CLAIMS

1. A method of analysing a patient with Hepatocellular Carcinoma (HCC), wherein the method comprises:

(a) detecting the expression levels of 5, 8, 7, 11, or 14 of genes selected from the group consisting of CLL5, CCR2, CEACAM8, CXCL10, IFNG, IL6, NCR3, TBX21, TLR3, TNF, CCL2, CD8A, FCGR1A, LTA, and TLR4 in a patient-derived tumor sample; and

(b) based on a gene signature as listed in Table 2A, Table 2B, Table 3, Table 4, Table 14, Table 15 and/or Table 16, determine one or more of the following: stratifying or classifying the patient to have HCC; providing a prognosis for the patient with HCC; monitoring disease progression of HCC; predicting efficacy of a therapeutic intervention, selecting treatment for the tumor, or evaluating the efficacy of a therapeutic intervention for a patient with HCC.

2. A method according to claim 1 wherein the method is a method of classifying a patient with HCC as having a poor or good prognosis comprising the steps of:

(a) detecting the expression levels of 5, 8, 7, 11, or 14 of genes selected from the group consisting of CLL5, CCR2, CEACAM8, CXCL10, IFNG, IL6, NCR3, TBX21, TLR3, TNF, CCL2, CD8A, FCGR1A, LTA, and TLR4 in a patient-derived tumor sample,; and

(b) based on a gene signature as listed in Table 2A, Table 2B, Table 3, Table 4, Table 14, Table 15 and/or Table 16, classifying the patient as having a short or long survival based on the expression levels determined in step (a), in which the patient has HCC.

3. A method according to claim 1 wherein the method is a method for evaluating the efficacy of a therapeutic intervention for treating HCC patients comprising the steps of:

(a) detecting the expression levels of 5, 8, 7, 11, or 14 of genes selected from the group consisting of CLL5, CCR2, CEACAM8, CXCL10, IFNG, IL6, NCR3, TBX21, TLR3, TNF, CCL2, CD8A, FCGR1A, LTA, and TLR4 in a patient-derived tumor sample; and

(b) based on a gene signature as listed in Table 2A, Table 2B, Table 3, Table 4, Table 14, Table 15 and/or Table 16, classifying the patient as having a short or long survival based on the expression levels determined in step (a), in which the patient has HCC, and in which classification of a patient by step (b) is monitored before, during and/or after the therapeutic intervention.

4. A method according to any one of the preceding claims wherein one or more of the following apply: (a) the expression levels are normalized expression levels and/or relative expression levels; (b) the patient-derived tumor sample comprises tumor infiltrating leukocytes (TIL), stroma and tumor cells; (c) the patient is human.

5. A method according to any one of the preceding claims wherein step (b) comprises deriving a value from the expression levels of the genes listed in Table 2A, 2B, 3, 4, 14, 15, or 16 (and optionally also from the expression levels of any one or more further marker genes which may be employed) and comparing the value with a threshold value wherein a determination that the derived value is below or above said threshold value indicates a particular prognosis (e.g. a good or poor prognosis),

and optionally wherein: (i) a poor prognosis is less than 3, 4, 5 or 6 years predicted survival and a good prognosis is more than or equal to 3, 4, 5 or 6 years predicted survival; or (ii) a poor prognosis is less than the median survival years of a given cohort and a good prognosis is more than the median survival years of a given cohort.

6. A method according to any one of claims 1 to 4 wherein an expression profile comprises the expression levels of said 5, 8, 7, 11, or 14 of genes selected from the group consisting of CLL5, CCR2, CEACAM8, CXCL10, IFNG, IL6, NCR3, TBX21, TLR3, TNF, CCL2, CD8A, FCGR1A, LTA, and TLR4 as listed in Table 2A, 2B, 3, 4, 14, 15, or 16 and wherein step (b) comprises determining the similarity of the expression profile to a good prognosis template and/or a poor prognosis template, wherein the degree of similarity to the good prognosis template and/or poor prognosis template indicates whether the patient has a good prognosis or poor prognosis.

7. A method according to claim 6 wherein step (b) comprises determining the similarity of the expression profile to a good prognosis template and/or a poor prognosis template, and wherein said patient is classified as having: (i) a good prognosis if said expression profile is similar to the good prognosis template and/or is dissimilar to the poor prognosis template; or (ii) a poor prognosis if said expression profile is dissimilar to the good prognosis template and/or is similar to the poor prognosis template, wherein the expression profile is determined as being similar or dissimilar to the template depending on whether the similarity is above or below a predetermined threshold value.

8. A method according to claim 5 wherein step (b) comprises determining the similarity of the expression profile to a good prognosis template and/or a poor prognosis template, and wherein said patient is classified as having: (i) a good prognosis if said expression profile has a higher similarity to said good prognosis template than to said poor prognosis template; or (ii) a poor prognosis if said expression profile has a higher similarity to said poor prognosis template than to said good prognosis template.

9. A method according to any one of the preceding claims in which step (b) is performed using at least one algorithm, and/or a computer.

10. A method according to claim 9 in which step (b) is performed using a Support Vector Machine (SVM) algorithm, a K-Nearest Neighbour (KNN) algorithm or a combination of an SVM and a KNN algorithm, and wherein:

(i) the genes signatures used are all of the genes listed in Table 2A ; all of the genes listed in Table 2B; ; all of the genes listed in Table 14; all of the genes listed in Table 15; all of the genes listed in Table 16, and step (b) is performed using the SVM algorithm; or

(ii) all of the genes listed in Table 3; all of the genes listed in Table 1, and wherein step (b) is performed using the KNN algorithm.

11. A method according to claim 9 in which step (b) is performed using a Nearest Template Prediction (NTP) algorithm, wherein the genes signature used are all of the genes listed in Table 4 or all of the genes listed in Table 1.

12. A method according to claim 9 or 10, wherein step (b) is performed by: (i) application of an SVM algorithm as described in Algorithm 1, classifies a patient as having a good or poor prognosis; or (ii) application of an KNN algorithm as described in Algorithm 2, classifies a patient as having a good or poor prognosis.

13. A method according to claim 9 or 11, wherein step (b) is performed by application of an NTP algorithm as described in Algorithm 3, classifies a patient as having a good or poor prognosis.

14. A method according to claim 1 wherein the method is a method for evaluating the efficacy of a therapeutic intervention for treating HCC patients and wherein the therapeutic intervention is a candidate agent.

15. A method as defined in claim 1 or 2 further comprising selecting the patient for therapy or follow-up on the basis of the patient having either a good or poor prognosis.

16. A method according to claim 1 or 3 wherein said therapeutic intervention is a neoadjuvant treatment.

17. A method according to any one of the preceding claims comprising use of a microarray kit or quantitative PCR to determine the expression level of any or all of the genes listed in Table 1, Table 2A, Table 2B, Table 3, Table 4, Table 14, Table 15 or Table 16.

18. A method according to any one of the preceding claims wherein the HCC is stage I or stage II.

19. A kit for use in any one of claims 1 to 18, wherein the kit comprises reagents for determining the expression of said 5, 8, 7, 11, or 14 of genes selected from the group consisting of CLL5, CCR2, CEACAM8, CXCL10, IFNG, IL6, NCR3, TBX21, TLR3, TNF, CCL2, CD8A, FCGR1A, LTA, and TLR4 and wherein the kit further optionally comprises instructions for use.

20. A computer program or computer software product for performing step (b) of a method according to any one of claims 1 to 18, or a computer system programmed to perform step (b) of a method according to any one of claims 1 to 18.

21. A microarray for use in a method according to any one of claims 1 to 18, wherein the microarray comprises a plurality of probes capable of hybridizing to the 5, 8, 7, 11, or 14 of genes selected from the group consisting of CLL5, CCR2, CEACAM8, CXCL10, IFNG, IL6, NCR3, TBX21, TLR3, TNF, CCL2, CD8A, FCGR1A, LTA, and TLR4.

22. A method of providing a an HCC human patient with a good or a poor prognosis, wherein the method comprises:

(a) determining the expression levels of 5, 8, 7, 11, or 14 of genes selected from the group consisting of CLL5, CCR2, CEACAM8, CXCL10, IFNG, IL6, NCR3, TBX21, TLR3, TNF, CCL2, CD8A, FCGR1A, LTA, and TLR4 in a tumor sample derived from said patient, which tumor sample comprises total tumor material,

(b) analysing the genes based on the gene signatures as listed in table Table 2A, Table 2B, Table 3, Table 4, Table 14, Table 15 and/or Table 16, ; and

(b) determining the similarity of an expression profile comprising the expression levels determined in step (a) to a good prognosis template which comprises gene expression levels characteristic of good prognosis patients and a poor prognosis template which comprises gene expression levels characteristic of poor prognosis patient, wherein a higher similarity of said expression profile to said good prognosis template indicates a poor prognosis and a higher similarity to said poor prognosis template than to said good prognosis template indicates a poor prognosis,

and wherein a poor prognosis is less than the median survival years of a given cohort and a good prognosis is more than the median survival years of a given cohort.

CLAIMS

1. A method of analysing a patient with Hepatocellular Carcinoma (HCC), wherein the method comprises:

(a) determining the expression levels of three or more genes in a patient-derived tumor sample wherein the said three or more genes are selected from the genes listed in Table 1; the genes listed in Table 2A or Table 2B; the genes listed in Table 3; the genes listed in Table 4, the genes listed in Table 14, the genes listed in Table 15, and/or the genes listed in Table 16; and

(b) using the expression levels determined in step (a) in one or more of the following: stratifying or classifying the patient, providing a prognosis, monitoring disease progression, predicting efficacy of a therapeutic intervention, selecting treatment for the tumor, or evaluating the efficacy of a therapeutic intervention.

2. A method according to claim 1 wherein the method is a method of classifying a patient with HCC as having a poor or good prognosis comprising the steps of:

(a) determining the expression levels of three or more genes (and preferably five or more genes) in a patient-derived tumor sample, wherein the genes are selected from the genes listed in Table 1; the genes listed in Table 2A or Table 2B; the genes listed in Table 3; the genes listed in Table 4, the genes listed in Table 14, the genes listed in Table 15, and/or the genes listed in Table 16; and

(b) classifying the patient as having a short or long survival based on the expression levels determined in step (a), in which the patient has HCC.

3. A method according to claim 1 wherein the method is a method for evaluating the efficacy of a therapeutic intervention for treating HCC patients comprising the steps of:

(a) determining the expression levels of three or more genes in a patient-derived tumor sample, wherein the genes are selected from the genes listed in Table 1; the genes listed in Table 2; the genes listed in Table 3; the genes listed in Table 4, the genes listed in Table 14, the genes listed in Table 15, and/or the genes listed in Table 16; and

(b) classifying the patient as having a short or long survival based on the expression levels determined in step (a), in which the patient has HCC, and in which classification of a patient by step (b) is monitored before, during and/or after the therapeutic intervention.

4. A method according to any one of claims 1, 2 or 3 in which:

(i) the three or more genes of Table 1 are at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and/or all the genes listed in Table 1 and/or any combination thereof;

(ii) the three or more genes of Table 2A are at least 3, 4, 5, 6 and/or all of the genes listed in Table 2A and/or any combination thereof;

(iii) the three or more genes of Table 2B are at least 3, 4, 5, 6 and/or all of the genes listed in Table 2B and/or any combination thereof;

(iv) the three or more genes of Table 3 are at least 3, 4, 5, 6, 7, 8, 9, 10 and/or all of the genes listed in Table 3 and/or any combination thereof;

(v) the three or more genes of Table 4 are at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and/or all of the genes listed in Table 4 and/or any combination thereof;

(vi) the three or more genes of Table 14 are at least 3, 4, 5, 6, 7 and/or all of the genes listed in Table 14 and/or any combination thereof;

(vii) the three or more genes of Table 15 are at least 3, 4 and/or all of the genes listed in Table 15 and/or any combination thereof;

(viii) the three or more genes of Table 16 are at least 3, 4 and/or all of the genes listed in Table 16 and/or any combination thereof;

(ix) wherein 14 genes are selected from Table 1;

(x) the three or more genes of Table 1 are between 4 to 15 genes, 4 to 14 genes, 5 to 15 genes, or 5 to 14 genes from Table 1; or

(xi) the three or more genes of Table 1 comprise: CCL2, CCL5 and CCR2; CCL5, CCL2 and CXCL10; or CCL5, CCL2, CXCL10 and CCR2.

5. A method according to claim 1 wherein step (b) uses additional information in stratifying or classifying the patient, providing a prognosis, monitoring disease progression, predicting efficacy of a therapeutic intervention, selecting treatment for the tumor, or evaluating the efficacy of a therapeutic intervention, and wherein such additional information is optionally staging information and/or the expression (present or absent, or the level of) of one or more further marker genes which are not found in Table 1, 2A, 2B, 3, 4, 14, 15 or 16 and which said one or more further marker genes is of predictive value for HCC prognosis.

6. A method according to any one of the preceding claims wherein one or more of the following apply: (a) the expression levels are normalized expression levels and/or relative expression levels; (b) the patient-derived tumor sample comprises tumor infiltrating leukocytes (TIL), stroma and tumor cells; (c) the patient is human.

7. A method according to any one of the preceding claims wherein step (b) comprises deriving a value from the expression levels of the three or more genes listed in Table 1, 2A, 2B, 3, 4, 14, 15, or 16 (and optionally also from the expression levels of any one or more further marker genes which may be employed) and comparing the value with a threshold value wherein a determination that the derived value is below or above said threshold value indicates a particular prognosis (e.g. a good or poor prognosis),

and optionally wherein: (i) a poor prognosis is less than 3, 4, 5 or 6 years predicted survival and a good prognosis is more than or equal to 3, 4, 5 or 6 years predicted survival; or (ii) a poor prognosis is less than the median survival years of a given cohort and a good prognosis is more than the median survival years of a given cohort.

8. A method according to any one of claims 1 to 6 wherein an expression profile comprises the expression levels of said three or more genes listed in Table 1, 2A, 2B, 3, 4, 14, 15, or 16 and wherein step (b) comprises determining the similarity of the expression profile to a good prognosis template and/or a poor prognosis template, wherein the degree of similarity to the good prognosis template and/or poor prognosis template indicates whether the patient has a good prognosis or poor prognosis.

9. A method according to claim 8 wherein step (b) comprises determining the similarity of the expression profile to a good prognosis template and/or a poor prognosis template, and

wherein said patient is classified as having: (i) a good prognosis if said expression profile is similar to the good prognosis template and/or is dissimilar to the poor prognosis template, or (ii) a poor prognosis if said expression profile is dissimilar to the good prognosis template and/or is similar to the poor prognosis template, wherein the expression profile is determined as being similar or dissimilar to the template depending on whether the similarity is above or below a predetermined threshold value.

10. A method according to claim 8 wherein step (b) comprises determining the similarity of the expression profile to a good prognosis template and/or a poor prognosis template, and wherein said patient is classified as having: (i) a good prognosis if said expression profile has a higher similarity to said good prognosis template than to said poor prognosis template; or (ii) a poor prognosis if said expression profile has a higher similarity to said poor prognosis template than to said good prognosis template.

11. A method according to any one of the preceding claims in which step (b) is performed using at least one algorithm, and/or a computer.

12. A method according to claim 11 in which step (b) is performed using a SVM algorithm, a KNN algorithm or a combination of an SVM and a KNN algorithm, and optionally wherein:

(i) the three or more genes of Table 2A are at least 3, 4, 5, 6 and/or all of the genes listed in Table 2A and/or any combination thereof; or the three or more genes of Table 2B are at least 3, 4, 5, 6 and/or all of the genes listed in Table 2B and/or any combination thereof; or the three or more genes of Table 1 are at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and/or all of the genes listed in Table 1 and/or any combination thereof; or the three or more genes of Table 14 are at least 3, 4, 5, 6, 7 and/or all of the genes listed in Table 14; or the three or more genes of Table 15 are at least 3, 4 and/or all of the genes listed in Table 15; or the three or more genes of Table 16 are at least 3, 4 and/or all of the genes listed in Table 16, and step (b) is performed using the SVM algorithm; or

(ii) the three or more genes of Table 3 are at least 3, 4, 5, 6, 7, 8, 9, 10 and/or all of the genes listed in Table 3 and/or any combination thereof; or the three or more genes of Table 1 are at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and/or all of the genes listed in Table 1 and/or any combination thereof, and wherein step (b) is performed using the KNN algorithm.

13. A method according to claim 11 in which step (b) is performed using an NTP algorithm, and optionally wherein the three or more genes of Table 4 are at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and/or all of the genes listed in Table 4 and/or any combination thereof or the three or more genes of Table 1 are at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and/or all of the genes listed in Table 1 and/or any combination thereof.

14. A method according to claim 11 or 12, wherein step (b) is performed by: (i) application of an SVM algorithm as described in Algorithm 1, classifies a patient as having a good or poor prognosis; or (ii) application of an KNN algorithm as described in Algorithm 2, classifies a patient as having a good or poor prognosis.

15. A method according to claim 11 or 13, wherein step (b) is performed by application of an NTP algorithm as described in Algorithm 3, classifies a patient as having a good or poor prognosis.

16. A method according to claim 1 wherein the method is a method for evaluating the efficacy of a therapeutic intervention for treating HCC patients and wherein the therapeutic intervention is a candidate agent.

17. A method as defined in claim 1 or 2 further comprising selecting the patient for therapy or follow-up on the basis of the patient having either a good or poor prognosis.

18. A method according to claim 1 or 3 wherein said therapeutic intervention is a neoadjuvant treatment.

19. A method according to any one of the preceding claims comprising use of a microarray kit or quantitative PCR to determine the expression level of any or all of the genes listed in Table 1, Table 2A, Table 2B, Table 3, Table 4, Table 14, Table 15 or Table 16.

20. A method according to any one of the preceding claims wherein the HCC is stage I or stage II.

21. A method of treating a patient characterised as a patient having either good or poor prognosis according to any one of the preceding claims, wherein said patient is to be administered with a hepatocellular-carcinoma immunotherapy or any other alternative treatments.

22. Use of immunotherapy or any other alternative treatment for hepatocellular-carcinoma in the preparation of a medicament for the treatment of patients characterized as having either good or poor prognosis according to the method of claim 1 or 2.

23. A kit for use in any one of claims 1 to 20 or 26, wherein the kit comprises reagents for determining the expression of said three or more genes selected from the genes listed in Table 1, the genes listed in Table 2A or Table 2B, the genes listed in Table 3, the genes listed in Table 4, the genes listed in Table 14, the genes listed in Table 15 and/or the genes listed in Table 16 and wherein the kit further optionally comprises instructions for use.

24. A computer program or computer software product for performing step (b) of a method according to any one of claims 1 to 20 or 26, or a computer system programmed to perform step (b) of a method according to any one of claims 1 to 24.

25. A microarray for use in a method according to any one of claims 1 to 20 or 26, wherein the microarray comprises a plurality of probes capable of hybridizing to the said three or more genes selected from the genes listed in Table 1, the genes listed in Table 2A or Table 2B, the genes listed in Table 3, the genes listed in Table 4, the genes listed in Table 14, the genes listed in Table 15, and/or the genes listed in Table 14.

26. A method of providing a an HCC human patient with a good or a poor prognosis, wherein the method comprises:

(a) determining the expression levels of five or more genes in a tumor sample derived from said patient, which tumor sample comprises total tumor material, wherein the said five or more genes are selected from at least one list of genes selected from the group consisting of the genes listed in Table 1; the genes listed in Table 2A; the genes listed in Table 2B; the genes listed in Table 3; the genes listed in Table 4, the genes listed in Table 14, the genes listed in Table 15, and the genes listed in Table 16, and wherein the expression levels may optionally be relative expression levels and/or normalized expression levels; and

(b) determining the similarity of an expression profile comprising the expression levels determined in step (a) to a good prognosis template which comprises gene expression levels characteristic of good prognosis patients and a poor prognosis template which comprises gene expression levels characteristic of poor prognosis patient, wherein a higher similarity of said expression profile to said good prognosis template indicates a poor prognosis and a higher similarity to said poor prognosis template than to said good prognosis template indicates a poor prognosis,

and wherein a poor prognosis is less than the median survival years of a given cohort and a good prognosis is more than the median survival years of a given cohort.