for inter-laboratory tests for predicting the survival and treatment response of patients with cancer.

In one aspect, there is provided a method for assessment of a number or density of immune cells in tumoral tissues comprising the steps consisting in: a. providing one or more immunostained slides of tissue section obtained by an automated slide-staining system by using antibodies binding specifically to antigens (markers) expressed by immune cells; b. proceeding to digitalisation of the slides of step a. by high resolution scan capture, whereby a high definition digital picture of the slide to be analysed is obtained; c. detecting the slide of tissue section on the digital picture; d. analyzing the slide of tissue section for defining (i) a tumour (CT) and (ii) an invasive margin of the tumour (IM); e. providing a size reference grid with uniformly distributed units having a same surface, said grid being adapted to the size of the tumour to be analysed; e1. checking the quality of immunostaining by comparison with a reference value; f. detecting and quantifying stained cells of each unit whereby the number or the density of immune cells stained of each unit is assessed. In one ~~aspect~~embodiment, there is provided a method for assessment of a number or density of immune cells in tumoral tissues comprising the steps consisting in: a. providing one or more immunostained slices of tissue section obtained by an automated slide-staining system by using antibodies binding specifically to antigens (markers) expressed by immune cells; b. proceeding to digitalisation of the slides of step a. by high resolution scan capture, whereby a high definition (4.6 µm/pixel or better) digital picture of the slide to be analysed is obtained; c. detecting the slice of tissue section on the digital picture; d. analyzing the slice of tissue section for defining (i) a tumour (CT) and (ii) an invasive margin of the tumour (IM); e. providing a size reference grid with uniformly distributed units having a same surface, said grid being adapted to the size of the tumour to be analysed; e1. checking the quality of immunostaining; f. detecting and quantifying stained cells of each unit whereby the number or the density of immune cells stained of each unit is assessed.

The new method has particularly fewer problems with repeatability and reproducibility when compared to the method of EP-A-EP1943520 and WO2007045996.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

A subject of the present application is therefore a method for assessment of a number or density of immune cells in tumoral tissues comprising the steps consisting in:

a. providing one or more immunostained slices of tissue section obtained by an automated slide-staining system by using antibodies binding specifically to antigens expressed by immune cells.

b. proceeding to digitalisation of the slides of step a. by high resolution scan capture, whereby a high definition (4.6 µm/pixel or better) digital picture of the slide to be analysed is obtained,

c. detecting the slice of tissue section on the digital picture

d. analyzing the slice of tissue section for defining (i) the tumour (CT) and (ii) the invasive margin of the tumour (IM),

e. providing a size reference grid with uniformly distributed units having a same surface, said grid being adapted to the size of the tumour to be analyzed,

f. detecting and quantifying stained cells of each unit whereby the number or the density of immune cells stained of each unit is assessed.

Typically, the antibodies are specific for a protein expressed by an immune cell and more particularly are specific for an immune cell surface marker. For example, the antibodies may be specific for a marker as described in WO2007045996, immune cells considered may be B cells and preferably T cells. For example, the T cells of the invention are T cells of sub groups CD3+ cells (T cells), CD8+ cells or GZMB+ cells (cytotoxic T cells), CD4+ cells (T helper cells), CD45RO+ cells (memory T cells). In a particular embodiment, the antibodies are specific for CD3, CD4, CD8, CD20, CD45RO, and GZMB markers.

What is claimed is:

1. A method for assessment of a number or density of immune cells in tumoral tissues comprising the steps consisting in:

a. providing one or more immunostained ~~slices~~ slides of tissue section obtained by an automated slide-staining system by using antibodies binding specifically to antigens (markers) expressed by immune cells,

b. proceeding to digitalisation of the slides of step a. by high resolution scan capture, whereby a high definition ~~(4.6 µm/pixel or better)~~ digital picture of the slide to be analysed is obtained,

c. detecting the ~~slice~~ slide of tissue section on the digital picture,

d. analyzing the ~~slice~~ slide of tissue section for defining (i) a tumour (CT) and (ii) an invasive margin of the tumour (IM),

e. providing a size reference grid with uniformly distributed units having a same surface, said grid being adapted to the size of the tumour to be analyzed,

e1. checking the quality of immunostaining by comparison with a reference value,

f. detecting and quantifying stained cells of each unit whereby the number or the density of immune cells stained of each unit is assessed.

2. A method according to claim 1, wherein detecting and quantifying stained cells of each unit is implemented separately in the tumour (CT) and the invasive margin of the tumour (IM).

~~3. A method according to claim 1, wherein no size reference grid is provided.~~

43. A method according to ~~one of~~ claims 1 ~~to 3~~or 2, wherein the antibodies are selected in the group consisting of antibodies specific for CD3, CD4, CD8, CD20, CD45RO, or GZMB markers.

~~54~~. A method according to one of claims 1 to 43, wherein two or three antibodies selected in the group consisting of the following combinations: CD3+CD8, CD3+CD45RO, CD3+CD20, CD3+GZMB, CD8+CD20, CD8+GZMB, CD8+CD45RO, CD20+GZMB, CD20+CD45RO, GZMB+CD45RO, CD4+CD8, CD4+CD45RO, CD4+GZMB, CD4+CD20, and all the combinations of 3 markers among the CD3, CD8, CD20, CD45RO, CD4 and GZMB markers are used.

~~6~~5. A method according to one of claims 1 to ~~5~~4, wherein an IHC automate is used for implementing step a).

~~7~~6. A method according to one of claims 1 to ~~6~~5, wherein step d. comprises analysing ~~analysis of~~ the ~~slice~~ slide of tissue section for defining the boundaries of (i) the tumour (CT) and (ii) the invasive margin of the tumour (IM) ~~is implemented~~ by ~~appropriate~~ software.

~~8~~7. A method according to one of claims 1,2 and 4 to ~~7~~6, wherein hexagonal, square or rectangular units having a surface area of 10 x 10^{-9} m^2 to 1000 x 10^{-9} m^2 are used.

~~9~~8. A method according to one of claims 1 to ~~8~~7, wherein the assessed number or density of immune cells stained of each unit is selected from values of group A:

- maximum number of stained cells
- maximum density of stained cells
- sum of the number of stained cells
- sum of the surface of stained cells
- sum of the densities of stained cells
- mean number of stained cells
- mean density of stained cells
- maximum number of stained cells
- median of the number of stained cells.

~~10~~9. A method according to one of claims 1,2 and 4 to ~~9~~8, further comprising detecting cells or units according to a criterion of Group B consisting of:

- single stained cells
- stained cells in clusters containing at least 3 stained cells
- single stained cells or clusters containing at least 3 stained cells
- all stained cells
- most stained units
- random units~~,~~.

~~11. A method according to one of claims 1 to 10, further comprising a step of segregating patients into groups according to their expected survival or to their response to a cancer treatment.~~

~~12~~10. A method according to one of claims 1 to ~~11~~9, ~~wherein the~~ further comprising provision of an output related to an immune score of the tissue section, the provision of an output related to an immune score of the tissue section comprising the steps of:

- for at least one of tumour regions chosen between the tumour (CT) and the invasive margin (IM) of the tumour, comparing a value of the number or the density of immune cells is compared, for a given marker, with a predetermined ~~reference~~ threshold value for the ~~said~~ given marker~~; which predetermined reference value is correlated with a specific prognosis of progression of said cancer and/or survival of the patient and/or response to a cancer treatment~~

- allotting a positive quotation to the tumour region if the value of the number or the density of immune cells is above the predetermined threshold value,

- allotting a negative quotation to the tumour region if the value of the number or the density of immune cells is below the predetermined threshold value,

- determining the immune score of the tissue section based on a number of positive quotations and a number of negative quotations.

11. The method according to claim 10, wherein the steps of the provision of an output related to an immune score are performed for each of the tumour regions.

12. The method according to claim 10 or 11, wherein the steps of the provision of an output related to an immune score are performed for a plurality of markers.

13. The method according to one of claims 10 to 12, further comprising a step of segregating patients into groups according to their immune score, each patient being assigned to one group of patients having the same immune score.

14. The method according to one of claims 1 to 13, wherein the step e1. of checking the quality of immunostaining comprises, for a given marker:

- measuring a distribution value representative of a distribution of stained cells in the tissue section,

- comparing the distribution value with the reference value, the tissue section being correctly stained if the distribution value is within a range around the reference values and the tissue section being not correctly stained if the distribution value is not within the range around the reference value.

15. The method according to claim 14, wherein the step e1. of checking the quality of immunostaining is performed before the step f. of detecting and quantifying stained cells, and

wherein after the step e1. of checking the quality of immunostaining, the step f. of detecting and quantifying stained cells is performed if the tissue section is correctly stained, and the step f. of detecting and quantifying stained cells is not performed if the tissue section is not correctly stained.

16. The method according to claim 14, wherein the step e1. of checking the quality of immunostaining is performed after the step f. of detecting and quantifying stained cells, and

wherein after the step e1. of checking the quality of immunostaining, the number or density of stained immune cells of each unit assessed during step f. of detecting and quantifying stained cells is validated if the tissue section is correctly stained, and the number or density of stained immune cells of each unit assessed during step f. of detecting and quantifying stained cells is not validated if the tissue section is not correctly stained.

17. The method according to one of claims 14 to 16, wherein the reference value is at least one of a mean value, a median value, a minimum value and a maximum value of staining intensity of stained cells of a reference tissue section having a known distribution of the given marker.

18. The method according to one of claims 14 to 17, wherein the range around the reference value is more or less 20 % of the reference value.