It has been proposed to enhance the immune response of a mammal to an antigen by engaging the OX40R through the use of an OX40R binding agent (WO 99/42585; Weinberg, 2000). Although the document refers generally to OX40-binding agents, the emphasis is on the use of OX40L or parts thereof; the disclosure of anti-OX40 antibodies is in the context of their being equivalent to OX40L. Indeed, when the Weinberg team translated the research to a study with non-human primates, they again deliberately chose an antibody that binds to the OX40L-binding site and generally mimics OX40L.

Al-Shamkhani et al. (Eur J Chem 1996; 26: 1695-1699) used an anti-OX40 antibody called OX86, which did not block OX40L-binding, in order to explore differential expression of OX40 on activated mouse T-cells; and Hirschhorn-Cymerman et al. (J Exp Med 2009; 206: 1103-1116) used OX86 together with cyclophosphamide in a mouse model as a potential chemoimmunotherapy. However, OX86 would not be expected to bind human OX40 and, when choosing an antibody that would be effective in humans, one would, in the light of the Weinberg work, choose an antibody that did bind at the OX40L-binding site.

In vivo ligation of the human CD134 receptor (by anti-human CD134-specific antibodies which interact with the OX40L binding domain on human CD134; US 2009/0214560 A1) in severe combined immunodeficient (SCID) mice enhances anti-tumour immunity, which leads to tumour growth inhibition of various human malignant tumour cell lines, *e.g.* lymphoma, prostate cancer, colon cancer, and breast cancer.

The exact mechanism of human CD134 ligation-mediated anti-tumour immune responses in humans is not yet elucidated, but is thought to be mediated via the CD134 transmembrane signalling pathway that is stimulated by the interaction with OX40L. This interaction is mediated by the binding of trimeric OX40L to CD134. In current anti-cancer therapies, the use of trimerized OX40 ligand is proposed as a more effective agent than anti-OX40 antibodies (Morris et al. Mol Immunol 2007; 44: 3112-3121).

Summary ~~of the Invention~~

It has now been surprisingly found by the applicants that, in order to induce T-cell mediated anti-tumour activity, the use of isolated binding molecules that bind to human CD134, wherein the binding molecule does not prevent human CD134 (CD134) receptor binding to OX40 ligand (OX40L), results in an enhanced immune response, characterised by enhancing the immunostimulator/effector function of T-effector cells and/or proliferating those cells

and/or down-regulation of the immunosuppressor function of T-regulatory cells.

The present ~~invention~~ disclosure therefore provides isolated binding molecules that bind to human CD134, wherein the binding molecule does not prevent human CD134 (OX40) receptor binding to OX40 ligand (OX40L). In one aspect, there is provided a binding molecule that binds to human CD134, wherein the binding molecule does not prevent human CD134 (OX40) receptor binding to OX40 ligand (OX40L).

In another aspect, there is provided a binding molecule comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:12 or a variant of that sequence having 1, 2 or 3 amino acid substitutions; and/or (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:13 or a variant of that sequence having 1, 2 or 3 amino acid substitutions.

In another aspect, there is provided a binding molecule that competes for human CD134 binding with an antibody comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:12; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:13.

In another aspect, there is provided a binding molecule comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:4 or a variant of that sequence having 1, 2 or 3 amino acid substitutions; and/or (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:5 or a variant of that sequence having 1, 2 or 3 amino acid substitutions.

In another aspect, there is provided a binding molecule that competes for human CD134 binding with an antibody comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:4; and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO:5.

In another aspect, there is provided a binding molecule that specifically binds to an epitope in an amino acid sequence of the extracellular domain of human CD134, to which an antibody as defined above binds.

In another aspect, there is provided a binding molecule that binds to human CD134, wherein the binding molecule does not prevent human CD134 binding to OX40 ligand (OX40L) and does not impede the immunostimulatory and/or proliferative responses of human OX40L on human CD134 expressing T-effector cells.

In another aspect, there is provided a binding molecule that binds to human CD134, wherein the binding molecule does not prevent human CD134 binding to OX40 ligand (OX40L) and does enhance the immunostimulatory and/or proliferative responses of human OX40L on human CD134 expressing T-effector cells

In another aspect, there is provided a binding molecule that binds to human CD134, wherein the binding molecule does not prevent human CD134 binding to OX40 ligand (OX40L) and does not impede suppressor function responses of human OX40L on human CD134 expressing T regulatory cells.

In another aspect, there is provided a binding molecule that binds to human CD134, wherein the binding molecule does not prevent human CD134 binding to OX40 ligand (OX40L) and does enhance suppressor function responses of human OX40L on human CD134 expressing T regulatory cells.

In another aspect, there is provided a binding molecule that binds to human CD134, wherein the binding molecule does not prevent human CD134 binding to OX40 ligand (OX40L) but does not impede the proliferative responses of human OX40L on human CD134 expressing T-regulatory cells

In another aspect, there is provided a binding molecule that binds to human CD134, wherein the binding molecule does not prevent human CD134 binding to OX40 ligand (OX40L) but does inhibit the proliferative responses of human OX40L on human CD134 expressing T-regulatory cells.

Such binding molecules include suitable anti-CD134 antibodies, antigen-binding fragments of the anti-CD134 antibodies, and derivatives of the anti-CD134 antibodies. In some embodiments the binding molecule binds to human CD134 with a K_d of 1 x 10^{-7} M or less. The binding molecule has agonist activity on human CD134 on T- effector cells and/or antagonistic activity on human CD134 on T-regulator cells. In some further embodiments, the binding molecule is a human monoclonal antibody that specifically binds human CD134 with a K_d of 100 nM or less, preferably less than 50nM, more preferably less than 20nM.

The present invention also provides a composition that comprises one or more of the binding molecules and a pharmaceutically acceptable carrier. In some embodiments, the binding molecule is a human monoclonal anti-CD134 antibody or an antigen-binding fragment thereof. The composition may further comprise additional pharmaceutical agents, such as immunotherapeutic agents, chemotherapeutic agents, and hormonal therapeutic agents.

In another aspect, there is provided a pharmaceutical composition comprising a binding moiety as defined above or produced as defined above together with one or more pharmaceutically acceptable diluents or excipients.

The present invention further provides diagnostic and therapeutic methods of using the binding molecules. In some embodiments is provided a method of treating or preventing cancer in a mammal, comprising administering to the mammal a therapeutically effective amount of a binding molecule or a composition comprising a binding molecule as disclosed herein. In some other embodiments, the disclosure provides a method of enhancing an immune response in a mammal, comprising administering to the mammal a therapeutically effective amount of a binding molecule or a composition comprising a binding molecule. In particular embodiments, the binding molecule used in the methods is a human monoclonal anti-CD134 antibody or an antigen-binding fragment thereof, which binds to human CD134, wherein the antibody does not prevent human CD134 (OX40) receptor binding to OX40 ligand (OX40L).

In another aspect, there is provided a binding molecule as defined above or produced as defined above, for use in preventing or treating cancer in a subject in need thereof.

In another aspect, there is provided a binding molecule as defined above or produced as defined above, and optionally a pharmaceutically acceptable carrier for use in enhancing an immune response in a human subject.

In another aspect, there is provided a binding molecule as defined above or produced as defined above for use in reducing the size of a tumour or inhibiting the growth of cancer cells in a subject or reducing or inhibiting the development of metastatic cancer in a subject suffering from cancer.

In another aspect, there is provided use of a binding molecule as defined above or produced as defined above in the preparation of a medicament for the treatment or prevention of cancer.

The present invention further provides nucleic acid molecules that encode an amino acid sequence of a binding molecule, vectors comprising such nucleic acids, host cells comprising the vectors, and methods of preparing the binding molecules.

In another aspect, there is provided a nucleic acid molecule encoding a binding molecule as defined above, provided that the binding moiety is a polypeptide.

In another aspect, there is provided a vector comprising at least one nucleic acid molecule as defined above.

In another aspect, there is provided a host cell comprising a vector as defined above.

In another aspect, there is provided a process for preparing a binding molecule as defined above, comprising the steps of (i) preparing CD134-binding molecules and (ii) screening the said molecules in order to identify and obtain binding molecules that do not prevent binding of OX40L to CD134.

The disclosure also provides other aspects, which will be apparent from the entire disclosure, including the claims.

38. A process according to Claim 36 or Claim 37 wherein the binding molecule is a monoclonal antibody and comprises immunizing an animal with human CD134, preparing hybridomas secreting anti-CD134 antibodies and screening for hybridomas producing anti-CD134 antibodies.

39. A binding molecule according to any of Claims 1 to 31 or produced according to any of Claims 36 to 38, for use in preventing or treating cancer in a subject in need thereof.

40. A binding molecule for use according to Claim 39, wherein the cancer is selected from the group consisting of lung cancer, prostate cancer, breast cancer, head and neck cancer, oesophageal cancer, stomach cancer, colon cancer, colorectal cancer, bladder cancer, cervical cancer, uterine cancer, ovarian cancer, liver cancer, hematological cancer, or any other disease or disorder characterized by uncontrolled cell growth.

41. A ~~method of enhancing an immune response in a human subject, comprising administering to the human subject a therapeutically effective amount of a~~ binding molecule according to any one of Claims 1 to 31 or produced according to any of Claims 36 to 38, and optionally a pharmaceutically acceptable carrier for use enhancing an immune response in a human subject.

42. A ~~method~~ binding molecule according to Claim 41 wherein the enhanced immune response comprises an increase in the immunostimulator/effector function of T-effector cells, optionally as a result of proliferation of those cells, and/or a down-regulation of the immunosuppressor function of T-regulatory cells, optionally without expansion in numbers of those cells.

~~43. A method of treating cancer in a human subject in need thereof, comprising administering to the human subject a therapeutically effective amount of a binding molecule according to any one of Claims 1 to 31 or produced according to any of Claims 36 to 38.~~

~~44. A method according to claim 43, wherein the cancer is selected from the group consisting of lung cancer, prostate cancer, breast cancer, head and neck cancer, oesophageal cancer, stomach cancer, colon cancer, colorectal cancer, bladder~~

~~cancer, cervical cancer, uterine cancer, ovarian cancer, liver cancer, hematological cancer, or any disease or disorder characterized by uncontrolled cell growth.~~

~~45.~~43. A ~~method of reducing the size of a tumour or inhibiting the growth of cancer cells in a subject or reducing or inhibiting the development of metastatic cancer in an subject suffering from cancer, comprising administering to the human subject a~~ binding molecule according to any one of Claims 1 to 31 or produced according to any of Claims 36 to 38 for use in reducing the size of a tumour or inhibiting the growth of cancer cells in a subject or reducing or inhibiting the development of metastatic cancer in a subject suffering from cancer.

~~46.~~44. The use of a binding molecule according to any one of Claims 1 to 31 or produced according to any of Claims 36 to 38 in the preparation of a medicament for the treatment or prevention of cancer.

~~47.~~45. A pharmaceutical composition comprising a binding moiety according to any one of Claims 1 to 31 or produced according to any of Claims 36 to 38 together with one or more pharmaceutically acceptable diluents or excipients.

~~48.~~46. A composition according to Claim ~~47~~ 45 wherein the composition is suitable for parenteral administration into the human body, for example by intravenous, intramuscular, intradermal, intraperitoneal, intratumour, intravesical, intra-arterial, intrathecal, intra-capsular, intra-orbital, intracardiac, transtracheal, intra-articular, subcapsular, subarachnoid, intraspinal, epidural, intrasternal or subcutaneous administration.