.g., FR1, FR2, FR3 and optionally FR4) together with three CDRs. V_H refers to the variable region of the heavy chain. V_L refers to the variable region of the light chain.

As used herein, the term "complementarity determining regions" (syn. CDRs; i.e., CDRI, 20 CDR2, and CDR3) refers to the amino acid residues of an antibody variable region the presence of which are major contributors to specific antigen binding. Each variable region domain (V_H or V_L) typically has three CDRs identified as CDRI, CDR2 and CDR3. In one example, the amino acid positions assigned to CDRs and FRs are defined according to Kabat *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, Md., 25 1987 and 1991 (also referred to herein as "the Kabat numbering system"). In another example, the amino acid positions assigned to CDRs and FRs are defined according to the Enhanced Chothia Numbering Scheme (<http://www.bioinfo.org.uk/mdex.html>). According to the numbering system of Kabat, V_H FRs and CDRs are positioned as follows: residues 1 to 30 (FR1), 31 to 35 (CDR1), 36 to 49 (FR2), 50 to 65 (CDR2), 66 to 94 (FR3), 95 to 102 (CDR3) and 30 103 to 113 (FR4). According to the numbering system of Kabat, V_L FRs and CDRs are positioned as follows: residues 1 to 23 (FR1), 24 to 34 (CDR1), 35 to 49 (FR2), 50 to 56 (CDR2), 57 to 88 (FR3), 89 to 97 (CDR3) and 98 to 107 (FR4). The present disclosure is not limited to FRs and CDRs as defined by the Kabat numbering system, but includes all 35 numbering systems, including the canonical numbering system or of Chothia and Lesk *J. Mol. Biol.* 196: 901-917, 1987; Chothia *et al.*, *Nature* 342: 877-883, 1989; and/or Al-Lazikani *et al.*, *J. Mol. Biol.* 273: 927-948, 1997; the numbering system of Honnegher and Plükthun *J. Mol. Biol.* 309: 657-670, 2001; or the IMGT system discussed in Giudicelli *et al.*, *Nucleic Acids Res.* 25:

206-211 1997. In one example, the CDRs are defined according to the Kabat numbering system. Optionally, heavy chain CDR2 according to the Kabat numbering system does not comprise the five C-terminal amino acids listed herein or any one or more of those amino acids are substituted with another naturally-occurring amino acid. In this regard, Padlan *et al.*,
5 *FASEB J.*, 9: 133-139, 1995 established that the five C-terminal amino acids of heavy chain CDR2 are not generally involved in antigen binding.

"Framework regions" (FRs) are those variable region residues other than the CDR residues.

As used herein, the term "Fv" shall be taken to mean any protein, whether comprised of
10 multiple polypeptides or a single polypeptide, in which a V_L and a V_H associate and form a complex having an antigen binding domain, i.e., capable of specifically binding to an antigen. The V_H and the V_L which form the antigen binding domain can be in a single polypeptide chain or in different polypeptide chains. Furthermore, an Fv of the disclosure (as well as any protein of the disclosure) may have multiple antigen binding domains which may or may not bind the
15 same antigen. This term shall be understood to encompass fragments directly derived from an antibody as well as proteins corresponding to such a fragment produced using recombinant means. In some examples, the V_H is not linked to a heavy chain constant domain (C_H) 1 and/or the V_L is not linked to a light chain constant domain (C_L). Exemplary Fv containing polypeptides or proteins include a Fab fragment, a Fab' fragment, a F(ab') fragment, a scFv, a diabody, a
20 triabody, a tetrabody or higher order complex, or any of the foregoing linked to a constant region or domain thereof, e.g., C_H2 or C_H3 domain, e.g., a minibody. A "Fab fragment" consists of a monovalent antigen-binding fragment of an immunoglobulin, and can be produced by digestion of a whole antibody with the enzyme papain, to yield a fragment consisting of an intact light chain and a portion of a heavy chain or can be produced using recombinant means.
25 A "Fab' fragment" of an antibody can be obtained by treating a whole antibody with pepsin, followed by reduction, to yield a molecule consisting of an intact light chain and a portion of a heavy chain comprising a V_H and a single constant domain. Two Fab' fragments are obtained per antibody treated in this manner. A Fab' fragment can also be produced by recombinant means. A "F(ab')2 fragment" of an antibody consists of a dimer of two Fab' fragments held
30 together by two disulfide bonds, and is obtained by treating a whole antibody molecule with the enzyme pepsin, without subsequent reduction. A "Fab₂" fragment is a recombinant fragment comprising two Fab fragments linked using, for example a leucine zipper or a C_H3 domain. A "single chain Fv" or "scFv" is a recombinant molecule containing the variable region fragment (Fv) of an antibody in which the variable region of the light chain and the variable region of the
35 heavy chain are covalently linked by a suitable, flexible polypeptide linker.

As used herein, the term "binds" in reference to the interaction of an IL-21-binding protein or an antigen binding domain thereof with an antigen means that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the antigen. For example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody binds to epitope "A", the presence of a molecule containing epitope "A" (or free, unlabeled "A"), in a reaction containing labeled "A" and the protein, will reduce the amount of labeled "A" bound to the antibody.

As used herein, the term "specifically binds" or "binds specifically" shall be taken to mean that an IL-21-binding protein of the disclosure reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular antigen or cell expressing same than it does with alternative antigens or cells. For example, an IL-21-binding protein binds to IL-21 (e.g., hIL-21) with materially greater affinity (e.g., 1.5 fold or 2 fold or 5 fold or 10 fold or 20 fold or 40 fold or 60 fold or 80 fold to 100 fold or 150 fold or 200 fold) than it does to other interleukins or to antigens commonly recognized by polyreactive natural antibodies (i.e., by naturally occurring antibodies known to bind a variety of antigens naturally found in humans). In an example of the present disclosure, an IL-21-binding protein that "specifically binds" to hIL-21 with an affinity at least 1.5 fold or 2 fold or greater (e.g., 5 fold or 10 fold or 20 fold or 50 fold or 100 fold or 200 fold) than it does to another interleukin. Generally, but not necessarily, reference to binding means specific binding, and each term shall be understood to provide explicit support for the other term.

As used herein, the term "does not detectably bind" shall be understood to mean that an IL-21-binding protein, e.g., an antibody, binds to a candidate antigen at a level less than 10%, or 8% or 6% or 5% above background. The background can be the level of binding signal detected in the absence of the protein and/or in the presence of a negative control protein (e.g., an isotype control antibody) and/or the level of binding detected in the presence of a negative control antigen. The level of binding is detected using biosensor analysis (e.g. Biacore) in which the IL-21-binding protein is immobilized and contacted with an antigen.

As used herein, the term "does not significantly bind" shall be understood to mean that the level of binding of an IL-21-binding protein of the disclosure to a polypeptide is not statistically significantly higher than background, e.g., the level of binding signal detected in the absence of the IL-21-binding protein and/or in the presence of a negative control protein (e.g., an isotype control antibody) and/or the level of binding detected in the presence of a negative control polypeptide. The level of binding is detected using biosensor analysis (e.g. Biacore) in which the IL-21binding protein is immobilized and contacted with an antigen.

For the purposes of clarification and as will be apparent to the skilled artisan based on the exemplified subject matter herein, reference to “affinity” in this specification is a reference to K_D of a protein or antibody.

For the purposes of clarification and as will be apparent to the skilled artisan based on 5 the description herein, reference to an “affinity of at least about” will be understood to mean that the affinity (or K_D) is equal to the recited value or higher (i.e., the value recited as the affinity is lower), i.e., an affinity of 2nM is greater than an affinity of 3nM. Stated another way, this term could be “an affinity of X or less”, wherein X is a value recited herein.

As used herein, the term “epitope” (*syn.* “antigenic determinant”) shall be understood to 10 mean a region of IL-21 to which an IL-21-binding protein comprising an antigen binding domain of an antibody binds. This term is not necessarily limited to the specific residues or structure to which the IL-21-binding protein makes contact. For example, this term includes the region spanning amino acids contacted by the IL-21-binding protein and 5-10 (or more) or 2-5 or 1-3 15 amino acids outside of this region. In some examples, the epitope comprises a series of discontinuous amino acids that are positioned close to one another when IL-21-binding protein is folded, i.e., a “conformational epitope”. The skilled artisan will also be aware that the term “epitope” is not limited to peptides or polypeptides. For example, the term “epitope” includes 20 chemically active surface groupings of molecules such as sugar side chains, phosphoryl side chains, or sulfonyl side chains, and, in certain examples, may have specific three dimensional structural characteristics, and/or specific charge characteristics.

The term “competitively inhibits” shall be understood to mean that an IL-21-binding protein of the disclosure (or an antigen binding domain thereof) reduces or prevents binding of a recited antibody or IL-21-binding protein to IL-21, e.g., to hIL-21. This may be due to the IL-21-binding protein (or antigen binding domain) and antibody binding to the same or an overlapping epitope. It will be apparent from the foregoing that the IL-21-binding protein need not completely inhibit binding of the antibody, rather it need only reduce binding by a statistically significant amount, for example, by at least about 10% or 20% or 30% or 40% or 25 50% or 60% or 70% or 80% or 90% or 95%. Preferably, the IL-21-binding protein reduces binding of the antibody by at least about 30%, more preferably by at least about 50%, more 30 preferably, by at least about 70%, still more preferably by at least about 75%, even more preferably, by at least about 80% or 85% and even more preferably, by at least about 90%. Methods for determining competitive inhibition of binding are known in the art and/or described herein. For example, the antibody is exposed to IL-21 either in the presence or absence of the IL-21-binding protein. If less antibody binds in the presence of the IL-21-binding protein than in 35 the absence of the IL-21-binding protein, the protein is considered to competitively inhibit

binding of the antibody. In one example, the competitive inhibition is not due to steric hindrance.

“Overlapping” in the context of two epitopes shall be taken to mean that two epitopes share a sufficient number of amino acid residues to permit an IL-21-binding protein (or antigen 5 binding domain thereof) that binds to one epitope to competitively inhibit the binding of an IL-21-binding protein (or antigen binding domain) that binds to the other epitope. For example, the “overlapping” epitopes share at least 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 20 amino acids.

As used herein, the term “agonize” shall be taken to mean that a protein is capable of 10 enhancing IL-21-mediated signaling in a cell. Methods for determining enhanced activity are known in the art and/or described herein.

As used herein, the term “condition” refers to a disruption of or interference with normal function, and is not to be limited to any specific condition, and will include diseases or disorders.

15 As used herein, the terms “preventing”, “prevent” or “prevention” include administering an IL-21-binding protein of the disclosure to thereby stop or hinder the development of at least one symptom of a condition. This term also encompasses treatment of a subject in remission to prevent or hinder relapse.

As used herein, the terms “treating”, “treat” or “treatment” include administering an IL-20 21-binding protein described herein to thereby reduce or eliminate at least one symptom of a specified disease or condition.

As used herein, the term “subject” shall be taken to mean any animal including humans, for example a mammal. Exemplary subjects include but are not limited to humans and non-human primates. For example, the subject is a human.

25

Antibodies

In one example, an IL-21-binding protein as described herein according to any example is an antibody.

Methods for generating antibodies are known in the art and/or described in Harlow and 30 Lane (editors) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, (1988). Generally, in such methods IL-21 (e.g., hIL-21) or a region thereof (e.g., an extracellular region) or immunogenic fragment or epitope thereof or a cell expressing and displaying same (i.e., an immunogen), optionally formulated with any suitable or desired carrier, adjuvant, or pharmaceutically acceptable excipient, is administered to a non-human animal, for example, a 35 mouse, chicken, rat, rabbit, guinea pig, dog, horse, cow, goat or pig. The immunogen may be

administered intranasally, intramuscularly, sub-cutaneously, intravenously, intradermally, intraperitoneally, or by other known route.

The production of polyclonal antibodies may be monitored by sampling blood of the immunized animal at various points following immunization. One or more further immunizations 5 may be given, if required to achieve a desired antibody titer. The process of boosting and titering is repeated until a suitable titer is achieved. When a desired level of immunogenicity is obtained, the immunized animal is bled and the serum isolated and stored, and/or the animal is used to generate monoclonal antibodies (mAbs).

Monoclonal antibodies are one exemplary form of antibody contemplated by the 10 present disclosure. The term "monoclonal antibody" or "mAb" refers to a homogeneous antibody population capable of binding to the same antigen(s), for example, to the same epitope within the antigen. This term is not intended to be limited with regard to the source of the antibody or the manner in which it is made.

For the production of mAbs any one of a number of known techniques may be used, 15 such as, for example, the procedure exemplified in US4196265 or Harlow and Lane (1988), *supra*.

For example, a suitable animal is immunized with an immunogen under conditions sufficient to stimulate antibody producing cells. Rodents such as rabbits, mice and rats are exemplary animals. Mice genetically-engineered to express human antibodies, for example, 20 which do not express murine antibodies, can also be used to generate an antibody of the present disclosure (e.g., as described in WO2002/066630).

Following immunization, somatic cells with the potential for producing antibodies, specifically B lymphocytes (B cells), are selected for use in the mAb generating protocol. These cells may be obtained from biopsies of spleens, tonsils or lymph nodes, or from a peripheral 25 blood sample. The B cells from the immunized animal are then fused with cells of an immortal myeloma cell, generally derived from the same species as the animal that was immunized with the immunogen.

Hybrids are amplified by culture in a selective medium comprising an agent that blocks the *de novo* synthesis of nucleotides in the tissue culture media. Exemplary agents are 30 aminopterin, methotrexate and azaserine.

The amplified hybridomas are subjected to a functional selection for antibody specificity and/or titer, such as, for example, by flow cytometry and/or immunohistochemistry and/or immunoassay (e.g. radioimmunoassay, enzyme immunoassay, cytotoxicity assay, plaque assay, dot immunoassay, and the like).

Alternatively, ABL-MYC technology (NeoClone, Madison WI 53713, USA) is used to produce cell lines secreting MAbs (e.g., as described in Largaespada *et al*, *J. Immunol. Methods*. 197: 85-95, 1996).

Antibodies can also be produced or isolated by screening a display library, e.g., a 5 phage display library, e.g., as described in US6300064 and/or US5885793. For example, the present inventors have isolated fully human antibodies from a phage display library.

The antibody of the present disclosure may be a synthetic antibody. For example, the antibody is a chimeric antibody, a humanized antibody, a human antibody synhumanized antibody, primatized antibody or a de-immunized antibody.

10

Deimmunized, Chimeric, CDR Grafted, Humanized, Synhumanized, Primatized, Human and Composite IL-21-Binding Proteins

The IL-21-binding proteins of the present disclosure may be CDR grafted proteins which include CDRs from an antibody from a non-human species (e.g., mouse or rat or non- 15 human primate) grafted onto or inserted into FRs from a human antibody or which include CDRs from an antibody from one type of antibody (e.g., one type of human antibody) grafted onto or inserted into FRs from another type of antibody (e.g., another type of human antibody). This term also encompasses a composite IL-21-binding protein comprising, for example, one or 20 more CDR grafted variable regions and one or more, e.g., human variable regions, chimeric variable regions, synhumanized variable regions or primatized variable regions.

The IL-21-binding proteins of the present disclosure may be a humanized protein.

The term "humanized protein" shall be understood to refer to a protein comprising a human-like variable region, which includes CDRs from an antibody from a non-human species (e.g., mouse or rat or non-human primate) grafted onto or inserted into FRs from a human 25 antibody (this type of antibody is falls within the class of "CDR-grafted antibody"). Humanized IL-21-binding proteins also include proteins in which one or more residues of the human protein are modified by one or more amino acid substitutions and/or one or more FR residues of the human protein are replaced by corresponding non-human residues. Humanized proteins may also comprise residues which are found in neither the human antibody or in the non-human 30 antibody. Any additional regions of the protein (e.g., Fc region) are generally human. Humanization can be performed using a method known in the art, e.g., US5225539, US6054297, US7566771 or US5585089. The term "humanized protein" also encompasses a super-humanized protein, e.g., as described in US7732578. This term also encompasses a composite protein comprising, for example, one or more humanized variable regions and one 35 or more, e.g., human variable regions, chimeric variable regions, synhumanized variable regions or primatized variable regions.

The IL-21-binding proteins of the present disclosure may be human IL-21-binding proteins. The term "human protein" as used herein refers to proteins having variable and, optionally, constant antibody regions found in humans, e.g. in the human germline or somatic cells or from libraries produced using such regions. The "human" proteins can include amino acid residues not encoded by human sequences, e.g. mutations introduced by random or site directed mutations *in vitro* (in particular mutations which involve conservative substitutions or mutations in a small number of residues of the protein, e.g. in 1, 2, 3, 4 or 5 of the residues of the protein). These "human proteins" do not necessarily need to be generated as a result of an immune response of a human, rather, they can be generated using recombinant means (e.g., screening a phage display library) and/or by a transgenic animal (e.g., a mouse) comprising nucleic acid encoding human antibody constant and/or variable regions and/or using guided selection (e.g., as described in US5565332). This term also encompasses affinity matured forms of such antibodies. For the purposes of the present disclosure, a human protein will also be considered to include a protein comprising FRs from a human antibody or FRs comprising sequences from a consensus sequence of human FRs and in which one or more of the CDRs are random or semi-random, e.g., as described in US6300064 and/or US6248516.

Optionally, the V_H is linked to a heavy chain constant region, e.g., an IgG4 heavy chain constant region. In one example, the heavy chain constant region lacks the C-terminal lysine residue.

20 Optionally, the V_L is linked to a light chain constant region.

The IL-21-binding proteins of the present disclosure may be synhumanized proteins. The term "synhumanized protein" refers to a protein prepared by a method described in WO2007/019620. A synhumanized IL-21-binding protein includes a variable region of an antibody, wherein the variable region comprises FRs from a New World primate antibody variable region and CDRs from a non-New World primate antibody variable region. For example, a synhumanized IL-21-binding protein includes a variable region of an antibody, wherein the variable region comprises FRs from a New World primate antibody variable region and CDRs from a mouse or rat antibody. In one example, the synhumanized IL-21-binding protein is a IL-21-binding antibody in which one or both of the variable regions are 25 synhumanized. This term also encompasses a composite protein comprising, for example, one or more synhumanized variable regions and one or more, e.g., human variable regions or humanized variable regions or chimeric variable regions.

The IL-21-binding proteins of the present disclosure may be primatized proteins. A "primatized protein" comprises variable region(s) from an antibody generated following 35 immunization of a non-human primate (e.g., a cynomolgus macaque). Optionally, the variable regions of the non-human primate antibody are linked to human constant regions to produce a primatized antibody. Exemplary methods for producing primatized antibodies are described in US6113898. This term also encompasses a composite protein comprising, for example, one or

more primatized variable regions and one or more, e.g., human variable regions or humanized variable regions or chimeric variable regions.

In one example an IL-21-binding protein of the disclosure is a chimeric protein. The term "chimeric proteins" refers to proteins in which an antigen binding domain is from a particular species (e.g., murine, such as mouse or rat) or belonging to a particular antibody class or subclass, while the remainder of the protein is from a protein derived from another species (such as, for example, human or non-human primate) or belonging to another antibody class or subclass. In one example, a chimeric protein is a chimeric antibody comprising a V_H and/or a V_L from a non-human antibody (e.g., a murine antibody) and the remaining regions of the antibody are from a human antibody. The production of such chimeric proteins is known in the art, and may be achieved by standard means (as described, e.g., in US6331415; US5807715; US4816567 and US4816397). This term also encompasses a composite protein comprising, for example, one or more chimeric variable regions and one or more, e.g., human variable regions or humanized variable regions or chimeric variable regions.

The present disclosure also contemplates a deimmunized IL-21-binding protein, e.g., as described in WO2000/34317 and WO2004/108158. De-immunized antibodies and proteins have one or more epitopes, e.g., B cell epitopes or T cell epitopes removed (i.e., mutated) to thereby reduce the likelihood that a subject will raise an immune response against the antibody or protein. For example, an IL-21-binding protein of the disclosure is analyzed to identify one or more B or T cell epitopes and one or more amino acid residues within the epitope is mutated to thereby reduce the immunogenicity of the IL-21-binding protein.

It will be apparent to the skilled artisan from the foregoing disclosure that a "composite" protein comprises one form of V_H (e.g., human) and another form of V_L (e.g., humanized). The present disclosure explicitly encompasses all combinations of forms of V_H and V_L .

25

Determination of Complementarity Determining Regions

The complementarity determining regions (CDRs) disclosed herein are based, in one example, on the numbering system of Lefranc and colleagues, referred to as the ImMunoGeneTics (IMGT) numbering system (Lefranc, Marie-Paule et al (1999) Nucl. Acids. Res. 27(1):209-212) which is a unified numbering system for immunoglobulin variable domain germ line sequences including antibody lambda and kappa light and heavy chain variable domains as well as T-cell receptor alpha, beta, gamma and delta chain variable domains.

Persons skilled in the art will appreciate that CDRs can also be determined using the numbering system of Kabat et al. (Kabat et al., Sequences of Proteins of Immunological Interest Washington DC United States Department of Health and Human Services, 1987 and/or 1991). According to this numbering system, the CDR regions were determined to be:

Variable Heavy Chain

CDR1 DYYIH
CDR2 WIDPESGDTEYAPKFQV
CDR3 GSGY

5

Variable Light Chain

CDR1 KSSQSLLSDSDGETYLN
CDR2 LVSKLDS
CDR3 WQGTHFPYT

10

Accordingly, the present disclosure also encompasses IL-21 binding proteins comprising an antigen binding domain of an antibody wherein the antigen-binding domain comprises heavy and light chain complementarity determining region sequences as described above according to the Kabat numbering system.

15

Antibody Binding Domain Containing Proteins*Single-Domain Antibodies*

In some examples, a protein of the disclosure is or comprises a single-domain antibody (which is used interchangeably with the term “domain antibody” or “dAb”). A single-domain antibody is a single polypeptide chain comprising all or a portion of the heavy chain variable region of an antibody. In certain examples, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., US6248516).

Diabodies, Triabodies, Tetrabodies

25 In some examples, a protein of the disclosure is or comprises a diabody, triabody, tetrabody or higher order protein complex such as those described in WO98/044001 and/or WO94/007921.

For example, a diabody is a protein comprising two associated polypeptide chains, each polypeptide chain comprising the structure V_L -X- V_H or V_H -X- V_L , wherein V_L is an antibody 30 light chain variable region, V_H is an antibody heavy chain variable region, X is a linker comprising insufficient residues to permit the V_H and V_L in a single polypeptide chain to associate (or form an Fv) or is absent, and wherein the V_H of one polypeptide chain binds to a V_L of the other polypeptide chain to form an antigen binding domain, i.e., to form a Fv molecule 35 capable of specifically binding to one or more antigens. The V_L and V_H can be the same in each polypeptide chain or the V_L and V_H can be different in each polypeptide chain so as to form a bispecific diabody (i.e., comprising two Fvs having different specificity).

Single Chain Fv (scFv)

The skilled artisan will be aware that scFvs comprise V_H and V_L regions in a single polypeptide chain and a polypeptide linker between the V_H and V_L which enables the scFv to 5 form the desired structure for antigen binding (i.e., for the V_H and V_L of the single polypeptide chain to associate with one another to form a Fv). For example, the linker comprises in excess of 12 amino acid residues with $(\text{Gly}_4\text{Ser})_3$ being one of the more favored linkers for a scFv.

The present disclosure also contemplates a disulfide stabilized Fv (or diFv or dsFv), in 10 which a single cysteine residue is introduced into a FR of V_H and a FR of V_L and the cysteine residues linked by a disulfide bond to yield a stable Fv.

Alternatively, or in addition, the present disclosure encompasses a dimeric scFv, i.e., a 15 protein comprising two scFv molecules linked by a non-covalent or covalent linkage, e.g., by a leucine zipper domain (e.g., derived from Fos or Jun). Alternatively, two scFvs are linked by a peptide linker of sufficient length to permit both scFvs to form and to bind to an antigen, e.g., as described in US20060263367.

Heavy Chain Antibodies

Heavy chain antibodies differ structurally from many other forms of antibodies, in so far 20 as they comprise a heavy chain, but do not comprise a light chain. Accordingly, these antibodies are also referred to as "heavy chain only antibodies". Heavy chain antibodies are found in, for example, camelids and cartilaginous fish (also called IgNAR).

The variable regions present in naturally occurring heavy chain antibodies are generally 25 referred to as " V_{HH} domains" in camelid antibodies and V-NAR in IgNAR, in order to distinguish them from the heavy chain variable regions that are present in conventional 4-chain antibodies (which are referred to as " V_H domains") and from the light chain variable regions that are present in conventional 4-chain antibodies (which are referred to as " V_L domains").

A general description of heavy chain antibodies from camelids and the variable regions thereof and methods for their production and/or isolation and/or use is found *inter alia* in the following references WO94/04678, WO97/49805 and WO 97/49805.

30 A general description of heavy chain antibodies from cartilaginous fish and the variable regions thereof and methods for their production and/or isolation and/or use is found *inter alia* in WO2005/118629.

Other Antibodies and Proteins Comprising Antigen Binding Domains Thereof

35 The present disclosure also contemplates other antibodies and proteins comprising antigen-binding domains thereof, such as:

(i) "key and hole" bispecific proteins as described in US5731168;

- (ii) heteroconjugate proteins, e.g., as described in US4676980;
- (iii) heteroconjugate proteins produced using a chemical cross-linker, e.g., as described in US4676980; and
- (iv) Fab₃ (e.g., as described in EP19930302894).

5

Mutations to Proteins

The present disclosure also provides an IL-21-binding protein or a nucleic acid encoding same having at least 80% identity to a sequence disclosed herein. In one example, a IL-21-binding protein or nucleic acid of the disclosure comprises sequence at least about 85% 10 or 90% or 95% or 97% or 98% or 99% identical to a sequence disclosed herein.

Alternatively, or additionally, the IL-21-binding protein comprises a CDR (e.g., three CDRs) at least about 80% or 85% or 90% or 95% or 97% or 98% or 99% identical to CDR(s) of a V_H or V_L as described herein according to any example.

In another example, a nucleic acid of the disclosure comprises a sequence at least about 15 80% or 85% or 90% or 95% or 97% or 98% or 99% identical to a sequence encoding an IL-21-binding protein having a function as described herein according to any example. The present disclosure also encompasses nucleic acids encoding an IL-21-binding protein of the disclosure, which differs from a sequence exemplified herein as a result of degeneracy of the genetic code.

The % identity of a nucleic acid or polypeptide is determined by GAP (Needleman and 20 Wunsch. *Mol. Biol.* 48, 443-453, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. The query sequence is at least 50 residues in length, and the GAP analysis aligns the two sequences over a region of at least 50 residues. For example, the query sequence is at least 100 residues in length and the GAP analysis aligns the two sequences over a region of at least 100 residues. For example, the two sequences are aligned 25 over their entire length.

The present disclosure also contemplates a nucleic acid that hybridizes under stringent hybridization conditions to a nucleic acid encoding an IL-21-binding protein described herein. A “moderate stringency” is defined herein as being a hybridization and/or washing carried out in 2 x SSC buffer, 0.1% (w/v) SDS at a temperature in the range 45°C to 65°C, or equivalent 30 conditions. A “high stringency” is defined herein as being a hybridization and/or wash carried out in 0.1 x SSC buffer, 0.1% (w/v) SDS, or lower salt concentration, and at a temperature of at least 65°C, or equivalent conditions. Reference herein to a particular level of stringency encompasses equivalent conditions using wash/hybridization solutions other than SSC known to those skilled in the art. For example, methods for calculating the temperature at which the 35 strands of a double stranded nucleic acid will dissociate (also known as melting temperature, or T_m) are known in the art. A temperature that is similar to (e.g., within 5°C or within 10°C) or equal to the T_m of a nucleic acid is considered to be high stringency. Medium stringency is to

be considered to be within 10°C to 20°C or 10°C to 15°C of the calculated Tm of the nucleic acid.

The present disclosure also contemplates mutant forms of an IL-21-binding protein of the disclosure comprising one or more conservative amino acid substitutions compared to a sequence set forth herein. In some examples, the IL-21-binding protein comprises 10 or fewer, e.g., 9 or 8 or 7 or 6 or 5 or 4 or 3 or 2 or 1 conservative amino acid substitutions. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain and/or hydrophobicity and/or hydrophilicity.

Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), β -branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Hydropathic indices are described, for example in Kyte and Doolittle *J. Mol. Biol.*, 157: 105-132, 1982 and hydrophylic indices are described in, e.g., US4554101.

The present disclosure also contemplates non-conservative amino acid changes. For example, of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or positively charged amino acids. In some examples, the IL-21-binding protein comprises 10 or fewer, e.g., 9 or 8 or 7 or 6 or 5 or 4 or 3 or 2 or 1 non-conservative amino acid substitutions.

In one example, the mutation(s) occur within a FR of an antigen binding domain of an IL-21-binding protein of the disclosure. In another example, the mutation(s) occur within a CDR of an IL-21-binding protein of the disclosure.

Exemplary methods for producing mutant forms of an IL-21-binding protein include:

- mutagenesis of DNA (Thie *et al.*, *Methods Mol. Biol.* 525: 309-322, 2009) or RNA (Kopsidas *et al.*, *Immunol. Lett.* 107:163-168, 2006; Kopsidas *et al.* *BMC Biotechnology*, 7: 18, 2007; and WO1999/058661);
- introducing a nucleic acid encoding the polypeptide into a mutator cell, e.g., XL-1Red, XL-mutS and XL-mutS-Kanr bacterial cells (Stratagene);
- DNA shuffling, e.g., as disclosed in Stemmer, *Nature* 370: 389-91, 1994; and
- site directed mutagenesis, e.g., as described in Dieffenbach (ed) and Dveksler (ed) (*In: PCR Primer: A Laboratory Manual*, Cold Spring Harbor Laboratories, NY, 1995).

Exemplary methods for determining biological activity of the mutant IL-21-binding proteins of the disclosure will be apparent to the skilled artisan and/or described herein, e.g., antigen binding. For example, methods for determining antigen binding, competitive inhibition of binding, affinity, association, dissociation and therapeutic efficacy are described herein.

Constant Regions

The present disclosure encompasses IL-21-binding proteins and/or antibodies described herein comprising a constant region of an antibody. This includes antigen binding fragments of an antibody fused to an Fc.

5 Sequences of constant regions useful for producing the proteins of the present disclosure may be obtained from a number of different sources. In some examples, the constant region or portion thereof of the protein is derived from a human antibody. The constant region or portion thereof may be derived from any antibody class, including IgM, IgG, IgD, IgA and IgE, and any antibody isotype, including IgG1, IgG2, IgG3 and IgG4. In one 10 example, the constant region is human isotype IgG4 or a stabilized IgG4 constant region.

In one example, the Fc region of the constant region has a reduced ability to induce effector function, e.g., compared to a native or wild-type human IgG1 or IgG3 Fc region. In one example, the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC) and/or antibody-dependent cell-mediated phagocytosis (ADCP) and/or complement-dependent 15 cytotoxicity (CDC). Methods for assessing the level of effector function of an Fc region containing protein are known in the art and/or described herein.

20 In one example, the Fc region is an IgG4 Fc region (i.e., from an IgG4 constant region), e.g., a human IgG4 Fc region. Sequences of suitable IgG4 Fc regions will be apparent to the skilled person and/or available in publically available databases (e.g., available from National Center for Biotechnology Information).

In one example, the constant region is a stabilized IgG4 constant region. The term 25 "stabilized IgG4 constant region" will be understood to mean an IgG4 constant region that has been modified to reduce Fab arm exchange or the propensity to undergo Fab arm exchange or formation of a half-antibody or a propensity to form a half antibody. "Fab arm exchange" refers to a type of protein modification for human IgG4, in which an IgG4 heavy chain and attached light chain (half-molecule) is swapped for a heavy-light chain pair from another IgG4 molecule. Thus, IgG4 molecules may acquire two distinct Fab arms recognizing two distinct antigens 30 (resulting in bispecific molecules). Fab arm exchange occurs naturally *in vivo* and can be induced *in vitro* by purified blood cells or reducing agents such as reduced glutathione. A "half antibody" forms when an IgG4 antibody dissociates to form two molecules each containing a single heavy chain and a single light chain.

In one example, a stabilized IgG4 constant region comprises a proline at position 241 of the hinge region according to the system of Kabat (Kabat *et al.*, Sequences of Proteins of Immunological Interest Washington DC United States Department of Health and Human 35 Services, 1987 and/or 1991). This position corresponds to position 228 of the hinge region according to the EU numbering system (Kabat *et al.*, Sequences of Proteins of Immunological

Interest Washington DC United States Department of Health and Human Services, 2001 and Edelman *et al.*, *Proc. Natl. Acad. USA*, 63, 78-85, 1969). In human IgG4, this residue is generally a serine. Following substitution of the serine for proline, the IgG4 hinge region comprises a sequence CPPC. In this regard, the skilled person will be aware that the “hinge region” is a proline-rich portion of an antibody heavy chain constant region that links the Fc and Fab regions that confers mobility on the two Fab arms of an antibody. The hinge region includes cysteine residues which are involved in inter-heavy chain disulfide bonds. It is generally defined as stretching from Glu226 to Pro243 of human IgG1 according to the numbering system of Kabat. Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain disulphide (S-S) bonds in the same positions (see for example WO2010/080538).

Additional examples of stabilized IgG4 antibodies are antibodies in which arginine at position 409 in a heavy chain constant region of human IgG4 (according to the EU numbering system) is substituted with lysine, threonine, methionine, or leucine (e.g., as described in WO2006/033386). The Fc region of the constant region may additionally or alternatively comprise a residue selected from the group consisting of: alanine, valine, glycine, isoleucine and leucine at the position corresponding to 405 (according to the EU numbering system). Optionally, the hinge region comprises a proline at position 241 (i.e., a CPPC sequence) (as described above).

In another example, the Fc region is a region modified to have reduced effector function, i.e., a “non-immunostimulatory Fc region”. For example, the Fc region is an IgG1 Fc region comprising a substitution at one or more positions selected from the group consisting of 268, 309, 330 and 331. In another example, the Fc region is an IgG1 Fc region comprising one or more of the following changes E233P, L234V, L235A and deletion of G236 and/or one or more of the following changes A327G, A330S and P331S (Armour *et al.*, *Eur J Immunol.* 29:2613-2624, 1999; Shields *et al.*, *J Biol Chem.* 276(9):6591-604, 2001). Additional examples of non-immunostimulatory Fc regions are described, for example, in Dall'Acqua *et al.*, *J Immunol.* 177 : 1129-1138 2006; and/or Hezareh *J Virol.* ;75: 12161-12168, 2001).

In another example, the Fc region is a chimeric Fc region, e.g., comprising at least one C_H2 domain from an IgG4 antibody and at least one C_H3 domain from an IgG1 antibody, wherein the Fc region comprises a substitution at one or more amino acid positions selected from the group consisting of 240, 262, 264, 266, 297, 299, 307, 309, 323, 399, 409 and 427 (EU numbering) (e.g., as described in WO2010/085682). Exemplary substitutions include 240F, 262L, 264T, 266F, 297Q, 299A, 299K, 307P, 309K, 309M, 309P, 323F, 399S, and 427F.

The present disclosure also contemplates additional modifications to an antibody or IL-21-binding protein comprising an Fc region or constant region.

For example, the antibody comprises one or more amino acid substitutions that increase the half-life of the protein. For example, the antibody comprises a Fc region 5 comprising one or more amino acid substitutions that increase the affinity of the Fc region for the neonatal Fc region (FcRn). For example, the Fc region has increased affinity for FcRn at lower pH, e.g., about pH 6.0, to facilitate Fc/FcRn binding in an endosome. In one example, the Fc region has increased affinity for FcRn at about pH 6 compared to its affinity at about pH 7.4, which facilitates the re-release of Fc into blood following cellular recycling. These amino acid 10 substitutions are useful for extending the half life of a protein, by reducing clearance from the blood.

Exemplary amino acid substitutions include T250Q and/or M428L or T252A, T254S and T266F or M252Y, S254T and T256E or H433K and N434F according to the EU numbering system. Additional or alternative amino acid substitutions are described, for example, in 15 US20070135620 or US7083784.

Protein Production

In one example, an IL-21-binding protein described herein according to any example is produced by culturing a hybridoma under conditions sufficient to produce the protein, e.g., as 20 described herein and/or as is known in the art.

Recombinant Expression

In another example, an IL-21-binding protein described herein according to any example is recombinant.

25 In the case of a recombinant protein, nucleic acid encoding same can be cloned into expression constructs or vectors, which are then transfected into host cells, such as *E. coli* cells, yeast cells, insect cells, or mammalian cells, such as simian COS cells, Chinese Hamster Ovary (CHO) cells, human embryonic kidney (HEK) cells, or myeloma cells that do not otherwise produce the protein. Exemplary cells used for expressing a protein are CHO cells, 30 myeloma cells or HEK cells. Molecular cloning techniques to achieve these ends are known in the art and described, for example in Ausubel *et al.*, (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) or Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989). A wide variety of cloning and *in vitro* amplification methods are 35 suitable for the construction of recombinant nucleic acids. Methods of producing recombinant antibodies are also known in the art, see, e.g., US4816567 or US5530101.

Following isolation, the nucleic acid is inserted operably linked to a promoter in an expression construct or expression vector for further cloning (amplification of the DNA) or for expression in a cell-free system or in cells.

As used herein, the term "promoter" is to be taken in its broadest context and includes

5 the transcriptional regulatory sequences of a genomic gene, including the TATA box or initiator element, which is required for accurate transcription initiation, with or without additional regulatory elements (e.g., upstream activating sequences, transcription factor binding sites, enhancers and silencers) that alter expression of a nucleic acid, e.g., in response to a developmental and/or external stimulus, or in a tissue specific manner. In the present context,

10 the term "promoter" is also used to describe a recombinant, synthetic or fusion nucleic acid, or derivative which confers, activates or enhances the expression of a nucleic acid to which it is operably linked. Exemplary promoters can contain additional copies of one or more specific regulatory elements to further enhance expression and/or alter the spatial expression and/or temporal expression of said nucleic acid.

15 As used herein, the term "operably linked to" means positioning a promoter relative to a nucleic acid such that expression of the nucleic acid is controlled by the promoter.

Many vectors for expression in cells are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, a sequence encoding a protein (e.g., derived from the information provided herein), an enhancer element, a

20 promoter, and a transcription termination sequence. The skilled artisan will be aware of suitable sequences for expression of a protein. Exemplary signal sequences include prokaryotic secretion signals (e.g., pelB, alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II), yeast secretion signals (e.g., invertase leader, α factor leader, or acid phosphatase leader) or mammalian secretion signals (e.g., herpes simplex gD signal).

25 Exemplary promoters active in mammalian cells include cytomegalovirus immediate early promoter (CMV-IE), human elongation factor 1- α promoter (EF1), small nuclear RNA promoters (U1a and U1b), α -myosin heavy chain promoter, Simian virus 40 promoter (SV40), Rous sarcoma virus promoter (RSV), Adenovirus major late promoter, β -actin promoter; hybrid regulatory element comprising a CMV enhancer/ β -actin promoter or an immunoglobulin promoter or active fragment thereof. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture; baby hamster kidney cells (BHK, ATCC CCL 10); or Chinese hamster ovary cells (CHO).

30 Typical promoters suitable for expression in yeast cells such as for example a yeast cell selected from the group comprising *Pichia pastoris*, *Saccharomyces cerevisiae* and *S. pombe*, include, but are not limited to, the *ADH1* promoter, the *GAL1* promoter, the *GAL4* promoter, the

CUP1 promoter, the *PHO5* promoter, the *nmt* promoter, the *RPR1* promoter, or the *TEF1* promoter.

Means for introducing the isolated nucleic acid or expression construct comprising same into a cell for expression are known to those skilled in the art. The technique used for a 5 given cell depends on the known successful techniques. Means for introducing recombinant DNA into cells include microinjection, transfection mediated by DEAE-dextran, transfection mediated by liposomes such as by using lipofectamine (Gibco, MD, USA) and/or cellfectin (Gibco, MD, USA), PEG-mediated DNA uptake, electroporation and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agracetus Inc., WI, 10 USA) amongst others.

The host cells used to produce the protein may be cultured in a variety of media, depending on the cell type used. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing mammalian cells. Media for 15 culturing other cell types discussed herein are known in the art.

Isolation of Proteins

Methods for isolating a protein are known in the art and/or described herein.

Where an IL-21-binding protein is secreted into culture medium, supernatants from 20 such expression systems can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants. Alternatively, or additionally, supernatants can be filtered and/or separated from cells expressing the protein, 25 e.g., using continuous centrifugation.

The IL-21-binding protein prepared from the cells can be purified using, for example, ion exchange, hydroxyapatite chromatography, hydrophobic interaction chromatography, gel electrophoresis, dialysis, affinity chromatography (e.g., protein A affinity chromatography or protein G chromatography), or any combination of the foregoing. These methods are known in 30 the art and described, for example in WO99/57134 or Ed Harlow and David Lane (editors) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, (1988).

The skilled artisan will also be aware that a protein can be modified to include a tag to facilitate purification or detection, e.g., a poly-histidine tag, e.g., a hexa-histidine tag, or a influenza virus hemagglutinin (HA) tag, or a Simian Virus 5 (V5) tag, or a FLAG tag, or a 35 glutathione S-transferase (GST) tag. The resulting protein is then purified using methods known in the art, such as, affinity purification. For example, a protein comprising a hexa-his tag

is purified by contacting a sample comprising the protein with nickel-nitrilotriacetic acid (Ni-NTA) that specifically binds a hexa-his tag immobilized on a solid or semi-solid support, washing the sample to remove unbound protein, and subsequently eluting the bound protein. Alternatively, or in addition a ligand or antibody that binds to a tag is used in an affinity 5 purification method.

Conjugates

In one example, an IL-21-binding protein of the present disclosure is conjugated to a compound. For example, the compound is selected from the group consisting of a radioisotope, 10 a detectable label, a therapeutic compound, a colloid, a toxin, a nucleic acid, a peptide, a protein, a compound that increases the half life of the IL-21-binding protein in a subject and mixtures thereof.

The other compound can be directly or indirectly bound to the IL-21-binding protein (e.g., can comprise a linker in the case of indirect binding). Examples of compounds include, a 15 radioisotope (e.g., iodine-131, yttrium-90 or indium-111), a detectable label (e.g., a fluorophore or a fluorescent nanocrystal or quantum dot), a therapeutic compound (e.g., a chemotherapeutic or an anti-inflammatory), a colloid (e.g., gold), a toxin (e.g., ricin or tetanus toxoid), a nucleic acid, a peptide (e.g., a serum albumin binding peptide), a protein (e.g., a protein comprising an antigen binding domain of an antibody or serum albumin), a compound 20 that increases the half life of the IL-21-binding protein in a subject (e.g., polyethylene glycol or other water soluble polymer having this activity) and mixtures thereof. Exemplary compounds that can be conjugated to a IL-21-binding protein of the disclosure and methods for such conjugation are known in the art and described, for example, in WO2010/059821.

The IL-21-binding protein may be conjugated to nanoparticles (for example as reviewed 25 in Kogan *et al.*, *Nanomedicine (Lond)*. 2: 287-306, 2007). The nanoparticles may be metallic nanoparticles.

The IL-21-binding protein may be comprised in antibody-targeted bacterial minicells (for example as described in PCT/IB2005/000204).

Some exemplary compounds that can be conjugated to a IL-21-binding protein of the 30 present disclosure are listed in Table 1.

Table 1. Compounds useful in conjugation.

Group	Detail
Radioisotopes (either directly or indirectly)	• ^{123}I , ^{125}I , ^{130}I , ^{133}I , ^{135}I , ^{47}Sc , ^{72}As , ^{72}Sc , ^{90}Y , ^{88}Y , ^{97}Ru , ^{100}Pd , $^{101\text{m}}\text{Rh}$, $^{101\text{m}}\text{Rh}$, ^{119}Sb , ^{128}Ba , ^{197}Hg , ^{211}At , ^{212}Bi , ^{153}Sm , ^{169}Eu , ^{212}Pb , ^{109}Pd , ^{111}In , ^{67}Cu , ^{68}Cu , ^{67}Cu , ^{75}Br , ^{76}Br , ^{77}Br ,

Group	Detail
	^{99m}Tc , ^{11}C , ^{13}N , ^{15}O , ^{18}I , ^{188}Rc , ^{203}Pb , ^{64}Cu , ^{105}Rh , ^{198}Au , ^{199}Ag or ^{177}Lu
Half life extenders	<ul style="list-style-type: none"> • Polyethylene glycol • Glycerol • Glucose
Fluorescent probes	<ul style="list-style-type: none"> • Phycoerythrin (PE) • Allophycocyanin (APC) • Alexa Fluor 488 • Cy5.5
Biologics	<ul style="list-style-type: none"> • fluorescent proteins such as Renilla luciferase, GFP • immune modulators or proteins, such as cytokines, e.g., an interferon • toxins • an immunoglobulin or antibody or antibody variable region • half life extenders such as albumin or antibody variable regions or peptides that bind to albumin
Chemotherapeutics	<ul style="list-style-type: none"> • Taxol • 5-FU • Doxorubicin • Idarubicin

Assaying Activity of an IL-21-Binding Protein

Binding to IL-21 and Mutants Thereof

It will be apparent to the skilled artisan from the disclosure herein that some IL-21-binding proteins of the present disclosure bind to the hIL-21 and to specific mutant forms of hIL-21. Methods for assessing binding to a protein are known in the art, e.g., as described in Scopes (*In: Protein purification: principles and practice*, Third Edition, Springer Verlag, 1994). Such a method generally involves immobilizing the IL-21-binding protein and contacting it with labeled antigen. Following washing to remove non-specific bound protein, the amount of label and, as a consequence, bound antigen is detected. Of course, the IL-21-binding protein can be labeled and the antigen immobilized. Panning-type assays can also be used. Alternatively, or additionally, surface plasmon resonance assays can be used.

Optionally, the dissociation constant (K_d), association constant (K_a) and/or affinity constant (K_D) of an immobilized IL-21-binding protein for IL-21 or an epitope thereof is

determined. The "Kd" or "Ka" or " K_D " for an IL-21-binding protein is in one example measured by a radiolabeled or fluorescently-labeled IL-21 binding assay. In the case of a "Kd", this assay equilibrates the IL-21-binding protein with a minimal concentration of labeled IL-21 in the presence of a titration series of unlabeled IL-21. Following washing to remove unbound IL-21, 5 the amount of label is determined, which is indicative of the Kd of the protein.

According to another example the Kd, Ka or K_D is measured by using surface plasmon resonance assays, e.g., using BIACore surface plasmon resonance (BIACore, Inc., Piscataway, NJ) with immobilized IL-21 or a region thereof or immobilized IL-21-binding protein.

In some examples, the IL-21-binding protein has a similar K_D or an improved K_D (i.e., a 10 K_D value lower than) than antibody 8E2, because they are likely to compete for binding to IL-21.

Determining Competitive Binding

Assays for determining a protein that competitively inhibits binding of antibody 2P2 will be apparent to the skilled artisan. One such method is exemplified herein.

15 For example, the antibody is conjugated to a detectable label, e.g., a fluorescent label or a radioactive label. The labeled antibody and the test IL-21-binding protein are then mixed and contacted with IL-21 or a region thereof or a cell expressing same. The level of labeled antibody is then determined and compared to the level determined when the labeled antibody is contacted with the IL-21, region or cells in the absence of the IL-21-binding protein. If the 20 level of labeled antibody is reduced in the presence of the test IL-21-binding protein compared to the absence of the IL-21-binding protein, the IL-21-binding protein is considered to competitively inhibit binding of the antibody to IL-21.

25 Optionally, the test IL-21-binding protein is conjugated to different label to the antibody. This alternate labeling permits detection of the level of binding of the test IL-21-binding protein to IL-21 or the region thereof or the cell.

Determining Agonist activity

In some examples of the present disclosure, a protein is capable of enhancing IL-21 activity.

30 Various assays are known in the art for assessing the ability of a protein to enhance signaling of a ligand through a receptor.

35 In one example, the IL-21-binding protein enhances proliferation of cells (e.g., BaF3 cells) expressing IL-21R and gp130 (e.g., cells modified to express the both proteins) which are cultured in the presence of IL-21. Cells (e.g., about 1×10^4 cell) are cultured in the presence of IL-21 (e.g., between about 0.5ng/mL to about 5ng/mL (such as 0.5ng/mL or 5ng/mL) for hIL-21 and the presence or absence of a test IL-21-binding protein. Methods for assessing cell

proliferation are known in the art and include, for example, MTT reduction and/or thymidine incorporation. An IL-21-binding protein that enhances the level of proliferation compared to the level observed in the absence of the IL-21-binding protein is considered to enhance or agonize IL-21 signaling.

5 A similar assay to that described in the foregoing paragraph can be performed with B9 cells or T10 cells (Dams-Kozlowska *et al.*, *BMC Biotechnol*, 12: 8, 2012; and Yokote *et al.*, *J AOAC*, 83: 1053-1057, 2000). In the case of an assay making use of T10 cells, proliferation can be measured by colorimetrically detecting reduction of the tetrazolium compound, 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1).

10 Other methods for assessing enhancement of IL-21 signaling are contemplated by the present disclosure.

Determining Half Life of IL-21

15 The half-life of IL-21 can be measured, for example, by pharmacokinetic studies, e.g., according to the method described by Kim *et al*, *Eur J of Immunol* 24:542, 1994. According to this method radiolabeled IL-21 is injected intravenously into mice and its plasma concentration is periodically measured as a function of time, for example at 3 minutes to 72 hours after the injection. The clearance curve thus obtained should be biphasic, that is, an alpha phase and beta phase. For the determination of the *in vivo* half-life of the protein, the clearance rate in 20 beta-phase is calculated and compared with that of the wild type or unmodified protein.

Assessing Therapeutic Efficacy

Assays for assessing therapeutic efficacy are described hereinabove in relation to determining neutralization by an IL-21-binding protein.

25 In another example, the efficacy of a protein to treat a condition is assessed using an *in vivo* assay.

For example, the IL-21-binding protein can be tested in a model of cancer, e.g., gastric cancer. For example, mice homologous for the Y757F mutant of gp130 (gp130^{Y757F/Y757F}) develop gastric tumors Jenkins *et al*, *Blood* 109: 2380-2388, 2007). Mice (e.g., eight week old 30 mice) are treated with an IL-21-binding protein and the number and/or weight of gastric polyps assessed. An IL-21-binding protein that reduces polyp size and/or weight is considered useful for treating cancer. A similar assay can be used to test for an effect on colon cancer, in which gp130^{Y757F/Y757F} mice are treated with azoxymethane (AOM) followed by dextran sodium sulfate (DSS) essentially as described in Greten (*et al*, *Cell*, 118: 285-296, 2004) to induce colon 35 cancer prior to treatment with the IL-21-binding protein.

The IL-21-binding protein can additionally or alternatively be tested in a model of cancer metastasis or cancer-related bone disease, e.g., as described in Li *et al.*, *Oncol. Lett.* 3: 802-806, 2012.

5 Conditions to be Treated

IL-21 is a CD4⁺ T cell-derived cytokine that is important for optimal CD8⁺ T cell mediated immunity, NK activation and optimal humoral responses such as antibody production and B cell maturation. IL-21 has been shown to induce a number of proinflammatory cytokines such as IL18, IL-15, IL-5, IL-6, IL-7A, IL-17F, TNFRII, sCD25 and RANTES.

10 The IL-21 binding proteins of the present disclosure are useful in the treatment of cancers. Exemplary cancers include cystic and solid tumors, bone and soft tissue tumors, including tumors in anal tissue, bile duct, bladder, blood cells, cone, bowel, brain, breast, carcinoid, cervix, eye, esophagus, head and neck, kidney, larynx, leukemia, liver, lung, lymph nodes, lymphoma, melanoma, mesothelioma, myeloma, ovary, pancreas, penis, prostate, skin, 15 sarcomas, stomach, testes, thyroid, vagina, vulva. Soft tissue tumors include Benign schwannoma Monosomy, Desmoid tumor, lipo-blastoma, lipoma, uterine leiomyoma, clear cell sarcoma, dermatofibrosarcoma, Ewing sarcoma, extraskeletal myxoid chondrosarcoma, liposarcoma myxoid, Alveolar rhabdomyosarcoma and synovial sarcoma. Specific bone tumors include nonossifying fibroma, unicameral bone cyst, enchondroma, aneurismal bone 20 cyst, osteoblastoma, chondroblastoma, chondromyxofibroma, ossifying fibroma and adamantinoma, Giant cell tumor, fibrous dysplasia, Ewing's sarcoma eosinophilic granuloma, osteosarcoma, chondroma, chondrosarcoma, malignant fibrous histiocytoma and metastatic carcinoma. Leukemias include acute lymphoblastic, acute myeloblastic, chronic lymphocytic and chronic myeloid.

25 The IL-21 binding proteins of the present disclosure are useful in the treatment and prevention of human viral infections. Examples of viral infections include infections caused by DNA viruses (e.g., Herpes Viruses such as Herpes Simplex viruses, Epstein-Barn virus, Cytomegalovirus; Pox viruses such as Variola (small pox) virus; Hepadnaviruses (e.g., Hepatitis B virus); Papilloma viruses; Adenoviruses); RNA Viruses (e.g., HIV I, II; HTLV I, II; 30 Poliovirus; Hepatitis A; coronaviruses, such as sudden acute respiratory syndrome (SARS); Orthomyxoviruses (e.g., Influenza viruses); Paramyxoviruses (e.g., Measles virus); Rabies virus; Hepatitis C virus), Flaviviruses, Influenza viruses; caliciviruses; rabies viruses, rinderpest viruses, Arena virus, and the like. Moreover, examples of the types of virus-related diseases for which IL-21 could be used include, but are not limited to: Acquired immunodeficiency; Hepatitis; 35 Gastroenteritis; Hemorrhagic diseases; Enteritis; Carditis; Encephalitis; Paralysis; Bronchiolitis;

Upper and lower respiratory disease; Respiratory Papillomatosis; Arthritis; Disseminated disease, Meningitis. Mononucleosis.

The IL-21 binding proteins of the present disclosure are also useful in the treatment of microbial infections including *chlamydiae*, *listeriae*, *helicobacter pylori*, *mycobacterium*, *mycoplasma*, *bacillus anthracis*, *salmonella*, and *shigella*.

The IL-21 binding proteins of the present disclosure are also useful as a monotherapy for acute and chronic viral infections and for immunocompromised patients. Methods that enhance immunity can accelerate the recovery time in patients with unresolved infections. Immunotherapies can have an even greater impact on subsets of immunocompromised patients such as the very young or elderly as well as patients that suffer immunodeficiencies acquired through infection, or induced following medical interventions such as chemotherapy or bone marrow ablation. Examples of the types of indications being treated via immune-modulation include; the use of IFN- α for chronic hepatitis, the use of IL-2 following HIV infection, and the use of either interferon for treating Epstein Barr Virus infections following transplantation.

The IL-21 binding proteins of the present disclosure are also useful as an allergy immunotherapy for the treatment of for example, allergic rhinitis, hay fever and bronchial asthma.

20 Compositions

In some examples, an IL-21-binding protein as described herein can be administered orally, parenterally, by inhalation spray, adsorption, absorption, topically, rectally, nasally, buccally, vaginally, intraventricularly, via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, or by any other convenient dosage form. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal, and intracranial injection or infusion techniques.

Methods for preparing an IL-21-binding protein into a suitable form for administration to a subject (e.g. a pharmaceutical composition) are known in the art and include, for example, methods as described in Remington's Pharmaceutical Sciences (18th ed., Mack Publishing Co., Easton, Pa., 1990) and U.S. Pharmacopeia: National Formulary (Mack Publishing Company, Easton, Pa., 1984).

The pharmaceutical compositions of this disclosure are particularly useful for parenteral administration, such as intravenous administration or administration into a body cavity or lumen of an organ or joint. The compositions for administration will commonly comprise a solution of an IL-21-binding protein dissolved in a pharmaceutically acceptable carrier, for example an

aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of an IL-21-binding protein of the present disclosure in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs. Exemplary carriers include water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as mixed oils and ethyl oleate may also be used. Liposomes may also be used as carriers. The vehicles may contain minor amounts of additives that enhance isotonicity and chemical stability, e.g., buffers and preservatives.

Upon formulation, an IL-21-binding protein of the present disclosure will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically/prophylactically effective. Formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but other pharmaceutically acceptable forms are also contemplated, e.g., tablets, pills, capsules or other solids for oral administration, suppositories, pessaries, nasal solutions or sprays, aerosols, inhalants, liposomal forms and the like. Pharmaceutical "slow release" capsules or compositions may also be used. Slow release formulations are generally designed to give a constant drug level over an extended period and may be used to deliver an IL-21-binding protein of the present disclosure.

WO2002/080967 describes compositions and methods for administering aerosolized compositions comprising antibodies for the treatment of, e.g., asthma, which are also suitable for administration of an IL-21-binding protein of the present disclosure.

Combination Therapies

In one example, an IL-21-binding protein of the present disclosure is administered in combination with one or more other compound useful for treating a condition described herein, either as combined or additional treatment steps or as additional components of a therapeutic formulation.

In another example, the other compound is a chemotherapy drug or other drug used for treating cancer.

In another example, the protein described herein is administered before or after radiotherapy for the treatment of cancer.

In another example, the IL-21 binding protein of the present invention is administered in combination with an immunotherapy.

Suitable immunotherapies include but are not limited to: checkpoint inhibitors; tyrosine kinase inhibitors, tumor necrosis factor receptor inhibitors, cytokines or interleukins; interferons; 5 granulocyte macrophage colony stimulating factor; vaccines (cancer vaccine or infectious disease); antiviral drugs; oncolytic viruses; polysaccharides such as beta glucan (Lentinan); and adoptive cell therapies (ACT) such as ex vivo cultured T cells to a cytotoxic T lymphocyte fate in a T helper cell independent manner or T cells genetically modified to express chimeric antigen receptors (CARs).

10 Suitable immunotherapies may be targeted to, but not limited to targeting the following: Programmed death 1 (PD1), Programmed death ligand 1 (PDL1) or Programmed death ligand 2 (PDL2), Cluster of Differentiation 80 (CD80), Cluster of Differentiation 80 (CD86), B7-related protein 1 (B7RP1), Cluster of Differentiation 276 (CD276), Cluster of Differentiation 274 (CD274), Herpesvirus entry mediator (HVEM), Cluster of Differentiation Ligand (CD137L), 15 Tumor Necrosis Factor Receptor Superfamily Member 4 Ligand (OX40L), Cluster of Differentiation (CD70), Cluster of Differentiation (CD40), Galectin 9 (GAL9), Cluster of Differentiation 28 (CD28), Cyotoxic T-lymphocyte activator-4 (CTLA4), Inducible T-cell costimulator (ICOS), B and T lymphocyte attenuator (BTLA), Killer cell immunoglobulin-like receptor (KIR), TCR, Lymphocyte-activation gene 3 (LAG3), Death receptor 5 (DR5), Cluster of Differentiation 137 (CD137), Tumor Necrosis Factor Receptor Superfamily Member 42 (OX40), 20 Cluster of Differentiation 27 (CD27), Cluster of Differentiation 40 Ligand (CD40L), adenosine A2a receptor (A2aR) and TIM3 (T-cell immunoglobulin-3).

Suitable checkpoint inhibitors include: the PD-1 inhibitors Nivolumab (Bristol-Myers Squibb/Ono Pharmaceutical), Lambrolizumab (MK-3475; Merck), Pidilizunab (CureTech/Teva) 25 and AMP-224 (Amplimmune/GlaxoSmithKline); the PD-L1 inhibitors RG-7446 (Roche) and MEDI-4736 (AstraZeneca); and the CTLA-4 inhibitor Ipilimumab. Suitable tyrosine kinase inhibitors include Sorafenib, Imatinib, Gefinitib, Palladia, Erlotinib, Lapatinib, Sunitinib, Nilotinib, 30 Suitable Tumor Necrosis Factor Receptors inhibitors include antibodies directed at DR5. Suitable cytokines or interleukins include IL-2, IL-7, IL-12. Suitable interferons include IFN-alpha, IFN-beta and IFN-gamma. A suitable Granulocyte Macrophage Colony Stimulating Factor includes Sargramostin. Suitable vaccines include Sipuleucel-T and suitable oncolytic viruses include Talimogene laherparepvec.

In another example, the IL-21 binding protein of the present invention is administered in combination with inhibitors, such as antibodies, directed at DR5 and/or CD137.

35 In another example, the IL-21 binding protein of the present invention is administered in combination with an immunomodulating drug.

Suitable immunomodulating drugs include Thalidomice (Thalomid), lenalidomide (Revlimid) and pomalidomide (Pomalyst).

Dosages and Timing of Administration

5 Suitable dosages of an IL-21-binding protein of the present disclosure will vary depending on the specific an IL-21-binding protein, the condition to be treated and/or the subject being treated. It is within the ability of a skilled physician to determine a suitable dosage, e.g., by commencing with a sub-optimal dosage and incrementally modifying the dosage to determine an optimal or useful dosage. Alternatively, to determine an appropriate
10 dosage for treatment/prophylaxis, data from the cell culture assays or animal studies are used, wherein a suitable dose is within a range of circulating concentrations that include the ED₅₀ of the active compound with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. A therapeutically/prophylactically effective dose can be estimated initially from cell culture assays.
15 A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration or amount of the compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma maybe measured, for example, by high performance liquid chromatography.
20 In some examples, a method of the present disclosure comprises administering a prophylactically or therapeutically effective amount of a protein described herein.

25 The term "therapeutically effective amount" is the quantity which, when administered to a subject in need of treatment, improves the prognosis and/or state of the subject and/or that reduces or inhibits one or more symptoms of a clinical condition described herein to a level that is below that observed and accepted as clinically diagnostic or clinically characteristic of that condition. The amount to be administered to a subject will depend on the particular characteristics of the condition to be treated, the type and stage of condition being treated, the mode of administration, and the characteristics of the subject, such as general health, other diseases, age, sex, genotype, and body weight. A person skilled in the art will be able to
30 determine appropriate dosages depending on these and other factors. Accordingly, this term is not to be construed to limit the present disclosure to a specific quantity, e.g., weight or amount of protein(s), rather the present disclosure encompasses any amount of the IL-21-binding protein(s) sufficient to achieve the stated result in a subject.

35 As used herein, the term "prophylactically effective amount" shall be taken to mean a sufficient quantity of a protein to prevent or inhibit or delay the onset of one or more detectable symptoms of a clinical condition. The skilled artisan will be aware that such an amount will vary

depending on, for example, the specific IL-21-binding protein(s) administered and/or the particular subject and/or the type or severity or level of condition and/or predisposition (genetic or otherwise) to the condition. Accordingly, this term is not to be construed to limit the present disclosure to a specific quantity, e.g., weight or amount of IL-21-binding protein(s), rather the 5 present disclosure encompasses any amount of the IL-21-binding protein(s) sufficient to achieve the stated result in a subject.

For *in vivo* administration of the IL-21-binding protein described herein, normal dosage amounts may vary from about 10ng/kg up to about 100mg/kg of an individual's body weight or more per day. For repeated administrations over several days or longer, depending on the 10 severity of the disease or disorder to be treated, the treatment can be sustained until a desired suppression of symptoms is achieved.

In some examples, the IL-21-binding protein is administered at an initial (or loading) dose of between about 1mg/kg to about 30mg/kg, such as from about 1mg/kg to about 10mg/kg, or about 1mg/kg or about 2mg/kg or 5mg/kg. The IL-21-binding protein can then be 15 administered at a lower maintenance dose of between about 0.01mg/kg to about 2mg/kg, such as from about 0.05mg/kg to about 1mg/kg, for example, from about 0.1mg/kg to about 1mg/kg, such as about 0.1mg/kg or 0.5mg/kg or 1mg/kg. The maintenance doses may be administered every 7-30 days, such as, every 10-15 days, for example, every 10 or 11 or 12 or 13 or 14 or 15 days.

20 In some examples, the IL-21-binding protein is administered at a dose of between about 0.01mg/kg to about 50mg/kg, such as between about 0.05mg/kg to about 30mg/kg, for example, between about 0.1mg/kg to about 20mg/kg, for example, between about 0.1mg/kg to about 10mg/kg, such as between about 0.1mg/kg to about 2mg/kg. For example, the IL-21-binding protein is administered at a dose of between about 0.01mg/kg to about 5mg/kg, such 25 as from about 0.1mg/kg to about 2mg/kg, such as about 0.2mg/kg or 0.3mg/kg or 0.5mg/kg or 1mg/kg or 1.5mg/kg (e.g., without a higher loading dose or a lower maintenance dose). In some examples, numerous doses are administered, e.g., every 7-30 days, such as, every 10-22 days, for example, every 10-15 days, for example, every 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 days. For example, the IL-21-binding protein is 30 administered every 7 days or every 14 days or every 21 days.

In some examples, at the time of commencing therapy, the mammal is administered the IL-21-binding protein on no more than 7 consecutive days or 6 consecutive days or 5 consecutive days or 4 consecutive days.

35 In the case of a mammal that is not adequately responding to treatment, multiple doses in a week may be administered. Alternatively, or in addition, increasing doses may be administered.

In another example, for mammals experiencing an adverse reaction, the initial (or loading) dose may be split over numerous days in one week or over numerous consecutive days.

Administration of an IL-21-binding protein according to the methods of the present disclosure can be continuous or intermittent, depending, for example, on the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of an IL-21-binding protein may be essentially continuous over a preselected period of time or may be in a series of spaced doses, e.g., either during or after development of a condition.

10

Kits

The present disclosure additionally comprises a kit comprising one or more of the following:

- (i) an IL-21-binding protein of the disclosure or expression construct(s) encoding same;
- 15 (ii) a cell of the disclosure;
- (iii) a complex of the disclosure; or
- (iii) a pharmaceutical composition of the disclosure.

In the case of a kit for detecting IL-21, the kit can additionally comprise a detection means, e.g., linked to a IL-21-binding protein of the disclosure.

20 In the case of a kit for therapeutic/prophylactic use, the kit can additionally comprise a pharmaceutically acceptable carrier.

Optionally a kit of the disclosure is packaged with instructions for use in a method described herein according to any example.

The present disclosure includes the following non-limiting Examples.

25

EXAMPLES

Methods

Human B-cell proliferation assay

5 Peripheral blood mononuclear cells (PBMC) were isolated from healthy human donors according to standard techniques and the PBMCs were stained with CFSE and then sorted into CD19+ CD27- IgD+ naïve B cells and CD19+ CD27+ IgD- memory B cells. Sorted cells were seeded into culture plate. Cells were then stimulated with 0.5 -50 ng/ml of human IL-21 with or without 2P2. After 5 days cellular proliferation was determined via CFSE dilution or 3H 10 thymidine proliferation assay.

In vivo half-life ELISA assay

To detect *in vivo* half-life of hIL-21/2P2 complex, mice were injected I.V. or S.C. with hIL-21/2P2 or hIL-21 alone, where 2P2 in the complex has been biotinylated using a 15 commercial biotinylation kit. After 1hr, 3hr, 8hr and 24hrs post injection, mice were bled and levels of hIL-21 in serum were measure by ELISA. ELISA was done by coating plate with unconjugated 3A3, followed by blocking and incubation with serum. Plate was subsequently incubated with biotinylated 2P2 and detected with Streptavidin-HRP and TMB substrates.

20 ELISA assay

To detect epitopes of 2P2 and 3A3, peptides consisting of different regions of hIL-21 were synthesized commercially and coated on an ELISA plate, followed by blocking of non-specific binding sites with 3% BSA in PBS. Biotinylated 2P2 or 3A3 were then added to the wells and developed with Streptavidin-HRP and TMB substrates.

25 Epitopes identified from previous experiments were then tested for the ability to block the binding of 2P2 or 3A3 to full length hIL-21. Full length hIL-21 was coated on EIA plate followed by blocking of non-specific binding sites with BSA. Biotinylated 2P2 or 3A3 were then spiked with the corresponding peptides and incubated on plate followed by detection with TMB substrates.

30

Example 1 Screening of the anti-IL-21 antibodies

A library of monoclonal antibodies to human IL-21 (hIL-21) was generated by the inventors. The clones were screened for their ability to enhance proliferation of cells of primary human B cells (BaF3 cells) expressing the human IL-21 receptor (BaF3-hIL-21R cells; Parrish-35 Novak J et al. (2000) *Nature* 408:57-63). Each monoclonal antibody was serially diluted 10-fold as shown in Figure 1 and mixed with 2ng/ml of hIL-21 and incubated with the BaF3-hIL-21R cells cultured in RPMI-1640 media respectively in a humidified chamber with 5% CO₂ at 37 C. For [³H]-labelled thymidine incorporation assay to measure the cell proliferation, BaF3-huIL-

21R cells were starved in the cultured media without cytokine for 18 h and then were incubated for 72 h with the addition of 1 μ Ci/well [3 H]-thymidine (Perkin Elmer, Waltham, MA, USA) for the last 18 h of culture in the presence of cytokine. Cells were harvested onto filter mats (Perkin Elmer) and incorporated radioactive nucleic acids counted using a Top Count NXT Scintillation Counter (Packard Biosciences, Meriden, CT, USA). Results were expressed as mean count per minute (CPM) for triplicate cultures.

As demonstrated in Figure 1, monoclonal antibody 2P2 increased the proliferation of BaF3-hIL-21R cells in a dose dependent manner. Accordingly, antibody 2P2 demonstrated an enhancing capability on cellular proliferation in the presence of the IL-21 cytokine suggesting that the antibody may function as an agonist of human IL-21. The Heavy and light CDRs of the VH and VL and are shown in Figure 2. The CDR sequences as determined by IMGT numbering scheme are underlined.

Example 2 mAB 2P2 potentiates hIL-21 through the IL-21 receptor

The inventors next determined whether the enhancing capability of the 2P2 monoclonal antibody was specific to the human IL-21 receptor. Primary human BaF3 cells, BaF3 cells expressing a murine IL-21 receptor (BaF3-mIL-21R) and BaF3 cells expressing a human IL-21 receptor (BaF3-hIL-21R) were plated in the presence of 0.1, 1.0 and 10.0 μ g/ml of 2P2 antibody (Figure 3A) and 2 ng/ml human IL-21. Coding sequences for human IL-21R (hIL-21R) and mouse IL-21R (mIL-21R) were cloned by PCR from human or mouse cDNA libraries respectively, sequenced to match NM_021798 and NM_021887. For [3 H]-labelled thymidine incorporation assay to measure the cell proliferation, Ba/F3-hIL-21R or Ba/F3-mIL-21R cells were starved in the cultured media without cytokine for 18 h and then were incubated for 72 h with the addition of 1 μ Ci/well [3 H]-thymidine (Perkin Elmer, Waltham, MA, USA) for the last 18 h of culture in the presence of cytokines with indicated concentrations. Cells were harvested onto filter mats (Perkin Elmer) and incorporated radioactive nucleic acids counted using a Top Count NXT Scintillation Counter (Packard Biosciences, Meriden, CT, USA). Results were expressed as mean count per minute (CPM) for triplicate cultures.

The results demonstrated that 2P2 specifically increased proliferation of Ba/F3-hIL-21R cells but not Ba/F3-mIL-21R or Ba/F3 cells indicating that the activity of the 2P2 antibody was specific to human IL-21 since it did not enhance the function of murine IL-21. Figure 3B shows the effect on cellular proliferation of Ba/F3-hIL-21R cells (closed circles) and BaF3-mIL-21R cells (open circles) by 2P2 plotted against human IL-12 (ng/ml). At a concentration of 10 μ g/ml 2P2, the bioactivity of human IL-21 was increased greater than 10-fold. However, there was no effect on the bioactivity of human IL-21 in the presence of cells expressing the murine IL-21 receptor.

Example 3 2P2 potentiates hIL-21 mediated human B cell proliferation

Human B cells were enriched from peripheral blood mononuclear cells of healthy donors using the markers CD19, CD27, CD4 and CD45. B cell proliferation was measured using flow cytometry according to standard methods. Cells were labelled with carboxyfluorescein succinimidyl ester (CFSE) and incubated in the presence of 0, 0.3, 1, 5, 20 and 100 ng/ml of human IL-21. The percentage of cells that had undergone at least one division was determined by flow cytometry. As shown in Figure 4A for example, at 20 ng/ml human IL-21, about 67% of the human B cells proliferated. In Figure 4B, the flow cytometry data is re-produced as the first six bars. The remaining four bars show percentage of proliferated B cells in the presence of 5 ng/ml of IL-21 and 0, 0.01, 0.1 and 1 µg/ml of 2P2 antibody. 5 ng/ml of hIL-21 plus 1 µg/ml of mAb 2P2 demonstrated a similar bioactivity to 100 ng/ml of hIL-21 in the absence of 2P2 antibody thus demonstrating that mAb 2P2 increased the bioactivity of hIL-21 by approximately 20-fold suggesting it was a potent agonist of IL-21 activity.

15 **Example 4 2P2 potentiates hIL-21 mediated human CD8 T cell activation**

Human CD8 T cells were incubated in the presence of IL-21 at the various concentrations shown in Figure 5. In the first three panels, cells were incubated in the presence of IL-21 alone (2 ng/ml and 25 ng/ml) and IL-21 plus 2P2 (2 ng/ml and 1 µg/ml respectively). In the second panel the cells were incubated in the presence of IL-21 alone (10 ng/ml and 100 ng/ml) and IL-21 plus 2P2 (10 ng/ml and 10 µg/ml respectively). The cells were stained for granzyme B and perforin which were indicative of activation of the CD8 T cells since expression of these cytotoxic molecules is important for anti-virus and anti-tumour immunity. The percentage of granzyme B/perforin double positive cells is shown in the upper right hand quadrant. Figure 5 shows that the 2 ng/ml of hIL-21 plus 1 µg/ml of mAb 2P2 demonstrated a better bioactivity on T cells than that of 25 ng/ml of hIL-21 alone (21.89% positive versus 20.29% positive) and 10 ng/ml of hIL-21 plus 10 µg/ml of mAb 2P2 demonstrated a better bioactivity than that of 10 ng/ml of hIL-21 alone (31.70% versus 25.77% positive), indicating over a 10-fold increase of hIL-21 bioactivity by mAb 2P2.

30 **Example 5 Monoclonal antibody 2P2 prolongs hIL-21 half-life in vivo**

Serum half-life of hIL-21/2P2 and hIL-21/3A3 complexes was determined in vivo according to an ELISA assay. Wild-type mice were injected intravenously with either 25 µg hIL-21; 25 µg hIL-21 and 125 µg 2P2-biotin; or 25 µg hIL-21 and 125 µg 3A3-biotin. Mice were bled from the cheek at 1, 3, 8 and 24 hrs and 100 µl of blood obtained. The blood was then incubated for 30 mins at room temperature to obtain serum. Measurement of the level of hIL-21 in the serum was determined by ELISA. The detection limit of the assay is 1 ng/ml. Figure 6 shows detection of hIL-21/2P2 complexes administered either intravenously (IV) or

subcutaneously (SC) from serum over 24 hrs. 2P2 administered via either route resulted in a significant increase in serum retention of hIL-21 at all time points tested when compared to hIL-12 administered alone. 2P2 enhanced serum AUC of hIL-21 54 fold when administered subcutaneously (SC IL-21 alone AUC 117; SC IL-21/2P2 AUC 6340) and 164 (IV IL-21 alone 5 AUC 91.36; IV IL-21/2P2 AUC 14993) when administered intravenously.

Example 6 Antibody 2P2 enhances IL-21 activity *in vivo*

The inventors then determined whether 2P2 enhances IL-21 activity *in vivo*. A mouse strain was generated by replacing the endogenous mIL-21 receptor with the hIL-21 receptor.

10 These mice have no endogenous IL-21 cytokine and are referred to as homozygous hIL-21R^{KI/KI} and hIL21^{KI/KI}. The mice have a normal immune system and physiological expression of hIL-21R so they can be used to test the function of hIL-21 and 2P2 *in vivo*.

Mice were injected intravenously with hIL-21 or hIL-21 and 2P2 at D0, D2 and D4. The treatment schedule is set out in Table 2 below.

15

Table 2 Treatment regimen

Group (n = 3)	Treatment
1	5 µg IL-21
2	25 µg IL-21
3	5 µg IL-21 + 125 µg 2P2
4	5 µg IL-21 + 125 µg 3A3

20 Antibody 3A3 (commercially available) which binds to human IL-21 but does not enhance the activity of IL-21 was used as a control. At D5 mice were sacrificed and their spleen and lymph nodes harvested. Administration of hIL-21/2P2 complex was found to enlarge the spleens to a greater extent compared to recombinant hIL-21 alone in hIL-21R knock-in mice, suggesting a better immunostimulatory function of 2P2 *in vivo*.

25 Single cell suspensions were prepared according to standard protocols. For B cell detection, the cell suspension was stained with antibodies and analyzed by FACS (flow cytometry LSR2 from BD). Populations of B cells were analyzed as followed:

follicular zone (FZ) B cells- CD3⁻B220⁺ IgD⁺;
marginal zone (MZ) B cells- CD3⁻B220⁺ IgM⁺ CD23⁻;
transitional B cells- CD3⁻B220⁺ IgM⁺ AA4.1⁺.

30 The results are shown in Figure 7 for FZ, MZ and transitional B cells from the lymph node and Figure 8 for FZ, MZ and transitional B cells from the spleen. Statistical analysis was performed using a One-Way ANOVA (Newman-Keuls test) for analysis of differences between

multiple groups. (Graphpad Prism Statistical software (Graphpad Software Inc, La Jolla, CA USA). The results showed that addition of 2P2 significantly increased transitional B cells in lymph node and spleen and significantly increase marginal zone B cells of the lymph node Table 4 and Table 5.

5

Table 4 One-Way ANOVA Summary

One-way ANOVA	FO B cells (LN)	MZ B cells (LN)	Transition B cells (LN)	FO B cells (spleen)	MZ B cells (spleen)	Transition B cells (spleen)
P value	0.0076	0.0003	0.0001	0.32.0	0.0016	0.0184
P value summary	**	***	***	ns	**	*

Table 5 Newman-Keuls Multiple Comparison test

Newman-Keuls Multiple Comparison test	FO B cells (LN)	MZ B cells (LN)	Transition B cells (LN)	FO B cells (spleen)	MZ B cells (spleen)	Transition B cells (spleen)
5µg v 25µg	ns	ns	*	-	**	*
5µg v 5µg+2P2	**	***	***	-	**	*
5µg v 5µg+3A3	ns	ns	**	-	ns	ns
25µg v 5µg+2P2	**	**	***	-	ns	ns
25µg v 5µg+3A3	ns	ns	Ns	-	**	*
5µg+2P2 v 5µg+3A3	ns	***	**	-	**	Ns

ns = not significant, *P < 0.05, **P < 0.01 and ***P < 0.001

10 Example 7 Epitope refinement

The inventors then sought to determine which residues of human IL-21 were bound by the 2P2 antibody. Figure 9 shows the sequence of human IL-21 which residues in bold predicted to be residues bound by antibody 2P2 according to structural simulation studies, underlined residues are located within IL-21 helix regions. The inventors generated 5 peptides of hIL-21 as follows and as shown in Figure 9:

15 Peptide 1 (P1, 30aa; SEQ ID NO 19): FLPAPEDVETNCEWSAFSCFQKAQLKSANT

Peptide 2 (P2, 27aa; SEQ ID NO 20): AQLKSANTGNNERIINVSIKKLKRKPP

Peptide 3 (P3, 30aa; SEQ ID NO 4): IKKLKRKPPSTNAGRRQKHRLTCPSCDSYE

20 Peptide 4 (P4, 23aa; SEQ ID NO 21): PPSTNAGRRQKHRLTCPSCDSYE

Peptide 5 (P5, 15aa; SEQ ID NO 22): PPSTNAGRRQKHRLT

An ELISA assay was used to examine binding between antibodies 2P2 and 3A3 and a Streptavidin-HRP secondary only control to each of the peptides P1 to P5, hIL-21 and OVA peptide. As shown in Figure 10, antibody 2P2 bound peptide 3 the strongest, followed by peptide 4 and peptide 5. Thus, the epitope recognized by antibody 2P2 comprised a minimal sequence of 9 mer to 15 mer, with the core sequence being STNAGRRQK (SEQ ID NO 23). Binding was stronger with the sequence CPSCD was also present and strongest binding was observed when the sequences IKKLK, CPSCD and STNAGRRQK were present. Strongest binding was observed with peptide 3 comprising the sequence IKKLKRKPPSTNAGRRQKHRLTCPSCDSYE (SEQ ID NO 4) (Summarized in Figure 10).

When pre-incubating 2P2 with peptide 3 2P2 failed to bind to full length hIL-21 (Figure 11A). Pre-incubating control antibody 3A3 with peptide 3 did not inhibit binding to full length hIL-21 (Figure 11B). This demonstrated that epitope consisting in peptide 3 is sufficient for the binding of 2P2 to hIL-21.

15 **Example 8 Binding Affinity of 2P2 to hIL-21**

Surface Plasma Resonance binding kinetics assay were performed on a Bio-rad XPR36 system by the direct amine coupling method. Briefly, a flow of mixture of EDC [N-ethyl-N'-(3-diethylamino-propyl) carbodiimide] and NHS (N-hydroxysuccinimide) was used to activate the cell surface of a GLC sensor chip before 25 µg/ml anti-IL-21 mAb was flowed through, then a shot flow of ethanolamine was used to block the remaining active sites on the cell surface. Afterwards, serial diluted solutions of IL-21 from 0 to 40 nM were flown over the cell surface to bind to the immobilized anti-IL-21 mAb molecules. Binding kinetics were performed at 25°C with a flow rate of 30 µL/min, association time of 3 min and dissociation time of 6 min. The buffer consisted of Hepes, NaCl, tween20, and 1 mg/mL BSA at pH 7.4. The flow cell regeneration buffer between cycles was 15 mM phosphoric acid. Double references were used to process the sensorgrams. The binding curves were globally fit to the 1:1 Langmuir binding model.

The final binding affinity of 2P2 with hIL-21 was calculated as 3.0E-09 M from the average of n = 5 measurements (table 6). The affinity of hIL-21 and 2P2 range from 2.47 X10⁻⁹ to 4.29 X10⁻⁹, with an average of 3.33 X10⁻⁹ and a SD of 0.74X10⁻⁹.

Table 6 Dissociation constant hIL-21/2P2 complex

Complex/ well number	KD (M)
IL-21/2P2 well 1	3.83X10 ⁻⁹
IL-21/2P2 well 2	3.29X10 ⁻⁹
IL-21/2P2 well 3	2.79X10 ⁻⁹

IL-21/2P2 well 4	4.29X10-9
IL-21/2P2 well 5	2.47X10-9

CLAIMS:

1. An IL-21-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-21 and enhances IL-21 activity.
- 5 2. The IL-21-binding protein of claim 1 wherein the IL-21-binding protein enhances IL-21-mediated proliferation of B cells *in vitro*.
3. The IL-21-binding protein of claim 1 or claim 2 wherein the IL-21-binding protein enhances IL-21-mediated cytotoxicity of CD8 T cells *in vitro*.
- 10 4. The IL-21-binding protein of any one of claims 1 to 3 wherein the IL-21-binding protein enhances IL-21 half-life *in vivo*.
- 15 5. The IL-21-binding protein of any one of claims 1 to 4 the antigen binding domain binds to an epitope set forth in SEQ ID NO: 23 or SEQ ID NO: 4.
- 20 6. An IL-21-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-21 and enhances IL-21 activity and wherein the protein competitively inhibits binding of antibody P2P (comprising a heavy chain comprising a sequence set forth in SEQ ID NO: 2 and a light chain comprising a sequence set forth in SEQ ID NO: 3).
- 25 7. An IL-21-binding protein comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to IL-21, wherein the antigen binding domain comprises at least one of:
 - (i) a V_H comprising a complementarity determining region (CDR) 1 comprising a sequence at least about 80% identical to a sequence set forth in SEQ ID NO: 5, a CDR2 comprising a sequence at least about 80% identical to a sequence set in SEQ ID NO: 6 and a CDR3 comprising a sequence at least about 80% identical to a sequence set forth in SEQ ID NO: 7;
 - 30 (ii) a V_H comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO: 2;
 - (iii) a V_L comprising a CDR1 comprising a sequence at least about 80 % identical to a sequence set forth in SEQ ID NO: 8, a CDR2 comprising a sequence at least about 80 % identical to a sequence set forth in SEQ ID NO: 9 and a CDR3 comprising a sequence at least 35 about 80% identical to a sequence set forth in SEQ ID NO: 10;

- (iv) a V_L comprising a sequence at least about 95% identical to a sequence set forth in SEQ ID NO: 3;
- (v) a V_H comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 5, a CDR2 comprising a sequence set forth between in SEQ ID NO: 6 and a CDR3 comprising a sequence set forth in SEQ ID NO: 7;
- 5 (vi) a V_H comprising a sequence set forth in SEQ ID NO: 2;
- (vii) a V_L comprising a CDR1 comprising a sequence set SEQ ID NO: 8, a CDR2 comprising a sequence set forth in SEQ ID NO: 9 and a CDR3 comprising a sequence set forth in SEQ ID NO: 10;
- 10 (viii) a V_L comprising a sequence set forth in SEQ ID NO: 3;
- (ix) a V_H comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 5, a CDR2 comprising a sequence set forth between in SEQ ID NO: 6 and a CDR3 comprising a sequence set forth in SEQ ID NO: 7; and a V_L comprising a CDR1 comprising a sequence set SEQ ID NO: 8, a CDR2 comprising a sequence set forth in SEQ ID NO: 9 and a CDR3 comprising a sequence set forth in SEQ ID NO: 10; and
- 15 (x) a V_H comprising a sequence set forth in SEQ ID NO: 2 and a V_L comprising a sequence set forth in SEQ ID NO: 3.

8. The IL-21-binding protein of any one of claims 1 to 7 comprising at least a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein the V_H and V_L bind to form a Fv comprising an antigen binding domain, wherein the V_H and the V_L are in a single polypeptide chain or the V_L and V_H are in separate polypeptide chains.

9. The IL-21-binding protein of claim 8, wherein if the V_H and V_L are in a single polypeptide chain, the protein is:

- (i) a single chain Fv fragment (scFv);
- (ii) a dimeric scFv (di-scFv);
- (iii) one of (i) or (ii) linked to a constant region of an antibody, Fc or a heavy chain constant domain (C_H) 2 and/or C_H 3; or
- 30 (iv) one of (i) or (ii) linked to a protein that binds to an immune effector cell, or if the V_H and V_L are in separate polypeptide chains the protein is:
 - (i) a diabody;
 - (ii) a triabody;
 - (iii) a tetrabody;
- 35 (iv) a Fab;
- (v) a $F(ab')_2$;

- (vi) a Fv;
- (vii) one of (i) to (vi) linked to a constant region of an antibody, Fc or a heavy chain constant domain (C_H) 2 and/or C_H3;
- (viii) one of (i) to (vi) linked to a protein that binds to an immune effector cell; or
- 5 (ix) an antibody.

10. 10. A complex comprising the IL-21-binding protein of any one of claims 1 to 9 and an IL-21 polypeptide.

10 11. The IL-21-binding protein of any one of claims 1 to 9 or the complex of claim 10 which is conjugated to another compound.

12. 12. A nucleic acid encoding the IL-21-binding protein of any one of claims 1 to 9.

15 13. 13. An expression construct comprising the nucleic acid of claim 12.

14. 14. An isolated or recombinant cell expressing the IL-21-binding protein of any one of claims 1 to 9.

20 15. 15. A composition comprising an IL-21-binding protein of any one of claims 1 to 9 or the complex of claim 10 and a pharmaceutically acceptable carrier.

16. 16. Use of the IL-21-binding protein of any one of claims 1 to 9 or the complex of claim 10 or the composition of claim 15 in medicine.

25

17. 17. A method for treating or preventing a chronic infection, an allergic immune response or cancer in a subject, the method comprising administering the IL-21-binding protein of any one of claims 1 to 9 or the complex of claim 10.

30 18. 18. Use of the IL-21-binding protein of any one of claims 1 to 9 or the complex of claim 10 in the manufacture of a medicament to treat or prevent a chronic infection, an allergic immune response or cancer.

35 19. 19. The IL-21-binding protein of any one of claims 1 to 9 or the complex of claim 10 or the composition of claim 15 for use in the treatment of a chronic infection, an allergic immune response or cancer.

20. The use or method of any one of 16 to 18 wherein the IL-21 binding protein is administered in combination with one or more immunotherapeutic compounds,
21. The use or method according to claim 20 wherein the immunotherapeutic compound is selected from a checkpoint inhibitor, tyrosine kinase inhibitor, tumor necrosis factor receptor inhibitor, cytokine or interleukin; interferon; granulocyte macrophage colony stimulating factor; vaccine (cancer vaccine or infectious disease); antiviral drug or an oncolytic virus.
22. The use or method of claim 21 wherein the checkpoint inhibitor is an inhibitor of Programmed death 1 (PD1), Programmed death ligand 1 (PDL1) or Programmed death ligand 2 (PDL2), Cluster of Differentiation 80 (CD80), Cluster of Differentiation 80 (CD86), B7-related protein 1 (B7RP1), Cluster of Differentiation 276 (CD276), Cluster of Differentiation 274 (CD274), Herpesvirus entry mediator (HVEM), Cluster of Differentiation Ligand (CD137L), Tumor Necrosis Factor Receptor Superfamily Member 4 Ligand (OX40L), Cluster of Differentiation (CD70), Cluster of Differentiation (CD40), Galectin 9 (GAL9), Cluster of Differentiation 28 (CD28), Cyotoxic T-lymphocyte activator-4 (CTLA4), Inducible T-cell costimulator (ICOS), B and T lymphocyte attenuator (BTLA), Killer cell immunoglobulin-like receptor (KIR), TCR, Lymphocyte-activation gene 3 (LAG3), Death receptor 5 (DR5), Cluster of Differentiation 137 (CD137), Tumor Necrosis Factor Receptor Superfamily Member 42 (OX40), Cluster of Differentiation 27 (CD27), Cluster of Differentiation 40 Ligand (CD40L), adenosine A2a receptor (A2aR) or TIM3 (T-cell immunoglobulin-3).
23. A method of culturing expanding a population of cells comprising immune system precursor cells, the method comprising culturing the population of cells *in vitro* or *ex vivo* in the presence of an IL-21 binding protein or complex of the present disclosure.

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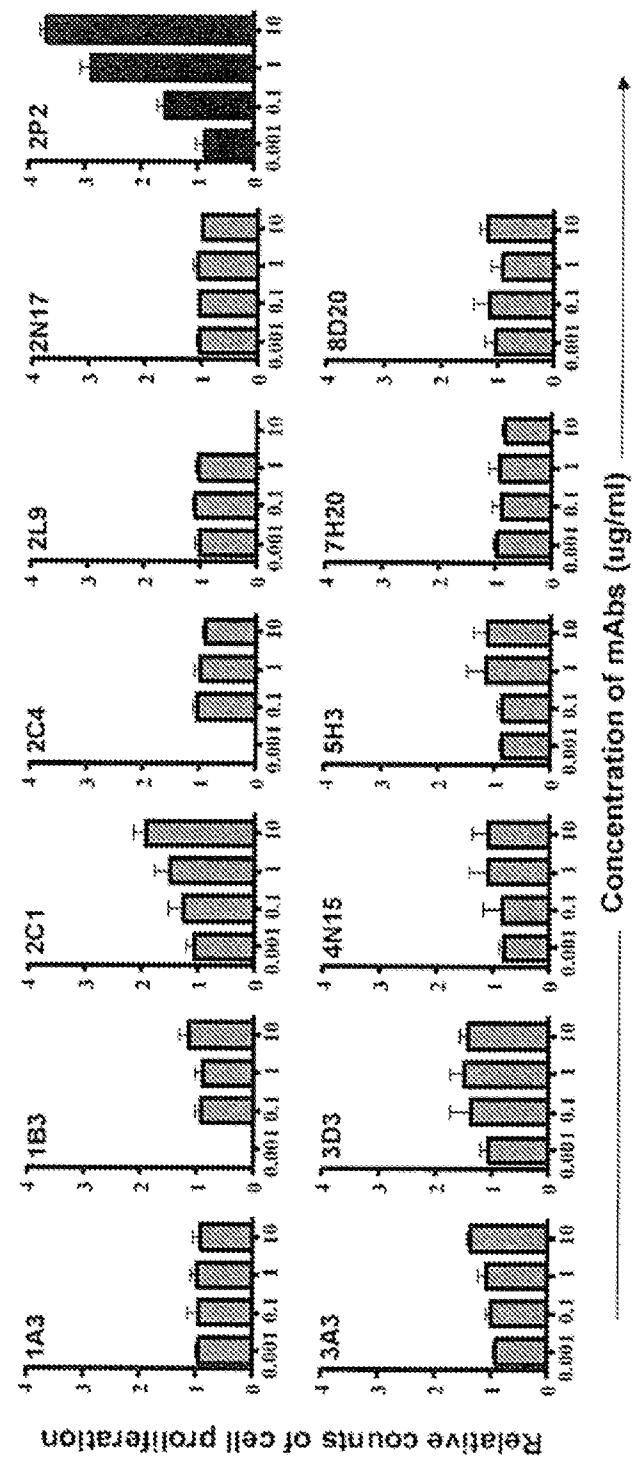


FIGURE 1

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Heavy chain

EVOLQOSGADLVRSGASVKLSCTASGENIK DYYIH WVKQRPEQGLELIG WIDPESGOTIEYAPKFQV

KATMVTADTSSNTAYLQLSSLTSEDTAVYYCND GSEY WGQGTTLTVSS

CDR1 CDR2 CDR3

Light chain

DVYMTQTPLTLSVTLGGPASISIC KSSQASLSDSDGETYLN WILQRPCQSPKRLLIS LMSKLDs

GVPDRFTGSSTGFTLKRSEVAEDLGVYYC WQGTHPPYT FGGSTKLEK

CDR1 CDR2 CDR3

FIGURE 2

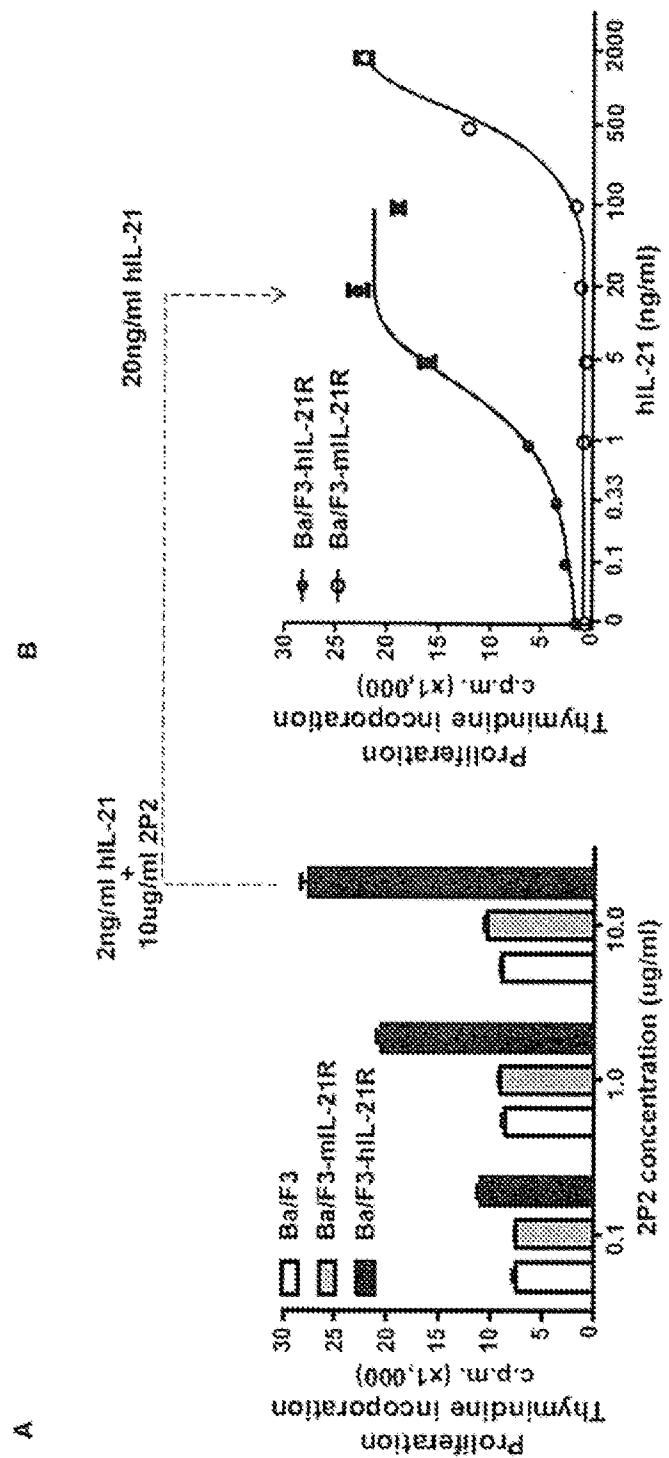


FIGURE 3

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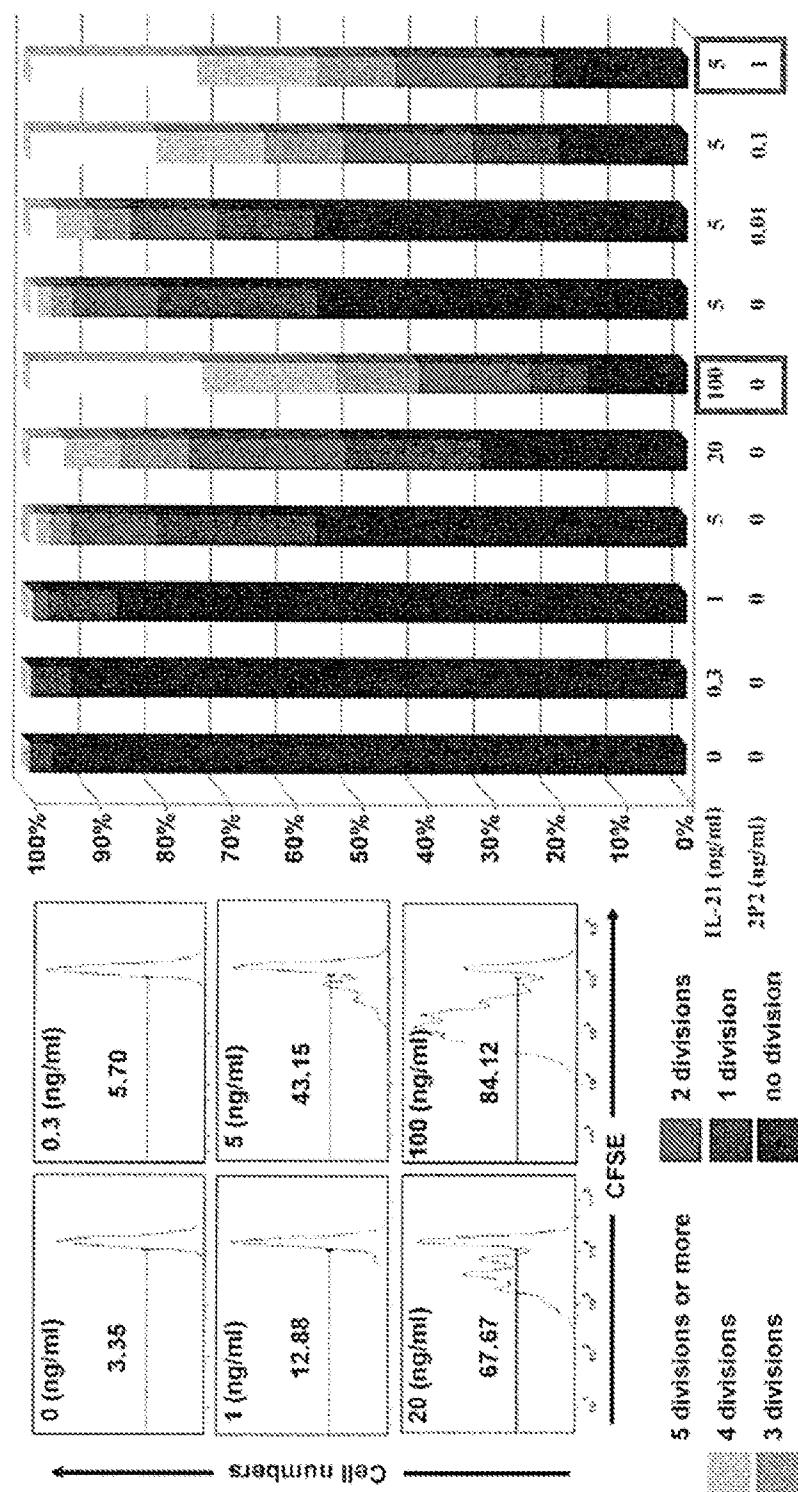


FIGURE 4

5/11

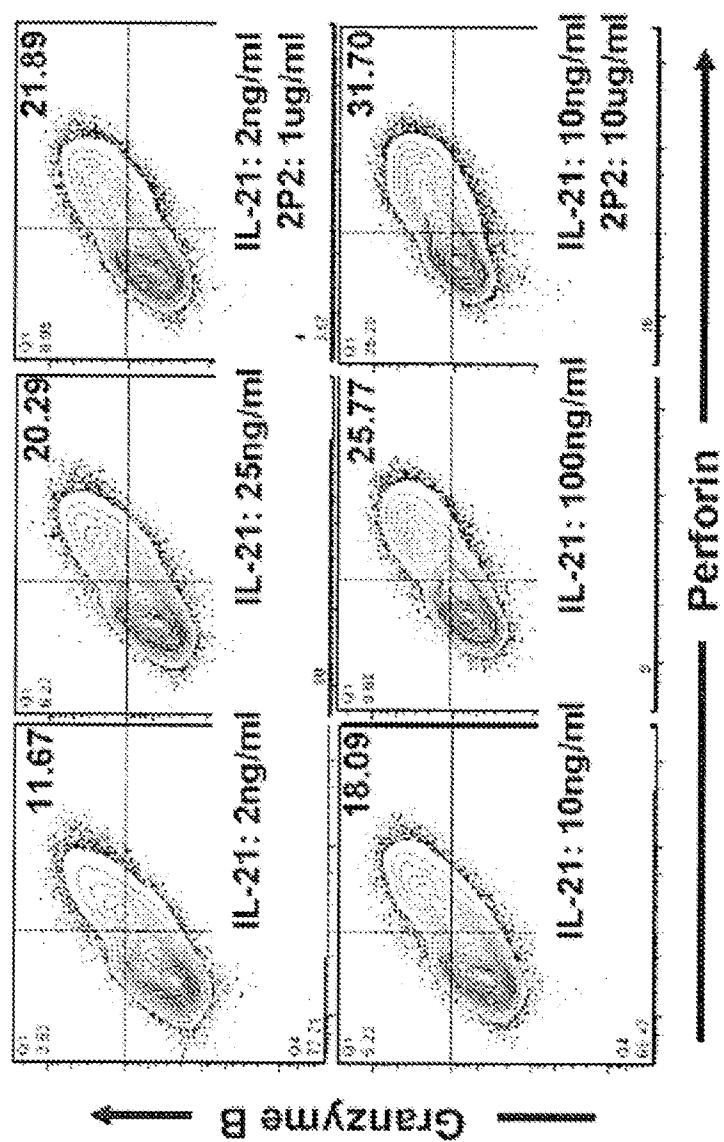
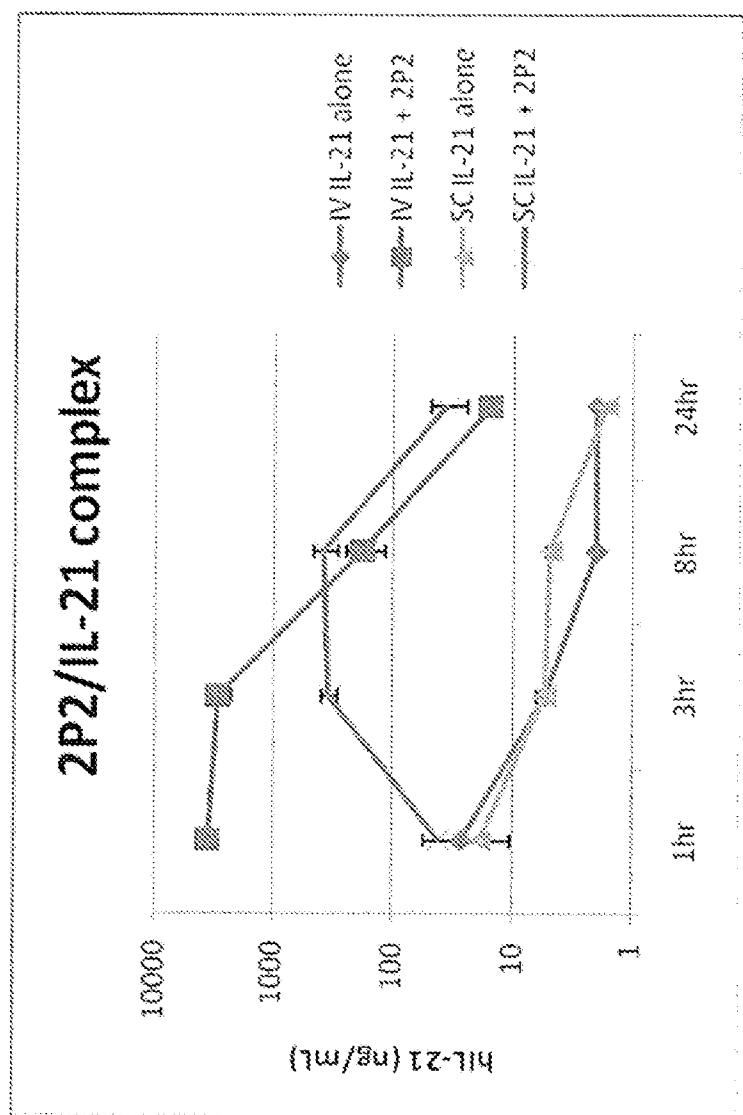


FIGURE 5

**FIGURE 6**

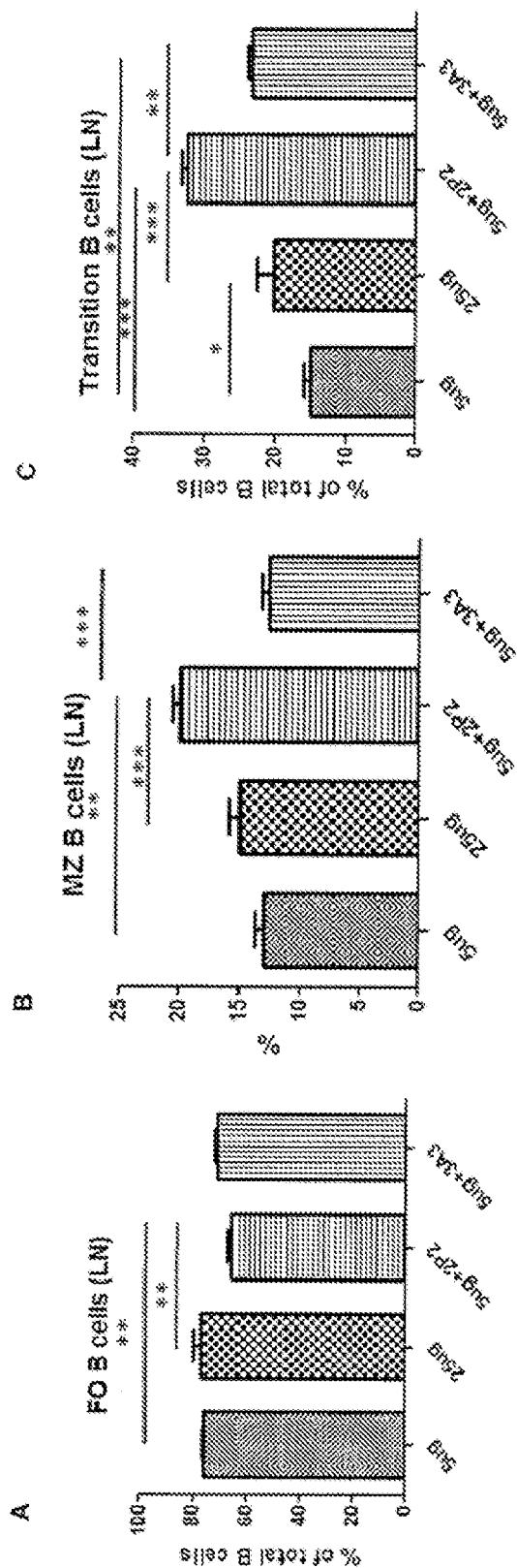


FIGURE 7

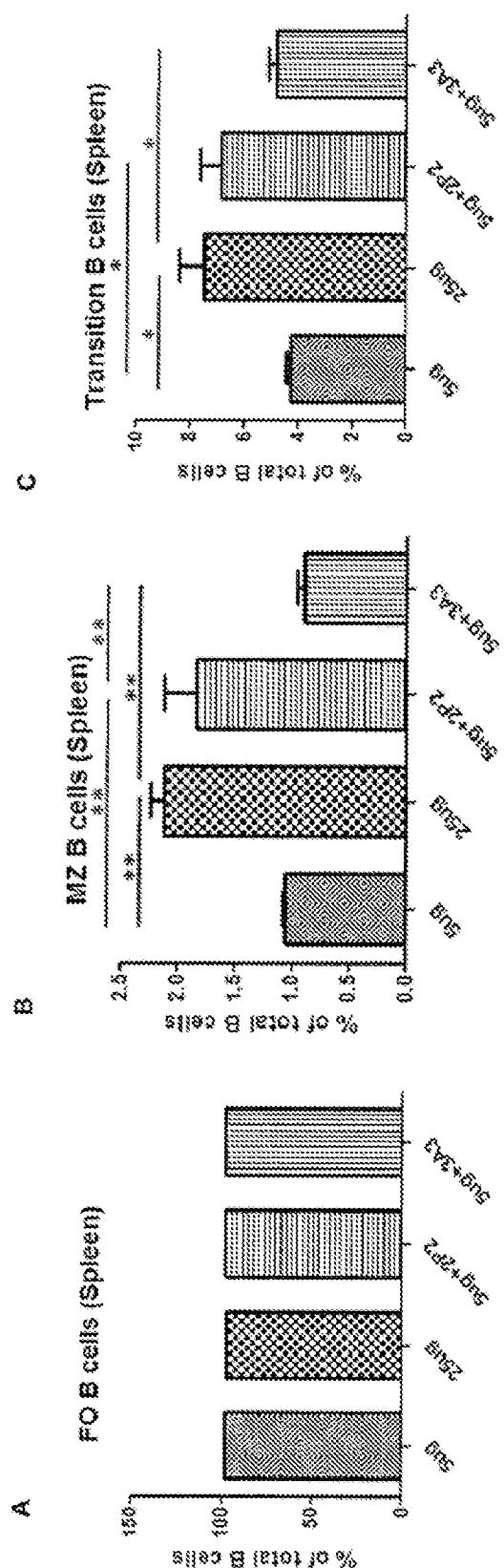


FIGURE 8

Human LL-21

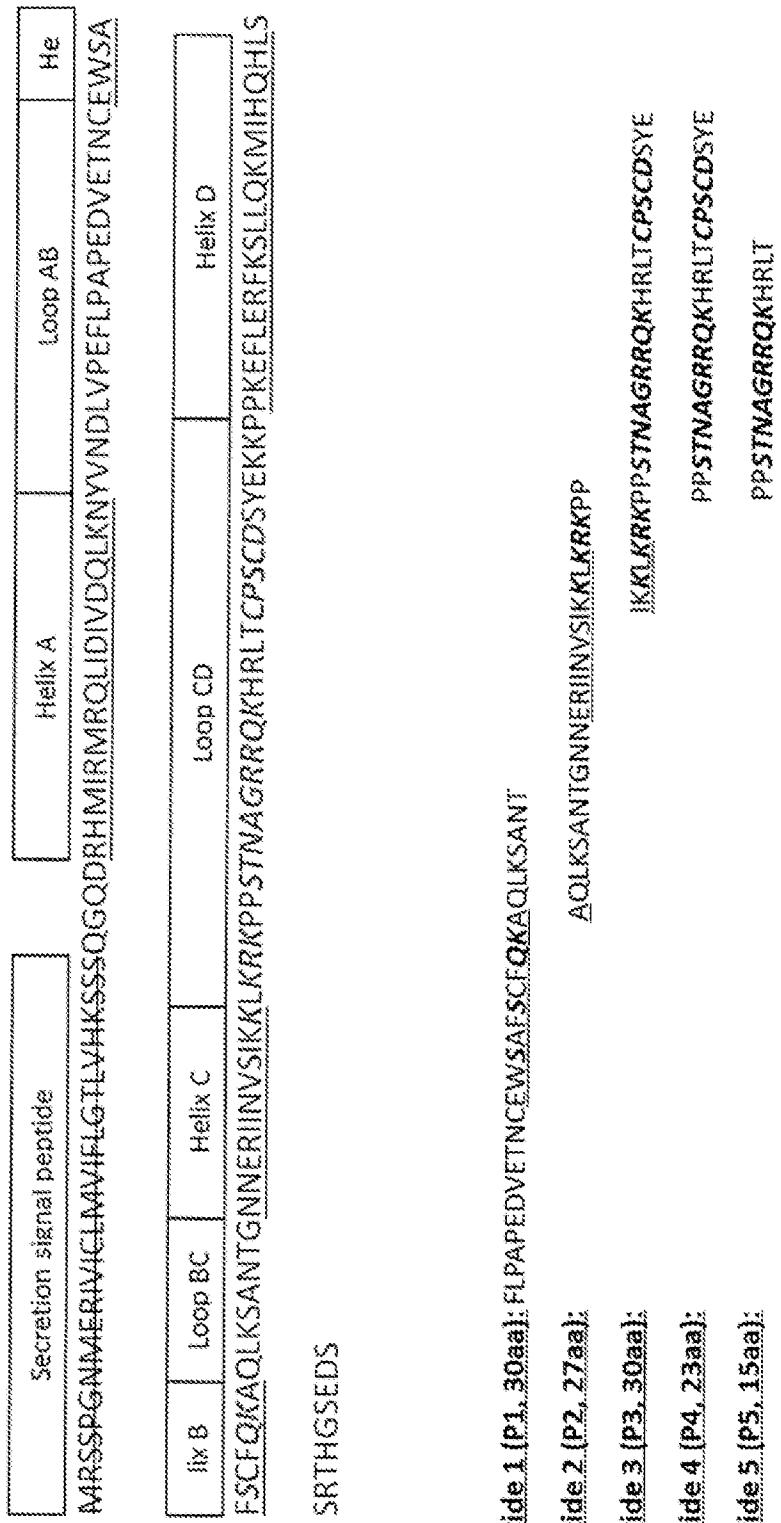


FIGURE 9

Peptide 1 (P1, 30aa): <u>FLPAPEDVETNCEWSAFSCFQKAQLKSANT</u>	No binding
Peptide 2 (P2, 27aa): <u>AQLKSANTGNNERIINVSIIKKLKRKPP</u>	No binding
Peptide 3 (P3, 30aa): <u>IKKLKRKPPSTNAGRRQKHHRLTCPSCDSYE</u>	Strongest binding
Peptide 4 (P4, 23aa): <u>PPSTNAGRRQKHHRLTCPSCDSYE</u>	weaker binding
Peptide 5 (P5, 15aa): <u>PPSTNAGRRQKHHRLT</u>	weakest binding
Refined epitope of 2p2: <u>IKKLKRKPPSTNAGRRQKHHRLTCPSCDSYE</u>	

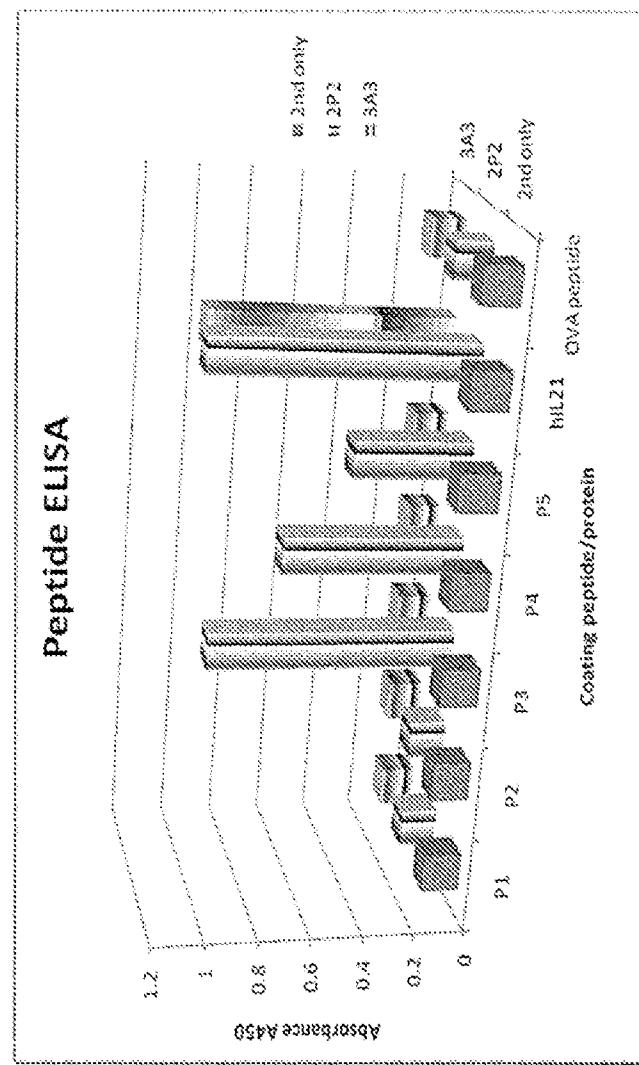


FIGURE 10

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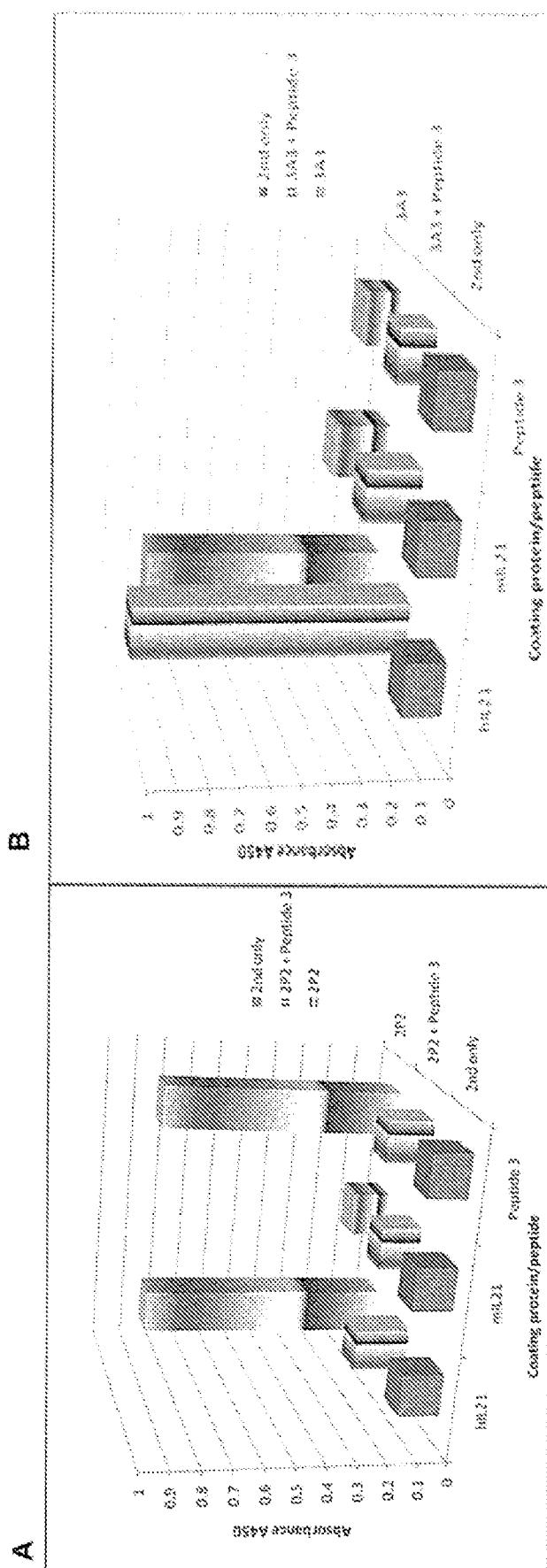


FIGURE 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2014/000673

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/24 (2006.01) A61K 39/395 (2006.01) A61P 35/00 (2006.01) A61P 37/02 (2006.01) A61P 37/08 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, Medline, HCaplus, Biosis: Interleukin-21, IL-21, immunoglobulin, antibody, antigen binding domain, agonist, infect, hypersensitive, allergy, cancer, checkpoint inhibitor and similar terms

GenomeQuest: SEQ ID NOS: 2-10, 23

PubMed, Google Patents, Espacenet, Patentscope: Applicant/Inventor Name Search.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
8 August 2014

Date of mailing of the international search report
08 August 2014

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INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2014/000673
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/100035 A2 (WYETH et al.) 13 August 2009 Abstract, paragraphs 13, 14, 78, 94, 107, 142, 144-147, 168, 169, 171-173, 201, 202	1-4, 8-22
X	WO 2006/105538 A2 (CENTOCOR, INC.) 05 October 2006 Paragraphs 7, 18, 19, 89, 90, 96, 98, 291	1-4, 8-22
X	WO 2005/112983 A2 (WYETH) 01 December 2005 Pages 20, 27-39	1-4, 8-22
X	WO 2008/112543 A2 (WYETH) 18 September 2008 Paragraphs 5, 64, claims 12, 14, 40-42	1-4, 8-14
A	SPOLSKI, R. and LEONARD, W.J. 'The Yin and Yang of Interleukin-21 in Allergy, Autoimmunity and Cancer', Current Opinion in Immunology, 2008, vol. 20, no. 3 pages 295-301 The whole document	1-22
A	VOGELZANG, A. et al. 'A Fundamental Role for Interleukin-21 in the Generation of T Follicular Helper Cells', Immunity, 2008, vol. 29, pages 127-137 The whole document	1-22
A	WO 2006/057027 A1 (GIULIANI INTERNATIONAL LIMITED) 01 June 2006 Pages 7 and 8	5
A	US 2012/0225065 A1 (JASPERS et al.) 06 September 2012 Example 17, paragraphs 274-285	5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2014/000673

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:
 - a. (means)
 on paper
 in electronic form
 - b. (time)
 in the international application as filed
 together with the international application in electronic form
 subsequently to this Authority for the purposes of search
2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:

because they relate to subject matter not required to be searched by this Authority, namely:

the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including

2. Claims Nos.: 23

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See Supplemental Box

3. Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

No protest accompanied the payment of additional search fees.

Supplemental Box**Continuation of Box II**

The claim does not comply with Rule 6.2(a) because it relies on references to the description and/or drawings.

INTERNATIONAL SEARCH REPORT Information on patent family members			International application No. PCT/AU2014/000673
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2009/100035 A2	13 August 2009	None	
WO 2006/105538 A2	05 October 2006	None	
WO 2005/112983 A2	01 December 2005	AU 2005244942 A1 BR PI0510996 A CA 2566333 A1 CN 1980698 A EC SP067014 A EP 1753458 A2 JP 2008501042 A KR 20070014181 A MX PA06013483 A NO 20065487 A RU 2006138704 A US 2006024268 A1 ZA 200609600 A	01 Dec 2005 04 Dec 2007 01 Dec 2005 13 Jun 2007 29 Dec 2006 21 Feb 2007 17 Jan 2008 31 Jan 2007 23 Jan 2007 18 Dec 2006 27 Jun 2008 02 Feb 2006 28 Apr 2010
WO 2008/112543 A2	18 September 2008	US 2008267910 A1	30 Oct 2008
WO 2006/057027 A1	01 June 2006	AU 2005308411 A1 BR PI0516661 A CA 2587889 A1 CN 101137664 A EA 200701092 A1 EP 1817327 A1 IT RM20040586 A1 JP 2008521797 A KR 20070084407 A MX 2007006355 A NO 20073175 A US 2009214549 A1	01 Jun 2006 16 Sep 2008 01 Jun 2006 05 Mar 2008 28 Feb 2008 15 Aug 2007 28 Feb 2005 26 Jun 2008 24 Aug 2007 09 Jul 2007 23 Aug 2007 27 Aug 2009
US 2012/0225065 A1	06 September 2012	US 8361470 B2	29 Jan 2013

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

Form PCT/ISA/210 (Family Annex)(July 2009)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2014/000673

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
		AU 2008364115 A1	20 May 2010
		AU 2008364115 B2	21 Nov 2013
		CA 2707026 A1	20 May 2010
		CN 102027011 A	20 Apr 2011
		CN 103772502 A	07 May 2014
		EP 2217268 A2	18 Aug 2010
		EP 2426146 A2	07 Mar 2012
		JP 2011506422 A	03 Mar 2011
		KR 20100097724 A	03 Sep 2010
		MX 2010006181 A	01 Jul 2010
		RU 2010123049 A	20 Jan 2012
		US 2009191214 A1	30 Jul 2009
		US 7883700 B2	08 Feb 2011
		US 2010041873 A1	18 Feb 2010
		US 8124089 B2	28 Feb 2012
		US 2012177655 A1	12 Jul 2012
		US 8226948 B1	24 Jul 2012
		US 2011130548 A1	02 Jun 2011
		US 8241629 B2	14 Aug 2012
		WO 2010055366 A2	20 May 2010

End of Annex