

Current technologies have utilized knock-in mouse models or human tumor cell lines that express only the mutant protein without the wildtype protein, which may not entirely reflect the role of the mutant protein in co-existence with the wildtype protein.

Attempts to generate antibodies against mutant p53 have been made over the decades, and monoclonal antibodies (mAb) against the mutant (PAb240) or the wild-type (PAb246) conformation of p53 are available (Gannon et al., EMBO J (1990) 9(5):1595-1602). However, these antibodies are not sufficiently specific and exhibit cross reactivity, and require very selective conditions for the detection of the correct conformations that have thus limited their use.

Vojtesek and Lane, J Cell Sci (1993), 105(3):607-612 describes an antibody designated DO-1 against the amino-terminal region of human p53, which shows a high level of specificity for human p53 without any cross-reactivity with mouse p53.

Summary

In a first aspect, the present invention provides a method for producing an antibody which is specific for a mutant p53 polypeptide over wildtype p53 polypeptide, comprising using as an immunogen a peptide or polypeptide comprising: (i) an antigen sequence, comprising an amino acid sequence of the mutant p53 polypeptide including the mutation and at least one amino acid immediately adjacent to the mutation, and (ii) a scaffold sequence for providing the antigen sequence in a solvent-accessible configuration. In another aspect, the present invention refers to a cancer vaccine, comprising an immunogen as described herein. In yet another aspect, the present invention refers to the use of an immunogen as described herein in the manufacture of a medicament for use in vaccination against a cancer.

In some examples, the scaffold sequence is derived from a peptide or polypeptide comprising a solvent-accessible sequence, and wherein the antigen sequence is inserted in, or substituted for all or part of, the solvent-accessible sequence of the peptide or polypeptide. In some examples, the peptide or polypeptide comprising a solvent-accessible sequence is a thioredoxin, and wherein the solvent-accessible sequence is the active site sequence of the thioredoxin. In some examples, the peptide or polypeptide used as an immunogen additionally comprises one or more linker sequences between the antigen sequence and the scaffold sequence. In some examples, the peptide or polypeptide used as an immunogen comprises at least two amino acid sequences of the mutant p53 polypeptide. In some examples, the peptide or polypeptide used as an immunogen additionally comprises

linker sequences between the at least two amino acid sequences of the mutant p53 polypeptide. In some examples, the at least two amino acid sequences of the mutant p53 polypeptide are non-identical. In some examples, the amino acid sequence of the mutant p53 polypeptide comprises at least 5 amino acids. In some examples, the mutant p53 polypeptide comprises a mutation in the DNA-binding domain (DBD). In some examples, the mutant p53 polypeptide comprises a mutation selected from one of R175H, R248Q, R273H, R248W, G245S, R273C, R282W, R249S, G245D, C176F, H179Y, H179R, Y220C, and R337H. In some examples, the mutant p53 polypeptide comprises, or consists of, the amino acid sequence of one of SEQ ID NOs: 3 to 16.

In another example, the present disclosure provides an antibody, or antigen binding fragment, obtained or obtainable by a method for producing an antibody according to the disclosure.

In another example, the present disclosure provides an antibody, or antigen binding fragment, which is specific for a mutant p53 polypeptide over wildtype p53 polypeptide.

In some examples, the mutant p53 polypeptide comprises a mutation in the DNA-binding domain (DBD). In some examples, the mutant p53 polypeptide comprises a mutation

selected from one of R175H, R248Q, R273H, R248W, G245S, R273C, R282W, R249S, G245D, C176F, H179Y, H179R, Y220C and R337H. In some examples, the mutant p53 polypeptide comprises, or consists of, the amino acid sequence of one of SEQ ID NOs: 3 to 16.

In another example, the present disclosure provides an antibody, or antigen binding fragment, which is capable of binding to R175H p53 polypeptide, optionally isolated, having the amino acid sequences i) to vi):

- i) LC-CDR1: QSLNSGNQKSX₁ (SEQ ID NO:31);
- ii) LC-CDR2: GAS (SEQ ID NO:20);
- iii) LC-CDR3: QNDHSYPLT (SEQ ID NO:21);
- iv) HC-CDR1: GX₂TFTEYT (SEQ ID NO:32);
- v) HC-CDR2: IX₃PX₄X₅GX₆T (SEQ ID NO:33);
- vi) HC-CDR3: ARWGGDYV (SEQ ID NO:27);

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, where X₁ = Y or N; X₂ = F or Y; X₃ = D or N; X₄ = N or Y; X₅ = N or S; and X₆ = V or G.

In some examples, LC-CDR1 is one of QSLNLSGNQKSY (SEQ ID NO:19) or QSLNLSGNQKSN (SEQ ID NO:23). In some examples, HC-CDR1 is one of GFTFTEYT (SEQ ID NO:25) or GYTFTEYT (SEQ ID NO:29). In some examples, HC-CDR2 is one of
5 IDPNNGVT (SEQ ID NO:26) or INPYSGGT (SEQ ID NO:30).

In some examples, the antibody, or antigen binding fragment, has at least one light chain variable region incorporating the following CDRs:

10 LC-CDR1: QSLNLSGNQKSX₁ (SEQ ID NO:31);
LC-CDR2: GAS (SEQ ID NO:20);
LC-CDR3: QNDHSYPLT (SEQ ID NO:21);

where X₁ = Y or N.

15 In some examples, the antibody, or antigen binding fragment has at least one light chain variable region incorporating the following CDRs:

LC-CDR1: QSLNLSGNQKSY (SEQ ID NO:19);
LC-CDR2: GAS (SEQ ID NO:20);
LC-CDR3: QNDHSYPLT (SEQ ID NO:21).

20 In some examples, the antibody, or antigen binding fragment has at least one light chain variable region incorporating the following CDRs:

LC-CDR1: QSLNLSGNQKSN (SEQ ID NO:23);
LC-CDR2: GAS (SEQ ID NO:20);
LC-CDR3: QNDHSYPLT (SEQ ID NO:21).

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In some examples, the antibody, or antigen binding fragment has at least one heavy chain variable region incorporating the following CDRs:

30 HC-CDR1: GX₂TFTEYT (SEQ ID NO:32);
HC-CDR2: IX₃PX₄X₅GX₆T (SEQ ID NO:33);
HC-CDR3: ARWG GDYV (SEQ ID NO:27);

where X₂ = F or Y; X₃ = D or N; X₄ = N or Y; X₅ = N or S; and X₆ = V or G.

In some examples, the antibody, or antigen binding fragment has at least one heavy chain variable region incorporating the following CDRs:

35 HC-CDR1: GFTFTEYT (SEQ ID NO:25);
HC-CDR2: IDPNNGVT (SEQ ID NO:26);

HC-CDR3: ARWGGDYV (SEQ ID NO:27).

In some examples, the antibody, or antigen binding fragment has at least one heavy chain variable region incorporating the following CDRs:

5 HC-CDR1: GYTFTEYT (SEQ ID NO:29);
HC-CDR2: INPYSGGT (SEQ ID NO:30);
HC-CDR3: ARWGGDYV (SEQ ID NO:27).

10 In another example, the present disclosure provides an isolated light chain variable region polypeptide, comprising the following the following CDRs:

LC-CDR1: QSLN₁SGNQK₁ (SEQ ID NO:31);
LC-CDR2: GAS (SEQ ID NO:20);
LC-CDR3: QNDHSYPLT (SEQ ID NO:21);

where $X_1 = Y$ or N .

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In some examples, LC-CDR1 is one of QSLN₁SGNQKSY (SEQ ID NO:19) or QSLN₁SGNQKSN (SEQ ID NO:23).

20 In another example, the present disclosure provides an isolated heavy chain variable region polypeptide, comprising the following the following CDRs:

HC-CDR1: GX₂TFTEYT (SEQ ID NO:32);
HC-CDR2: IX₃PX₄X₅GX₆T (SEQ ID NO:33);
HC-CDR3: ARWGGDYV (SEQ ID NO:27);

where $X_2 = F$ or Y ; $X_3 = D$ or N ; $X_4 = N$ or Y ; $X_5 = N$ or S ; and $X_6 = V$ or G .

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In some examples, HC-CDR1 is one of GFTTFTEYT (SEQ ID NO:25) or GYTFTEYT (SEQ ID NO:29). In some examples, HC-CDR2 is one of IDPNNGVT (SEQ ID NO:26) or INPYSGGT (SEQ ID NO:30).

30 In another example, the present disclosure provides an isolated light chain variable region polypeptide as described herein in combination with a heavy chain variable region polypeptide as described herein.

In another example, the present disclosure provides an antibody, or antigen binding fragment, which is capable of binding to R175H p53 polypeptide, comprising a light chain and a heavy chain variable region sequence, wherein:

the light chain comprises a LC-CDR1, LC-CDR2, LC-CDR3, having at least 85% overall sequence identity to LC-CDR1: one of QSLNLSGNQKSX₁ (SEQ ID NO:31), QSLNLSGNQKSY (SEQ ID NO:19) or QSLNLSGNQKSN (SEQ ID NO:23); LC-CDR2: GAS (SEQ ID NO:20); LC-CDR3: QNDHSYPLT (SEQ ID NO:21); and the heavy chain comprises a HC-CDR1, HC-CDR2, HC-CDR3, having at least 85% overall sequence identity to HC-CDR1: one of GX₂TFTEYT (SEQ ID NO:32), GFTFTEYT (SEQ ID NO:25) or GYTFTEYT (SEQ ID NO:29); HC-CDR2: one of IX₃PX₄X₅GX₆T (SEQ ID NO:33), IDPNNGVT (SEQ ID NO:26) or INPYSGGT (SEQ ID NO:30); HC-CDR3: ARWGGDYV (SEQ ID NO:27); where X₁ = Y or N; X₂ = F or Y; X₃ = D or N; X₄ = N or Y; X₅ = N or S; and X₆ = V or G.

In another example, the present disclosure provides an antibody, or antigen binding fragment, which is capable of binding to R175H p53 polypeptide, comprising a light chain and a heavy chain variable region sequence, wherein:

the light chain sequence has at least 85% sequence identity to the light chain sequence of SEQ ID NO:18 or 22 (Figure 24), or SEQ ID NO:237 or 94 (Figure 49), and;

the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence of SEQ ID NO:24 or 28 (Figure 25), or SEQ ID NO:239, 240 or 241 (Figure 50).

In another example, the present disclosure provides an antibody, or antigen binding fragment, optionally isolated, which is capable of binding to R175H p53 polypeptide, which is a bispecific antibody or a bispecific antigen binding fragment comprising (i) an antigen binding fragment or polypeptide as described herein, and (ii) an antigen binding fragment capable of binding to a polypeptide other than R175H p53 polypeptide.

In another example, the present disclosure provides an *in vitro* complex, optionally isolated, comprising an antibody, antigen binding fragment, or polypeptide as described herein bound to R175H p53 polypeptide or a fragment thereof.

In another example, the present disclosure provides an antibody, or antigen binding fragment, which is capable of binding to R248Q p53 polypeptide, optionally isolated, having the amino acid sequences i) to vi):

i) LC-CDR1: QSLLYSDGKTY (SEQ ID NO:41);

- ii) LC-CDR2: LVS (SEQ ID NO:42);
- iii) LC-CDR3: WQGTHFPLT (SEQ ID NO:43);
- iv) HC-CDR1: GYTFTDYY (SEQ ID NO:46);
- v) HC-CDR2: IX₇PKNGGT (SEQ ID NO:52);
- 5 vi) HC-CDR3: AKX₈GGX₉DDY (SEQ ID NO:53);

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, where X₇ = H or D; X₈ = M or Q; and X₉ = Y or F.

- 10 In some examples, HC-CDR2 is one of IHPKNGGT (SEQ ID NO:47) or IDPKNGGT (SEQ ID NO:50). In some examples, HC-CDR3 is one of AKMGGYDDY (SEQ ID NO:48) or AKQGGFDDY (SEQ ID NO:51).

- In some examples, the antibody, or antigen binding fragment, has at least one light chain
15 variable region incorporating the following CDRs:

LC-CDR1: QSLLYSDGKTY (SEQ ID NO:41)
LC-CDR2: LVS (SEQ ID NO:42)
LC-CDR3: WQGTHFPLT (SEQ ID NO:43).

- 20 In some examples, the antibody, or antigen binding fragment, has at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1: GYTFTDYY (SEQ ID NO:46);
HC-CDR2: IX₇PKNGGT (SEQ ID NO:52);
HC-CDR3: AKX₈GGX₉DDY (SEQ ID NO:53);

- 25 where X₇ = H or D; X₈ = M or Q; and X₉ = Y or F.

In some examples, the antibody, or antigen binding fragment, has at least one heavy chain variable region incorporating the following CDRs:

- 30 HC-CDR1: GYTFTDYY (SEQ ID NO:46);
HC-CDR2: IHPKNGGT (SEQ ID NO:47);
HC-CDR3: AKMGGYDDY (SEQ ID NO:48).

In some examples, the antibody, or antigen binding fragment, has at least one heavy chain variable region incorporating the following CDRs:

- 35 HC-CDR1: GYTFTDYY (SEQ ID NO:46);
HC-CDR2: IDPKNGGT (SEQ ID NO:50);

HC-CDR3: AKQGGFDDY (SEQ ID NO:51).

In another example, the present disclosure provides an isolated light chain variable region polypeptide, comprising the following the following CDRs:

5 LC-CDR1: QSLLYSDGKTY (SEQ ID NO:41)
LC-CDR2: LVS (SEQ ID NO:42)
LC-CDR3: WQGTHFPLT (SEQ ID NO:43).

10 In another example, the present disclosure provides an isolated heavy chain variable region polypeptide, comprising the following the following CDRs:

HC-CDR1: GYTFTDYY (SEQ ID NO:46);
HC-CDR2: IX₇PKNGGT (SEQ ID NO:52);
HC-CDR3: AKX₈GGX₉DDY (SEQ ID NO:53);

where X₇ = H or D; X₈ = M or Q; and X₉ = Y or F.

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In some examples, HC-CDR2 is one of IHPKNGGT (SEQ ID NO:47) or IDPKNGGT (SEQ ID NO:50). In some examples, HC-CDR3 is one of AKMGGYDDY (SEQ ID NO:48) or AKQGGFDDY (SEQ ID NO:51).

20 In another example, the present disclosure provides an isolated light chain variable region polypeptide as described herein in combination with a heavy chain variable region polypeptide as described herein.

25 In another example, the present disclosure provides an antibody, or antigen binding fragment, which is capable of binding to R248Q p53 polypeptide, comprising a light chain and a heavy chain variable region sequence, wherein:

the light chain comprises a LC-CDR1, LC-CDR2, LC-CDR3, having at least 85% overall sequence identity to LC-CDR1: QSLLYSDGKTY (SEQ ID NO:41); LC-CDR2: LVS (SEQ ID NO:42); LC-CDR3: WQGTHFPLT (SEQ ID NO:43); and

30 the heavy chain comprises a HC-CDR1, HC-CDR2, HC-CDR3, having at least 85% overall sequence identity to HC-CDR1: GYTFTDYY (SEQ ID NO:46); HC-CDR2: one of IX₇PKNGGT (SEQ ID NO:52), IHPKNGGT (SEQ ID NO:47) or IDPKNGGT (SEQ ID NO:50); HC-CDR3: one of AKX₈GGX₉DDY (SEQ ID NO:53), AKMGGYDDY (SEQ ID NO:48) or AKQGGFDDY (SEQ ID NO:51); where X₇ = H or D; X₈ = M or Q; and X₉
35 = Y or F.

In another example, the present disclosure provides an antibody, or antigen binding fragment, which is capable of binding to R248Q p53 polypeptide, comprising a light chain and a heavy chain variable region sequence, wherein:

- the light chain sequence has at least 85% sequence identity to the light chain sequence of SEQ ID NO:40 or 44 (Figure 28), or SEQ ID NO:251 or 252 (Figure 57), and;
the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence of SEQ ID NO:45 or 49 (Figure 29), or SEQ ID NO:253 or 254 (Figure 58).

In another example, the present disclosure provides an antibody, or antigen binding fragment, optionally isolated, which is capable of binding to R248Q p53 polypeptide, which is a bispecific antibody or a bispecific antigen binding fragment comprising (i) an antigen binding fragment or polypeptide as described herein, and (ii) an antigen binding fragment capable of binding to a polypeptide other than R248Q p53 polypeptide.

In another example, the present disclosure provides an *in vitro* complex, optionally isolated, comprising an antibody, antigen binding fragment, or polypeptide as described herein bound to R248Q p53 polypeptide or a fragment thereof.

In another example, the present disclosure provides an antibody, or antigen binding fragment, which is capable of binding to R273H p53 polypeptide, optionally isolated, having the amino acid sequences i) to vi):

- i) LC-CDR1: QSIVHNNGDTY (SEQ ID NO:59);
- ii) LC-CDR2: KVS (SEQ ID NO:60);
- iii) LC-CDR3: FQGSHLPLT (SEQ ID NO:61);
- iv) HC-CDR1: GFSFSDYY (SEQ ID NO:63);
- v) HC-CDR2: ISVGGTYT (SEQ ID NO:64);
- vi) HC-CDR3: VRDGNKGKFLG (SEQ ID NO:65);

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.

In some examples, the antibody, or antigen binding fragment, has at least one light chain variable region incorporating the following CDRs:

- LC-CDR1: QSIVHNNGDTY (SEQ ID NO:59)
- LC-CDR2: KVS (SEQ ID NO:60)
- LC-CDR3: FQGSHLPLT (SEQ ID NO:61).

In some examples, the antibody, or antigen binding fragment, has at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1: GFSFSDYY (SEQ ID NO:63)
HC-CDR2: ISVGGTYT (SEQ ID NO:64)
5 HC-CDR3: VRDGNDGKFLG (SEQ ID NO:65).

In another example, the present disclosure provides an isolated light chain variable region polypeptide, comprising the following the following CDRs:

10 LC-CDR1: QSIVHNNGDTY (SEQ ID NO:59)
LC-CDR2: KVS (SEQ ID NO:60)
LC-CDR3: FQGSHLPLT (SEQ ID NO:61).

In another example, the present disclosure provides an isolated heavy chain variable region polypeptide, comprising the following the following CDRs:

15 HC-CDR1: GFSFSDYY (SEQ ID NO:63)
HC-CDR2: ISVGGTYT (SEQ ID NO:64)
HC-CDR3: VRDGNDGKFLG (SEQ ID NO:65).

20 In another example, the present disclosure provides an isolated light chain variable region polypeptide as described herein in combination with a heavy chain variable region polypeptide as described herein.

In another example, the present disclosure provides an antibody, or antigen binding fragment, which is capable of binding to R273H p53 polypeptide, comprising a light chain
25 and a heavy chain variable region sequence, wherein:

the light chain comprises a LC-CDR1, LC-CDR2, LC-CDR3, having at least 85% overall sequence identity to LC-CDR1: QSIVHNNGDTY (SEQ ID NO:59); LC-CDR2: KVS (SEQ ID NO:60); LC-CDR3: FQGSHLPLT (SEQ ID NO:61); and
the heavy chain comprises a HC-CDR1, HC-CDR2, HC-CDR3, having at least 85%
30 overall sequence identity to HC-CDR1: GFSFSDYY (SEQ ID NO:63); HC-CDR2: ISVGGTYT (SEQ ID NO:64); HC-CDR3: VRDGNDGKFLG (SEQ ID NO:65).

In another example, the present disclosure provides an antibody, or antigen binding fragment, which is capable of binding to R273H p53 polypeptide, comprising a light chain
35 and a heavy chain variable region sequence, wherein:

the light chain sequence has at least 85% sequence identity to the light chain sequence of SEQ ID NO:58 (Figure 32) or SEQ ID NO:247 (Figure 53), and;

the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence of SEQ ID NO:62 (Figure 33) or SEQ ID NO:248 (Figure 54).

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In another example, the present disclosure provides an antibody, or antigen binding fragment, optionally isolated, which is capable of binding to R273H p53 polypeptide, which is a bispecific antibody or a bispecific antigen binding fragment comprising (i) an antigen binding fragment or polypeptide as described herein, and (ii) an antigen binding fragment

10 capable of binding to a polypeptide other than R273H p53 polypeptide.

In another example, the present disclosure provides an *in vitro* complex, optionally isolated, comprising an antibody, antigen binding fragment, or polypeptide as described herein bound to R273H p53 polypeptide or a fragment thereof.

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In another example, the present disclosure provides an antibody, antigen binding fragment, or polypeptide as described herein conjugated to a drug moiety or a detectable moiety.

In another example, the present disclosure provides a chimeric antigen receptor (CAR) comprising an antigen binding fragment or polypeptide as described herein.

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In another example, the present disclosure provides a cell comprising a chimeric antigen receptor (CAR) as described herein.

In another example, the present disclosure provides a composition comprising the antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein and at least one pharmaceutically-acceptable carrier.

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In another example, the present disclosure provides an isolated nucleic acid encoding the antibody, antigen binding fragment, polypeptide, conjugate, or chimeric antigen receptor (CAR) as described herein.

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In another example, the present disclosure provides a vector comprising the nucleic acid as described herein.

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In another example, the present disclosure provides a host cell comprising the vector as described herein.

5 In another example, the present disclosure provides a method for making an antibody, antigen binding fragment, polypeptide, conjugate, or chimeric antigen receptor (CAR) as described herein, comprising culturing the host cell under conditions suitable for the expression of a vector encoding the antibody, antigen binding fragment, polypeptide or conjugate, and recovering the antibody, antigen binding fragment, polypeptide or conjugate

10 In another example, the present disclosure provides an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein for use in therapy, or in a method of medical treatment.

15 In another example, the present disclosure provides an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein in the manufacture of a medicament for use in a method of medical treatment.

20 In another example, the present disclosure provides a method of treating a disease, the method comprising administering an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein to a patient suffering from a disease.

25 In another example, the present disclosure provides an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein for use in the treatment or prevention of cancer.

30 In another example, the present disclosure provides an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein in the manufacture of a medicament for use in the treatment or prevention of cancer.

In another example, the present disclosure provides a method of treating cancer comprising administering an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein to a patient suffering from a cancer.

35 In another example, the present disclosure provides a method of preventing cancer or preventing a recurrence of cancer, the method comprising administering an antibody,

antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein to a patient considered to be at risk of cancer or at risk of recurrence of cancer.

5 In another example, the present disclosure provides a method, optionally an *in vitro* method, comprising contacting a sample containing, or suspected to contain, a mutant p53 polypeptide with an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein and detecting the formation of a complex of the antibody, antigen binding fragment, polypeptide, conjugate, CAR or cell with the
10 mutant p53 polypeptide.

In another example, the present disclosure provides a method of diagnosing a disease or condition in a subject, the method comprising contacting, *in vitro*, a sample from the subject with an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen
15 receptor (CAR) or cell as described herein and detecting the formation of a complex of the antibody, antigen binding fragment, polypeptide, conjugate, CAR or cell with the mutant p53 polypeptide.

In another example, the present disclosure provides a method of selecting or stratifying a
20 subject for treatment with a mutant p53 polypeptide-targeted agent, the method comprising contacting, *in vitro*, a sample from the subject with an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein and detecting the formation of a complex of the antibody, antigen binding fragment, polypeptide, conjugate, CAR or cell with the mutant p53 polypeptide.

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In another example, the present disclosure provides the use of an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein for the detection of a mutant p53 polypeptide *in vitro* or *in vivo*.

30 In another example, the present disclosure provides the use of an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein as an *in vitro* or *in vivo* diagnostic or prognostic agent.

In another example, the present disclosure provides the use of an antibody, antigen binding
35 fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein in a method for detecting, localizing or imaging a cancer *in vivo*.

In another example, the present disclosure provides a method for detecting, localizing or imaging a cancer *in vivo*, comprising administering an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell as described herein to a subject, and detecting the antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR) or cell.

In another example, the present disclosure provides a vaccine, optionally a cancer vaccine, comprising an immunogen as described herein.

In another example, the present disclosure provides an immunogen as described herein for use in a method of vaccination, optionally against a cancer.

In another example, the present disclosure provides the use of an immunogen as described herein in the manufacture of a medicament for use in the vaccination of a subject, optionally against a cancer.

In another example, the present disclosure provides a method for vaccinating a subject, optionally against a cancer, comprising administering to the subject an immunogen as described herein, thereby vaccinating the subject against a cancer.

In some examples in accordance with the present disclosure, the cancer is a cancer comprising a cell or cells expressing a mutant p53 polypeptide, or comprising nucleic acid encoding a mutant p53 polypeptide. The mutant p53 polypeptide may correspond to the selected antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR), cell or immunogen.

Description

Mutant p53 polypeptides and wildtype p53 polypeptides

The p53 tumour suppressor is a protein which is encoded in humans by the TP53 gene. p53 plays a crucial role in cellular response to DNA damage and other genomic aberrations. Activation of p53 leads to cell cycle arrest, DNA repair or apoptosis. Mutations of the gene encoding p53 have been implicated in the development and progression of a variety of cancers (Muller and Vousden, Cancer Cell (2014) 25:304-317).

Certain p53 mutations, including the hotspot mutations (R175H, R248W and R273H), not only result in the loss of p53-dependent tumor suppressor activity, but also result in the acquisition of oncogenic activity.

- 5 A wildtype p53 polypeptide as referred to herein may comprise or consist of a reference amino acid sequence for p53 for a given animal. Reference amino acid sequences for p53 can be retrieved from databases known to the person skilled in the art. Such databases

Claims:

1. A method for producing an antibody which is specific for a mutant p53 polypeptide over wildtype p53 polypeptide, comprising using as an immunogen a peptide or polypeptide comprising: (i) an antigen sequence, comprising an amino acid sequence of the mutant p53 polypeptide including the mutation and at least one amino acid immediately adjacent to the mutation, and (ii) a scaffold sequence for providing the antigen sequence in a solvent-accessible configuration.
2. The method according to claim 1, wherein the scaffold sequence is derived from a peptide or polypeptide comprising a solvent-accessible sequence, and wherein the antigen sequence is inserted in, or substituted for all or part of, the solvent-accessible sequence of the peptide or polypeptide.
3. The method according to claim 2, wherein the peptide or polypeptide comprising a solvent-accessible sequence is a thioredoxin, and wherein the solvent-accessible sequence is the active site sequence of the thioredoxin.
4. The method according to any one of claims 1 to 3, wherein the peptide or polypeptide used as an immunogen additionally comprises one or more linker sequences between the antigen sequence and the scaffold sequence.
5. The method according to any one of claims 1 to 4, wherein the peptide or polypeptide used as an immunogen comprises at least two amino acid sequences of the mutant p53 polypeptide.
6. The method according to claim 5, wherein the peptide or polypeptide used as an immunogen additionally comprises linker sequences between the at least two amino acid sequences of the mutant p53 polypeptide.
7. The method according to claim 5 or claim 6, wherein the at least two amino acid sequences of the mutant p53 polypeptide are non-identical.
8. The method according to any one of claims 1 to 7, wherein the amino acid sequence of the mutant p53 polypeptide comprises at least 5 amino acids.

9. The method according to any one of claims 1 to 8, wherein the mutant p53 polypeptide comprises a mutation in the DNA-binding domain (DBD).

10. The method according to any one of claims 1 to 9, wherein the mutant p53 polypeptide comprises a mutation selected from one of R175H, R248Q, R273H, R248W, G245S, R273C, R282W, R249S, G245D, C176F, H179Y, H179R, Y220C and R337H.

11. The method according to any one of claims 1 to 10, wherein the mutant p53 polypeptide comprises, or consists of, the amino acid sequence of one of SEQ ID NOs: 3 to 16.

12. A cancer vaccine, comprising an immunogen as defined in any one of claims 1 to 11.

13. Use of an immunogen as defined in any one of claims 1 to 11, in the manufacture of a medicament for use in vaccination against a cancer.

14. The cancer vaccine, or the use of any one of claims 12 to 13, wherein the cancer is a cancer comprising a cell or cells expressing a mutant p53 polypeptide, or comprising nucleic acid encoding a mutant p53 polypeptide.