

Claims:

What is claimed is:

1. A method for prognosis of a cancerous solid tumour in a patient, comprising
providing a sample of tumour core tissue obtained from the patient,
determining one or more of the following biomarkers in said sample:
 - (i) ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,
 - (ii) mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell,
 - (iii) proportion of FOXP3 positive cells which are ICOS positive, and
 - (iv) density of ICOS positive cells, andproviding a prognosis for the patient based on said one or more biomarkers, wherein a shorter duration of patient survival, recurrence free survival (RFS), progression free survival (PFS) or time to progression (TTP) is indicated by
 - a greater ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,
 - a shorter mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell,
 - a higher proportion of FOXP3 positive cells which are ICOS positive, and/or
 - a higher density of ICOS positive cells.
2. A method according to claim 1, comprising determining the ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells, and
comparing said ratio against a reference value, wherein
a ratio higher than the reference value indicates a prognosis of shorter duration of survival, RFS, PFS or TTP.
3. A method according to claim 2, wherein the tumour is hepatocellular carcinoma and the reference value is a ratio of 0.1 ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells.

4. A method according to any of claims 1 to 3, comprising determining the mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell, and
comparing said distance against a reference value, wherein
a distance less than the reference value indicates a prognosis of shorter duration of survival, RFS, PFS or TTP.
5. A method according to claim 4, wherein the tumour is hepatocellular carcinoma and the reference value is a mean distance of 105 μm between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell.
6. A method according to any of claims 1 to 5, comprising determining the proportion of FOXP3 positive cells which are ICOS positive, and
comparing said proportion against a reference value, wherein
a proportion higher than the reference value indicates a prognosis of shorter duration of survival, RFS, PFS or TTP.
7. A method according to claim 6, wherein the tumour is hepatocellular carcinoma and the reference value is a half of FOXP3 positive cells being ICOS positive.
8. A method according to any of claims 1 to 7, comprising determining the density of ICOS positive cells, and
comparing said density against a reference value, wherein
a density higher than the reference value indicates a prognosis of shorter duration of survival, RFS, PFS or TTP.
9. A method according to claim 7, wherein the tumour is hepatocellular carcinoma and the reference value is a density of 120 ICOS positive cells per mm^2 .
10. A method according to claim 7, wherein the tumour is hepatocellular carcinoma associated with hepatitis B virus infection or is stage 2 or later hepatocellular carcinoma, and wherein the reference value is a density of 100 ICOS positive cells per mm^2 .
11. A method according to any of claims 1 to 10, comprising determining from said one or more biomarkers that the patient has a prognosis of shorter duration of survival, RFS, PFS or TTP.

12. A method according to claim 11, comprising determining the ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells, and
- comparing said ratio against a reference value, wherein a ratio higher than the reference value indicates a prognosis of shorter duration of survival, RFS, PFS or TTP,
 - determining that said ratio is higher than the reference value, and
 - providing a prognosis of shorter duration of survival, RFS, PFS or TTP.
13. A method according to any of claims 1 to 10, comprising determining from said one or more biomarkers that the patient has a prognosis of longer duration of survival, RFS, PFS or TTP.
14. A method according to claim 13, comprising determining the ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells, and
- comparing said ratio against a reference value, wherein a ratio higher than the reference value indicates a prognosis of shorter duration of survival, RFS, PFS or TTP,
 - determining that said ratio is lower than the reference value, and
 - providing a prognosis of longer duration of survival, RFS, PFS or TTP.
15. Use of a biomarker for prognosis of a cancerous solid tumour in a patient, wherein the biomarker is one or more of the following as determined in tumour core tissue from the patient:
- (i) ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,
 - (ii) mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell,
 - (iii) proportion of FOXP3 positive cells which are ICOS positive, and
 - (iv) density of ICOS positive cells.
16. A method of determining likelihood of a cancerous solid tumour in a patient to respond to an anti-ICOS and/or anti-TReg immunotherapeutic agent, comprising
- providing a sample of tumour core tissue obtained from the patient, and
 - determining one or more of the following biomarkers in said sample:
- (i) ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,
 - (ii) mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell,

- (iii) proportion of FOXP3 positive cells which are ICOS positive, and
 - (iv) density of ICOS positive cells, wherein
 - a greater likelihood of the patient to respond to the immunotherapeutic agent is indicated by
 - a greater ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,
 - a shorter mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell,
 - a higher proportion of FOXP3 positive cells which are ICOS positive, and/or
 - a higher density of ICOS positive cells.
17. A method according to claim 16, comprising determining the ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells, and
 - comparing said number against a reference value, wherein
 - a ratio higher than the reference value indicates an increased likelihood of responding to the immunotherapeutic agent.
18. A method according to claim 17, wherein the tumour is hepatocellular carcinoma and the reference value is a ratio of 0.1 ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells.
19. A method according to any of claims 16 to 18, comprising determining the mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell, and
 - comparing said distance against a reference value, wherein
 - a distance less than the reference value indicates an increased likelihood of responding to the immunotherapeutic agent.
20. A method according to claim 19, wherein the tumour is hepatocellular carcinoma and the reference value is a mean distance of 105 μm between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell.
21. A method according to any of claims 16 to 20, comprising determining the proportion of FOXP3 positive cells which are ICOS positive, and
 - comparing said proportion against a reference value, wherein

a proportion higher than the reference value indicates an increased likelihood of responding to the immunotherapeutic agent.

22. A method according to claim 21, wherein the tumour is hepatocellular carcinoma and the reference value is a half of FOXP3 positive cells being ICOS positive.

23. A method according to any of claims 16 to 22, comprising determining the density of ICOS positive cells, and
comparing said density against a reference value, wherein
a density higher than the reference value indicates an increased likelihood of responding to the immunotherapeutic agent.

24. A method according to claim 23, wherein the tumour is hepatocellular carcinoma and the reference value is a density of 120 ICOS positive cells per mm².

25. A method according to claim 23, wherein the tumour is hepatocellular carcinoma associated with hepatitis B virus infection or is stage 2 or later hepatocellular carcinoma, and wherein the reference value is a density of 100 ICOS positive cells per mm².

26. A method according to any of claims 16 to 25, comprising identifying the patient as having an increased likelihood of responding to the immunotherapeutic agent and optionally thereby selecting an anti-ICOS and/or anti-TReg immunotherapeutic agent for treatment of said patient.

27. A method according to claim 26, comprising determining the ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,
comparing said ratio against a reference value, wherein a ratio higher than the reference value indicates an increased likelihood of responding to the immunotherapeutic agent,
determining that said ratio is higher than the reference value, and thereby identifying the patient as having an increased likelihood of responding to the immunotherapeutic agent.

28. A method according to claim 26 or claim 27, comprising administering the anti-ICOS and/or anti-TReg immunotherapeutic agent to said patient.

29. Use of a biomarker for determining likelihood of a cancerous tumour in a patient to respond to an anti-ICOS and/or anti-TReg immunotherapeutic agent, wherein the biomarker is one or more of the following as determined in tumour core tissue from the patient:

- (i) ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,
- (ii) mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell,
- (iii) proportion of FOXP3 positive cells which are ICOS positive, and
- (iv) density of ICOS positive cells.

30. A method of treating a cancerous solid tumour in a patient, wherein the tumour has been determined to comprise one or more of the following biomarkers:

ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells, wherein said ratio is higher than a reference value,

a mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell, wherein said distance is less than a reference value,

a proportion of FOXP3 positive cells which are ICOS positive, wherein said proportion is higher than a reference value, and

a density of ICOS positive cells, wherein said density is higher than a reference value,

the method comprising administering an anti-ICOS and/or anti-TReg immunotherapeutic agent to the patient.

31. An anti-ICOS and/or anti-TReg immunotherapeutic agent for use in a method of treating a cancerous solid tumour in a patient, wherein the tumour has been determined to comprise one or more of the following biomarkers:

a ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells, wherein said ratio is higher than a reference value,

a mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell, wherein said distance is less than a reference value,

a proportion of FOXP3 positive cells which are ICOS positive, wherein said proportion is higher than a reference value, and

a density of ICOS positive cells, wherein said density is higher than a reference value.

32. A method according to claim 30, or an agent for use according to claim 31, wherein the tumour is hepatocellular carcinoma and the tumour has been determined to comprise the following biomarkers:

a ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells, wherein said ratio is higher than 0.1,

a mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell, wherein said distance is less than 105 μm ,

a proportion of FOXP3 positive cells which are ICOS positive, wherein said proportion is higher than half, and

a density of ICOS positive cells, wherein said density is higher than 120 cells per mm^2 .

33. A method according to claim 30, or an agent for use according to claim 31, wherein the tumour is hepatocellular carcinoma associated with hepatitis B virus infection or is grade 2 or later hepatocellular carcinoma, and wherein the tumour has been determined to comprise the following biomarkers:

a ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells, wherein said ratio is higher than 0.1,

a mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell, wherein said distance is less than 105 μm ,

a proportion of FOXP3 positive cells which are ICOS positive, wherein said proportion is higher than half, and

a density of ICOS positive cells, wherein said density is higher than 100 cells per mm^2 .

34. An anti-ICOS and/or anti-TReg immunotherapeutic agent for use in a method of treating a cancerous solid tumour in a patient, the method comprising

selecting an anti-ICOS and/or anti-TReg immunotherapeutic agent for treatment of a patient as defined in claim 26, and

administering the anti-ICOS and/or anti-TReg immunotherapeutic agent to the patient.

35. A method of monitoring a patient's response to an anti-ICOS and/or anti-TReg immunotherapeutic agent for a cancerous solid tumour, comprising
- providing a sample of tumour core tissue obtained from a patient who has received an anti-ICOS and/or anti-TReg immunotherapeutic agent,
 - determining one or more of the following biomarkers in said sample:
 - (i) ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,
 - (ii) mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell,
 - (iii) proportion of FOXP3 positive cells which are ICOS positive, and
 - (iv) density of ICOS positive cells,
 - comparing said one or more biomarkers in said sample against the same one or more biomarkers in a sample of tumour core tissue obtained from the patient prior to administration of said immunotherapeutic agent, and
 - determining whether a change has occurred in said one or more biomarkers and thereby assessing whether the patient is responding to the immunotherapeutic agent, wherein response is indicated by one or more of the following changes from the sample prior to administration of said immunotherapeutic agent:
 - a reduction in the ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,
 - an increase in the mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell,
 - a reduction in the proportion of FOXP3 positive cells which are ICOS positive, and/or
 - a reduction in the density of ICOS positive cells.
36. A method according to claim 35, comprising
- measuring a reduction in the ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells compared with prior to therapy, and
 - prescribing continued treatment with the anti-ICOS and/or anti-TReg immunotherapeutic agent for the patient.
37. A method according to claim 35 or claim 36, comprising
- measuring an increase in the mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell, and

prescribing continued treatment with the anti-ICOS and/or anti-TReg immunotherapeutic agent for the patient.

38. A method according to any of claims 35 to 37, comprising
measuring a reduction in the proportion of FOXP3 positive cells which are ICOS positive,
and

prescribing continued treatment with the anti-ICOS and/or anti-TReg immunotherapeutic agent for the patient.

39. A method according to any of claims 35 to 38, comprising
measuring a reduction in the density of ICOS positive cells, and
prescribing continued treatment with the anti-ICOS and/or anti-TReg immunotherapeutic agent for the patient.

40. Use of a biomarker for monitoring a patient's response to an anti-ICOS and/or anti-TReg immunotherapeutic agent for a cancerous solid tumour, wherein the biomarker is one or more of the following as determined in tumour core tissue from the patient:

- (i) ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,
- (ii) mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell,
- (iii) proportion of FOXP3 positive cells which are ICOS positive, and
- (iv) density of ICOS positive cells,

wherein response to the immunotherapeutic agent is indicated by one or more of the following changes as compared with a sample of tumour core tissue obtained prior to administration of the immunotherapeutic agent:

a reduction in the ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,

an increase in the mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell,

a reduction in the proportion of FOXP3 positive cells which are ICOS positive, and

a reduction in the density of ICOS positive cells.

41. A method of identifying a reference value for classifying patients with cancerous solid tumours according to their predicted prognosis or predicted response to an anti-ICOS and/or anti-TReg immunotherapeutic agent, comprising
- providing samples of tumour core tissue obtained from each of a population of patients with cancerous solid tumours of the same type for whom disease outcome is known or response to the anti-ICOS and/or anti-TReg immunotherapeutic agent is known,
 - determining one or more of the following biomarkers in each of said samples:
 - (i) ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells,
 - (ii) mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell,
 - (iii) proportion of FOXP3 positive cells which are ICOS positive, and
 - (iv) density of ICOS positive cells,
 - pairing data for each of said one or more biomarkers with data for disease outcome or response to the anti-ICOS and/or anti-TReg immunotherapeutic agent in each patient, and
 - grouping data for each of said one or more biomarkers to identify a numerical cut-off which defines two groups of statistically significant data for disease outcome or response to the anti-ICOS and/or anti-TReg immunotherapeutic agent,
 - wherein said cut-off represents a reference value for classifying patients with the same type of cancerous solid tumours according to their predicted prognosis or predicted response to the anti-ICOS and/or anti-TReg immunotherapeutic agent.
42. A method according to claim 41, wherein the samples are obtained from a population of patients with liver cancer, renal cell cancer, head and neck cancer, melanoma, non-small cell lung cancer, bladder cancer, ovarian cancer, cervical cancer, gastric cancer, pancreatic cancer, breast cancer (including triple negative breast cancer), carcinoma, leiomyosarcoma, anal cancer, squamous cell cancer and oesophageal cancer.
43. A method according to claim 42, wherein the samples are obtained from a population of patients with hepatocellular carcinoma.
44. A method, use or an immunotherapeutic agent for use according to any of claims 16 to 43, wherein the immunotherapeutic agent is an anti-ICOS antibody.
45. A method, use or an immunotherapeutic agent for use according to any of claims 16 to 44, wherein the immunotherapeutic agent selectively depletes or inhibits TRegs.

46. A method, use or an immunotherapeutic agent for use according to any of claims 16 to 45, wherein the immunotherapeutic agent is an antibody which binds TRegs and mediates cellular effector functions.
47. A method, use or immunotherapeutic agent for use according to claim 46, wherein the antibody is an IgG comprising an Fc region which mediates cellular effector functions.
48. A method, use or immunotherapeutic agent for use according to any of claims 45 to 47, wherein the immunotherapeutic agent is an antibody which binds ICOS, CD25, CCR8, CTLA-4, GITR or an MHC displayed epitope of FOXP3.
49. A method, use or immunotherapeutic agent for use according to claim 48, wherein the antibody binds ICOS.
50. A method, use or immunotherapeutic agent for use according to claim 44 or claim 49, wherein the one or more biomarkers comprise the density of ICOS positive cells.
51. A method, use or immunotherapeutic agent for use according to claim 49 or claim 50, wherein the antibody comprises an ICOS binding site comprising the CDRs of KY1044, wherein
HCDR1 is SEQ ID NO: 1
HCDR2 is SEQ ID NO: 2
HCDR3 is SEQ ID NO: 3
LCDR1 is SEQ ID NO: 8
LCDR2 is SEQ ID NO: 9 and
LCDR3 is SEQ ID NO: 10.
52. A method, use or immunotherapeutic agent for use according to any of claims 49 to 51, wherein the antibody comprises a VH domain amino acid sequence at least 90 % identical to the KY1044 VH domain SEQ ID NO: 5 and a VL domain amino acid sequence at least 90 % identical to the KY1044 VL domain SEQ ID NO: 12.
53. A method, use or immunotherapeutic agent for use according to claim 52, wherein antibody comprises the KY1044 VH domain SEQ ID NO: 5 and the KY1044 VL domain SEQ ID NO: 12.

54. A method, use or immunotherapeutic agent for use according to claim 53, wherein the antibody is a human IgG1 κ comprising the KY1044 heavy chain SEQ ID NO: 7 and the KY1044 light chain SEQ ID NO: 14.

55. A method, use or immunotherapeutic agent according to claim 48, wherein the antibody binds CD25, CCR8, CTLA-4, GITR or an MHC displayed epitope of FOXP3.

56. A method, use or immunotherapeutic agent according to claim 55, wherein the one or more biomarkers comprise the ratio of the number of ICOS FOXP3 double positive cells within a defined radius of influence around ICOS single positive cells to the total number of ICOS single positive cells, the mean distance between each ICOS positive FOXP3 negative cell and its nearest ICOS FOXP3 double positive cell, and/or the proportion of FOXP3 positive cells which are ICOS positive.

57. Use of the spatial arrangement of immune cells expressing ICOS and/or FOXP3 in the TME as a biomarker for disease prognosis or for response to treatment with an anti-ICOS and/or anti-TReg immunotherapeutic agent.