The technical problem underlying the present invention is the provision of methods and means for the prevention, treatment or diagnosis of HNSCC using anti-CD44 antibodies and, in some aspects particular, unlabeled anti-CD44 antibodies.

The technical problem is solved by provision of the embodiments characterized in the claims.

SUMMARY ~~OF THE INVENTION~~

The present disclosure~~invention~~ relates to novel methods for preventing, treating or diagnosing head and neck squamous cell carcinoma (HNSCC) comprising the use of anti-CD44 antibodies. ~~In certain aspects t~~The disclosure~~invention~~ may encompass~~es~~ the use of anti-CD44 antibodies that bind to the amino terminal domain of the extracellular domain of CD44 and/or that immunospecifically bind to one or more, preferably multiple human variants CD44 as expressed in humans. ~~In particular aspects, t~~The disclosure~~invention~~ may encompass~~es~~ the use of the anti-CD44 antibody produced by the hybridoma deposited with the ATCC having accession number PTA-4621, and variants and/or derivatives thereof, *e.g.*, chimeric and humanized versions of PTA-4621. The disclosure~~invention~~ may further encompass~~es~~ the use of antibodies and antigen binding fragments thereof that compete for binding with PTA-4621. In a first aspect, there is provided the use of a monoclonal anti-CD44 antibody, or antigen binding fragment thereof, for the manufacture of a medicament for the treatment of a head and neck squamous cell carcinoma (HNSCC) in a mammal, wherein said HNSCC is characterized by the expression of CD44, wherein said antibody comprises

(a) a V_H CDR1 having the amino acid sequence of SEQ ID NO:3, a V_H CDR2 having the amino acid sequence of SEQ ID NO:4, a V_H CDR3 having the amino acid sequence of SEQ ID NO:5, a V_L CDR1 having the amino acid sequence of SEQ ID NO:6, a V_L CDR2 having the amino acid sequence of SEQ ID NO:7 and a V_L CDR3 having the amino acid sequence of SEQ ID NO:8;

or

(b) a V_H domain having an amino acid sequence that is at least 85% identical to the V_H domain having the amino acid sequence of SEQ ID NO:9 or SEQ NO:10 and a V_L domain having an amino acid sequence that is at least 85% identical to the V_L domain having the amino acid sequence of SEQ ID NO:11;

and wherein said antibody competes for binding to CD44 with the antibody produced by the hybridoma deposited with the ATCC with Accession number PTA-4621.

The antibodies and fragments used according to the methods of the disclosure~~invention~~ are specific for CD44, in particular CD44 as expressed on the surface of a cell, and preferably, specific for the amino terminal extracellular domain of one or more variants of human CD44, *e.g.*, as expressed on the surface of a HNSCC cancer cell. ~~In certain aspects t~~The antibodies for use according to the methods disclosed herein may be~~are~~ humanized or chimeric versions of the antibody produced by the hybridoma deposited with the ATCC having accession number PTA-4621. In specific embodiments the antibodies for use according to the methods disclosed herein, or antigen binding fragments thereof, comprise one or more of

a heavy chain variable domain (V_H) CDR1 having the amino acid sequence of RYWMS (SEQ ID NO:3),

a V_H CDR2 having the amino acid sequence of EVNPDSTSINYTPSLKD (SEQ ID NO:4),

a V_H CDR3 having the amino acid sequence of PNYYGSRYHYYAMDY (SEQ ID NO:5),

a light chain variable domain (V_L) CDR1 having the amino acid sequence of RASQDINNYLN (SEQ ID NO:6),
a V_L CDR2 having the amino acid sequence of YTSRLHS (SEQ ID NO:7); and
a V_L CDR3 having the amino acid sequence of QQGSTLPFT (SEQ ID NO:8).

The antibodies as encompassed by the disclosure~~invention~~ may also comprise two or more of, three or more of, four or more of, five or more of, and, in certain embodiment~~aspects~~, all of a V_H CDR1 having the amino acid sequence of SEQ ID NO:3, a V_H CDR2 having the amino acid sequence of SEQ ID NO:4, a V_H CDR3 having the amino acid sequence of SEQ ID NO:5, a V_L CDR1 having the amino acid sequence of SEQ ID NO:6, a V_L CDR2 having the amino acid sequence of SEQ ID NO:7 and a V_L CDR3 having the amino acid sequence of SEQ ID NO:8.

The antibodies and antigen binding fragments for use according to the methods of the disclosure~~invention~~ may comprise

a V_H domain having the amino acid sequence of
EVKLLESGGGLVQPGGSLKLSCATSGFDFSRYWMSWVRQAPGKGL
EWIGEVNPDSTSINYTPSLKDQFIISRDNAKNTLDLQMSKVSSEDTA
LYYCTRPNYYGSRYHYYAMDYWGQGTSVTVSS (SEQ ID NO:1)

and/or a V_L domain having the amino acid sequence of
DIQMTQTTSSLSVSLGDRVTINCRASQDINNYLNWYQQKPDGTVKL
LIYYTSRLHSGVPSRFSGSGSGTDFSLTISNLEKEDVATYFCQQGSTL
PFTFGSGTKLEIK (SEQ ID NO:2).

~~In certain aspects t~~The disclosure may~~invention~~ encompasses the use of an anti-CD44 antibody or antigen binding fragment comprising a V_H domain having the amino acid sequence of SEQ ID NO:1 and a V_L domain having the amino acid sequence of SEQ ID NO:2.

The disclosure~~invention~~ further encompasses the use of an anti-CD44 antibody, or antigen binding fragments thereof, for treating, preventing or diagnosing an HNSCC, wherein the anti-CD44 antibody is humanized. ~~In some aspects, t~~The humanized anti-CD44 antibody or antigen binding fragment may comprises

a V_H domain having the amino acid sequence of

EVQLVESGGGLVQPGGSLRLSCAASGFDFSRYWMSWVRQAPGKGL
VWVGEVNPDSTSINYTPSLKDRFTISRDNAKNTLYLQMNSLRAEDT
AVYYCTRPNYYGSRYHYYAMDYWGQGTLVTVSS (SEQ ID NO:9)
or
EVQLVESGGGLVQPGGSLRLSCATSGFDFSRYWMSWVRQAPGKGL
VWIGEVNPDSTSINYTPSLKDQFTISRDNAKNTLYLQMNSLRAEDTA
VYYCTRPNYYGSRYHYYAMDYWGQGTLVTVSS (SEQ ID NO:10)

and/or a V_L domain having the sequence of
DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKL
LIYYTSRLHSGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQGSTLP
FTFGQGTKLEIK (SEQ ID NO:11).

~~In a particular aspect, t~~The disclosure may~~invention~~ provides for a humanized anti-CD44 antibody or antigen binding fragment thereof for use in preventing, treating or diagnosis HNSCC in a subject, wherein said humanized anti-CD44 antibody comprises a V_H domain having the amino acid sequence of SEQ ID NO:9 and a V_L domain having the amino acid sequence of SEQ ID NO:11, or wherein said anti-CD44 antibody comprises a V_H domain having the amino acid sequence of SEQ ID NO:10 and a V_L domain having the amino acid sequence of SEQ ID NO:11. In a second aspect, there is provided the use of a humanized anti-CD44 antibody, or antigen binding fragment thereof, for the manufacture of a medicament for the treatment of a head and neck squamous cell carcinoma (HNSCC) in a human, wherein said HNSCC is characterized by the expression of CD44 and wherein said humanized antibody comprises a V_H domain having the amino acid sequence of SEQ ID NO:9 and a V_L domain having the amino acid sequence of SEQ ID NO:11, or comprises a V_H domain having the amino acid sequence of SEQ ID NO:10 and a V_L domain having the amino acid sequence of SEQ ID NO:11. In particular embodiment~~aspects~~ the subject is a human. The disclosure~~invention~~ also provides for the use of a humanized anti-CD44 antibody or antigen binding fragment thereof for the prevention, treatment or diagnosis of HNSCC in a subject, wherein said humanized anti-CD44 antibody comprises a V_H domain having the amino acid sequence of SEQ ID NO:9 and a V_L domain having the amino acid sequence of SEQ ID NO:11, or wherein said anti-CD44 antibody comprises a V_H domain having the amino acid sequence of SEQ ID NO:10 and a V_L domain having the amino acid sequence of SEQ ID NO:11.

~~In certain aspects, t~~The antibody or antibody fragment for use in accordance with the methods of the disclosure may~~invention~~ recognizes the epitope within the amino terminal domain of the extracellular region of CD44 having the amino acid sequence of AFDGPITITIV (SEQ ID NO:12).

The anti-CD44 antibodies for use according to the disclosure~~invention~~ may be conjugated to active moieties such as therapeutic or reporter moieties. The anti-CD44 antibodies may also be conjugated to hematogeneous cells from the subject. ~~In certain aspects~~

~~t~~The therapeutic moieties may be~~are~~ moieties known in the art to be beneficial for the treatment, prevention or diagnosis of HNSCC, such as, but not limited to, a cytotoxin, an enzyme, a radioactive element, a cytokine, an antimetabolite or an interferon.

The disclosure~~invention~~ also encompasses the use of anti-CD44 antibodies or antigen binding fragments thereof as single agent therapies or in combination with one or more other chemotherapeutic or diagnostic agents and/or therapies. Accordingly, the disclosure~~invention~~ encompasses the use of anti-CD44 antibodies (*e.g.*, humanized or chimeric antibodies), or antigen binding fragments thereof, in combination with a standard or experimental treatment regimen for HNSCC (e.g., chemotherapy, radioimmunotherapy, or radiotherapy). Examples of therapeutic agents that are particularly useful in combination with a CD44-specific antibody or an antigen-binding fragment thereof, according to the methods disclosed herein for the prevention, treatment, or diagnosis HNSCC, include, but are not limited to chemotherapeutics as known in the art, alkylating agents, antimetabolites, natural products, and hormones. The combination therapies as disclosed herein may enable lower dosages of an anti-CD44 antibody or an antigen-binding fragment thereof and/or less frequent administration of anti-CD44 antibody or an antigen-binding fragment thereof to a subject with a HNSCC, to achieve a therapeutic or prophylactic effect. According to the methods disclosed herein, the anti-CD44 antibodies or antigen-binding fragments thereof may be co-administered, concurrently administered, and/or sequentially administered with the one or more other chemotherapeutic or diagnostic agents, or may be administered in conjunction with radiation therapy or surgery. Administration of the antibodies, fragments and/ or additional agents or therapies, may be by any means known in the art to be suitable for delivery of the particular agent or therapy to the tumor site. Such administration may be parenteral (*e.g.*, IV, intramuscular or subcutaneous administration), oral or, in certain aspects, direct delivery to the tumor site or to the site of suspected tumor occurrence or recurrence. The antibodies, fragments and/or compositions may also be administered as a sustained release formulation.

The disclosure~~invention~~ further provides a pharmaceutical composition for the prevention, treatment or diagnosis of HNSCC in a subject comprising (i) a therapeutically effective amount of an anti-CD44 antibody as disclosed herein (*e.g.*, a chimeric or humanized antibody), or an antigen binding fragment thereof; and (ii) a pharmaceutically acceptable carrier.

Also disclosed herein are methods of diagnosis of HNSCC in a subject comprising suspected of having such disease comprising: (i) contacting a biological sample from said subject with an effective amount of an anti-CD44 antibody as disclosed herein or antigen binding fragment thereof; and (ii) detecting binding of said antibody or a fragment thereof, wherein detection of said antibody or fragment above a background or standard level indicates that said subject has HNSCC. The detection of the anti-CD44 antibody or antigen binding fragment thereof may be aided by the use of a detectable marker as is known in the art. The disclosure~~invention~~ also encompasses a diagnostic or prognostic kit for detection of HNSCC in a tissue sample from a subject known or suspected to comprise HNSCC cells. ~~In certain aspects, t~~The kit may comprises a means for detecting CD44, in particular as expressed by HNSCC cells and/or a tissue sample from a subject known or suspected to contain HNSCC cells. The kit may comprise (1) the monoclonal antibody produced by the hybridoma deposited with the ATCC as accession number PTA-4621, (2) an antibody that competes for binding with the monoclonal antibody produced by the hybridoma deposited with the ATCC as accession number PTA-4621; (3) an antibody comprising one or more of a V_H CDR1 having the amino acid sequence of SEQ ID NO:3, a V_H CDR2 having the amino acid sequence of SEQ ID NO:4, a V_H CDR3 having the amino acid sequence of SEQ ID NO:5, a V_L CDR1 having the amino acid sequence of SEQ ID NO:6, a V_L CDR2 having the amino acid sequence of SEQ ID NO:7 and a V_L CDR3 having the amino acid sequence of SEQ ID NO:8; or (4) an antigen binding fragment of the antibody of (1), (2) or (3). The kit may also comprise one or more of: a substrate or container for holding a biological sample, reference standard(s) of biomarker(s) in solution or solid form (*e.g.*, CD44 or peptide specifically bound by the antibody produced by antibody PTA-4621), one or more antibodies specific for the biomarkers (*e.g.*, antibody PTA-4621, and antigen binding fragment thereof, or a variant or derivative thereof), and directions for carrying out detection assay(s) for the biomarkers with the contents of the kit.

It is further an objective of the embodiments disclosed herein~~invention~~ to teach a method of treatment of HNSCC using antibody PTA-4621 or antigen-binding fragments thereof. It is another objective of the embodiments disclosed herein~~invention~~ to teach the diagnosis of HNSCC using antibody PTA-4621 or antigen-binding fragments thereof. It is a further objective of the embodiments disclosed herein~~invention~~ to teach the use of PTA-4621 or antigen-binding fragments thereof for the prognosis or staging of HNSCC. It is a further objective of the embodiments disclosed herein~~invention~~ to teach

the use of PTA-4621 or antigen-binding fragments thereof for the selection of a patient for the treatment of a HNSCC.

~~In certain aspects, t~~The anti-CD44 antibodies and antigen binding fragments thereof for use according to the methods disclosed herein may comprise an amino acid sequence of a V_H domain and/or V_L domain that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of the V_H domain and/or V_L domain of the mouse monoclonal antibody produced by the hybridoma deposited with the ATCC having accession number PTA-4621, provided that the anti-CD44 antibodies or fragments exhibit binding specificity to CD44 and/or compete for binding with PTA-4621. The disclosure~~invention~~ also encompasses the use of anti-CD44 antibodies or antigen binding fragments thereof that comprise an amino acid sequence of a V_H domain and/or V_L domain that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the V_H domain having the amino acid sequence of SEQ ID NO:1 and/or the V_L domain having the amino acid sequence of SEQ ID NO:2, provided that the anti-CD44 antibodies or fragments exhibit binding specificity to CD44 and/or compete for binding with PTA-4621. ~~In certain aspects, t~~The present disclosure may~~invention~~ ~~encompasses~~ antibodies or fragments thereof that specifically bind CD44 and that comprise an amino acid sequence of one or more CDRs that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of one or more CDRs of the mouse monoclonal antibody produced by the hybridoma deposited with the ATCC having accession number PTA-4621, provided that said antibodies or fragments exhibit binding specificity to CD44 and/or compete for binding with PTA-4621. The disclosure~~invention~~ also encompasses the use of anti-CD44 antibodies or antigen binding fragments thereof that comprise an amino acid sequence of one or more CDRs that are at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to one or more of a V_H CDR1 having the amino acid sequence of SEQ ID NO:3, a V_H CDR2 having the amino acid sequence of SEQ ID NO:4, a V_H CDR3 having the amino acid sequence of SEQ ID NO:5, a V_L CDR1 having the amino acid sequence of SEQ ID NO:6, a V_L CDR2 having the amino acid sequence of SEQ ID NO:7 and a V_L CDR3 having the amino acid sequence of SEQ ID NO:8, provided

that provided that said antibodies or fragments exhibit binding specificity to CD44 and/or competes for binding with PTA-4621.

The present disclosure~~invention~~ also encompasses the use of humanized antibodies and antibody fragments specific for CD44 or that compete for binding with antibody PTA-4621, in which one or more regions of one or more CDRs of the heavy and/or light chain variable regions of a human antibody (the recipient antibody) have been substituted by analogous parts of one or more CDRs of a donor monoclonal antibody which specifically binds CD44, *e.g.*, of the murine monoclonal antibody produced by clone PTA-4621. Preferably, the humanized antibody specifically binds to the same epitope as the donor murine antibody. It will be appreciated by one skilled in the art that the disclosure~~invention~~ encompasses CDR grafting of antibodies in general. ~~In particular aspects, t~~The present disclosure may~~invention~~ encompasses the use of humanized antibodies or antibody fragments that specifically bind CD44 and/or compete for binding with PTA-4621, which humanized antibodies or antibody fragments comprise an amino acid sequence of a V_H domain and/or V_L domain that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the V_H domain having the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10 and/or the V_L domain having the amino acid sequence of SEQ ID NO:11.

The anti-CD44 antibodies and antigen binding fragments thereof for use according to the methods disclosed herein may be encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence encoding a V_H domain, the complete heavy chain, a V_L domain or the complete light chain of the murine monoclonal antibody PTA-4621. ~~In other aspects, t~~The disclosure may~~invention~~ encompasses the use of a an anti-CD44 antibody or antigen binding fragment thereof comprising a V_H domain that is encoded by a nucleic acid sequence that hybridizes under stringent conditions to a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1, SEQ ID NO:9 or SEQ ID NO:10. ~~In other aspects, t~~The disclosure may~~invention~~ encompasses the use of a an anti-CD44 antibody or antigen binding fragment thereof comprising a V_L domain that is encoded by a nucleic acid sequence that hybridizes under stringent conditions to a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:11. The disclosure~~invention~~ encompasses the use of a an anti-CD44 antibody or antigen binding fragment thereof comprising one or more CDRs encoded by a nucleotide sequence

that hybridizes under stringent conditions to the nucleotide sequence encoding one or more CDRs of the murine monoclonal antibody PTA-4621, or the nucleic acid sequence encoding one or more of V_H CDR1 having the amino acid sequence of SEQ ID NO:3, a V_H CDR2 having the amino acid sequence of SEQ ID NO:4, a V_H CDR3 having the amino acid sequence of SEQ ID NO:5, a V_L CDR1 having the amino acid sequence of SEQ ID NO:6, a V_L CDR2 having the amino acid sequence of SEQ ID NO:7 and a V_L CDR3 having the amino acid sequence of SEQ ID NO:8. Stringent hybridization conditions include, but are not limited to, (i) hybridization to filter-bound DNA in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC/0.1% SDS at about 50-65°C; (ii) highly stringent conditions such as hybridization to filter-bound DNA in 6X SSC at about 45°C, followed by one or more washes in 0.1X SSC/0.2% SDS at about 60°C, or (iii) any other stringent hybridization conditions known to those skilled in the art.

The disclosure~~invention~~ also relates to the use of a vector comprising the nucleic acids disclosed herein (*i.e.*, nucleic acids encoding V_H domains, V_L domains and/or one or more CDR of anti-CD44 antibody or antigen binding fragments). In certain embodiments, said vector is an expression vector and the nucleic acid sequences encoding the amino acid sequences as described herein are operably joined with nucleic acid regulatory sequences or sequences encoding amino acid regulatory sequences (*e.g.*, transcription, translation, translocation signals) necessary for the proper expression of the encoded amino acid sequences. The disclosure~~invention~~ further provides host cells containing the vectors or nucleotide sequences encoding the antibodies or antibody fragments disclosed herein.

Other objects and advantages of this disclosure~~invention~~ will become apparent from the following description wherein are set forth, by way of illustration and example, certain embodiments of this disclosure~~invention~~.

DEFINITIONS

In general, the following words or phrases have the indicated definition when used in the summary, description, examples, and claims.

The term "antibody" is used in the broadest sense and specifically covers, for example, single monoclonal antibodies (including agonist, antagonist, and