

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 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), and

having at least one heavy chain variable region incorporating the following CDRs:

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

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In another aspect, the present disclosure refers to an *in vitro* complex, optionally isolated, comprising an antibody, or antigen binding fragment bound to PRL3 as disclosed herein.

10 In one example, 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) QQSNEDPFT (SEQ ID NO:6)
 iv) GYTFTNYYMH (SEQ ID NO:1)
 15 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.

20 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
 25 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.

30 In a further aspect, the present disclosure refers to the use of a humanised antibody or 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
 35 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 sequences i) to iii), or the amino acid sequences iv) to vi), or preferably the amino acid sequences i) to vi):

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|----|------|-------------------|---------------|
| 5 | i) | KASQSVEDDGENYMN | (SEQ ID NO:4) |
| | ii) | AASNLES | (SEQ ID NO:5) |
| | iii) | QQSNEDPFT | (SEQ ID NO:6) |
| | iv) | GYTFTNYYMH | (SEQ ID NO:1) |
| 10 | 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.

Claims

1. A humanised antibody or antigen binding fragment that binds PRL3, the antibody 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), and

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).

2. The humanised antibody or antigen binding fragment of claim 1, wherein the antibody or antibody 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. An *in vitro* complex, optionally isolated, comprising an antibody, or antigen binding fragment, according to any one of the preceding claims bound to PRL3.

5. 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.

6. 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.

7. 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.

8. 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.

9. The use according to claim 8, wherein the PRL3 expressing cancer is gastric cancer or metastasis of gastric cancer.
10. The use according to claim 8, wherein the humanised antibody or antigen binding fragment is to be administered intravenously.
11. The use according to claim 8, wherein the humanised antibody is to be administered at a location distant to the PRL3 expressing cancer to be treated.
12. The use according to claim 8, 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.
13. The use according to claim 12, wherein the antimetabolite therapy is 5-FU.
14. The use according to claim 8, wherein the patient has been determined not to have an impaired immune system.
15. 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 1-3 or 5, and wherein expression of PRL3 at the cell surface indicates that the cell is cancerous.
16. The method of claim 15, wherein the expression of PRL3 at the cell surface is 2-fold increased as compared to a control cell.
17. The method of claim 15, 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.