

inflammatory factor 1 (AIF1), epithelial cell adhesion molecule (EpCAM) and cyclin-dependent kinase inhibitor 1B (CDKN1B) are indicative of the presence of liver cancer. Therefore, the detection of autoantibodies immunologically specific for any one of these tumour marker proteins can be used for the diagnosis of liver cancer.

In one aspect, the present disclosure refers to a method of detecting liver cancer in a mammalian subject by detecting an antibody in a test sample comprising a bodily fluid from the mammalian subject, wherein the antibody is an autoantibody immunologically specific for a tumour marker protein selected from the group consisting of MMP9 (matrix metalloproteinase 9), AIF1 (allograft inflammatory factor 1), EpCAM (epithelial cell adhesion molecule) and CDKN1B (cyclin-dependent kinase inhibitor 1B), which method comprises the steps of:

- (a) contacting the test sample with a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B; and
 - (b) determining the presence or absence of complexes of the tumour marker antigen bound to autoantibodies present in the test sample;
- whereby the presence of said complexes is indicative of the presence of liver cancer.

According to an example of the disclosure there is provided a method of detecting an antibody in a test sample comprising a bodily fluid from a mammalian subject, wherein the antibody is an autoantibody immunologically specific for a tumour marker protein selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B, which method comprises the steps of:

- (a) contacting the test sample with a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B; and
- (b) determining the presence or absence of complexes of the tumour marker antigen bound to autoantibodies present in the test sample.

Within this example the subject is preferably suspected of having liver cancer.

According to an example of the disclosure there is provided a method of detecting liver cancer in a mammalian subject by detecting an antibody in a test sample comprising a bodily fluid from the mammalian subject, wherein the antibody is an autoantibody immunologically specific for a tumour marker protein selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B, which method comprises the steps of:

- (a) contacting the test sample with a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B; and
 - (b) determining the presence or absence of complexes of the tumour marker antigen bound to autoantibodies present in the test sample;
- whereby the presence of said complexes is indicative of the presence of liver cancer.

(d) administering a liver cancer treatment to the diagnosed subject.

In another aspect, the present disclosure refers to a method of predicting response to an anti-liver cancer treatment, the method comprising detecting an antibody in a test sample comprising a bodily fluid from a mammalian subject, wherein the antibody is an autoantibody immunologically specific for a tumour marker protein selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B, which method comprises the steps of:

- (a) contacting the test sample with a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B;
 - (b) determining the presence or absence of complexes of the tumour marker antigen bound to autoantibodies present in the test sample;
 - (c) detecting the amount of specific binding between the tumour marker antigen and autoantibodies present in the test sample; and
 - (d) comparing the amount of specific binding between the tumour marker antigen and the autoantibody with a previously established relationship between the amount of binding and the likely outcome of treatment;
- whereby a change in the amount of specific binding, when compared to controls, predicts that the patient will or will not respond to the anti-liver cancer treatment.

Within this example of the disclosure the anti-liver cancer treatment may be selected from the group consisting of chemotherapy, radiofrequency ablation, liver resection, liver transplant, vaccination, anti-growth factor or signal transduction therapies, endocrine therapy, human antibody therapy, transcatheter arterial chemoembolization, percutaneous ethanol injection, microwave ablation, sorafenib administration and radioembolisation.

In another example, the present disclosure refers to the use of a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B in a method of detecting liver cancer in a mammalian subject by detecting an autoantibody immunologically specific for MMP9, AIF1, EpCAM or CDKN1B in a test sample comprising a bodily fluid from the mammalian subject, which method comprises the steps of:

- (a) contacting the test sample with a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B; and
 - (b) determining the presence or absence of complexes of the tumour marker antigen bound to autoantibodies present in the test sample;
- whereby the presence of said complexes is indicative of the presence of liver cancer.

In another example, the present disclosure refers to a kit for the detection of liver cancer, wherein the kit comprises:

- (a) a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B; and
- (b) a reagent capable of detecting complexes of the tumour marker antigen bound to autoantibodies present in the test sample;
optionally further comprising:
- (c) means for contacting the tumour marker antigen with a test sample comprising a bodily fluid from a mammalian subject.

In one aspect, the present disclosure refers to a kit for the detection of liver cancer, wherein the kit comprises:

- (a) a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B; and
- (b) a reagent capable of detecting complexes of the tumour marker antigen bound to autoantibodies present in a test sample;
wherein the tumour marker antigen is present within a panel of two or more tumour marker antigens; and
wherein the panel of two or more tumour marker antigens:
 - (i) comprises or consists of CAGE, NY-ESO-1, MMP9, transferrin, MAGE A4, RalA, HSPA4, SALL4, Cyclin B1, EpCAM, DDX3X, and AIF1;
 - (ii) comprises or consists of CAGE, NY-ESO-1, MMP9, transferrin, MAGE A4, RalA, HSPA4, SALL4, Cyclin B1, EpCAM, DDX3X, AIF1, SOX2 and AFP;
 - (iii) comprises or consists of MMP9, AIF1, EpCAM, NY-ESO-1, HSPA4, vimentin, HNRNP-L and transferrin;
 - (iv) comprises or consists of AIF1, EpCAM, HSPA4 and CPS1, optionally wherein the subject is female;
 - (v) comprises or consists of AIF1, CAGE, HSPD1, SOX2, SALL4, HSPA4 and transferrin, optionally wherein the subject is female;
 - (vi) comprises or consists of EpCAM, NY-ESO-1, vimentin, HSPA2, HSPA4 and HNRNP-L, optionally wherein the subject is male;
 - (vii) comprises or consists of EpCAM, CAGE, SOX2, RalA, MAGE A4, DDX3X and NY-ESO-1, optionally wherein the subject is male;
 - (viii) comprises or consists of MMP9, AIF1, EpCAM, DDX3X, SALL4, MAGE A4, NY-ESO-1, CAGE, RalA and SOX2;
 - (ix) comprises NY-ESO-1, vimentin, HSPA4, transferrin, HNRNP-L, HSPD1, HNRNP-A2, SALL4, Cyclin B1, AFP, NPM1, YWHAZ, DDX3X, p62, CAGE, MAGE A4, RalA, GBU4-5, SOX2, AKR1B10, ApoA1, BCL2, CD44, CK18, CPS1, FUCA1, GLUL, HSPA2, IL-8, MDM2, PEBP1, prolactin, RGN, SPP1, SSX2 and TGFB1;
 - (x) comprises or consists of MMP9, AIF1, EpCAM, CDKN1B, NY-ESO-1, vimentin, HSPA4, transferrin, HNRNP-L, HSPD1, HNRNP-A2, SALL4, Cyclin B1, AFP, NPM1, YWHAZ, DDX3X, p62, CAGE, MAGE A4, RalA, GBU4-5, SOX2, AKR1B10, ApoA1, BCL2, CD44, CK18, CPS1, FUCA1, GLUL, HSPA2, IL-8, MDM2, PEBP1, prolactin, RGN, SPP1, SSX2 and TGFB1; or
 - (xi) comprises or consists of MMP9, AIF1, EpCAM and CDKN1B.

According to an example of the disclosure there is provided a kit for the detection of autoantibodies in a test sample comprising a bodily fluid from a mammalian subject comprising:

Claims

1. A method of detecting liver cancer in a mammalian subject by detecting an antibody in a test sample comprising a bodily fluid from the mammalian subject, wherein the antibody is an autoantibody immunologically specific for a tumour marker protein selected from the group consisting of MMP9 (matrix metalloproteinase 9), AIF1 (allograft inflammatory factor 1), EpCAM (epithelial cell adhesion molecule) and CDKN1B (cyclin-dependent kinase inhibitor 1B), which method comprises the steps of:

(a) contacting the test sample with a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B; and

(b) determining the presence or absence of complexes of the tumour marker antigen bound to autoantibodies present in the test sample;

whereby the presence of said complexes is indicative of the presence of liver cancer.

2. The method of claim 1,

wherein the mammalian subject has tested positive for alpha-fetoprotein (AFP), des-gamma carboxyprothrombin (DCP) or lectin-reactive alpha-fetoprotein (AFP-L3); or

wherein the mammalian subject has tested positive for liver cancer using ultrasound surveillance; or

wherein the mammalian subject has liver cirrhosis, non-alcoholic fatty liver disease, alcoholic liver disease, Wilson's disease, hereditary hemochromatosis, autoimmune hepatitis, hepatitis B, hepatitis C, documented aflatoxin exposure, schistosomiasis or diabetes mellitus; or

wherein the liver cancer is hepatocellular carcinoma (HCC).

3. A method of predicting response to an anti-liver cancer treatment, the method comprising detecting an antibody in a test sample comprising a bodily fluid from a mammalian subject, wherein the antibody is an autoantibody immunologically specific for a tumour marker protein selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B, which method comprises the steps of:

(a) contacting the test sample with a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B;

(b) determining the presence or absence of complexes of the tumour marker antigen bound to autoantibodies present in the test sample;

(c) detecting the amount of specific binding between the tumour marker antigen and autoantibodies present in the test sample; and

(d) comparing the amount of specific binding between the tumour marker antigen and the autoantibody with a previously established relationship between the amount of binding and the likely outcome of treatment;

whereby a change in the amount of specific binding, when compared to controls, predicts that the patient will or will not respond to the anti-liver cancer treatment.

4. The method of claim 3, wherein the liver cancer treatment is selected from the group consisting of chemotherapy, radiofrequency ablation, liver resection, liver transplant, vaccination, anti-growth factor or signal transduction therapies, endocrine therapy, human antibody therapy, transcatheter arterial chemoembolization, percutaneous ethanol injection, microwave ablation, sorafenib administration and radioembolisation.

5. The method of any one of claims 1-4, wherein two or more autoantibodies are detected, and wherein the method comprises the step of:

(a) contacting the test sample with a panel of two or more tumour marker antigens comprising a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B and one or more further tumour marker antigens immunologically specific for at least one of said autoantibodies; optionally

wherein the panel comprises two or more tumour marker antigens which are distinct antigens; optionally

wherein the panel comprises two or more antigen variants of one or more of the distinct antigens.

6. The method of claim 5, wherein the panel of two or more tumour marker antigens comprises MMP9, AIF1, EpCAM and CDKN1B.

7. The method of claim 5, wherein the panel of two or more tumour marker antigens comprises one or more tumour marker antigens selected from the group consisting of NY-ESO-1, vimentin, HSPA4, transferrin, HNRNP-L, HSPD1, HNRNP-A2, SALL4, Cyclin B1, AFP, NPM1, YWHAZ, DDX3X, p62, CAGE, MAGE A4, RalA, GBU4-5, SOX2, AKR1B10, ApoA1, BCL2, CD44, CK18, CPS1, FUCA1, GLUL, HSPA2, IL-8, MDM2, PEBP1, prolactin, RGN, SPP1, SSX2 and TGFB1.

8. The method of claim 7, wherein the panel of two or more tumour marker antigens:

(i) comprises or consists of CAGE, NY-ESO-1, MMP9, transferrin, MAGE A4, RalA, HSPA4, SALL4, Cyclin B1, EpCAM, DDX3X, and AIF1;

(ii) comprises or consists of CAGE, NY-ESO-1, MMP9, transferrin, MAGE A4, RalA, HSPA4, SALL4, Cyclin B1, EpCAM, DDX3X, AIF1, SOX2 and AFP;

(iii) comprises or consists of MMP9, AIF1, EpCAM, NY-ESO-1, HSPA4, vimentin, HNRNP-L and transferrin;

(iv) comprises or consists of AIF1, EpCAM, HSPA4 and CPS1, optionally wherein the subject is female;

- (v) comprises or consists of AIF1, CAGE, HSPD1, SOX2, SALL4, HSPA4 and transferrin, optionally wherein the subject is female;
- (vi) comprises or consists of EpCAM, NY-ESO-1, vimentin, HSPA2, HSPA4 and HNRNP-L, optionally wherein the subject is male;
- (vi) comprises or consists of EpCAM, CAGE, SOX2, RalA, MAGE A4, DDX3X and NY-ESO-1, optionally wherein the subject is male;
- (viii) comprises or consists of MMP9, AIF1, EpCAM, DDX3X, SALL4, MAGE A4, NY-ESO-1, CAGE, RalA and SOX2;
- (ix) comprises NY-ESO-1, vimentin, HSPA4, transferrin, HNRNP-L, HSPD1, HNRNP-A2, SALL4, Cyclin B1, AFP, NPM1, YWHAZ, DDX3X, p62, CAGE, MAGE A4, RalA, GBU4-5, SOX2, AKR1B10, ApoA1, BCL2, CD44, CK18, CPS1, FUCA1, GLUL, HSPA2, IL-8, MDM2, PEBP1, prolactin, RGN, SPP1, SSX2 and TGFB1; or
- (x) comprises or consists of MMP9, AIF1, EpCAM, CDKN1B, NY-ESO-1, vimentin, HSPA4, transferrin, HNRNP-L, HSPD1, HNRNP-A2, SALL4, Cyclin B1, AFP, NPM1, YWHAZ, DDX3X, p62, CAGE, MAGE A4, RalA, GBU4-5, SOX2, AKR1B10, ApoA1, BCL2, CD44, CK18, CPS1, FUCA1, GLUL, HSPA2, IL-8, MDM2, PEBP1, prolactin, RGN, SPP1, SSX2 and TGFB1.

9. An *in vitro* method of determining an antibody profile of an individual suffering from liver cancer in a test sample comprising a bodily fluid from the mammalian subject wherein the antibody is an autoantibody immunologically specific for a tumour marker protein selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B, which method comprises the steps of:

- a) contacting the test sample with a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B; and
- b) determining the presence or absence of complexes of the tumour marker antigen bound to autoantibodies present in the test sample, wherein the method is repeated to build up a profile of antibody production.

10. The method of any one of the preceding claims, wherein the tumour marker antigen is a naturally occurring protein or polypeptide, a recombinant protein or polypeptide, a synthetic protein or polypeptide, a synthetic peptide, a peptide mimetic, a polysaccharide or a nucleic acid; or

wherein the bodily fluid is selected from the group consisting of plasma, serum, whole blood, urine, sweat, lymph, faeces, cerebrospinal fluid, ascites fluid, pleural effusion, seminal fluid, sputum, nipple aspirate, post-operative seroma, saliva, amniotic fluid, tears and wound drainage fluid.

11. The method of any one of the preceding claims, wherein the method further comprises detecting alpha-fetoprotein (AFP), des-gamma carboxyprothrombin (DCP) or lectin-reactive alpha-fetoprotein (AFP-L3) in a test sample comprising a bodily fluid from the mammalian subject.

12. The method of claim 11, wherein the method comprises detecting alpha-fetoprotein (AFP) in a test sample comprising a bodily fluid from the mammalian subject, optionally wherein autoantibodies immunologically specific for the tumour marker proteins CAGE, NY-ESO-1, MMP9, transferrin, MAGE A4, RalA, HSPA4, SALL4, Cyclin B1, EpCAM, DDX3X, and AIF1 are detected in a test sample comprising a bodily fluid from the mammalian subject; or optionally

wherein autoantibodies immunologically specific for the tumour marker proteins CAGE, NY-ESO-1, MMP9, transferrin, MAGE A4, RalA, HSPA4, SALL4, Cyclin B1, EpCAM, DDX3X, AIF1, SOX2 and AFP are detected in a test sample comprising a bodily fluid from the mammalian subject.

13. A kit for the detection of liver cancer, wherein the kit comprises:

(a) a tumour marker antigen selected from the group consisting of MMP9, AIF1, EpCAM and CDKN1B; and

(b) a reagent capable of detecting complexes of the tumour marker antigen bound to autoantibodies present in a test sample;

wherein the tumour marker antigen is present within a panel of two or more tumour marker antigens; and

wherein the panel of two or more tumour marker antigens:

(i) comprises or consists of CAGE, NY-ESO-1, MMP9, transferrin, MAGE A4, RalA, HSPA4, SALL4, Cyclin B1, EpCAM, DDX3X, and AIF1;

(ii) comprises or consists of CAGE, NY-ESO-1, MMP9, transferrin, MAGE A4, RalA, HSPA4, SALL4, Cyclin B1, EpCAM, DDX3X, AIF1, SOX2 and AFP;

(iii) comprises or consists of MMP9, AIF1, EpCAM, NY-ESO-1, HSPA4, vimentin, HNRNP-L and transferrin;

(iv) comprises or consists of AIF1, EpCAM, HSPA4 and CPS1, optionally wherein the subject is female;

(v) comprises or consists of AIF1, CAGE, HSPD1, SOX2, SALL4, HSPA4 and transferrin, optionally wherein the subject is female;

(vi) comprises or consists of EpCAM, NY-ESO-1, vimentin, HSPA2, HSPA4 and HNRNP-L, optionally wherein the subject is male;

(vii) comprises or consists of EpCAM, CAGE, SOX2, RalA, MAGE A4, DDX3X and NY-ESO-1, optionally wherein the subject is male;

- (viii) comprises or consists of MMP9, AIF1, EpCAM, DDX3X, SALL4, MAGE A4, NY-ESO-1, CAGE, RalA and SOX2;
- (ix) comprises NY-ESO-1, vimentin, HSPA4, transferrin, HNRNP-L, HSPD1, HNRNP-A2, SALL4, Cyclin B1, AFP, NPM1, YWHAZ, DDX3X, p62, CAGE, MAGE A4, RalA, GBU4-5, SOX2, AKR1B10, ApoA1, BCL2, CD44, CK18, CPS1, FUCA1, GLUL, HSPA2, IL-8, MDM2, PEBP1, prolactin, RGN, SPP1, SSX2 and TGFB1;
- (x) comprises or consists of MMP9, AIF1, EpCAM, CDKN1B, NY-ESO-1, vimentin, HSPA4, transferrin, HNRNP-L, HSPD1, HNRNP-A2, SALL4, Cyclin B1, AFP, NPM1, YWHAZ, DDX3X, p62, CAGE, MAGE A4, RalA, GBU4-5, SOX2, AKR1B10, ApoA1, BCL2, CD44, CK18, CPS1, FUCA1, GLUL, HSPA2, IL-8, MDM2, PEBP1, prolactin, RGN, SPP1, SSX2 and TGFB1; or
- (xi) comprises or consists of MMP9, AIF1, EpCAM and CDKN1B.

14. The kit of claim 13 further comprising:

(c) means for contacting the tumour marker antigen with a test sample comprising a bodily fluid from a mammalian subject, optionally wherein the means for contacting the tumour marker antigen with a test sample comprising a bodily fluid from a mammalian subject comprises the tumour marker antigen immobilised on a chip, slide, plate, wells of a microtitre plate, bead, membrane or nanoparticle.

15. The kit of claim 13 or 14, wherein the panel of two or more tumour marker antigens comprises one or more tumour marker antigens selected from the group consisting of NY-ESO-1, vimentin, HSPA4, transferrin, HNRNP-L, HSPD1, HNRNP-A2, SALL4, Cyclin B1, AFP, NPM1, YWHAZ, DDX3X, p62, CAGE, MAGE A4, RalA, GBU4-5, SOX2, AKR1B10, ApoA1, BCL2, CD44, CK18, CPS1, FUCA1, GLUL, HSPA2, IL-8, MDM2, PEBP1, prolactin, RGN, SPP1, SSX2 and TGFB1.

16. The kit of claim 14 or 15, wherein the bodily fluids is selected from the group consisting of plasma, serum, whole blood, urine, sweat, lymph, faeces, cerebrospinal fluid, ascites fluid, pleural effusion, seminal fluid, sputum, nipple aspirate, post-operative seroma, saliva, amniotic fluid, tears and wound drainage fluid.