

The present invention thus seeks to provide improved antibodies for use in the diagnosis and treatment of diseases and conditions associated with the IL1RAP biomarker and/or responsive to inhibition of IL-1 and/or IL-33 signalling

Summary

In one aspect, the present invention refers to a monoclonal antibody or an antigen-binding fragment thereof with binding specificity for human interleukin-1 receptor accessory protein (IL1RAP) comprising (i) a heavy chain variable region comprising the three CDRs of a variable region as defined by SEQ ID NO:1, or of a humanised version thereof as defined by SEQ ID NO: 8, 9, 10 or 11 and (ii) a light chain variable region comprising the three CDRs of a variable region as defined by SEQ ID NO:2, or of a humanised version thereof as defined by SEQ ID NO: 15, 16 or 17.

In another aspect, the present invention refers to a pharmaceutical composition comprising an effective amount of an antibody or antigen-binding fragment thereof according to the present invention and a pharmaceutically-acceptable diluent, carrier or excipient.

In yet another aspect, the present invention refers to an *in vitro* method for the detection of cancer cells in a subject, the method comprising:

- (a) providing a sample of cells from a subject to be tested;
- (b) optionally, extracting and/or purifying the cells present in the sample;
- (c) contacting an antibody or antigen-binding fragment thereof according to any one of Claims 1 to 7 with cells present in the sample;
- (d) determining whether the antibody or antigen-binding fragment thereof binds to the cells

wherein the binding of the antibody or antigen-binding fragment thereof to the cells is indicative of the presence of cancer cells in the tissue of a subject.

In another aspect, the present invention refers to an *in vitro* method for identifying a patient with cancer who would benefit from treatment with an antibody or antigen-binding fragment thereof according to the present invention, the method comprising:

- (a) providing a sample of cancer cells from a patient to be tested;
- (b) optionally, extracting and/or purifying the cells present in the sample;

- (c) contacting an antibody or antigen-binding fragment thereof according to the present invention with cells present in the sample;
- (d) determining whether the antibody or antigen-binding fragment thereof binds to the cells

wherein the binding of the antibody or antigen-binding fragment thereof to the cancer cells is indicative of a patient who would benefit from treatment with an antibody or antigen-binding fragment thereof according to the present invention.

A first example of the disclosure provides an antibody or an antigen-binding fragment thereof ('antibody polypeptides') with binding specificity for interleukin-1 receptor accessory protein ('IL1RAP'), wherein the antibody or antigen-binding fragment is capable of inhibiting the binding of reference antibody 'CAN04' to human IL1RAP.

By "interleukin-1 receptor accessory protein", "IL1RAP" and "IL1-RAP" we specifically include the human IL1RAP protein, for example as described in GenBank Accession No. AAB84059, NCBI Reference Sequence: NP_002173.1 and UniProtKB/Swiss-Prot Accession No. Q9NPH3-1 (see also Huang *et al.*, 1997, *Proc. Natl. Acad. Sci. USA*. **94** (24), 12829-12832, the disclosures of which are incorporated herein by reference). IL1RAP is also known in the scientific literature as IL1R3, C3orf13, FLJ37788, IL-1RAcP and EG3556.

Thus, the antibody polypeptides of the disclosure have specificity for IL1RAP. By "specificity" we mean that the antibody polypeptide is capable of binding to IL1RAP *in vivo*, *i.e.* under the physiological conditions in which IL1RAP exists within the human body. Preferably, the antibody polypeptide does not bind to any other protein *in vivo*. Such binding specificity may be determined by methods well known in the art, such as ELISA, immunohistochemistry, immunoprecipitation, Western blots and flow cytometry using transfected cells expressing IL1RAP. Advantageously, the antibody polypeptide is capable of binding selectively to IL1RAP, *i.e.* it bind at least 10-fold more strongly to IL1RAP than to any other proteins.

By "reference antibody 'CAN04'" we include an intact IgG antibody comprising heavy and light chain variable regions having the amino acid sequences of SEQ ID NOS: 1 and 2, respectively. Unless otherwise stated, references herein to "CAN04" refer to an intact IgG antibody comprising (a) a heavy chain comprising a variable region as defined by SEQ ID NO:1 and a constant region as defined by SEQ ID NO: 19, and (b) a light chain comprising a variable region as defined by SEQ ID NO:2 and a constant region as defined by SEQ ID

NO: 18. Alternatively, a humanised version of CAN04 ('hCAN04') may be used as the reference antibody. For example, the reference antibody may be an intact IgG antibody comprising (a) a heavy chain comprising a variable region as defined by any one of SEQ ID NOS:8 to 11 and a constant region as defined by SEQ ID NO: 19, and (b) a light chain comprising a variable region as defined by any one of SEQ ID NOS:15 to 17 and a constant region as defined by SEQ ID NO: 18.

As discussed below, the reference antibody 'CAN04' binds to domain 2 of IL1RAP. Thus, it will be appreciated that the antibody or an antigen-binding fragment of the disclosure also binds to domain 2 of IL1RAP

By "capable of inhibiting the binding of reference antibody 'CAN04' to human IL1RAP" we mean that the presence of the antibody polypeptides of the disclosure inhibits, in whole or in part, the binding of 'CAN04' to human IL1RAP. Such competitive binding inhibition can be determined using assays and methods well known in the art, for example using BIAcore chips with immobilised IL1RAP and incubating with the reference antibody 'CAN04' with and without an antibody polypeptide to be tested. Alternatively, a pair-wise mapping approach can be used, in which the reference antibody 'CAN04' is immobilised to the surface of the BIAcore chip, IL1RAP antigen is bound to the immobilised antibody, and then a second antibody is tested for simultaneous IL1RAP-binding ability (see 'BIAcore Assay Handbook', GE Healthcare Life Sciences, 29-0194-00 AA 05/2012; the disclosures of which are incorporated herein by reference).

In a further alternative, competitive binding inhibition can be determined using flow cytometry. For example, to test whether a test antibody is able to inhibit the binding of the CAN04 reference antibody to a cell surface antigen, cells expressing the antigen can be pre-incubated with the test antibody for 20 min before cells are washed and incubated with the reference CAN04 antibody conjugated to a fluorophore, which can be detected by flow cytometry. If the pre-incubation with the test antibody reduces the detection of the reference CAN04 antibody in flow cytometry, the test antibody inhibits the binding of the reference antibody to the cell surface antigen. If the antibody to be tested exhibits high affinity for IL1RAP, then a reduced pre-incubation period may be used (or even no pre-incubation at all).

In a further alternative, competitive binding inhibition can be determined using an ELISA (e.g. as described in Example J).

By "an antibody or an antigen-binding fragment thereof" we include substantially intact antibody molecules, as well as chimaeric antibodies, humanised antibodies, isolated human antibodies, single chain antibodies, bispecific antibodies, antibody heavy chains, antibody light chains, homodimers and heterodimers of antibody heavy and/or light chains, and antigen-binding fragments and derivatives of the same. Suitable antigen-binding fragments and derivatives include, but are not necessarily limited to, Fv fragments (e.g. single chain Fv and disulphide-bonded Fv), Fab-like fragments (e.g. Fab fragments, Fab' fragments and F(ab)₂ fragments), single variable domains (e.g. V_H and V_L domains) and domain antibodies (dAbs, including single and dual formats [i.e. dAb-linker-dAb]). The potential advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments may lead to improved pharmacological properties, such as better penetration of solid tissue. Moreover, antigen-binding fragments such as Fab, Fv, ScFv and dAb antibody fragments can be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of the said fragments.

The phrase "an antibody or an antigen-binding fragment thereof" is also intended to encompass antibody mimics (for example, non-antibody scaffold structures that have a high degree of stability yet allow variability to be introduced at certain positions). Those skilled in the art of biochemistry will be familiar with many such molecules, as discussed in Gebauer & Skerra, 2009, *Curr Opin Chem Biol* **13**(3): 245–255 (the disclosures of which are incorporated herein by reference). Exemplary antibody mimics include: affibodies (also called Trinetins; Nygren, 2008, *FEBS J*, 275, 2668-2676); CTLDs (also called Tetranectins; *Innovations Pharmac. Technol.* (2006), 27-30); adnectins (also called monobodies; *Meth. Mol. Biol.*, 352 (2007), 95-109); anticalins (*Drug Discovery Today* (2005), 10, 23-33); DARPins (ankyrins; *Nat. Biotechnol.* (2004), 22, 575-582); avimers (*Nat. Biotechnol.* (2005), 23, 1556-1561); microbodies (*FEBS J*, (2007), 274, 86-95); peptide aptamers (*Expert. Opin. Biol. Ther.* (2005), 5, 783-797); Kunitz domains (*J. Pharmacol. Exp. Ther.* (2006) 318, 803-809); affilins (*Trends. Biotechnol.* (2005), 23, 514-522); affimers (Avacta Life Sciences, Wetherby, UK).

Also included within the scope of the disclosure are chimeric T-cell receptors (also known as chimeric T cell receptors, chimeric immunoreceptors, and chimeric antigen receptors or CARs) (see Pule *et al.*, 2003, *Cytotherapy* **5**(3):211–26, the disclosures of which are incorporated herein by reference). These are engineered receptors, which graft an arbitrary specificity onto an immune effector cell. Typically, CARs are used to graft the

specificity of a monoclonal antibody onto a T cell; with transfer of their coding sequence facilitated by retroviral vectors. The most common form of such molecules is fusions comprising a single-chain variable fragment (scFv) derived from a monoclonal antibody fused to CD3-zeta transmembrane and endodomain. When T cells express this fusion molecule, they recognize and kill target cells that express the transferred monoclonal antibody specificity.

Persons skilled in the art will further appreciate that the disclosure also encompasses modified versions of antibodies and antigen-binding fragments thereof, whether existing now or in the future, e.g. modified by the covalent attachment of polyethylene glycol or another suitable polymer (see below).

Methods of generating antibodies and antibody fragments are well known in the art. For example, antibodies may be generated via any one of several methods which employ induction of *in vivo* production of antibody molecules, screening of immunoglobulin libraries (Orlandi. *et al*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86:3833-3837; Winter *et al.*, 1991, *Nature* 349:293-299, the disclosures of which are incorporated herein by reference) or generation of monoclonal antibody molecules by cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the Epstein-Barr virus (EBV)-hybridoma technique (Kohler *et al.*, 1975. *Nature* 256:495-497; Kozbor *et al.*, 1985. *J. Immunol. Methods* 81:31-42; Cote *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80:2026-2030; Cole *et al.*, 1984. *Mol. Cell. Biol.* 62:109-120, the disclosures of which are incorporated herein by reference).

Suitable methods for the production of monoclonal antibodies are also disclosed in "Monoclonal Antibodies: A manual of techniques", H Zola (CRC Press, 1988, the disclosures of which are incorporated herein by reference) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications", J G R Hurrell (CRC Press, 1982, the disclosures of which are incorporated herein by reference).

Likewise, antibody fragments can be obtained using methods well known in the art (see, for example, Harlow & Lane, 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, New York, the disclosures of which are incorporated herein by reference). For example, antibody fragments according to the present disclosure can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* or mammalian cells (e.g. Chinese hamster ovary cell culture or other protein expression systems) of DNA

encoding the fragment. Alternatively, antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods.

The antibodies of the disclosure are defined by reference to the variable regions of a murine-derived antibody, designated 'CAN04', which comprises:

(a) a heavy chain variable region having the amino acid sequence of SEQ ID NO: 1:

Q V Q L Q Q S G P E L L K P G A S V K I S C K A S
G Y A F S S S W M N W V K Q R P G K G L E W I G
R I Y P G D G N T H Y S G K F K G K A T L T A D K
S S S I A Y M Q L S S L T S E D S A V Y F C G E G
Y L D P M D Y W G Q G T S V T V S S

[SEQ ID NO:1]

and

(b) a light chain variable region having the amino acid sequence of SEQ ID NO: 2:

D I Q M T Q T T S S L S A S L G D R V T I S C S A
S Q G I N N Y L N W Y Q Q K P D G T V K L L I H Y
T S G L H A G V P S R F S G S G S G T D Y S L T I
S N L E P E D V A T Y Y C Q Q Y S I L P W T F G G
G T K L E I K R

[SEQ ID NO:2]

The term "amino acid" as used herein includes the standard twenty genetically-encoded amino acids and their corresponding stereoisomers in the 'D' form (as compared to the natural 'L' form), omega-amino acids and other naturally-occurring amino acids, unconventional amino acids (e.g. α,α -disubstituted amino acids, N-alkyl amino acids, *etc.*) and chemically derivatised amino acids (see below).

When an amino acid is being specifically enumerated, such as "alanine" or "Ala" or "A", the term refers to both L-alanine and D-alanine unless explicitly stated otherwise. Other unconventional amino acids may also be suitable components for polypeptides of the present disclosure, as long as the desired functional property is retained by the polypeptide. For the peptides shown, each encoded amino acid residue, where appropriate, is

represented by a single letter designation, corresponding to the trivial name of the conventional amino acid.

In one example, the antibody polypeptides as defined herein comprise or consist of L-amino acids.

It will be appreciated by persons skilled in the art that any intact IgG antibody comprising the above variable regions may be used as the reference antibody to identify antibody polypeptides of the disclosure that competitively inhibit CAN04 binding to IL1RAP.

Thus, in one example, the CAN04 antibody used as a reference to determined competitive binder is an intact IgG antibody comprising:

(a) a heavy chain comprising a variable domain of SEQ ID NO:1 grafted on to a murine IgG1 or IgG2a constant region

(b) a light chain comprising a variable domain of SEQ ID NO:2 grafted on to a murine kappa constant region.

Alternatively, the reference antibody may be a chimeric, intact IgG antibody comprising:

(a) a heavy chain comprising a variable domain of SEQ ID NO:1 grafted on to an human IgG1 constant region (for example, as encoded by the pFUSEss-CHlg-hG1 vector InvivoGen, San Diego, USA)

(b) a light chain comprising a variable domain of SEQ ID NO:2 grafted on to a human kappa constant region (for example, as encoded by the pFUSE2ss-CLlg-hk vector InvivoGen, San Diego, USA).

Competitive binding typically arises because the test antibody binds at, or at least very close to, the epitope on the antigen to which binds the reference antibody (in this case, CAN04). However, it will be appreciated by persons skilled in the art that competitive binding may also arise by virtue of steric interference; thus, the test antibody may bind at an epitope different from that to which the reference antibody binds but may still be of sufficient size or configuration to hinder the binding of the reference antibody to the antigen.

The antibodies and antigen-binding fragments of the present disclosure were identified after extensive screening of a large number of anti-IL1RAP antibodies, on the basis of exhibiting properties that make them particularly suitable as diagnostic and therapeutic agents for cancer.

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Thus, in one example, the antibody or antigen-binding fragment exhibits one or more of the following properties:

- 10 (a) a binding affinity (K_D) for human IL1RAP of 200 pM or greater, *i.e.* the $K_D \leq 200$ pM (for example, as determined in Example A);
- (b) cross-reactivity with IL1RAP from *Macaca fascicularis* (for example, as determined in Example D);
- (c) an inhibitory action on IL1 signalling (IL-1 α and/or IL-1 β ; for example, as determined in Example E);
- 15 (d) capability of inducing antibody-dependent cell-mediated cytotoxicity (ADCC) in one or more cancer cell lines (such as a CML, ALL and/or melanoma cell lines) (for example, as determined in Example F); and/or
- (e) capability of internalisation upon binding to one or more cancer cell lines (such as a CML, AML and/or melanoma cell line) (for example, as determined in
- 20 Example G).

Advantageously, the antibody or antigen-binding fragment exhibits all of the above properties.

- 25 In an alternative example, the antibody or antigen-binding fragment exhibits one or more of properties (a), (b), (c) and (e) above, but is *not* capable of inducing ADCC.

In one example, the antibody or antigen-binding fragment is capable of binding to an epitope on the extracellular domain of IL1RAP which overlaps, at least in part, with the
30 epitope on IL1RAP to which reference antibody CAN04 is capable of binding. Thus, the antibody or antigen-binding fragment may be capable of binding to an epitope located at/within domain 2 of IL1RAP (see Wang *et al.*, 2010, *Nature Immunology*, 11:905-912, the disclosures of which are incorporated herein by reference), *i.e.* within amino acids 135 to 234 of IL1RAP (see Accession No. Q9NPH3 within UniProtKB/Swiss-Prot). For example,
35 the epitope to which the antibody or antigen-binding fragment may be located

within amino acids 135 to 154, 155 to 174, 175 to 194, 195 to 214 or between amino acids 215 to 234 of IL1RAP. However, it will be appreciated that the epitope may be non-linear.

5 In one example, the antibody polypeptide of the disclosure comprises or consists of an intact antibody (such as an IgG1 antibody).

In an alternative example, the antibody polypeptide of the disclosure comprises or consists of an antigen-binding fragment selected from the group consisting of Fv fragments (e.g. single chain Fv and disulphide-bonded Fv), Fab-like fragments (e.g. Fab fragments, 10 Fab' fragments and F(ab)₂ fragments) and domain antibodies (e.g. single V_H variable domains or V_L variable domains).

In a further example, as discussed above, the polypeptide of the disclosure comprises or consists of an antibody mimic selected from the group comprising or consisting of affibodies, 15 tetranectins (CTLDs), adnectins (monobodies), anticalins, DARPins (ankyrins), avimers, iMabs, microbodies, peptide aptamers, Kunitz domains and affilins.

In a preferred example, the antibody or antigen-binding fragment thereof according to the first example of the disclosure comprises a heavy chain variable region comprising the 20 following CDRs:

- a) GYAFSSS [SEQ ID NO: 3] or an amino acid sequence having at least 60% sequence identity therewith, for example at least 70%, 80%, or 90% sequence identity;
- 25 b) YPGDGN [SEQ ID NO: 4] or an amino acid sequence having at least 60% sequence identity therewith, for example at least 70%, 80%, or 90% sequence identity; and/or
- c) GYLDPMDY [SEQ ID NO: 5] or an amino acid sequence having at least 60% sequence identity therewith, for example at least 70%, 80%, or 90% sequence 30 identity.

Thus, the antibody or antigen-binding fragment thereof may comprise a heavy chain variable region comprising the CDRs of SEQ ID NOs 3, 4 and 5.

For example, the antibody or antigen-binding fragment thereof may comprise a heavy 35 chain variable region having the amino acid sequence of the corresponding region of the CAN04 reference antibody, i.e. SEQ ID NO:1.

However, it will be appreciated that a low level of mutation (typically, just one or two amino acids) within a CDR sequence may be tolerated without loss of the specificity of the antibody or antigen-binding fragment for IL1RAP.

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Percent identity can be determined by, for example, the LALIGN program (Huang and Miller, *Adv. Appl. Math.* (1991) 12:337-357, the disclosures of which are incorporated herein by reference) at the Expasy facility site (http://www.ch.embnet.org/software/LALIGN_form.html) using as parameters the global alignment option, scoring matrix BLOSUM62, opening gap penalty -14, extending gap penalty -4. Alternatively, the percent sequence identity between two polypeptides may be determined using suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group and it will be appreciated that percent identity is calculated in relation to polypeptides whose sequence has been aligned optimally.

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The alignment may alternatively be carried out using the Clustal W program (as described in Thompson *et al.*, 1994, *Nucl. Acid Res.* 22:4673-4680, which is incorporated herein by reference). The parameters used may be as follows:

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- Fast pair-wise alignment parameters: K-tuple(word) size; 1, window size; 5, gap penalty; 3, number of top diagonals; 5. Scoring method: x percent.
- Multiple alignment parameters: gap open penalty; 10, gap extension penalty; 0.05.
- Scoring matrix: BLOSUM.

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Alternatively, the BESTFIT program may be used to determine local sequence alignments.

In a further preferred example, the antibody or antigen-binding fragment thereof according to the first example of the disclosure comprises a heavy chain variable region comprising the following CDRs:

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- a) GYAFSSSWMN [SEQ ID NO: 6] or an amino acid sequence having at least 60% sequence identity therewith, for example at least 70%, 80%, or 90% sequence identity;

Methods for humanising non-human antibodies are well known in the art. Generally, the humanised antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues, often referred to as imported residues, are typically taken from an imported variable domain. Humanisation can be essentially performed as described (see, for example, Jones *et al.*, 1986, *Nature* 321:522-525; Reichmann *et al.*, 1988, *Nature* 332:323-327; Verhoeyen *et al.*, 1988, *Science* 239:1534-1536; US 4,816,567, the disclosures of which are incorporated herein by reference) by substituting human complementarity determining regions with corresponding rodent complementarity determining regions. Accordingly, such humanised antibodies are chimaeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanised antibodies may be typically human antibodies in which some complementarity determining region residues and possibly some framework residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be identified using various techniques known in the art, including phage display libraries (see, for example, Hoogenboom & Winter, 1991, *J. Mol. Biol.* 227:381; Marks *et al.*, 1991, *J. Mol. Biol.* 222:581; Cole *et al.*, 1985, In: *Monoclonal antibodies and Cancer Therapy*, Alan R. Liss, pp. 77; Boerner *et al.*, 1991, *J. Immunol.* 147:86-95, the disclosures of which are incorporated herein by reference).

Thus, the antibody or antigen-binding fragment thereof of the disclosure may be humanised, for example it may comprise a heavy chain variable region having one of the following amino acid sequence of any one of SEQ ID NOs: 8 to 11 or an amino acid sequence having at least 90% sequence identity therewith:

a) Q V Q L V Q S G A E V K K P G S S V K V S C K
A S G Y A F S S S W M N W V R Q A P G Q G L E
W M G R I Y P G D G N T H Y A Q K F Q G R V T
L T A D K S T S T A Y M E L S S L R S E D T A V
Y Y C G E G Y L D P M D Y W G Q G T L V T V S
S [SEQ ID NO: 8];

b) Q V Q L V Q S G A E V K K P G S S V K V S C K
 A S G Y A F T S S W M N W V R Q A P G Q G L E
 W M G R I Y P G D G N T H Y A Q K F Q G R V T
 L T A D K S T S T A Y M E L S S L R S E D T A V
 Y Y C G E G Y L D P M D Y W G Q G T L V T V S
 S

[SEQ ID NO: 9];

c) Q V Q L V Q S G A E V K K P G S S V K V S C K
 A S G Y T F T S S W M N W V R Q A P G K G L E
 W M G R I Y P G D G Q T H Y A Q K F Q G R V T
 L T A D K S T S T A Y M E L S S L R S E D T A V
 Y Y C G E G Y L D P M D Y W G Q G T L V T V S
 S

[SEQ ID NO: 10]; or

d) Q V Q L V Q S G A E V K K P G S S V K V S C K
 A S G Y T F T S S W M N W V R Q A P G K G L E
 W M G R I Y P G D G Q T H Y A Q K F Q G R V T
 I T A D K S T S T A Y M E L S S L R S E D T A V
 Y Y C G E G Y L D P M D Y W G Q G T L V T V S
 S

[SEQ ID NO: 11].

For example, the antibody or antigen-binding fragment thereof may comprise a heavy chain variable region having an amino acid sequence of any one of SEQ ID NOs: 8 to 11.

In a related preferred example, the antibody or antigen-binding fragment thereof comprises a light chain variable region comprising the following CDRs:

a) SASQGINNYLN [SEQ ID NO: 12] or an amino acid sequence having at least 60% sequence identity therewith, for example at least 70%, 80%, or 90% sequence identity;

b) YTSGLHA [SEQ ID NO: 13] or an amino acid sequence having at least 60% sequence identity therewith, for example at least 70%, 80%, or 90% sequence identity; and/or

c) QQYSILPWT [SEQ ID NO: 14] or an amino acid sequence having at least 60% sequence identity therewith, for example at least 70%, 80%, or 90% sequence identity.

5 Thus, the antibody or antigen-binding fragment thereof may comprise a light chain variable region comprising the CDRs of SEQ ID NOs 12, 13 and 14.

For example, the antibody or antigen-binding fragment thereof may comprise a light chain variable region having the amino acid sequence of the corresponding region of the murine
10 CAN04 reference antibody, *i.e.* SEQ ID NO:2.

As in the case of the heavy chain variable region detailed above, it will be appreciated that the light chain variable region of the antibody polypeptide of the disclosure may be humanised in order to produce agents more suitable for use in humans. For example, the
15 CDRs of SEQ ID NOs 12, 13 and 14 may be engrafted into a human variable region framework.

Thus, the antibody or antigen-binding fragment thereof may comprise a light chain variable region which comprises or consists of the amino acid sequence of any one of SEQ ID
20 NOs: 15 to 17 or an amino acid sequence having at least 90% sequence identity therewith:

a) D I Q M T Q S P S S L S A S V G D R V T I T C S
A S Q G I N N Y L N W Y Q Q K P G K A P K L L I
H Y T S G L H A G V P S R F S G S G S G T D Y
25 T L T I S S L Q P E D V A T Y Y C Q Q Y S I L P
W T F G G G T K V E I K R [SEQ ID NO: 15];

b) D I Q M T Q S P S S L S A S V G D R V T I T C Q
A S Q G I N N Y L N W Y Q Q K P G K A P K L L I
30 H Y T S G L H A G V P S R F S G S G S G T D Y
T L T I S S L E P E D V A T Y Y C Q Q Y S I L P
W T F G G G T K V E I K R [SEQ ID NO: 16]; or

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c) D I Q M T Q S P S S L S A S V G D R V T I T C Q
 A S Q G I N N Y L N W Y Q Q K P G K A P K L L I
 H Y T S G L H A G V P S R F S G S G S G T D F
 T L T I S S L E P E D V A T Y Y C Q Q Y S I L P
 W T F G G G T K V E I K R [SEQ ID NO: 17].

For example, the antibody or antigen-binding fragment thereof may comprise a light chain variable region having an amino acid sequence of any one of SEQ ID NOs: 15 to 17.

In one example, the antibody or antigen-binding fragment thereof comprises a murine heavy chain variable region which comprises or consists of the amino acid sequence of any one of SEQ ID NO: 1 and a murine light chain variable region which comprises or consists of the amino acid sequence of any one of SEQ ID NO: 2.

Alternatively, the antibody or antigen-binding fragment thereof may comprise a humanised heavy chain variable region which comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 8 to 11 and a humanised light chain variable region which comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 15 to 17.

For example, the antibody or antigen-binding fragment thereof may comprise:

- a) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 8 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 15;
- b) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 9 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 15;
- c) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 15;
- d) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 11 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 15;
- e) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 8 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 16;

- f) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 9 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 16;
- 5 g) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 16;
- h) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 11 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 16;
- 10 i) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 8 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 17;
- j) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 9 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 17;
- 15 k) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 17; or
- l) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 11 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 17.
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It will be appreciated by persons skilled in the art that the above-defined humanised antibodies or antigen-binding fragments of the disclosure may further comprise a heavy chain constant region, or part thereof (see below).

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In one example, the antibody polypeptide comprises a CH1, CH2 and/or CH3 region of an IgG heavy chain (such as an IgG1, IgG2, IgG3 or IgG4 heavy chain). Thus, the antibody polypeptide may comprise part or all of the constant regions from an IgG1 heavy chain.

30 For example, the antibody polypeptide may be a Fab fragment comprising CH1 and CL constant regions, combined with any of the above-defined heavy and light variable regions respectively.

Likewise, the above-defined antibodies or antigen-binding fragments of the disclosure may further comprise a light chain constant region, or part thereof (see below). For example, the antibody polypeptide may comprise a CL region from a kappa or lambda light chain.

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For example, the antibody polypeptide may comprise the following constant regions:

(a) Ig kappa chain C region (Homo sapiens) (UnitProt Accession No. P01834)

5 TVAAPSVFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN
 SQESVTEQDS KDSTYSLSST LTLISKADYEK HKVYACEVTH QGLSSPVTKS
 FNRGEC **[SEQ ID NO: 18]**

(b) Ig gamma-1 chain C region (Homo sapiens) (UnitProt Accession No. P01857)

15 ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
 HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP
 KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS
20 HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
 EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC
 LVKGFYPSDI AVEWESNGQP ENNYKTTTPV LDSDGSFFLY SKLTVDKSRW
25 QQGNVFSCSV MHEALHNHYT QKSLSLSPGK **[SEQ ID NO: 19]**

30 In an alternative example, naturally occurring variants of the above constant regions may be utilised (e.g. see Jefferis & Lefranc, 2009, *MAbs* 1(4):332–8, the disclosures of which are incorporated herein by reference). For example, the light chain constant region may comprise or consist of SEQ ID NO: 18 having a W40R and/or V83L mutation and/or the heavy chain constant region may comprise or consist of SEQ ID NO: 19 having a K97R, D239E and/or L241M mutation, or without the C-terminal lysine/K (wherein the position of
35 the amino acid mutations is defined using the Eu Numbering Scheme, which differs from the numbering in SEQ ID NOS: 18 and 19; see Edelman *et al.*, 1969, *Proc. Natl. Acad. Sci. USA*, 63:78–85, the disclosures of which are incorporated herein by reference).

Thus, exemplary antibody polypeptides of the disclosure comprise:

(a) a heavy chain comprising a variable region of SEQ ID NO: 1, 8, 9, 10 or 11 together with a constant region of SEQ ID NO: 19; and

(b) a light chain comprising a variable region of SEQ ID NO: 2, 15, 16 or 17 together with a constant region of SEQ ID NO: 18.

In a related example, the antibody polypeptide may comprise an antibody Fc-region (e.g. the CH2 and CH3 regions of an IgG heavy chain). It will be appreciated by a skilled person that the Fc portion may be from an IgG antibody, or from a different class of antibody (such as IgM, IgA, IgD or IgE). In one example, the Fc region is from an IgG1, IgG2, IgG3 or IgG4 antibody.

The Fc region may be naturally-occurring (e.g. part of an endogenously produced antibody) or may be artificial (e.g. comprising one or more point mutations relative to a naturally-occurring Fc region).

As is well documented in the art, the Fc region of an antibody mediates its serum half-life and effector functions, such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP).

Engineering the Fc region of a therapeutic monoclonal antibody or Fc fusion protein allows the generation of molecules that are better suited to the pharmacology activity required of them (Strohl, 2009, *Curr Opin Biotechnol* **20**(6):685-91, the disclosures of which are incorporated herein by reference).

(a) Engineered Fc regions for increased half-life

One approach to improve the efficacy of a therapeutic antibody is to increase its serum persistence, thereby allowing higher circulating levels, less frequent administration and reduced doses.

The half-life of an IgG depends on its pH-dependent binding to the neonatal receptor FcRn. FcRn, which is expressed on the surface of endothelial cells, binds the IgG in a pH-dependent manner and protects it from degradation.

CLAIMS

1. A monoclonal antibody or an antigen-binding fragment thereof with binding specificity for human interleukin-1 receptor accessory protein (IL1RAP) comprising
 - (i) a heavy chain variable region comprising the three CDRs of a variable region as defined by SEQ ID NO:1, or of a humanised version thereof as defined by SEQ ID NO: 8, 9, 10 or 11and
 - (ii) a light chain variable region comprising the three CDRs of a variable region as defined by SEQ ID NO:2, or of a humanised version thereof as defined by SEQ ID NO: 15, 16 or 17.
2. An antibody or antigen-binding fragment thereof according to Claim 1 wherein the antibody or antigen-binding fragment exhibits the following properties:
 - a) a binding affinity (K_D) for human IL1RAP of 200 pM or greater;
 - b) cross-reactivity with IL1RAP from *Macaca fascicularis*;
 - c) an inhibitory action on IL1 signalling;
 - d) capability of inducing ADCC in one or more cancer cell lines; and
 - e) capability of internalisation upon binding to one or more cancer cell lines.
3. An antibody or antigen-binding fragment thereof according to Claim 1 or 2 comprising a heavy chain variable region SEQ ID NO:1 and a light chain variable region having the amino acid sequence of SEQ ID NO:2.
4. An antibody or antigen-binding fragment thereof according to Claim 1 or 2 comprising:
 - a) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 8 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 15;
 - b) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 9 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 15;

- c) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 15;
 - d) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 11 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 15;
 - e) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 8 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 16;
 - f) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 9 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 16;
 - g) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 16;
 - h) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 11 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 16;
 - i) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 8 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 17;
 - j) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 9 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 17;
 - k) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 17; or
 - l) a heavy chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 11 and a light chain variable region which comprises or consists of the amino acid sequence of SEQ ID NO: 17.
5. An antibody or antigen-binding fragment thereof according to any one of the preceding claims comprising a heavy chain constant region, or part thereof, and a light chain constant region, or part thereof.
6. An antibody or antigen-binding fragment thereof according to any one of the preceding claims comprising an Fc region.

7. An antibody or antigen-binding fragment thereof according to any one of the preceding claims further comprising a cytotoxic moiety and/or a detectable moiety.
8. A pharmaceutical composition comprising an effective amount of an antibody or antigen-binding fragment thereof according to any one of Claims 1 to 7 and a pharmaceutically-acceptable diluent, carrier or excipient.
9. An antibody or antigen-binding fragment thereof according to any one of Claims 1 to 7 for use in medicine.
10. An antibody or antigen-binding fragment thereof according to any one of Claims 1 to 7 for use in the treatment of a neoplastic disorder in a subject, wherein the neoplastic disorder is associated with cells expressing IL1RAP.
11. An antibody or antigen-binding fragment thereof for use in the treatment of a neoplastic disorder according to Claim 10 wherein the neoplastic disorder is a neoplastic hematologic disorder or is associated with the formation of solid tumours within the subject's body.
12. An antibody or antigen-binding fragment thereof for use in the treatment of a neoplastic disorder according to Claim 11 wherein the neoplastic disorder is selected from the group consisting of (a) chronic myeloid leukemia (CML), myeloproliferative disorders (MPD), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) or (b) prostate cancer, breast cancer, lung cancer, colorectal cancer, melanomas, bladder cancer, brain/CNS cancer, cervical cancer, oesophageal cancer, gastric cancer, head/neck cancer, kidney cancer, liver cancer, lymphomas, ovarian cancer, pancreatic cancer, and sarcomas.
13. An antibody or antigen-binding fragment thereof according to any one of Claims 1 to 7 for use in the treatment of a disease or condition susceptible to treatment with an inhibitor of IL-1 signalling.

14. An *in vitro* method for the detection of cancer cells in a subject, the method comprising:

- (a) providing a sample of cells from a subject to be tested;
- (b) optionally, extracting and/or purifying the cells present in the sample;
- (c) contacting an antibody or antigen-binding fragment thereof according to any one of Claims 1 to 7 with cells present in the sample;
- (d) determining whether the antibody or antigen-binding fragment thereof binds to the cells

wherein the binding of the antibody or antigen-binding fragment thereof to the cells is indicative of the presence of cancer cells in the tissue of a subject.

15. An *in vitro* method for identifying a patient with cancer who would benefit from treatment with an antibody or antigen-binding fragment thereof according to any one of Claims 1 to 7, the method comprising:

- (a) providing a sample of cancer cells from a patient to be tested;
- (b) optionally, extracting and/or purifying the cells present in the sample;
- (c) contacting an antibody or antigen-binding fragment thereof according to any one of Claims 1 to 7 with cells present in the sample;
- (d) determining whether the antibody or antigen-binding fragment thereof binds to the cells

wherein the binding of the antibody or antigen-binding fragment thereof to the cancer cells is indicative of a patient who would benefit from treatment with an antibody or antigen-binding fragment thereof according to any one of Claims 1 to 7.