Epigenomics 7:100), ovarian cancer (Teschendorff et al, 2009; PLos ONE 4: e8274) and/or association with chemotherapeutic response in ovarian cancer (Ref. 25).

[12] Hence, there is still a need, from one or more of the above or perspectives, for improved methods to determine the presence or absence of one or more cancers in human individuals (such as ovarian cancer in a woman), preferably in a non-invasive manner, such as by the use of (eg cell-free) DNA of said human individual (eg cell-free DNA isolated from a sample of a circulatory fluid). Preferably, such methods will have improved ability to discriminate ovarian cancer from benign pelvic mass, and/or high-grade serious (HGS) ovarian cancer from less severe or aggressive forms of ovarian cancer, such as by having improved specificity and/or sensitivity for the phenotype/disease to be detected. Such methods would provide a significant shift in the clinical paradigm for early-detection, diagnosis (eg by an *in-vitro* method) and/or management of cancers (especially ovarian cancer, and in particular of HGS ovarian cancer and or chemotherapy-responsive ovarian cancer), and in particular providing the potential for individualisation of treatment for women of men suffering from cancer (eg women suffering from ovarian cancer).

[13] Accordingly, it is an object of the present invention to provide alternative, improved, simpler, cheaper and/or integrated methods, means, compounds, compositions, kits and other aspects that address one or more of these or other problems (such as those set forth elsewhere herein). Such an object underlying the present invention is solved by the subject matter as disclosed or defined anywhere herein, for example by the subject matter of the numbered itemised embodiments or the claimed embodiments.

SUMMARY

[14] Generally, and by way of brief description, the main aspects of the present invention can be described as follows:

[14a] In one aspect, the present disclosure refers to **a method** of determining the presence or absence of, or response to therapy against, a cancer in a human individual, said method comprising the steps: - providing a biological sample from said human individual, said sample comprising cell-free DNA of said human individual; and - determining, in at least one molecule of said cell-free DNA, the methylation status at two or more CpGs located within one or more of the nucleotide sequences independently selected from the group consisting of: SEQ ID NOs: 3 (DMR #228), 4 (DMR #144), 2 (DMR #204), 1 (DMR #141), 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 and 31, or a complementary sequence of said nucleotide sequence(s), wherein, the presence in at least one of said cell-free DNA molecules of two or more: (i) methylated CpGs associated with one or more of said nucleotide sequences independently selected from: SEQ ID NOs: 3 (DMR #228), 4 (DMR #144), 2 (DMR #204), 1 (DMR #141), 10, 12, 14, 15, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28, 29 and 30; and/or (ii) un-methylated CpGs associated with one or more of said nucleotide sequences independently selected from: SEQ ID NOs: 5, 6, 7, 8, 9, 11, 13, 16, 17, 22 and 31, indicates the presence of, or a reduced response to therapy against, the cancer in said human individual; and wherein: (a) when the human individual is a woman the cancer is selected from the group consisting of: breast cancer, lung cancer, colon cancer, pancreatic cancer, uterine (endometrial) cancer, cervical cancer and ovarian cancer; or (b) when the human individual is a man the cancer is selected from the group consisting of: lung cancer, colon cancer, pancreatic cancer and prostate cancer.

[14b] In another aspect, the present disclosure refers to **use of a chemotherapeutic agent**, such as one selected from the list consisting of: carboplatin, paclitaxel, docetaxel, cisplatin, liposomal doxorubicin, gemcitabine, trabectedin, etoposide, cyclophosphamide, an angiogenesis inhibitor and a PARP inhibitor, in the manufacture of a medicament for treating cancer in a human individual, wherein said chemotherapeutic agent is to be administered to a human individual within about 3 months of said human individual having been determined, using the method as disclosed herein, to not respond to a therapy against the cancer.

[14c] In yet another aspect, the present disclosure refers to **a nucleic acid** comprising at least 50 contiguous bases comprised in a sequence selected from the group consisting of: SEQ ID NOs 34, 33, 32, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 59, 60, 61 and 62, wherein said nucleic acid sequence includes one or more of the bases identified by "Y" therein is a U or T and, preferably, where one or more of the bases identified by "Y" therein is a C, or a complementary sequence of said nucleotide sequence.

[14d] In yet another aspect, the present disclosure refers to **a nucleic acid probe** complementary to a nucleic acid sequence as disclosed herein, preferably for detection of said nucleic acid

[14e] In yet another aspect, the present disclosure refers to **a nucleic acid primer pair** for amplifying a nucleic acid sequence consisting of at least 50 contiguous bases comprised in a sequence selected from the group consisting of: SEQ ID NOs: SEQ ID NOs 65, 64, 63, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 90, 91, 92 and 93, or a complementary sequence of said nucleotide sequence(s), preferably wherein at least one primer of said pair includes a sequence corresponding to at least one bisulphite-converted CpG present in said nucleotide sequence(s).

[14f] In yet another aspect, the present disclosure refers to **a kit**, preferably for determining the presence or absence of, or response to therapy against, a cancer in a human individual, said kit comprising: - one or more nucleic acid sequences as disclosed herein and/or nucleic acid probes as disclosed herein and/or primer pairs as disclosed herein; and - optionally, said kit further comprising: (i) a printed manual or computer readable memory comprising instructions to use said nucleic acid sequence(s), nucleic acid probe(s), primer pair(s) and/or plurality of nucleic acids to practice a method as disclosed herein and/or to produce or detect the nucleic acid sequence(s) as disclosed herein; and/or (ii) one or more other claim, component or reagent useful for the practice of a method as disclosed herein and and/or the production or detection of the nucleic acid sequence(s) as disclosed herein, including any such item, component or reagent disclosed herein useful for such practice, production or detection.

[14g] In yet another aspect, the present disclosure refers to **a computer program product** comprising: a computer readable medium encoded with a plurality of instructions for controlling a computing system to perform and/or manage an operation for determining the presence or absence of, or response to therapy against, a cancer in a human individual, from a biological sample from said human individual, said sample comprising cell-free DNA of said human individual, and determining, in at least one molecule of said cell-free DNA, the methylation status at two or more CpGs located within one or more nucleotide sequences in accordance with a method as disclosed herein; said operation comprising the steps of: - receiving a first signal representing the number of molecules of said cell-free DNA comprising two or more methylated and/or un-methylated CpGs (as applicable), and/or comprising one or more pattern of methylation and/or un-methylation as set forth in TABLE 2B for the respective nucleotide sequence(s), located within one or more of the nucleotide sequences independently selected from the group consisting of: SEQ ID NOs: 3, 4, 2, 1, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 and 31, or a complementary sequence of said nucleotide sequence(s); and - determining a classification of the presence or absence of, or response to therapy against, the cancer in said human individual based on their being at least one molecules of said cell-free DNA comprising two or more of said methylated and/or un-methylated CpGs (as applicable), and/or comprising one or more said pattern of methylation and/or un-methylation, located within one or more of said nucleotide sequences.

[14h] In yet another aspect, the present disclosure refers to **a use** of a nucleic acid sequences as disclosed herein and/or a nucleic acid probes as disclosed herein and/or a primer pair as disclosed herein and/or a kit as disclosed herein and/or a computer program product as disclosed herein, in each case for determining the presence or absence of, or response to therapy against, a cancer in a human individual.

[15] In a first example, and as may be further described, defined, claimed or otherwise disclosed herein, the disclosure relates to **a method of determining** the presence or absence of, or response to therapy against, a cancer in a human individual, said method comprising the steps:

- Providing a biological sample from said human individual, said sample comprising (eg cell-free) DNA of said human individual; and
- Determining, in at least one molecule of said (eg cell-free) DNA, the methylation status at one or more CpGs located within one or more of the nucleotide sequences comprised in one or more of the respective DMRs of the present disclosure independently selected from the group consisting of DMR#: #141, #204, #228, #144, #123, #129, #137, #148, #150, #154, #158, #164, #176, #178, #180, #186, #188, #190, #192, #200, #202, #208,#210, #213, #214, #219, #222, #223, #224, #225 and #226, or a nucleotide sequence present within about 2,000bp (such as within about 200bp) 5' or 3' thereof, or an allelic variant and/or complementary sequence of any of said nucleotide sequences,

wherein, the presence in at least one of said (eg cell-free) DNA molecules of one or more: (i) methylated CpGs associated with one or more of the hyper-methylated DMRs of the present disclosure; and/or (ii) un-methylated CpGs associated with one or more of the hypo-methylated DMRs of the present disclosure, indicates the presence of, or a reduced response to therapy against, such cancer in said human individual.

[16] In a second example, and as may be further described, defined, claimed or otherwise disclosed herein, the disclosure relates to **a chemotherapeutic agent**, such as one selected from the group consisting of: carboplatin, paclitaxel, docetaxel, cisplatin, liposomal doxorubicin, gemcitabine, trabectedin, etoposide, cyclophosphamide, an angiogenesis inhibitor (such as bevacizumab) and a PARP inhibitor (such as olaparib), for use in a method of therapy of a cancer in a human individual, wherein said chemotherapeutic agent is administered to a human individual within about 3 months of said human individual having been predicted and/or determined, using a method of the first example, to not respond to a therapy against such cancer.

[17] In a third example, and as may be further described, defined, claimed or otherwise disclosed herein, the disclosure relates to **a nucleic acid** comprising a nucleic acid sequence consisting of at least about 10 contiguous bases (preferably at least about 15 contiguous bases for any DMR other than DMR #222) comprised in a sequence producible by bisulphite conversion of a sequence comprised within a DMR selected from the group consisting of DMR#: #141, #204, #228, #144, #123, #129, #137, #148, #150, #154, #158, #164, #176, #178, #180, #186, #188, #190, #192, #200, #202, #208,#210, #213, #214, #219, #222, #223, #224, #225 and #226, or an allelic variant and/or complementary sequence of any of said nucleotide sequences.

[18] In other examples, the disclosure also relates to a **nucleic acid probe**, **a nucleic acid PCR primer pair**, **a population of nucleic acids** of the disclosure, a **kit** and a **computer program product**, in each case as may be described, defined, claimed or otherwise disclosed herein, and in each case related to use within or in connection with a method of the disclosure and/or to detect one or more a nucleic acid of the disclosure.

[19] The figures show:

[20] FIGURE 1 depicts the study design. Using two different epigenome-wide technologies, 711 human tissue samples have been analysed to identify a total of 31 regions whose methylation status has been analysed in two sets consisting of 151 serum samples. Three markers have been validated in two independent settings: (1) Serum Set 3 which consisted of 250 serum samples from women with various benign and malignant conditions of the female genital tract; and (2) NACT (NeoAdjuvant ChemoTherapy) Set consisting of serial samples from women with advanced stage ovarian cancer before and during chemotherapy. Samples obtainable from the UKCTOCS (United Kingdom Collaborative Trial of Ovarian Cancer Screening) sample collection may be included in a third validation set to include serum samples from those women in the control arm who developed ovarian cancer within 2 years; for each case, a number of (such as three) control women who did not develop ovarian cancer within 5 years of sample donation can been matched to those women who did so develop ovarian cancer.

[21] FIGURE 2 depicts the principles of methylation pattern discovery in tissue and analyses in serum. Reduced Representation Bisulfite Sequencing (RRBS) was used in tissue samples in order to identify those CpG regions for which methylation patterns discriminate ovarian cancer from other tissues, in particular blood cells which were deemed to be the most abundant source of cell-free DNA. An example of region #141 is provided which is a 136 base-pair long region containing 7 linked CpGs. A cancer pattern may consist of reads in which all linked CpGs are methylated, indicated by "1111111" (Panel A). The tissue RRBS data have been processed through a bioinformatic pipeline in order to identify the most promising markers (Panel B). The principles of the serum DNA methylation assay are demonstrated in Panel C.

[22] FIGURE 3 depicts serum DNA methylation analysis in women with benign and malignant conditions of the female genital tract. Pattern frequencies for the different regions and CA125 levels analysed in Serum Set 3 samples are shown and horizontal bars denote the mean (Panels A-C; ns not significant; *p<0.05, **p<0.01, ***p<0.001; Mann-Whitney U test compared to HGS; H, Healthy; BPM, benign pelvic mass; BOT, borderline tumours; NET, non-epithelial tumours; OCM other cancerous malignancies; NHGS, non-high grade serous ovarian cancers; HGS, high grade serous; OC ovarian cancers). Based on Set 1&2 analyses cut-off thresholds of 0.0008, 0.0001 and 0.0001 for regions #141, #204 and #228, respectively, to discriminate HGS OC from H or BPM women were chosen and validated in Set 3; combining Sets 1-3 the cut-off thresholds have been refined for regions #204 and #228 so that the final cut-offs were 0.0008, 0.00003 and 0.00001, respectively; the sample was called positive if at least one of the three regions showed a pattern frequency above the cut-off; sensitivities and specificities to discriminate HGS from H&BPM are shown in Panel E. The overlap between CA125 positive samples (cut-off >35IU/mL) and the three DNA methylation (DNAme) marker panel in cases and controls is shown in Panel F.

[23] FIGURE 4 depicts the dynamics of serum DNA methylation markers and CA125 as a function of exposure to carboplatin-based chemotherapy. The changes in pattern frequency of the three markers as well as CA125 is shown

CLAIMS

1. **A method** of determining the presence or absence of, or response to therapy against, a cancer in a human individual, said method comprising the steps:
   - providing a biological sample from said human individual, said sample comprising cell-free DNA of said human individual; and
   - determining, in at least one molecule of said cell-free DNA, the methylation status at two or more CpGs located within one or more of the nucleotide sequences independently selected from the group consisting of: SEQ ID NOs: 3 (DMR #228), 4 (DMR #144), 2 (DMR #204), 1 (DMR #141), 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 and 31, or a complementary sequence of said nucleotide sequence(s),

   wherein, the presence in at least one of said cell-free DNA molecules of two or more: (i) methylated CpGs associated with one or more of said nucleotide sequences independently selected from: SEQ ID NOs: 3 (DMR #228), 4 (DMR #144), 2 (DMR #204), 1 (DMR #141), 10, 12, 14, 15, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28, 29 and 30; and/or (ii) un-methylated CpGs associated with one or more of said nucleotide sequences independently selected from: SEQ ID NOs: 5, 6, 7, 8, 9, 11, 13, 16, 17, 22 and 31, indicates the presence of, or a reduced response to therapy against, the cancer in said human individual; and

   wherein: (a) when the human individual is a woman the cancer is selected from the group consisting of: breast cancer, lung cancer, colon cancer, pancreatic cancer, uterine (endometrial) cancer, cervical cancer and ovarian cancer; or (b) when the human individual is a man the cancer is selected from the group consisting of: lung cancer, colon cancer, pancreatic cancer and prostate cancer.

2. The method of claim 1, wherein said biological sample is liquid biological sample selected from the group consisting of: a blood sample, a plasma sample and a serum sample.

3. The method of claim 1 or 2, wherein the methylation status is determined at a number being two, three, four, five, six, seven, eight, nine, ten, 12, 15, 20, 25 or more of said CpGs located within said nucleotide sequence; wherein, the presence in at least one of said cell-free DNA molecules of at least two, such as up to said number, of: (i) methylated CpGs associated with one or more of said nucleotide sequences independently selected from: SEQ ID NOs: 3, 4, 2,

1, 10, 12, 14, 15, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28, 29 and 30; and/or (ii) un-methylated CpGs associated with one or more of said nucleotide sequences independently selected from: SEQ ID NOs: 5, 6, 7, 8, 9, 11, 13, 16, 17, 22 and 31, indicates the presence of, or a reduced response to therapy against, the cancer in said human individual.

4. The method of any one of claims 1 to 3, wherein said nucleotide sequence(s) is/are associated with
DMR(s) #228 and/or #144 and/or #204 and/or #141 (eg, SEQ ID NOs: 3, 4, 2 and/or 1), or a complementary sequence of said nucleotide sequence(s).

5. The method of any one of claims 1 to 4, comprising the step of treating said cell-free DNA with an agent that differentially modifies said cell-free DNA based on the methylation status of one or more CpGs located within; preferably a methylation sensitive restriction enzyme and/or bisulphite.

6. The method of claim 5, wherein (a) said agent is bisulphite and said determining step comprises the detection of at least one bisulphite-converted un-methylated cytosine within one or more of the nucleotide sequences independently selected from those set forth in TABLE 2A (eg, independently selected the group consisting of: SEQ ID NOs 34, 35, 33, 32, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61 and 62), wherein one or more of the bases identified by "Y" therein is a U or T and, preferably, where one or more of the bases identified by "Y" in a CpG therein is a C, or a complementary sequence of said nucleotide sequence(s); and/or.
(b) wherein said agent is bisulphite and said determining step comprises the detection of at least one bisulphite-converted un-methylated cytosine within a nucleotide sequence having a length of at least 50bp comprised in SEQ ID NO 34 and/or SEQ ID NO 35 and/or SEQ ID NO 33 and/or SEQ ID NO 32, wherein one or more of the bases identified by "Y" therein is a U or T and, preferably, where one or more of the bases identified by "Y" in a CpG therein is a C, or a complementary sequence of said nucleotide sequence(s).

7. The method of any one of claims 1 to 6, wherein the methylation status of said CpG(s) is determined in multiple molecules of said cell-free DNA and/or amplified DNA representing each of said nucleotide sequences.

8. The method of claim 7, wherein a fraction or ratio of, or an absolute number of, cell-free DNA molecules in said sample having said methylated and/or un-methylated CpG(s) (as applicable) located within said nucleotide sequence(s), and/or having said pattern of methylation and/or un-methylation as set forth in TABLE 2B for the respective nucleotide sequence(s), is estimated.

9. The method of claim 8, further comprising a step of comparing said fraction or ratio with a standard or cut-off value; wherein a fraction or ratio greater than the standard or cut-off value indicates the presence of or a reduced response to therapy against, the cancer in said human individual.

10. The method of any one of claims 1 to 9, for determining the response of a human individual suffering from a cancer to a therapy comprising chemotherapeutic agent(s) against said cancer.

11. **Use of a chemotherapeutic agent**, such as one selected from the list consisting of: carboplatin, paclitaxel, docetaxel, cisplatin, liposomal doxorubicin, gemcitabine, trabectedin, etoposide, cyclophosphamide, an angiogenesis inhibitor and a PARP inhibitor, in the manufacture of a medicament for treating cancer in a human individual, wherein said chemotherapeutic agent is to be administered to a human individual within about 3 months of said human individual having been determined, using the method of claim 10, to not respond to a therapy against the cancer.

12. **A nucleic acid** comprising at least 50 contiguous bases comprised in a sequence selected from the group consisting of: SEQ ID NOs 34, 33, 32, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 59, 60, 61 and 62, wherein said nucleic acid sequence includes one or more of the bases identified by "Y" therein is a U or T and, preferably, where one or more of the bases identified by "Y" therein is a C, or a complementary sequence of said nucleotide sequence.

13. The nucleic acid sequence of claim 12, comprising at least 50 contiguous bases comprised in a sequence of SEQ ID NO 34, SEQ ID NO 33 or SEQ ID NO 32, wherein said nucleic acid sequence includes one or more of the bases identified by "Y" therein is a U or T and,

preferably, where one or more of the bases identified by "Y" therein is a C, or a complementary sequence of said nucleotide sequence.

14. **A nucleic acid probe** complementary to a nucleic acid sequence recited in any one of claims 12 to 13, preferably for detection of said nucleic acid.

15. **A nucleic acid primer pair** for amplifying a nucleic acid sequence consisting of at least 50 contiguous bases comprised in a sequence selected from the group consisting of: SEQ ID NOs: SEQ ID NOs 65, 64, 63, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 90, 91, 92 and 93, or a complementary sequence of said nucleotide sequence(s), preferably wherein at least one primer of said pair includes a sequence corresponding to at least one bisulphite-converted CpG present in said nucleotide sequence(s).

16. The primer pair of claim 15, selected from the group of primer-pairs set forth in each row of TABLE 3.

17. **A kit**, preferably for determining the presence or absence of, or response to therapy against, a cancer in a human individual, said kit comprising:
    - one or more nucleic acid sequences of claim 12 or 13 and/or nucleic acid probes of claim 14 and/or primer pairs of claim 15 or 16; and
    - optionally, said kit further comprising:

    (i) a printed manual or computer readable memory comprising instructions to use said nucleic acid sequence(s), nucleic acid probe(s), primer pair(s) and/or plurality of nucleic acids to practice a method of any one of claims 1 to 10 and/or to produce or detect the nucleic acid sequence(s) of any one of claim 12 or 13; and/or

    (ii) one or more other claim, component or reagent useful for the practice of a method of any one of claims 1 to 10 and and/or the production or detection of the nucleic acid sequence(s) of claim 12 or 13, including any such item, component or reagent disclosed herein useful for such practice, production or detection.

18. **A computer program product** comprising: a computer readable medium encoded with a plurality of instructions for controlling a computing system to perform and/or manage an operation for determining the presence or absence of, or response to therapy against, a cancer in a human individual, from a biological sample from said human individual, said

sample comprising cell-free DNA of said human individual, and determining, in at least one molecule of said cell-free DNA, the methylation status at two or more CpGs located within one or more nucleotide sequences in accordance with a method as set forth in any one of claims 1 to 10; said operation comprising the steps of:

- receiving a first signal representing the number of molecules of said cell-free DNA comprising two or more methylated and/or un-methylated CpGs (as applicable), and/or comprising one or more pattern of methylation and/or un-methylation as set forth in TABLE 2B for the respective nucleotide sequence(s), located within one or more of the nucleotide sequences independently selected from the group consisting of: SEQ ID NOs: 3, 4, 2, 1, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 and 31, or a complementary sequence of said nucleotide sequence(s); and
- determining a classification of the presence or absence of, or response to therapy against, the cancer in said human individual based on their being at least one molecules of said cell-free DNA comprising two or more of said methylated and/or un-methylated CpGs (as applicable), and/or comprising one or more said pattern of methylation and/or un-methylation, located within one or more of said nucleotide sequences.

19. **A use** of a nucleic acid sequences of claim 12 or 13 and/or a nucleic acid probes of claim 14 and/or a primer pair of claim 15 or 16 and/or a kit of claim 17 and/or a computer program product of claim 18, in each case for determining the presence or absence of, or response to therapy against, a cancer in a human individual.