

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 disclosure 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~~ three amino acid sequences of the mutant p53 polypeptide, wherein the amino acid sequences of the mutant p53 polypeptide including the mutation and at least one amino acid immediately adjacent to the mutation, and ~~wherein the mutant p53 polypeptide is~~ are 5-20 amino acids in length, and (ii) a scaffold sequence derived from a thioredoxin for providing the antigen sequence in a solvent-accessible configuration, and wherein the immunogen additionally comprises one or more flexible linker sequences between the antigen sequence and the scaffold sequence. In another aspect, the present disclosure refers to a ~~cancer vaccine, comprising an immunogen as defined in any one of claims 1 to 9, comprising a peptide or polypeptide comprising: (i) an antigen sequence, comprising three amino acid sequences of the mutant p53 polypeptide, wherein the amino acid sequences of the mutant p53 polypeptide include the mutation and at least one amino acid immediately adjacent to the mutation, and are 5-20 amino acids in length, and (ii) a scaffold sequence derived from a thioredoxin for providing the antigen sequence in a solvent-accessible configuration, and wherein the immunogen additionally comprises one or more flexible linker sequences between the antigen sequence and the scaffold sequence~~ a cancer vaccine, comprising an immunogen as described herein. In yet another aspect, the present disclosure 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

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 ~~three~~ amino acid sequences of the mutant p53 polypeptide, wherein the amino acid sequences of the mutant p53 polypeptide including ~~include~~ the mutation and at least one amino acid immediately adjacent to the mutation, and wherein the mutant p53 polypeptide is ~~are~~ 5-20 amino acids in length, and (ii) a scaffold sequence derived from a thioredoxin for providing the antigen sequence in a solvent-accessible configuration, and wherein the immunogen additionally comprises one or more flexible linker sequences between the antigen sequence and the scaffold sequence.

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 active site sequence of the thioredoxin from which the scaffold sequence is derived 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 comprises at least two amino acid sequences of the mutant p53 polypeptide.~~

~~53.~~ The method according to claim 41 or claim 2, 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.

~~64.~~ The method according to any one of claims 4 or claim 51 to 3, wherein the at least two amino acid sequences of the mutant p53 polypeptide are non-identical.

~~75.~~ The method according to any one of claims 1 to ~~64~~, wherein the mutant p53 polypeptide comprises a mutation in the DNA-binding domain (DBD).

86. The method according to any one of claims 1 to 75, 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.

97. The method according to any one of claims 1 to 86, wherein the mutant p53 polypeptide comprises, or consists of, the amino acid sequence of one of SEQ ID NOs: 3 to 16.

~~108. A cancer vaccine, comprising an immunogen as defined in any one of claims 1 to 9, comprising a peptide or polypeptide comprising: (i) an antigen sequence, comprising three amino acid sequences of the mutant p53 polypeptide, wherein the amino acid sequences of the mutant p53 polypeptide include the mutation and at least one amino acid immediately adjacent to the mutation, and are 5-20 amino acids in length, and (ii) a scaffold sequence derived from a thioredoxin for providing the antigen sequence in a solvent-accessible configuration, and wherein the immunogen additionally comprises one or more flexible linker sequences between the antigen sequence and the scaffold sequence.~~

~~9. The immunogen according to claim 8, wherein the antigen sequence is inserted in, or substituted for all or part of, the active site sequence of the thioredoxin from which the scaffold sequence is derived.~~

~~10. The immunogen according to claim 8 or claim 9, wherein the peptide or polypeptide used as an immunogen additionally comprises linker sequences between the amino acid sequences of the mutant p53 polypeptide.~~

~~11. The immunogen according to any one of claims 8 to 10, wherein the amino acid sequences of the mutant p53 polypeptide are non-identical.~~

~~12. The immunogen according to any one of claims 8 to 11, wherein the mutant p53 polypeptide comprises a mutation in the DNA-binding domain (DBD).~~

~~13. The immunogen according to any one of claims 8 to 12, 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.~~

14. The immunogen according to any one of claims 8 to 13, wherein the mutant p53 polypeptide comprises, or consists of, the amino acid sequence of one of SEQ ID NOs: 3 to 16.

5 1115. Use of an immunogen as defined in any one of claims 1-8 to 914, in the manufacture of a medicament for use in vaccination against a cancer.

1216. The ~~cancer vaccine, or the use of any one of~~according to claims ~~10-15~~or 11, wherein the cancer is a cancer comprising a cell or cells expressing a mutant p53 polypeptide, or
10 comprising nucleic acid encoding a mutant p53 polypeptide.