

antibodies (PRL3-zumab) instead of mouse or chimeric antibodies; 2) targeting of human cancer cell lines instead of mouse cancer cell lines; 3) the development of more clinically-relevant orthotopic gastric tumor models instead of mouse tail vein metastatic models; and 4) the identification of a potential surrogate biomarker for monitoring of PRL3-zumab therapeutic efficacy. We demonstrate the first example of a new class of humanized antibody to block gastric tumorigenesis. Our findings reveal the potential of targeting intracellular oncoproteins with antibody therapy, ushering in a new era of cancer therapeutics.

Summary

Off-target effects are major clinical concerns for cancer therapies. We generated a first-in-class humanized antibody (PRL3-zumab) against tumor-specific intracellular PRL-3, an oncogenic phosphatase upregulated in multiple human cancers. We focused on gastric cancer (GC), providing independent evidence that elevated *PRL-3* mRNA levels significantly correlate with shortened overall survival of GC patients. PRL-3 protein was overexpressed in 85% of fresh-frozen GC tumors, but not in patient-matched normal gastric tissues examined. Using human GC cell lines, we established clinically relevant orthotopic gastric tumor models and demonstrated that PRL3-zumab specifically blocked growth of PRL-3-positive (PRL-3⁺), but not PRL-3-negative (PRL-3⁻) tumors. PRL-3-zumab had better therapeutic efficacy as a monotherapy than in combination with 5-fluorouracil (5-FU), or 5-FU alone. PRL3-zumab was specifically enriched in PRL-3⁺ tumor tissues and promoted immune cell recruitment to PRL-3⁺ tumor microenvironments. Unexpectedly, we found secreted PRL-3 oncoprotein in 62% of multiple types of human cancer urines and in 100% of cancer urines derived from PRL-3⁺, but not PRL-3⁻ tumor-bearing mice. Furthermore, urinary PRL-3 levels were significantly reduced after effective treatment with PRL3-zumab. The Urinary PRL-3 could be considered as a potential diagnostic and a surrogate biomarker for therapeutic response monitoring of PRL3-zumab therapy in multiple cancer types in future.

We also investigated the mechanism of action (MOA) to address how PRL-3 antibody could possibly bind to its intracellular PRL-3 antigen, and conclude that indeed 'Intracellular oncoprotein' can be re-localized to the cell surface as 'Extracellular oncoprotein' in cancer, thus follow a rational basis for tumor elimination via antibody conventional pathways against Extracellular Oncotargets.

Consistently, we found that PRL3-zumab blocks tumors expressing PRL-3 'Intracellular' antigen, requiring host FcγII/III receptor interaction, full antibody activities, and increased M1 (but not M2) macrophages, B lymphocytes, natural killer cells to enhance host immunity. These results suggest the MOA of antibody targeting 'Intracellular oncoprotein' is indeed following the similar principles of targeting 'Extracellular Oncoprotein' via classical antibody-dependent cell cytotoxicity (ADCC) or phagocytotic (ADCP) pathways to eliminate tumors.

Finally, using 110 precious fresh-frozen human tumors or their matched normal tissues, we further showed that PRL-3 is an excellent tumor-specific oncotarget broadly overexpressed on an average $\geq 78\%$ from 9 different human cancer types: liver, lung, colon, breast, stomach, bladder, prostate, AML, and kidney patient tumor samples, but not in matched normal tissues. PRL-3 may therefore be a useful biomarker of cancer, and a near-universal target for cancer therapy. PRL-3 may therefore provide a useful biomarker for solid cancers.

The present disclosure is concerned with antibodies, or antigen binding fragments, that bind to PRL3. Heavy and light chain polypeptides are also disclosed. The antibodies, antigen binding fragments and polypeptides may be provided in isolated and/or purified form and may be formulated into compositions suitable for use in research, therapy and diagnosis. In particular, the disclosure is concerned with humanized antibodies that bind PRL3, and in particular PRL3 antagonist antibodies.

In some cases, the antibodies of the disclosure inhibit a function of PRL3. In some cases, the antibodies inhibit a protein tyrosine phosphatase (PTP) function of PRL3. In some cases, the antibodies induce ADCC and/or ADCP. In some cases, the antibodies are capable of binding to Fc receptors, such as FcγRII and/or FcγRIII. In some cases, binding of the antibody to the cell leads to the recruitment of immune cells to the cell, such as B cells, NK cells, or macrophages, preferably M1 macrophages.

In one aspect, the present disclosure refers to a humanised antibody or antigen binding fragment that binds PRL3, the antibody having the amino acid sequences i) to vi):

- i) KASQSVEDDGENYMN (SEQ ID NO:4)
- ii) AASNLES (SEQ ID NO:5)
- iii) QQSNEPFT (SEQ ID NO:6)
- iv) GYTFTNYMH (SEQ ID NO:1)
- v) WIYPGNVNTYYNEKFRG (SEQ ID NO:2)

vi) EEKNYPWFAY (SEQ ID NO:3)

or a variant thereof in which one, two or three amino acids in one or more of the sequences i) to vi) are replaced with another amino acid.

5 In another aspect, the present disclosure refers to an antibody or antigen binding fragment comprising heavy chain having the sequence of any one of SEQ ID NO: 16-25 and a light chain having the sequence of any one of SEQ ID NO: 7-15. In another aspect, the present disclosure refers to the use of a humanised antibody, antigen binding fragment according to any one of the preceding claims in the manufacture of a
10 medicament for the treatment of a PRL3 expressing cancer. In another aspect, the present disclosure refers to a humanised antibody or antigen binding fragment according to any one of the preceding claims for use in the treatment of a PRL3 expressing cancer.

In a further aspect, the present disclosure refers to the use of a humanised antibody or
15 antigen binding fragment as described herein in the manufacture of a medicament for the treatment of a PRL3 expressing cancer, wherein said use comprises that the humanised antibody or antigen binding fragment is to be administered to a patient. In a further aspect, the present disclosure refers to an *in vitro* method comprising determining the cellular localisation of PRL3 in a cell obtained from an individual, wherein the method is
20 an immunoassay using an antibody as described herein, and wherein expression of PRL3 at the cell surface indicates that the cell is cancerous.

In one example of the present disclosure an antibody, or antigen binding fragment, is provided, the amino acid sequence of the antibody may comprise the amino acid
25 sequences i) to iii), or the amino acid sequences iv) to vi), or preferably the amino acid sequences i) to vi):

- | | | | |
|----|------|-------------------|---------------|
| | i) | KASQSVEDDGENYMN | (SEQ ID NO:4) |
| | ii) | AASNLES | (SEQ ID NO:5) |
| | iii) | QQSNEDPFT | (SEQ ID NO:6) |
| 30 | iv) | GYTFTNYMH | (SEQ ID NO:1) |
| | v) | WIYPGNVNTYYNEKFRG | (SEQ ID NO:2) |
| | vi) | EEKNYPWFAY | (SEQ ID NO:3) |

or a variant thereof in which one or two or three amino acids in one or more of the sequences (i) to (vi) are replaced with another amino acid.

The antibody, or antigen binding fragment, may comprise at least one light chain variable region incorporating the following CDRs:

LC-CDR1: KASQSVEDDGENYMN (SEQ ID NO:4)
 LC-CDR2: AASNLES (SEQ ID NO:5)
 5 LC-CDR3: QQSNEDPFT (SEQ ID NO:6)

The antibody, or antigen binding fragment, may comprise at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1: GYTFTNYMH (SEQ ID NO:1)
 10 HC-CDR2: WIYPGNVNTYYNEKFRG (SEQ ID NO:2)
 HC-CDR3: EEKNYPWFAY (SEQ ID NO:3)

The antibody may comprise at least one light chain variable region incorporating the CDRs shown in Figure 7. The antibody may comprise at least one heavy chain variable
 15 region incorporating the CDRs shown in Figure 7.

The antibody may comprise at least one light chain variable region (V_L) comprising the one of the amino acid sequences shown in Figure 7 or an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%,
 20 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to one of the amino acid sequences of the V_L chain amino acid sequence shown in Figure 7. The antibody may have a V_L chain amino acid sequence having least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to one of the amino acid sequences
 25 shown in Figure 7 and comprise the following CDR sequences:

LC-CDR1: KASQSVEDDGENYMN (SEQ ID NO:4)
 LC-CDR2: AASNLES (SEQ ID NO:5)
 LC-CDR3: QQSNEDPFT (SEQ ID NO:6)

30 The antibody may comprise at least one heavy chain variable region (V_H) comprising the one of the amino acid sequences shown in Figure 7 or an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to one of amino acid sequences of the V_H chain amino acid sequence shown in Figure 7. The
 35 antibody may have a V_H chain amino acid sequence having least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99%, or 100% sequence identity to one of the amino acid sequences shown in Figure 7 and comprise the following CDR sequences:

HC-CDR1: GYTFTNYMH (SEQ ID NO:1)

HC-CDR2: WIYPGNVNTYYNEKFRG (SEQ ID NO:2)

5 HC-CDR3: EEKNYPWFAY (SEQ ID NO:3)

The antibody may comprise at least one light chain variable region comprising one of the amino acid sequences shown in Figure 7 (or an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%
10 or 100%, sequence identity to one of the amino acid sequences of the V_L chain amino acid sequence shown in Figure 7) and at least one heavy chain variable region comprising one of the amino acid sequence shown in Figure 7 (or an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%,
15 sequence identity to one of the amino acid sequences of the V_H chain amino acid sequence shown in Figure 7).

The antibody may bind PRL3. The antibody may optionally have amino acid sequence components as described above. The antibody may be an IgA, IgD, IgE, IgM or IgM,
20 preferably an IgG. In one aspect, an *in vitro* complex, optionally isolated, comprising an antibody, or antigen binding fragment, as described herein, bound to PRL3 is provided.

In one example of the present disclosure an isolated heavy chain variable region polypeptide is provided, the heavy chain variable region polypeptide comprising the
25 following CDRs:

HC-CDR1: GYTFTNYMH (SEQ ID NO:1)

HC-CDR2: WIYPGNVNTYYNEKFRG (SEQ ID NO:2)

HC-CDR3: EEKNYPWFAY (SEQ ID NO:3)

30 In one example of the present disclosure an antibody, or antigen binding fragment, is provided, the antibody, or antigen binding fragment, comprising a heavy chain and a light chain variable region sequence, wherein:

the heavy chain comprises a HC-CDR1, HC-CDR2, HC-CDR3, having at least 85% overall sequence identity to HC-CDR1 sequence (SEQ ID NO:1), HC-CDR2
35 sequence (SEQ ID NO:2), HC-CDR3 sequence (SEQ ID NO:3), respectively, and

the light chain comprises a LC-CDR1, LC-CDR2, LC-CDR3,, having at least 85% overall sequence identity to LC-CDR1 sequence (SEQ ID NO:4), LC-CDR2 sequence (SEQ ID NO:5), LC-CDR3 sequence (SEQ ID NO:6), respectively.

In some examples the degree of sequence identity may be one of 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

In another example of the present disclosure an antibody, or antigen binding fragment, optionally isolated, is provided comprising a heavy chain and a light chain variable region sequence, wherein:

the heavy chain sequence has at least 85% sequence identity to a heavy chain sequence set out in Figure 7, and

the light chain sequence has at least 85% sequence identity to a light chain sequence set out in Figure 7

In some examples the degree of sequence identity may be one of 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

In some examples the antibody, antigen binding fragment, or polypeptide further comprises variable region heavy chain framework sequences between the CDRs according to the arrangement HCFR1:HC-CDR1:HCFR2:HC-CDR2:HCFR3:HC-CDR3:HCFR4. The framework sequences may be derived from human consensus framework sequences.

In some cases, the antibody, antigen binding fragment, or polypeptide comprises a heavy chain sequence selected from:

VQSGAEVKKPGASVKVSCKASGYTFTNYYMHWV (SEQ ID NO: 29);
WIYPGNVNTYYNEKFR (SEQ ID NO: 30);
ASTAYMELSSLRSE (SEQ ID NO: 31); and/or
ASEEKNYPWFAYWGQGTLVT (SEQ ID NO: 32).

In one example of the present disclosure an isolated light chain variable region polypeptide, optionally in combination with a heavy chain variable region polypeptide as described herein, is provided, the light chain variable region polypeptide comprising the following CDRs:

LC-CDR1: KASQSVEDDGENYMN (SEQ ID NO:4)

LC-CDR2: AASNLES (SEQ ID NO:5)

LC-CDR3: QQSNEDPFT (SEQ ID NO:6)

In some examples the antibody, antigen binding fragment, or polypeptide further comprises variable region light chain framework sequences between the CDRs according to the arrangement LCFR1:LC-CDR1:LCFR2:LC-CDR2:LCFR3:LC-CDR3:LCFR4. The framework sequences may be derived from human consensus framework sequences. In some cases, the antibody, antigen binding fragment, or polypeptide comprises a light chain sequence selected from:

QSPSSLSASVGDRVT (SEQ ID NO: 26);
 KASQSVEDDGENYMNWYQQK (SEQ ID NO: 27); and/or
 SGSGSGTDFTLTISLQPEDFATYYCQQSNEDPFT (SEQ ID NO: 28).

In some cases, the antibody, antigen binding fragment, or polypeptide comprises 2, 3, 4, 5, 6, or all of the amino acid sequences selected from:

VQSGAEVKKPGASVKVSCKASGYTFTNYYMHV;
 WIYPGNVNTYYNEKFR;
 ASTAYMELSSLRSE;
 ASEEKNYPWFAYWGQGTSLV;
 QSPSSLSASVGDRVT;
 KASQSVEDDGENYMNWYQQK; and/or
 SGSGSGTDFTLTISLQPEDFATYYCQQSNEDPFT.

The antibody may comprise at least one light chain variable region (V_L) and/or a heavy chain variable region (V_H) comprising the one of the amino acid sequences shown in Figure 7 or an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to one of the amino acid sequences shown in Figure 7, and comprise the following CDR sequences:

LC-CDR1: KASQSVEDDGENYMN (SEQ ID NO:4)
 LC-CDR2: AASNLES (SEQ ID NO:5)
 LC-CDR3: QQSNEDPFT (SEQ ID NO:6)
 HC-CDR1: GYTFTNYYMH (SEQ ID NO:1)
 HC-CDR2: WIYPGNVNTYYNEKFRG (SEQ ID NO:2)
 HC-CDR3: EEKNYPWFAY (SEQ ID NO:3)

and contain at least one of the following sequences:

VQSGAEVKKPGASVKVSCKASGYTFTNYYMHV;
 WIYPGNVNTYYNEKFR;

ASTAYMELSSLRSE;
 ASEEKNYPWFAYWGQGTSLVT;
 QSPSSLSASVGDRVT;
 KASQSVEDDGENYMNWYQQK; and/or
 5 SGSGSGTDFTLTISLQPEDFATYYCQQSNEDPFT.

The antibody may comprise at least one light chain variable region (V_L) and/or a heavy chain variable region (V_H) comprising the one of the amino acid sequences shown in Figure 7 or an amino acid sequence having at least 70%, more preferably one of at least
 10 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to one of the amino acid sequences shown in Figure 7.

The antibody may comprise at least on light chain variable region (V_H) selected from:
 15 SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 or SEQ ID NO: 25, or an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to amino acid sequence SEQ ID NO: 16, SEQ ID NO: 17, SEQ
 20 ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 or SEQ ID NO: 25.

Preferably, the antibody comprises a light chain variable region (V_H) selected from: SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID
 25 NO: 21 or SEQ ID NO: 22, or an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to amino acid sequence SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21 or SEQ ID NO: 22.

The antibody may comprise at least on light chain variable region (V_L) selected from: SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15, or an amino acid sequence
 35 having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to amino acid sequence SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID

NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15.

Preferably, the antibody comprises a light chain variable region (V_L) selected from: SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, or an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to amino acid sequence SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12.

The antibody may comprise the following CDR sequences:

LC-CDR1: KASQSVEDDGENYMN (SEQ ID NO:4)

LC-CDR2: AASNLES (SEQ ID NO:5)

LC-CDR3: QQSNEDPFT (SEQ ID NO:6)

HC-CDR1: GYTFTNYMH (SEQ ID NO:1)

HC-CDR2: WIYPGNVNTYYNEKFRG (SEQ ID NO:2)

HC-CDR3: EEKNYPWFAY (SEQ ID NO:3)

and contain at least one of the following sequences:

VQSGAEVKKPGASVKVSCKASGYTFTNYYMHVW;

WIYPGNVNTYYNEKFR;

ASTAYMELSSLRSE;

ASEEKNYPWFAYWGQGTLVT;

QSPSSLSASVGDRVT;

KASQSVEDDGENYMNWYQQK; and/or

SGSGSGTDFLTISLQPEDFATYYCQQSNEDPFT;

and be capable of binding to PRL3, and antagonising a biological function of PRL3.

In some examples, the antibody, or antibody binding fragment, may further comprise a human constant region. For example selected from one of IgG1, IgG2, IgG3 and IgG4.

In some examples, the antibody, or antibody binding fragment, may further comprise a murine constant region. For example, selected from one of IgG1, IgG2A, IgG2B and IgG3.

The antibody is preferably a whole antibody, or an antibody or antibody fragment that includes an Fc domain. The antibody or antibody fragment may include one or both of a

CH1 and a CH2 domain. Preferably, the antibody includes a CH2 domain. The antibody may contain both a CH1 and a CH2 domain. Preferably, the antibody is not a Fab', F(ab)'₂ fragment, and/or is not an scFv and/or is not a minibody. Preferably, the antibody is an IgG immunoglobulin.

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In some example s, the individual to be treated is immunocompetent. The individual may have been determined to be immunocompetent. The individual may have been determined to produce NK cells, and/or B cells. The individual may be treated to stimulate the production and/or activation of NK cells and/or B cells, such as through the administration of cytokines, or by stopping the administration of agents known to reduce the production and/or activation of NK cells and/or B cells.

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In another example of the present disclosure, a composition, e.g. a pharmaceutical composition or medicament, is provided. The composition may comprise an antibody, antigen binding fragment, or polypeptide as described herein and at least one pharmaceutically-acceptable carrier, excipient, adjuvant or diluent.

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In another example of the present disclosure an isolated nucleic acid encoding an antibody, antigen binding fragment, or polypeptide as described herein is provided. The nucleic acid encode a sequence set out in Figure 7, or a coding sequence which is degenerate as a result of the genetic code, or may have a nucleotide sequence having at least 70% identity thereto, optionally one of 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

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The antibody may bind to PRL3. The antibody may bind to an epitope comprising the amino acid sequence KAKFYN and/or HTHKTR. The antibody may be capable of binding both sequences.

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In one example of the present disclosure there is provided a vector comprising a nucleic acid described herein. In another example of the present disclosure, there is provided a host cell comprising the vector. For example, the host cell may be eukaryotic, or mammalian, e.g. Chinese Hamster Ovary (CHO), or human or may be a prokaryotic cell, e.g. *E. coli*.

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In one example of the present disclosure a method for making an antibody, or antigen binding fragment or polypeptide as described herein is provided, the method comprising culturing a host cell as described herein under conditions suitable for the expression of a

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vector encoding the antibody, or antigen binding fragment or polypeptide, and recovering the antibody, or antigen binding fragment or polypeptide.

5 In another example of the present disclosure an antibody, antigen binding fragment or polypeptide is provided for use in therapy, or in a method of medical treatment. In another example of the present disclosure an antibody, antigen binding fragment or polypeptide as described herein is provided for use in the treatment of a T-cell dysfunctional disorder. In another example of the present disclosure, the use of an antibody, antigen binding fragment or polypeptide as described herein in the manufacture
10 of a medicament or pharmaceutical composition for use in the treatment of a T-cell dysfunctional disorder is provided.

In another example of the present disclosure a method is provided, the method comprising contacting a sample containing, or suspected to contain, PRL3 with an
15 antibody or antigen binding fragment, as described herein, and detecting the formation of a complex of antibody, or antigen binding fragment, and PRL3.

In another example of the present disclosure a method of diagnosing a disease or condition in a subject is provided, the method comprising contacting, *in vitro*, a sample
20 from the subject with an antibody, or antigen binding fragment, , as described herein, and detecting the formation of a complex of antibody, or antigen binding fragment, and PRL3.

In a further example of the present disclosure the use of an antibody, or antigen binding fragment, as described herein, for the detection of PRL3 *in vitro* is provided. In another
25 example of the present disclosure the use of an antibody, or antigen binding fragment, as described herein, as an *in vitro* diagnostic agent is provided.

In methods of the present disclosure the antibody, antigen binding fragment or polypeptide may be provided as a composition as described herein.

30 In any example of the present disclosure the antibody preferably specifically binds PRL3 over other PRL phosphatases, such as PRL1 or PRL2.

The antibody may be an IgG. It may have a molecular weight of about 140 to 160kDa, preferably about 150kDa.
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In some examples the antibody may be PRL3-ZUMAB.

Also disclosed herein is the use of a humanised antibody or antigen binding fragment as disclosed herein for the manufacture of a medicament for the treatment of cancer.

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In other example s, there is provided a humanised antibody or antibody binding fragment for use in a method of treating cancer. The antibody may be useful for inhibiting tumor formation, and/or inhibiting metastasis of tumor. The antibody may be useful for reducing the size of tumors. A treated individual may for example show a 1%, 2%, 3%, 4%, 5%,
10 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more decrease in tumour size of a particular tumour, or decrease in tumour number, or both, compared to an individual who has not been treated, or compared to that same individual prior to treatment.

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Also provided are method of treating cancer comprising administering a humanised antibody or antibody binding fragment as disclosed herein.

The cancer may be a PRL3 expressing or overexpressing cancer. The cancer may be gastric cancer.

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The humanised antibody or antibody binding fragment may be administered intravenously. It may be administered at a location distant to the cancer to be treated.

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In some methods the patient has not previously received chemotherapy, particularly antimetabolite therapy, such as 5-FU. In some cases, the patient has not previously received such therapy before, or has not received such treatment for their cancer, such as for their gastric cancer. In some cases, the antibody is not co-administered with another agent (i.e. antibody monotherapy). In some cases, the antibody is not co-administered with 5-FU.

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In some methods, the patient has been determined not to have an impaired immune system. In particular, the patient may have been determined to have a white blood cell count within normal range. In particular, the patient may have been determined to not have leukopenia. The patient may have been determined to have neturophil, lymphocyte,
35 monocyte, red blood cell or platelet counts within normal range. The patient may have a white blood count, neturophil, lymphocyte, monocyte, red blood cell or platelet count that

is not significantly different to a control, such as the count from an individual known to not have an impaired immune system, or to established normal values. For example, the patient may be determined to have between about 4,500 and about 10,000 white blood cells per microliter of blood.

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In some example s, the disclosure provides a method for selecting a patient for treatment with a humanised anti-PRL3 antibody or antibody fragment, the method comprising determining, in a sample of urine from the patient, the presence of PRL3. In some cases, the method involves determining the level of PRL3 in a urine sample from the patient. In some cases, the patient may have gastric, nasopharyngeal, bladder, lung, breast or prostate cancer.

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In some cases, the individual has a family history of PRL3 overexpressing cancer, or has been identified as having a likelihood of developing a PRL3 overexpressing cancer. In some cases, the individual has a PRL3 overexpressing cancer, and is considered to be at risk of metastasis of that cancer.

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In another example, provided herein are method involving determining the cellular localisation of PRL3. An increased proportion of cellular PRL3 on the cell surface may indicate that the individual has cancer. Provided herein is a method comprising determining the cellular localisation of PRL3 in a cell, wherein expression of PRL3 at the cell surface indicates that the cell is cancerous.

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Methods include methods for the diagnosis of cancer, wherein the presence of, or an increase in PRL3 on the surface of a cell may indicate that the individual has cancer. In some cases, the amount of PRL3 in the cell is the same as a non-cancerous control sample, but the localisation of that PRL3 may be changed as compared to the non-cancerous control.

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Other methods include a method for determining whether or not a cell is cancerous, the method comprising determining the presence of PRL3 at the surface of the cell. An increase in the level or proportion of PRL3 as compared to a control cell may indicate that the individual is, or will become, cancerous.

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Methods may involve the selection of an individual for an anti-cancer therapy, based on the cellular localisation of PRL3 in the sample. In some cases, the methods involve administration of an anti-cancer therapy to an individual so selected.

5 In some cases, the method may comprise determining the cellular localisation of PRL3 in two or more samples from the patient, taken at two or more time points. A change in the amount of PRL3 on the surface of the cell may indicate an increase or decrease in the cancer in the individual. An increase in cell surface PRL3 over time may indicate that the individual has developed cancer, or the cancer has worsened. A decrease in cell surface
10 PRL3 over time may indicate that the cancer has reduced, or that the therapy has resulted in treatment of the cancer. An increased or unchanged level of PRL3 at the cell surface may indicate that additional or alternative anti-cancer therapy is required. The level of PRL3 at the cell surface may therefore be used to select an individual for a further, or alternative, anti-cancer therapy.

15 An increase of 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold more PRL3 at the cell surface may indicate that the individual has cancer, and/or the cell is cancerous, or that the individual should be selected for treatment. The level of PRL3 at the cell surface may be compared to a control.

20 The sample may be a blood sample or a serum sample. The sample may be a urine sample. The cancer may be a sample of the tumor or of the tissue surrounding the tumor. The method may involve obtaining the sample, or the method may be performed on a sample previously obtained from the individual.

25 Methods of diagnosis and detection may be performed in vitro, or ex vivo, and in some cases do not involve the step of obtaining a sample from an individual.

Description

Antibodies

Claims

1. A humanised antibody or antigen binding fragment that binds PRL3, the antibody having the amino acid sequences i) to vi):

- i) KASQSVEDDGENYMN (SEQ ID NO:4)
- ii) AASNLES (SEQ ID NO:5)
- iii) QQSNEDPFT (SEQ ID NO:6)
- iv) GYTFTNYMH (SEQ ID NO:1)
- v) WIYPGNVNTYYNEKFRG (SEQ ID NO:2)
- vi) EEKNYPWFAY (SEQ ID NO:3)

or a variant thereof in which one, two or three amino acids in one or more of the sequences i) to vi) are replaced with another amino acid.

2. The humanised antibody or antigen binding fragment of claim 1, wherein the antibody or antigen binding fragment contains a CH1 and a CH2 domain.

3. The humanised antibody or antigen binding fragment according to claim 1 which binds to an epitope comprising the amino acid sequence KAKFYN and/or to HTHKTR.

4. The humanised antibody, or antigen binding fragment, of any one of the preceding claims, having at least one light chain variable region incorporating the following CDRs:

- LC-CDR1: KASQSVEDDGENYMN (SEQ ID NO:4)
- LC-CDR2: AASNLES (SEQ ID NO:5)
- LC-CDR3: QQSNEDPFT (SEQ ID NO:6)

5. The humanised antibody, or antigen binding fragment, of any one of the preceding claims, having at least one heavy chain variable region incorporating the following CDRs:

- HC-CDR1: GYTFTNYMH (SEQ ID NO:1)
- HC-CDR2: WIYPGNVNTYYNEKFRG (SEQ ID NO:2)
- HC-CDR3: EEKNYPWFAY (SEQ ID NO:3)

6. An *in vitro* complex, optionally isolated, comprising an antibody, or antigen binding fragment, according to any one of the preceding claims bound to PRL3.

7. An antibody or antigen binding fragment comprising heavy chain having the sequence of any one of SEQ ID NO: 16-25 and a light chain having the sequence of any one of SEQ ID NO: 7-15.
8. Use of a humanised antibody, antigen binding fragment according to any one of the preceding claims in the manufacture of a medicament for the treatment of a PRL3 expressing cancer.
9. A humanised antibody or antigen binding fragment according to any one of the preceding claims for use in the treatment of a PRL3 expressing cancer.
10. Use of a humanised antibody or antigen binding fragment according to claim 1 in the manufacture of a medicament for the treatment of a PRL3 expressing cancer, wherein said use comprises that the humanised antibody or antigen binding fragment is to be administered to a patient.
11. The use according to claim 10, wherein the PRL3 expressing cancer is gastric cancer or metastasis of gastric cancer.
12. The use according to claim 10, wherein the humanised antibody or antigen binding fragment is to be administered intravenously.
13. The use according to claim 10, wherein the humanised antibody is to be administered at a location distant to the PRL3 expressing cancer to be treated.
14. The use according to claim 10, wherein said use comprises that the humanized anti-PRL3 antibody is to be administered to a patient with a PRL3 expressing gastric cancer, wherein the patient has not previously received antimetabolite therapy for gastric cancer, or has not previously received antimetabolite therapy.
15. The use according to claim 14, wherein the antimetabolite therapy is 5-FU.
16. The use according to claim 10, wherein the patient has been determined not to have an impaired immune system.
17. An *in vitro* method comprising determining the cellular localisation of PRL3 in a cell obtained from an individual, wherein the method is an immunoassay using an antibody according to any one of claims 1-5 or 7, and wherein expression of PRL3 at the cell surface indicates that the cell is cancerous.
18. The method of claim 17, wherein the expression of PRL3 at the cell surface is 2-fold increased as compared to a control cell.

19. The method of claim 17, wherein the individual is determined to have cancer, or the individual is selected for anti-cancer therapy if PRL3 is expressed at the cell surface.