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[Continued on next page]

- (54) **Title:** HOMOLOGOUS RECOMBINATION DEFICIENCY TO PREDICT NEOADJUVANT CHEMOTHERAPY NECESSITY IN BLADDER CANCER

(57) **Abstract:** Methods and materials involved in assessing samples (e.g., cancer cells) are provided for the presence of homologous recombination deficiency (HRD) or an HRD signature. For example, methods and materials for determining whether or not a cancer cell (e.g., a bladder cancer cell) contains an HRD signature are provided. Materials and methods for identifying cancer cells (e.g., bladder cancer cells) having a deficiency in homology directed repair (HDR) as well as materials and methods for identifying cancer patients likely to respond to a particular cancer treatment regimen (e.g., neoadjuvant chemotherapy) also are provided.

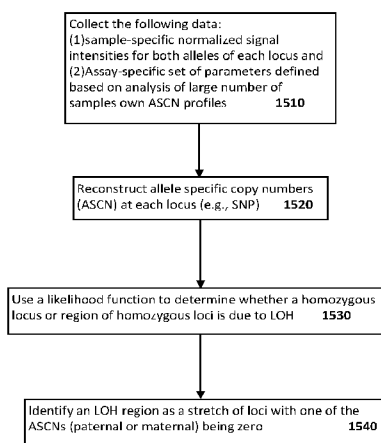


Figure 2



(84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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HOMOLOGOUS RECOMBINATION DEFICIENCY TO PREDICT NEOADJUVANT CHEMOTHERAPY NECESSITY IN BLADDER CANCER

BACKGROUND

[0001] This application claims priority to U.S. provisional application number 62/311,231, filed March 21, 2016, and U.S. provisional application number 62/332,526, filed May 5, 2016, the entire contents of both of which are hereby incorporated by reference.

[0002] Cancer is a serious public health problem, with 562,340 people in the United States of America dying of cancer in 2009 alone. *American Cancer Society, Cancer Facts & Figures 2009* (available at American Cancer Society website). Urothelial cell carcinoma (UCC) of the urinary bladder is the 4th most common cancer in the United States, with an estimated 73,510 new cases and 14,880 deaths from bladder cancer in 2012, alone. *American Cancer Society, Facts and Figures 2012*. Each year in the U.S. and EU, bladder cancer is diagnosed in >160,000 men and results in >48,000 deaths. While the five-year survival rate for early-stage bladder cancer is high, over 25% of patients present with advanced disease and around 70% experience recurrence or progression following treatment.

[0003] The prognosis for patients with progressive or recurrent invasive bladder cancer is generally poor. Management of recurrent cancers depends on the prior therapy used, site of recurrence, and patient-specific considerations. One of the primary challenges in cancer treatment is discovering relevant, clinically useful characteristics of a patient's own cancer and then, based on these characteristics, administering a treatment plan best suited to the patient's cancer. Given the limitations of current methodologies, there is a clear need for more sensitive methods for novel and improved tools for predicting response to particular therapy regimens.

SUMMARY

[0004] In general, one aspect of this invention features an *in vitro* method of predicting patient response to a bladder cancer treatment regimen. The method comprises a DNA damaging agent, anthracycline, topoisomerase I inhibitor, or PARP inhibitor, the method comprising: (1) determining, in a sample comprising a bladder cancer cell, the number of Indicator CA Regions comprising at least two types chosen from Indicator LOH Regions, Indicator TAI Regions, or Indicator LST Regions in at least one pair of human chromosomes of a bladder cancer cell of said

cancer patient; and (2) diagnosing a patient in whose sample said number of Indicator LOH Regions, Indicator TAI Regions, and/or Indicator LST Regions is greater than a reference number as having an increased likelihood of responding to said bladder cancer treatment regimen. In another embodiment, said at least one pair of human chromosomes is representative of the entire genome. In another embodiment, said Indicator CA Regions are determined in at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 pairs of human chromosomes. In another embodiment, the reference number of Indicator LOH Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, the reference number of Indicator TAI Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, and the reference number of Indicator LST Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more. In another embodiment, said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but less than a either a complete chromosome or a complete chromosome arm, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but not extending across a centromere, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length. In an embodiment, said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci. In another embodiment, said DNA damaging agent is cisplatin, carboplatin, oxaloplatin, nedaplatin, iproplatin, or picoplatin, said anthracycline is epirubicin or doxorubicin, said topoisomerase I inhibitor is camptothecin, topotecan, or irinotecan, or said PARP inhibitor is iniparib, olaparib or velapirib. In another embodiment, said bladder cancer treatment regimen is administered to said patient diagnosed as having an increased likelihood of responding to said

bladder cancer treatment regimen. In another embodiment, mutations in RB1 and/or TP53 are identified.

[0005] In other embodiment, an *in vitro* method of predicting patient response to a bladder cancer treatment regimen comprising a platinum agent is provided. The method comprises (1) determining, in a sample comprising a bladder cancer cell, the number of Indicator CA Regions comprising at least two types chosen from Indicator LOH Regions, Indicator TAI Regions, and/or Indicator LST Regions in at least one pair of human chromosomes of a bladder cancer cell of said cancer patient; and (3) diagnosing a patient in whose sample said number of Indicator LOH Regions, Indicator TAI Regions, or Indicator LST Regions is greater than a reference number as having an increased likelihood of responding to said cancer treatment regimen. In another embodiment, at least one pair of human chromosomes is representative of the entire genome. In another embodiment, said Indicator CA Regions are determined in at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 pairs of human chromosomes. In another embodiment, the reference number of Indicator LOH Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, the reference number of Indicator TAI Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, and the reference number of Indicator LST Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more. In another embodiment, said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but less than a either a complete chromosome or a complete chromosome arm, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but not extending across a centromere, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length. In an embodiment, said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, and said

Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci. In another embodiment, said DNA damaging agent is platinum, cisplatin, carboplatin, oxaloplatin, nedaplatin, iproplatin, or picoplatin, said anthracycline is epirubicin or doxorubicin, said topoisomerase I inhibitor is camptothecin, topotecan, or irinotecan, or said PARP inhibitor is iniparib, olaparib or velapirib. In another embodiment, the method further comprises identifying mutations in RB1 and/or TP53.

[0006] In an embodiment, an *in vitro* method of predicting patient response to a bladder cancer treatment regimen comprising a DNA damaging agent, anthracycline, topoisomerase I inhibitor, or PARP inhibitor is provided. The method comprises (1) determining, in a sample comprising a bladder cancer cell, the number of Indicator CA Regions comprising at least two types chosen from Indicator LOH Regions, Indicator TAI Regions, or Indicator LST Regions in at least one pair of human chromosomes of a bladder cancer cell of said cancer patient; (2) providing a test value derived from the number of said Indicator CA Regions; (3) comparing said test value to one or more reference values derived from the number of said Indicator CA Regions in a reference population; and (4) diagnosing a patient in whose sample said test value is greater than said one or more reference numbers as having an increased likelihood of responding to said cancer treatment regimen. In another embodiment, said at least one pair of human chromosomes is representative of the entire genome. In another embodiment, said Indicator CA Regions are determined in at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 pairs of human chromosomes. In another embodiment, the reference number of Indicator LOH Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, the reference number of Indicator TAI Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, and the reference number of Indicator LST Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more. In another embodiment, said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but less than a either a complete chromosome or a complete chromosome arm, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,

30, 35, 40, 45, 50 or more megabases in length but not extending across a centromere, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length. In an embodiment, said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci. In another embodiment, said DNA damaging agent is cisplatin, carboplatin, oxalaplatin, nedaplatin, iproplatin, or picoplatin, said anthracycline is epirubicin or doxorubicin, said topoisomerase I inhibitor is camptothecin, topotecan, or irinotecan, or said PARP inhibitor is iniparib, olaparib or velapirib. In another embodiment, the method further comprises diagnosing a patient in whose sample said test value is not greater than said one or more reference numbers as not having an increased likelihood of responding to said cancer treatment regimen and recommending, prescribing, initiating or continuing a bladder treatment regimen not comprising a DNA damaging agent, anthracycline, topoisomerase I inhibitor, or PARP inhibitor in said patient diagnosed as not having an increased likelihood of responding to said bladder cancer treatment regimen. In another embodiment, said test value is derived by calculating the arithmetic mean of the numbers of Indicator LOH Regions, Indicator TAI Regions and Indicator LST Regions in said sample as follows:

$$\text{Test Value} = \frac{(\# \text{ of Indicator LOH Regions}) + (\# \text{ of Indicator TAI Regions}) + (\# \text{ of Indicator LST Regions})}{3}$$

3

and said one or more reference values were derived by calculating the arithmetic mean of the numbers of Indicator LOH Regions, Indicator TAI Regions and Indicator LST Regions in samples from said reference population as follows:

$$\text{Test Value} = \frac{(\# \text{ of Indicator LOH Regions}) + (\# \text{ of Indicator TAI Regions}) + (\# \text{ of Indicator LST Regions})}{3}$$

3.

In another embodiment, the method comprises diagnosing a patient in whose sample said test value is at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10-fold greater, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 standard

deviations greater, or at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% greater than said one or more reference numbers as having an increased likelihood of responding to said bladder cancer treatment regimen. In another embodiment, the method further comprises identifying mutations in RB1 and/or TP53.

[0007] In an embodiment, a method of treating bladder cancer patients is provided. The method comprises (1) determining, in a sample comprising a cancer cell, the number of Indicator CA Regions comprising Indicator LOH Regions, Indicator TAI Regions, and Indicator LST Regions in at least one pair of human chromosomes of a bladder cancer cell of said cancer patient; (2) providing a test value derived from the number of said Indicator CA Regions; (3) comparing said test value to one or more reference values derived from the number of said Indicator CA Regions in a reference population; and either (4)(a) recommending, prescribing, initiating or continuing a bladder treatment regimen comprising a DNA damaging agent, anthracycline, topoisomerase I inhibitor, or PARP inhibitor in a patient in whose sample the test value is greater than at least one said reference value; or (4)(b) recommending, prescribing, initiating or continuing a treatment regimen comprising a DNA damaging agent, anthracycline, topoisomerase I inhibitor, or PARP inhibitor in a patient in whose sample the test value is not greater than at least one said reference value. In another embodiment, said at least one pair of human chromosomes is representative of the entire genome. In another embodiment, said Indicator CA Regions are determined in at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 pairs of human chromosomes. In another embodiment, the reference number of Indicator LOH Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, the reference number of Indicator TAI Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, and the reference number of Indicator LST Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more. In another embodiment, said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but less than either a complete chromosome or a complete chromosome arm, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but not extending across a centromere, and said Indicator LST Regions are

defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length. In an embodiment, said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci. In another embodiment, said DNA damaging agent is cisplatin, carboplatin, oxaloplatin, nedaplatin, iproplatin, or picoplatin, said anthracycline is epirubicin or doxorubicin, said topoisomerase I inhibitor is camptothecin, topotecan, or irinotecan, or said PARP inhibitor is iniparib, olaparib or velapirib. In another embodiment, said test value is derived by calculating the arithmetic mean of the numbers of Indicator LOH Regions, Indicator TAI Regions and Indicator LST Regions in said sample as follows:

$$\text{Test Value} = \frac{(\# \text{ of Indicator LOH Regions}) + (\# \text{ of Indicator TAI Regions}) + (\# \text{ of Indicator LST Regions})}{3}$$

and said one or more reference values were derived by calculating the arithmetic mean of the numbers of Indicator LOH Regions, Indicator TAI Regions and Indicator LST Regions in samples from said reference population as follows:

$$\text{Test Value} = \frac{(\# \text{ of Indicator LOH Regions}) + (\# \text{ of Indicator TAI Regions}) + (\# \text{ of Indicator LST Regions})}{3}.$$

In another embodiment, the method comprises diagnosing a patient in whose sample said test value is at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10-fold greater, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 standard deviations greater, or at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% greater than said one or more reference numbers as having an increased likelihood of responding to said bladder cancer treatment regimen. In another embodiment, the method further comprises identifying mutations in RB1 and/or TP53.

[0008] The details of one or more embodiments of the invention are set forth in the description and accompanying drawings below. The materials, methods, and examples are

illustrative only and not intended to be limiting. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

[0009] **Figure 1** illustrates a graph identifying patients with a likelihood of responding to adjuvant therapy.

[0010] **Figure 2** illustrates an exemplary process by which a computing system (or a computer program (*e.g.*, software) containing computer-executable instructions) can identify LOH loci or regions from genotype data as described herein, which can be adapted to use in determining TAI and LST as will be apparent to those skilled in the art.

[0011] **Figure 3** is a flow chart of an example process for assessing the genome of a cell (*e.g.*, a cancer cell) for an HRD signature.

[0012] **Figure 4** is a diagram of an example of a computer device and a mobile computer device that can be used to implement the techniques described herein.

[0013] **Figure 5** illustrates the distribution of HRD scores in the cohort of Example 2.

[0014] **Figure 6** illustrates the distribution of mutations of the patients in the cohort of Example 2.

[0015] **Figure 7** illustrates the correlations between HRD with RB1 and TP53 mutations.

[0016] **Figure 8** illustrates the correlations between HRD with RB1 and TP53 response.

[0017] **Figure 9** illustrates a Kaplan-Meier recurrence-free survival analysis based on HRD score.

DETAILED DESCRIPTION

[0018] In general, one aspect of this invention features a method for assessing HRD in a cancer cell or DNA (*e.g.*, genomic DNA) derived therefrom. In some embodiments, the method comprises, or consists essentially of, (a) detecting, in a sample or DNA derived therefrom, CA Regions in at least one pair of human chromosomes or DNA derived therefrom; and (b) determining the number, size (*e.g.*, length), and/or character of said CA Regions. In some embodiments, the method further comprises recommending, prescribing, initiating or continuing a treatment.

[0019] As used herein, “chromosomal aberration” or “CA” means a somatic change in a cell’s chromosomal DNA that falls into at least one of three partially overlapping categories: LOH, TAI, or LST. Polymorphic loci within the human genome (*e.g.*, single nucleotide polymorphisms (SNPs)) are generally heterozygous within an individual’s germline since that individual typically receives one copy from the biological father and one copy from the biological mother. Somatic, however, this heterozygosity can change (via mutation) to homozygosity. This change from heterozygosity to homozygosity is called loss of heterozygosity (LOH). LOH may result from several mechanisms. For example, in some cases, a locus of one chromosome can be deleted in a somatic cell. The locus that remains present on the other chromosome (the other non-sex chromosome for males) is an LOH locus as there is only one copy (instead of two copies) of that locus present within the genome of the affected cells. This type of LOH event results in a copy number reduction. In other cases, a locus of one chromosome (*e.g.*, one non-sex chromosome for males) in a somatic cell can be replaced with a copy of that locus from the other chromosome, thereby eliminating any heterozygosity that may have been present within the replaced locus. In such cases, the locus that remains present on each chromosome is an LOH locus and can be referred to as a copy neutral LOH locus. LOH and its use in determining HRD is described in detail in International Application no. PCT/US2011/040953 (published as WO/2011/160063), the entire contents of which are incorporated herein by reference.

[0020] A broader class of chromosomal aberration, which generally encompasses LOH, is allelic imbalance. Allelic imbalance occurs when the relative copy number (*i.e.*, copy proportion) at a particular locus in somatic cells differs from the germline. For example, if the germline has one copy of allele A and one copy of allele B at a particular locus, and a somatic cell has two copies of A and one copy of B, there is allelic imbalance at the locus because the copy proportion of the somatic cell (2:1) differs from the germline (1:1). LOH is an example of allelic imbalance since the somatic cell has a copy proportion (1:0 or 2:0) that differs from the germline (1:1). But allelic imbalance encompasses more types of chromosomal aberration, *e.g.*, 2:1 germline going to 1:1 somatic; 1:0 germline going to 1:1 somatic; 1:1 germline going to 2:1 somatic, etc. Analysis of regions of allelic imbalance encompassing the telomeres of chromosomes is particularly useful in the invention. Thus, a “telomeric allelic imbalance region” or “TAI Region” is defined as a region with allelic imbalance that (a) extends to one of the subtelomeres and (b) does not cross the centromere. TAI and its use in determining HRD is described in detail in International Application no.

PCT/US2011/048427 (published as WO/2012/027224), the entire contents of which are incorporated herein by reference.

[0021] A class of chromosomal aberrations that is broader still, which generally encompasses LOH and TAI, is referred to herein as large scale transition (“LST”). LST refers to any somatic copy number transition (*i.e.*, breakpoint) along the length of a chromosome where it is between two regions of at least some minimum length (*e.g.*, at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more megabases) after filtering out regions shorter than some maximum length (*e.g.*, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4 or more megabases). For example, if after filtering out regions shorter than 3 megabases the somatic cell has a copy number of 1:1 for, *e.g.*, at least 10 megabases and then a breakpoint transition to a region of, *e.g.*, at least 10 megabases with copy number 2:2, this is an LST. An alternative way of defining the same phenomenon is as an LST Region, which is genomic region with stable copy number across at least some minimum length (*e.g.*, at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 megabases) bounded by breakpoints (*i.e.*, transitions) where the copy number changes for another region also at least this minimum length. For example, if after filtering out regions shorter than 3 megabases the somatic cell has a region of at least 10 megabases with copy number of 1:1 bounded on one side by a breakpoint transition to a region of, *e.g.*, at least 10 megabases with copy number 2:2, and bounded on the other side by a breakpoint transition to a region of, *e.g.*, at least 10 megabases with copy number 1:2, then this is two LSTs. Notice that this is broader than allelic imbalance because such a copy number change would not be considered allelic imbalance (because the copy proportions 1:1 and 2:2 are the same, *i.e.*, there has been no change in copy proportion). LST and its use in determining HRD is described in detail in Popova *et al.*, *Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation*, *CANCER RES.* (2012) 72:5454-5462.

[0022] Different cutoffs for LST score may be used for “near-diploid” and “near-tetraploid” tumors to separate BRCA1/2 intact and deficient samples. LST score sometimes increases with ploidy both within intact and deficient samples. As an alternative to using ploidy-specific cutoffs, some embodiments may employ a modified LST score adjusting it by ploidy: $LST_m = LST - kP$, where P is ploidy and k is a constant. Based on multivariate logistic regression analysis with deficiency as an outcome and LST and P as predictors, $k=15.5$ provided the best separation between intact and deficient samples (though one skilled in the art can envisage other values for k).

[0023] Chromosomal aberrations can extend across a plurality of loci to define a region of chromosomal aberration, referred to herein as a “CA Region.” Such CA Regions can be any length (*e.g.*, from a length less than about 1.5 Mb up to a length equal to the entire length of the chromosome). An abundance of CA Regions of a certain size and/or character (“Indicator CA Regions”) indicate a deficiency in the homology-dependent repair (HDR) mechanism of a cell. The definition of a region of CA, and what constitutes an “Indicator” region, for each type of CA (*e.g.*, LOH, TAI, LST) depends on the particular character of the CA. For example, an “LOH Region” means at least some minimum number of consecutive loci exhibiting LOH or some minimum stretch of genomic DNA having consecutive loci exhibiting LOH. A “TAI Region,” on the other hand, means at least some minimum number of consecutive loci exhibiting allelic imbalance extending from the telomere into the rest of the chromosome (or some minimum stretch of genomic DNA extending from the telomere into the rest of the chromosome having consecutive loci exhibiting allelic imbalance). LST is already defined in terms of a region of genomic DNA of at least some minimum size, so “LST” and “LST Region” are used interchangeably in this document to refer to a minimum number of consecutive loci (or some minimum stretch of genomic DNA) having the same copy number bounded by a breakpoint or transition from that copy number to a different one. In some embodiments a CA Region (whether an LOH Region, TAI region, or LST Region) is an Indicator CA Region (whether an Indicator LOH Region, Indicator TAI region, or Indicator LST Region) if it is at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, or 100 consecutive loci.

[0024] In some embodiments a CA Region (whether an LOH Region, TAI region, or LST Region) is an Indicator CA Region (whether an Indicator LOH Region, Indicator TAI region, or Indicator LST Region) if it is at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, or 100 megabases or more in length. In some embodiments, Indicator LOH Regions are LOH Regions that are longer than about 1.5, 5, 12, 13, 14, 15, 16, 17 or more (preferably 14, 15, 16 or more, more preferably 15 or more) megabases but shorter than the entire length of the respective chromosome within which the LOH Region is located. Alternatively or additionally, the total combined length of such Indicator LOH Regions may be determined. In some embodiments, Indicator TAI Regions are TAI Regions with allelic imbalance that (a) extend to one of the subtelomeres, (b) do not cross the centromere and (c) are longer than 1.5, 5, 12, 13, 14, 15, 16, 17 or more (preferably 10, 11, 12 or more, more preferably 11 or more) megabases.

Alternatively or additionally, the total combined length of such Indicator TAI Regions may be determined. Because the concept of LST already involves regions of some minimum size (such minimum size being determined based on its ability to differentiate HRD from HDR intact samples), Indicator LST Regions as used herein are the same as LST Regions. Furthermore, an LST Region Score can be either derived from the number of regions showing LST as described above or the number of LST breakpoints. In some embodiments the minimum length of the region of stable copy number bounding the LST breakpoint is at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 megabases (preferably 8, 9, 10, 11 or more megabases, more preferably 10 megabases) and the maximum region remaining unfiltered is less than 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4 or fewer megabases (preferably 2, 2.5, 3, 3.5, or 4 or fewer megabases, more preferably fewer than 3 megabases).

[0025] As used herein, a sample has an “HRD signature” if such sample has a number of Indicator CA Regions (as described herein) or a CA Region Score (as described herein) exceeding a reference as described herein, wherein a number or score exceeding such reference indicates homologous recombination deficiency.

[0026] Thus the invention generally involves detecting and quantifying Indicator CA Regions in a sample to determine whether cells in the sample (or cells from which DNA in the sample are derived) have an HRD signature. Often this comprises comparing the number of Indicator CA Regions (or a test value or score derived or calculated therefrom and corresponding to such number) to a reference, or index number (or score).

[0027] The various aspects of the present invention comprise using a combined analysis of two or more types of CA Regions (including two or more types of Indicator CA Regions) to assess (*e.g.*, detect, diagnose) HRD in a sample. Thus, in one aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining the total number (or combined length) of Indicator LOH Regions in the sample; (2) determining the total number (or combined length) of Indicator TAI Regions in the sample; and (3) determining the presence or absence of (*e.g.*, detecting, diagnosing) HRD in the sample based at least in part on the determinations made in (1) and (2). In another aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining the total number (or combined length) of Indicator LOH Regions in the sample; (2) determining the total number (or

combined length) of Indicator LST Regions in the sample; and (3) determining the presence or absence of (*e.g.*, detecting, diagnosing) HRD in the sample based at least in part on the determinations made in (1) and (2). In another aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining the total number (or combined length) of Indicator TAI Regions in the sample; (2) determining the total number (or combined length) of Indicator LST Regions in the sample; and (3) determining the presence or absence of (*e.g.*, detecting, diagnosing) HRD in the sample based at least in part on the determinations made in (1) and (2). In another aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining the total number (or combined length) of Indicator LOH Regions in the sample; (2) determining the total number of Indicator TAI Regions in the sample; (3) determining the total number (or combined length) of Indicator LST Regions in the sample; and (4) determining the presence or absence of (*e.g.*, detecting, diagnosing) HRD in the sample based at least in part on the determinations made in (1), (2) and (3).

[0028] The various aspects of the present invention comprise using a combined analysis of the averages of three different CA Regions to assess (*e.g.*, detect, diagnose) HRD in a sample. Thus, in one aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining the total number of LOH Regions of a certain size or character (*e.g.*, “Indicator LOH Regions”, as defined herein) in the sample; (2) determining the total number of TAI Regions of a certain size or character (*e.g.*, “Indicator TAI Regions”, as defined herein) in the sample; (3) determining the total number of LST Regions of a certain size or character (*e.g.*, “Indicator LST Regions”, as defined herein) in the sample; (4) calculating the average (*e.g.*, arithmetic mean) of the determinations made in (1), (2), and (3); and (5) assessing HRD in the sample based at least in part on the calculated average (*e.g.*, arithmetic mean) made in (4).

[0029] As used herein, “CA Region Score” means a test value or score derived or calculated from (*e.g.*, representing or corresponding to) Indicator CA Regions detected in a sample (*e.g.*, a score or test value derived or calculated from the number of Indicator CA Regions detected in a sample). Analogously, as used herein, “LOH Region Score” is a subset of CA Region Scores and means a test value or score derived or calculated from (*e.g.*, representing or corresponding to) Indicator LOH Regions detected in a sample (*e.g.*, a score or test value derived or calculated from the number of Indicator LOH Regions detected in a sample), and so on for TAI Region Score and LST Region Score. Such a score may in some embodiments be simply the number of Indicator CA

Regions detected in a sample. In some embodiments the score is more complicated, factoring in the lengths of each Indicator CA Region or a subset of Indicator CA Regions detected.

[0030] As discussed above, the invention will generally involve combining the analysis of two or more types of CA Region Scores (which may include the number of such regions). Thus, in one aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining an LOH Region Score for the sample; (2) determining a TAI Region Score for the sample; and (3)(a) detecting (or diagnosing) HRD in the sample based at least in part on either the LOH Region Score exceeding a reference or the TAI Region Score exceeding a reference; or optionally (3)(b) detecting (or diagnosing) an absence of HRD in the sample based at least in part on both the LOH Region Score not exceeding a reference and the TAI Region Score not exceeding a reference. In another aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining an LOH Region Score for the sample; (2) determining an LST Region Score for the sample; and (3)(a) detecting (or diagnosing) HRD in the sample based at least in part on either the LOH Region exceeding a reference or the LST Region Score exceeding a reference; or optionally (3)(b) detecting (or diagnosing) an absence of HRD in the sample based at least in part on both the LOH Region Score not exceeding a reference and the LST Region Score not exceeding a reference. In another aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining a TAI Region Score for the sample; (2) determining an LST Region Score for the sample; and (3)(a) detecting (or diagnosing) HRD in the sample based at least in part on either the TAI Region Score exceeding a reference or the LST Region Score exceeding a reference; or optionally (3)(b) detecting (or diagnosing) an absence of HRD in the sample based at least in part on both the TAI Region Score not exceeding a reference and the LST Region Score not exceeding a reference. In another aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining an LOH Region Score for the sample; (2) determining a TAI Region Score for the sample; (3) determining an LST Region Score for the sample; and (4)(a) detecting (or diagnosing) HRD in the sample based at least in part on either the LOH Region Score exceeding reference, the TAI Region Score exceeding a reference or the LST Region Score exceeding a reference; or optionally (4)(b) detecting (or diagnosing) an absence of HRD in the sample based at least in part on the LOH Region Score not exceeding a reference, the TAI Region Score not exceeding a reference and the LST Region Score not exceeding a reference.

[0031] In some embodiments the CA Region Score is a combination of scores derived or calculated from (*e.g.*, representing or corresponding to) two or more of (1) the detected LOH Regions (“LOH Region Score”, as defined herein), (2) the detected TAI Regions (“TAI Region Score”, as defined herein), and/or (3) the detected LST Regions (“LST Region Score”, as defined herein). In some embodiments the LOH Region Score and TAI Region Score are combined as follows to yield a CA Region Score:

$$CA\ Region\ Score = A*(LOH\ Region\ Score) + B*(TAI\ Region\ Score)$$

In some embodiments the LOH Region Score and TAI Region Score are combined as follows to yield a CA Region Score:

$$CA\ Region\ Score = 0.32*(LOH\ Region\ Score) + 0.68*(TAI\ Region\ Score)$$

OR

$$CA\ Region\ Score = 0.34*(LOH\ Region\ Score) + 0.66*(TAI\ Region\ Score)$$

In some embodiments the LOH Region Score and LST Region Score are combined as follows to yield a CA Region Score:

$$CA\ Region\ Score = A*(LOH\ Region\ Score) + B*(LST\ Region\ Score)$$

In some embodiments an LOH Region Score for a sample and an LST Region Score for a sample are combined to yield a CA Region Score as follows:

$$CA\ Region\ Score = 0.85*(LOH\ Region\ Score) + 0.15*(LST\ Region\ Score)$$

In some embodiments the TAI Region Score and LST Region Score are combined as follows to yield a CA Region Score:

$$CA\ Region\ Score = A*(TAI\ Region\ Score) + B*(LST\ Region\ Score)$$

In some embodiments the LOH Region Score, TAI Region Score and LST Region Score are combined as follows to yield a CA Region Score:

$$CA\ Region\ Score = A*(LOH\ Region\ Score) + B*(TAI\ Region\ Score) + C*(LST\ Region\ Score)$$

In some embodiments the LOH Region Score, TAI Region Score and LST Region Score are combined as follows to yield a CA Region Score:

$$CA \text{ Region Score} = 0.21*(LOH \text{ Region Score}) + 0.67*(TAI \text{ Region Score}) + 0.12*(LST \text{ Region Score})$$

OR

$$CA \text{ Region Score} = [0.24]*(LOH \text{ Region Score}) + [0.65]*(TAI \text{ Region Score}) + [0.11]*(LST \text{ Region Score})$$

OR

$$CA \text{ Region Score} = [0.11]*(LOH \text{ Region Score}) + [0.25]*(TAI \text{ Region Score}) + [0.12]*(LST \text{ Region Score})$$

[0032] In some embodiments the CA Region Score is a combination of scores derived or calculated from (*e.g.*, representing or corresponding to) the average (*e.g.*, arithmetic mean) of (1) the detected LOH Regions (“LOH Region Score”, as defined herein), (2) the detected TAI Regions (“TAI Region Score”, as defined herein), and/or (3) the detected LST Regions (“LST Region Score”, as defined herein) to yield a CA Region Score calculated from one of the following formulae:

$$CA \text{ Region Score} = \frac{A*(LOH \text{ Region Score}) + B*(TAI \text{ Region Score}) + C*(LST \text{ Region Score})}{3}$$

$$CA \text{ Region Score} = \frac{A*(LOH \text{ Region Score}) + B*(TAI \text{ Region Score})}{2}$$

$$CA \text{ Region Score} = \frac{A*(LOH \text{ Region Score}) + C*(LST \text{ Region Score})}{2}$$

$$CA \text{ Region Score} = \frac{B*(TAI \text{ Region Score}) + C*(LST \text{ Region Score})}{2}$$

In some embodiments, including some specifically illustrated herein, one or more of these coefficients (*i.e.*, A, B, or C, or any combination thereof) is 1 and in some embodiments all three coefficients (*i.e.*, A, B, and C) are 1. Thus, in some embodiments the *CA Region Score* = (*LOH Regions Score*) + (*TAI Region Score*) + (*LST Region Score*), wherein the LOH Region Score is the number of Indicator LOH Regions (or the total length of LOH), the TAI Region Score is the number of Indicator TAI Regions (or the total length of TAI), and the LST Region Score is the number of Indicator LST Regions (or the total length of LST).

[0033] In some cases a formula may not have all of the specified coefficients (and thus not incorporate the corresponding variable(s)). For example, the embodiment mentioned immediately previously may be applied to formula (2) where A in formula (2) is 0.95 and B in formula (2) is 0.61. C and D would not be applicable as these coefficients and their corresponding variables are not found in formula (2) (though the clinical variables are incorporated into the clinical score found in formula (2)). In some embodiments A is between 0.9 and 1, 0.9 and 0.99, 0.9 and 0.95, 0.85 and 0.95, 0.86 and 0.94, 0.87 and 0.93, 0.88 and 0.92, 0.89 and 0.91, 0.85 and 0.9, 0.8 and 0.95, 0.8 and 0.9, 0.8 and 0.85, 0.75 and 0.99, 0.75 and 0.95, 0.75 and 0.9, 0.75 and 0.85, or between 0.75 and 0.8. In some embodiments B is between 0.40 and 1, 0.45 and 0.99, 0.45 and 0.95, 0.55 and 0.8, 0.55 and 0.7, 0.55 and 0.65, 0.59 and 0.63, or between 0.6 and 0.62. In some embodiments C is, where applicable, between 0.9 and 1, 0.9 and 0.99, 0.9 and 0.95, 0.85 and 0.95, 0.86 and 0.94, 0.87 and 0.93, 0.88 and 0.92, 0.89 and 0.91, 0.85 and 0.9, 0.8 and 0.95, 0.8 and 0.9, 0.8 and 0.85, 0.75 and 0.99, 0.75 and 0.95, 0.75 and 0.9, 0.75 and 0.85, or between 0.75 and 0.8. In some embodiments D is, where applicable, between 0.9 and 1, 0.9 and 0.99, 0.9 and 0.95, 0.85 and 0.95, 0.86 and 0.94, 0.87 and 0.93, 0.88 and 0.92, 0.89 and 0.91, 0.85 and 0.9, 0.8 and 0.95, 0.8 and 0.9, 0.8 and 0.85, 0.75 and 0.99, 0.75 and 0.95, 0.75 and 0.9, 0.75 and 0.85, or between 0.75 and 0.8.

[0034] In some embodiments A is between 0.1 and 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.2 and 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.3 and 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.4 and 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.5 and 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.6 and 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.7 and 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.8 and 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.9 and 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 1 and 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 1.5 and 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 2 and 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 2.5 and 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 3 and 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 3.5 and 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 4 and 4.5, 5, 6, 7, 8, 9, 10, 11, 12,

13, 14, 15, or 20; or between 4.5 and 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 5 and 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 6 and 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 7 and 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 8 and 9, 10, 11, 12, 13, 14, 15, or 20; or between 9 and 10, 11, 12, 13, 14, 15, or 20; or between 10 and 11, 12, 13, 14, 15, or 20; or between 11 and 12, 13, 14, 15, or 20; or between 12 and 13, 14, 15, or 20; or between 13 and 14, 15, or 20; or between 14 and 15, or 20; or between 15 and 20; B is between 0.1 and 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.2 and 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.3 and 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.4 and 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.5 and 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.6 and 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.7 and 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.8 and 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.9 and 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 1 and 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 1.5 and 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 2 and 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 2.5 and 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 3 and 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 3.5 and 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 4 and 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 4.5 and 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 5 and 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 6 and 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 7 and 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 8 and 9, 10, 11, 12, 13, 14, 15, or 20; or between 9 and 10, 11, 12, 13, 14, 15, or 20; or between 10 and 11, 12, 13, 14, 15, or 20; or between 11 and 12, 13, 14, 15, or 20; or between 12 and 13, 14, 15, or 20; or between 13 and 14, 15, or 20; or between 14 and 15, or 20; or between 15 and 20; C is, where applicable, between 0.1 and 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.2 and 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.3 and 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.4 and 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 0.5 and 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6,

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12, 13, 14, 15, or 20; or between 7 and 8, 9, 10, 11, 12, 13, 14, 15, or 20; or between 8 and 9, 10, 11, 12, 13, 14, 15, or 20; or between 9 and 10, 11, 12, 13, 14, 15, or 20; or between 10 and 11, 12, 13, 14, 15, or 20; or between 11 and 12, 13, 14, 15, or 20; or between 12 and 13, 14, 15, or 20; or between 13 and 14, 15, or 20; or between 14 and 15, or 20; or between 15 and 20. In some embodiments, A, B, and/or C is within rounding of any of these values (*e.g.*, A is between 0.45 and 0.54, etc.).

[0035] Thus, in one aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining an LOH Region Score for the sample; (2) determining a TAI Region Score for the sample; and (3)(a) detecting (or diagnosing) HRD in the sample based at least in part on a combination of the LOH Region Score and the TAI Region Score (*e.g.*, a Combined CA Region Score) exceeding a reference; or optionally (3)(b) detecting (or diagnosing) an absence of HRD in the sample based at least in part on a combination of the LOH Region Score and the TAI Region Score (*e.g.*, a Combined CA Region Score) not exceeding a reference. In another aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining an LOH Region Score for the sample; (2) determining an LST Region Score for the sample; and (3)(a) detecting (or diagnosing) HRD in the sample based at least in part on a combination of the LOH Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score) exceeding a reference; or optionally (3)(b) detecting (or diagnosing) an absence of HRD in the sample based at least in part on a combination of the LOH Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score) not exceeding a reference. In another aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining a TAI Region Score for the sample; (2) determining an LST Region Score for the sample; and (3)(a) detecting (or diagnosing) HRD in the sample based at least in part on a combination of the TAI Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score) exceeding a reference; or optionally (3)(b) detecting (or diagnosing) an absence of HRD in the sample based at least in part on a combination of the TAI Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score) not exceeding a reference. In another aspect the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining an LOH Region Score for the sample; (2) determining a TAI Region Score for the sample; (3) determining an LST Region Score for the sample; and (4)(a) detecting (or diagnosing) HRD in the sample based at least in part on a

combination of the LOH Region Score, the TAI Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score) exceeding a reference; or optionally (4)(b) detecting (or diagnosing) an absence of HRD in the sample based at least in part on the LOH Region Score, the TAI Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score) not exceeding a reference.

[0036] Thus another aspect of the invention provides a method of assessing (*e.g.*, detecting, diagnosing) HRD in a sample comprising (1) determining the total number of LOH Regions of a certain size or character (*e.g.*, “Indicator LOH Regions”, as defined herein) in the sample; (2) determining the total number of TAI Regions of a certain size or character (*e.g.*, “Indicator TAI Regions”, as defined herein) in the sample; (3) determining the total number of LST Regions of a certain size or character (*e.g.*, “Indicator LST Regions”, as defined herein) in the sample; (4) calculating the average (*e.g.*, arithmetic mean) of the determinations made in (1), (2), and (3); and (5) assessing HRD in the sample based at least in part on the calculated average (*e.g.*, arithmetic mean) made in (4).

[0037] In some embodiments, the reference (or index) discussed above for the CA Region Score (*e.g.*, the number of Indicator CA Regions) may be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20 or greater, preferably 5, preferably 8, more preferably 9 or 10, most preferably 10. The reference for the total (*e.g.*, combined) length of Indicator CA Regions may be about 75, 90, 105, 120, 130, 135, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 megabases or greater, preferably about 75 megabases or greater, preferably about 90 or 105 megabases or greater, more preferably about 120 or 130 megabases or greater, and more preferably about 135 megabases or greater, and most preferably about 150 megabases or greater. In some embodiments, the reference discussed above for the Combined CA Region Score (*e.g.*, the combined number of Indicator LOH Regions, Indicator, TAI Regions and/or Indicator LST Regions) may be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50 or greater, preferably 5, preferably 10, preferably 15, preferably 20, preferably 25, preferably 30, preferably 35, preferably 40-44, most preferably ≥ 42 . The reference for the total (*e.g.*, combined) length of Indicator LOH Regions, Indicator TAI Regions and/or Indicator LST Regions may be about 75, 90, 105, 120, 130, 135, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 megabases or greater, preferably about 75 megabases or greater, preferably about 90 or 105 megabases or greater, more preferably about 120 or 130 megabases or greater, and more preferably about 135 megabases or greater, and most preferably about 150 megabases or greater.

[0038] In some embodiments, the invention provides a method for detecting an HRD signature in a sample. Thus, another aspect of the invention provides a method for detecting an HRD signature in a sample comprising (1) determining the total number of LOH Regions of a certain size or character (*e.g.*, “Indicator LOH Regions”, as defined herein) in the sample; (2) determining the total number of TAI Regions of a certain size or character (*e.g.*, “Indicator TAI Regions”, as defined herein) in the sample; (3) determining the total number of LST Regions of a certain size or character (*e.g.*, “Indicator LST Regions”, as defined herein) in the sample; (4) combining the determinations made in (1), (2), and (3) (*e.g.*, calculating or deriving a Combined CA Region Score); and (5) characterizing a sample in which the Combined CA Region Score is greater than a reference value as having an HRD signature. In some embodiments, the reference value is 42. Thus, in some embodiments a sample is characterized as having an HRD signature when the reference value is 42. In some embodiments, the reference discussed above for the Combined CA Region Score (*e.g.*, the combined number of Indicator LOH Regions, Indicator, TAI Regions and/or Indicator LST Regions) may be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50 or greater, preferably 5, preferably 10, preferably 15, preferably 20, preferably 25, preferably 30, preferably 35, preferably 40-44, most preferably ≥ 42 .

[0039] In some embodiments, the number of Indicator CA Regions (or the combined length, a CA Region Score or a Combined CA Region Score) in a sample is considered “greater” than a reference if it is at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10-fold greater than the reference while in some embodiments, it is considered “greater” if it is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 standard deviations greater than the reference. Conversely, in some embodiments the number of Indicator CA Regions (or the combined length, a CA Region Score or a Combined CA Region Score) in a sample is considered “not greater” than a reference if it is not more than 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10-fold greater than the reference while in some embodiments, it is considered “not greater” if it is not more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 standard deviations greater than the reference.

[0040] In some embodiments the reference number (or length, value or score) is derived from a relevant reference population. Such reference populations may include patients (a) with the same cancer, such as bladder cancer, as the patient being tested, (b) with the same cancer sub-type, (c) with cancer having similar genetic or other clinical or molecular features, (d) who responded to a particular treatment, (e) who did not respond to a particular treatment, (f) who are apparently healthy (*e.g.*, do not have any cancer or at least do not have the tested patient’s cancer), etc. The reference

number (or length, value or score) may be (a) representative of the number (or length, value or score) found in the reference population as a whole, (b) an average (mean, median, etc.) of the number (or length, value or score) found in the reference population as a whole or a particular sub-population, (c) representative of the number (or length, value or score) (*e.g.*, an average such as mean or median) found in terciles, quartiles, quintiles, etc. of the reference population as ranked by (i) their respective number (or length, value or score) or (ii) the clinical feature they were found to have (*e.g.*, strength of response, prognosis (including time to cancer-specific death), etc.), or (d) selected to have a high sensitivity for detecting HRD for predicting response to a particular therapy (*e.g.*, platinum, PARP inhibitor, etc.).

[0041] In some embodiments the reference or index that, if exceeded by the test value or score from the sample, indicates HRD is the same as the reference that, if not exceeded by the test value or score from the sample, indicates the absence of HRD (or functional HDR). In some embodiments they are different.

[0042] In another aspect, the present invention provides a method of predicting the status of BRCA1 and BRCA2 genes in a sample. Such method is analogous to the methods described above and differs in that the determination of CA Regions, LOH Regions, TAI Regions, LST Regions, or scores incorporating these are used to assess (*e.g.*, detect) BRCA1 and/or BRCA2 deficiency in the sample.

[0043] In another aspect, this invention provides a method of predicting a cancer patient's response (*e.g.*, a bladder cancer patient's response) to a cancer treatment regimen comprising a DNA damaging agent, an anthracycline, a topoisomerase I inhibitor, radiation, and/or a PARP inhibitor. Such method is analogous to the methods described above and differs in that the determination of CA Regions, LOH Regions, TAI Regions, LST Regions, or scores incorporating these, including high HRD scores (*e.g.*, an HRD signature or a high combined CA Region Score), are used to predict the likelihood that the cancer patient will respond to the cancer treatment regimen.

[0044] In some embodiments, the patients are treatment naïve patients. In another aspect, this invention provides a method of treating cancer, *e.g.*, bladder cancer. Such method is analogous to the methods described above and differs in that a particular treatment regimen is administered (recommended, prescribed, etc.) based at least in part on the determination of CA Regions, LOH Regions, TAI Regions, LST Regions, or scores incorporating these.

[0045] In another aspect, this invention features the use of one or more drugs selected from the group consisting of DNA damaging agents, anthracyclines, topoisomerase I inhibitors, and PARP inhibitors, in the manufacture of a medicament useful for treating a cancer in a patient identified as having (or as having had) a cancer cell (*e.g.*, a bladder cancer cell) determined to have high levels of HRD (*e.g.*, an HRD signature) as described herein.

[0046] In another aspect, this document features a method for assessing a sample for the presence of a mutation within a gene from an HDR pathway. Such method is analogous to the methods described above and differs in that the determination of CA Regions, LOH Regions, TAI Regions, LST Regions, or scores incorporating these are used to detect (or not) the presence of a mutation within a gene from an HDR pathway.

[0047] In another aspect, this document features a method for assessing cancer cells (*e.g.*, bladder cancer cells) of a patient for the presence of an HRD signature. The method comprises, or consists essentially of, (a) detecting the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell (*e.g.*, a bladder cancer cell) of the cancer patient (*e.g.*, a bladder cancer patient), and (b) identifying the patient as having cancer cells with the HRD signature. In another aspect, this document features a method for assessing cancer cells (*e.g.*, bladder cancer cells) of a patient for the presence of an HDR deficient status. The method comprises, or consists essentially of, (a) detecting the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell (*e.g.*, bladder cancer cell) of the bladder cancer patient, and (b) identifying the patient as having cancer cells with the HDR deficient status. In another aspect, this document features a method for assessing cancer cells (*e.g.*, bladder cancer cells) of a patient for having an HRD signature. The method comprises, or consists essentially of, (a) detecting the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell of the cancer patient (*e.g.*, a bladder cancer patient), and (b) identifying the patient as having cancer cells (*e.g.*, bladder cancer cells) with an HRD signature. In another aspect, this document features a method for assessing cancer cells (*e.g.*, bladder cancer cell) of a patient for the presence of a genetic mutation within a gene from an HDR pathway. The method comprises, or consists essentially of, (a) detecting the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell of the cancer patient (*e.g.*, a bladder cancer patient), and (b) identifying the patient as having cancer cells (*e.g.*, bladder cancer cells) with the genetic mutation. In

another aspect, this invention features methods further comprising identifying mutations in RB1 and/or TP53.

[0048] In another aspect, this document features a method for determining if a patient (*e.g.*, a bladder cancer patient) is likely to respond to a cancer treatment regimen comprising administering radiation or a drug selected from the group consisting of DNA damaging agents, anthracyclines, topoisomerase I inhibitors, and PARP inhibitors. The method comprises, or consists essentially of, (a) detecting the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell (*e.g.*, a bladder cancer cell) of the cancer patient, and (b) identifying the patient as being likely to respond to the cancer treatment regimen. In another aspect, this document features a method for assessing a patient. The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells having an HRD signature, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell of the cancer patient indicates that the cancer cells have the HRD signature, and (b) diagnosing the patient as having cancer cells (*e.g.*, bladder cancer cells) with the HRD signature. In another aspect, this document features a method for assessing a patient. The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells (*e.g.*, bladder cancer cells) having an HDR deficiency status, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell (*e.g.*, a bladder cancer cell) of the cancer patient indicates that the cancer cells have the HDR deficiency status, and (b) diagnosing the patient as having cancer cells with the HDR deficient status. In another aspect, this document features a method for assessing a patient. The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells (*e.g.*, bladder cancer cells) having an HDR deficient status, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell (*e.g.*, a bladder cancer cell) of the cancer patient (*e.g.*, a bladder cancer patient) indicates that the cancer cells have high HDR, and (b) diagnosing the patient as having cancer cells with an HDR deficient status. In another aspect, this document features a method for assessing a patient (*e.g.*, a bladder cancer patient). The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells (*e.g.*, bladder cancer cells) having a genetic mutation within a gene from an HDR pathway, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell (*e.g.*, bladder cancer cell) of the cancer patient

indicates that the cancer cells have the genetic mutation, and (b) diagnosing the patient as having cancer cells with the genetic mutation. In another aspect, this document features a method for assessing a patient (*e.g.*, a bladder cancer patient) for a likelihood to respond to a cancer treatment regimen comprising administering radiation or a drug selected from the group consisting of DNA damaging agents, anthracyclines, topoisomerase I inhibitors, and PARP inhibitors. The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells (*e.g.*, bladder cancer cells) having an HRD signature, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell of the cancer patient indicates that the cancer cells have the HRD signature, and (b) diagnosing, based at least in part on the presence of the HRD signature, the patient as being likely to respond to the cancer treatment regimen. In another aspect, this document features a method for assessing a patient (*e.g.*, a bladder cancer patient) for a likelihood to respond to a cancer treatment regimen comprising administering radiation or a drug selected from the group consisting of DNA damaging agents, anthracyclines, topoisomerase I inhibitors, and PARP inhibitors. The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells (*e.g.*, bladder cancer cells) having an HRD signature, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell of the cancer patient indicates that the cancer cells have an HRD signature, and (b) diagnosing, based at least in part on the presence of the HRD signature, the patient as being likely to respond to the cancer treatment regimen. In another aspect, this invention features methods further comprising identifying mutations in RB1 and/or TP53.

[0049] In another aspect, this document features a method for performing a diagnostic analysis of a cancer cell (*e.g.*, a bladder cancer cell) of a patient (*e.g.*, a bladder cancer patient). The method comprises, or consists essentially of, (a) detecting the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of the cancer cell, and (b) identifying or classifying the patient as having cancer cells with an HRD signature. In another aspect, this document features a method for performing a diagnostic analysis of a cancer cell (*e.g.*, a bladder cancer cell) of a patient (*e.g.*, a bladder cancer patient). The method comprises, or consists essentially of, (a) detecting the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of the cancer cell, and (b) identifying or classifying the patient as having cancer cells with a HDR deficient status. In another aspect, this document features

a method for performing a diagnostic analysis of a cancer cell (*e.g.*, a bladder cancer cell) of a patient (*e.g.*, a bladder cancer patient). The method comprises, or consists essentially of, (a) detecting the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of the cancer cell, and (b) identifying or classifying the patient as having cancer cells with an HDR deficient status. In another aspect, this document features a method for performing a diagnostic analysis of a cancer cell (*e.g.*, a bladder cancer cell) of a patient (*e.g.*, a bladder cancer patient). The method comprises, or consists essentially of, (a) detecting the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of the cancer cell that are longer, and (b) identifying or classifying the patient as having cancer cells with a genetic mutation within a gene from an HDR pathway. In another aspect, this document features a method for performing a diagnostic analysis of a cancer cell (*e.g.*, a bladder cancer cell) of a patient (*e.g.*, a bladder cancer patient) to determine if the cancer patient is likely to respond to a cancer treatment regimen comprising administering radiation or a drug selected from the group consisting of DNA damaging agents, anthracyclines, topoisomerase I inhibitors, and PARP inhibitors. The method comprises, or consists essentially of, (a) detecting the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of the cancer cell, and (b) identifying or classifying the patient as being likely to respond to the cancer treatment regimen. In another aspect, this invention features methods further comprising identifying mutations in RB1 and/or TP53.

[0050] In another aspect, this document features a method for diagnosing a patient (*e.g.*, a bladder cancer patient) as having cancer cells (*e.g.*, bladder cancer cells) having an HRD signature. The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells having the HRD signature, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell of the cancer patient indicates that the cancer cells have the HRD signature, and (b) diagnosing the patient as having cancer cells with the HRD signature. In another aspect, this document features a method for diagnosing a patient (*e.g.*, a bladder cancer patient) as having cancer cells (*e.g.*, bladder cancer cells) with an HDR deficient status. The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells having the HDR deficiency status, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell of the cancer patient indicates that the cancer cells have the HDR deficiency status, and

(b) diagnosing the patient as having cancer cells with the HDR deficient status. In another aspect, this document features a method for diagnosing a patient (*e.g.*, a bladder cancer patient) as having cancer cells (*e.g.*, bladder cancer cells) with an HDR deficient status. The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells having the HDR deficient status, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell of the cancer patient indicates that the cancer cells have the HDR deficient status, and (b) diagnosing the patient as having cancer cells with the HDR deficient status. In another aspect, this document features a method for diagnosing a patient (*e.g.*, a bladder cancer patient) as having cancer cells (*e.g.*, bladder cancer cells) with a genetic mutation within a gene from an HDR pathway. The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells having the genetic mutation, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell of the cancer patient indicates that the cancer cells have the genetic mutation, and (b) diagnosing the patient as having cancer cells with the genetic mutation. In another aspect, this document features a method for diagnosing a patient (*e.g.*, a bladder cancer patient) as being a candidate for a cancer treatment regimen comprising administering radiation or a drug selected from the group consisting of DNA damaging agents, anthracyclines, topoisomerase I inhibitors, and PARP inhibitors. The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells (*e.g.*, bladder cancer cells) having an HDR signature, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell of the cancer patient indicates that the cancer cells have the HDR signature, and (b) diagnosing, based at least in part on the presence of the HDR signature, the patient as being likely to respond to the cancer treatment regimen. In another aspect, this document features a method for diagnosing a patient (*e.g.*, a bladder cancer patient) as being a candidate for a cancer treatment regimen comprising administering radiation or a drug selected from the group consisting of DNA damaging agents, anthracyclines, topoisomerase I inhibitors, and PARP inhibitors. The method comprises, or consists essentially of, (a) determining that the patient comprises cancer cells (*e.g.*, bladder cancer cells) having high an HDR signature, wherein the presence of more than a reference number of Indicator CA Regions in at least one pair of human chromosomes of a cancer cell of the cancer patient indicates that the cancer cells have an HDR signature, and (b) diagnosing, based at least in part on the presence of the HDR signature, the patient as being likely to respond to

the cancer treatment regimen. In another aspect, this invention features methods further comprising identifying mutations in RB1 and/or TP53.

[0051] In another aspect, the invention provides a method for assessing a patient (*e.g.*, a bladder cancer patient). The method comprises, or consists essentially of, (a) determining whether the patient has (or had) cancer cells (*e.g.*, bladder cancer cells) with more than a reference number of Indicator CA Regions (or, *e.g.*, a CA Region Score exceeding a reference CA Region Score); and (b)(1) diagnosing the patient as having cancer cells with HRD if it is determined that the patient has (or had) cancer cells with more than a reference number of CA Regions (or, *e.g.*, a CA Region Score exceeding a reference CA Region Score); or (b)(2) diagnosing the patient as not having cancer cells with HRD if it is determined that the patient does not have (or has not had) cancer cells with more than a reference number of CA Regions (or, *e.g.*, the patient does not have (or has not had) cancer cells with a CA Region Score exceeding a reference CA Region Score). In another aspect, this invention features methods further comprising identifying mutations in RB1 and/or TP53 in addition to detecting HRD.

[0052] In another aspect, this invention features the use of a plurality of oligonucleotides capable of hybridizing to a plurality of polymorphic regions of human genomic DNA, in the manufacture of a diagnostic kit useful for determining the total number or combined length of CA Regions in at least a chromosome pair (or DNA derived therefrom) in a sample obtained from a cancer patient (*e.g.*, a bladder cancer patient), and for detecting (a) HRD, high HRD, or likelihood of HRD (each, *e.g.*, an HRD signature) in the sample, (b) deficiency (or likelihood of deficiency) in a BRCA1 or BRCA2 gene in the sample, or (c) an increased likelihood that the cancer patient will respond to a cancer treatment regimen comprising a DNA damaging agent, an anthracycline, a topoisomerase I inhibitor, radiation, or a PARP inhibitor. In another aspect, this invention features methods further comprising identifying mutations in RB1 and/or TP53.

[0053] In another aspect, this invention features a system for detecting HRD (*e.g.*, an HRD signature) in a sample. The system comprises, or consists essentially of, (a) a sample analyzer configured to produce a plurality of signals about genomic DNA of at least one pair of human chromosomes (or DNA derived therefrom) in the sample, and (b) a computer sub-system programmed to calculate, based on the plurality of signals, the number or combined length of CA Regions in the at least one pair of human chromosomes. The computer sub-system can be

programmed to compare the number or combined length of CA Regions to a reference number to detect (a) HRD, high HRD, or likelihood of HRD (each, *e.g.*, an HRD signature) in the sample, (b) deficiency (or likelihood of deficiency) in a BRCA1 or BRCA2 gene in the sample, or (c) an increased likelihood that the cancer patient will respond to a cancer treatment regimen comprising a DNA damaging agent, an anthracycline, a topoisomerase I inhibitor, radiation, or a PARP inhibitor. The system can comprise an output module configured to display (a), (b), or (c). The system can comprise an output module configured to display a recommendation for the use of the cancer treatment regimen. In another aspect, this invention features methods further comprising identifying mutations in RB1 and/or TP53.

[0054] In another aspect, the invention provides a computer program product embodied in a computer readable medium that, when executing on a computer, provides instructions for detecting the presence or absence of any CA Region along one or more of human chromosomes other than the human X and Y sex chromosomes (the CA Regions optionally being Indicator CA Regions); and determining the total number or combined length of the CA Regions in the one or more chromosome pairs. The computer program product can include other instructions.

[0055] In another aspect, the present invention provides a diagnostic kit. The kit comprises, or consists essentially of, at least 500 oligonucleotides capable of hybridizing to a plurality of polymorphic regions of human genomic DNA (or DNA derived therefrom); and a computer program product provided herein. The computer program product can be embodied in a computer readable medium that, when executing on a computer, provides instructions for detecting the presence or absence of any CA Region along one or more of human chromosomes other than the human X and Y sex chromosomes (the CA Regions optionally being Indicator CA Regions); and determining the total number or combined length of the CA Regions in the one or more chromosome pairs. The computer program product can include other instructions. In another aspect, this invention features methods further comprising identifying mutations in RB1 and/or TP53.

[0056] In some embodiments of any one or more of the aspects of the invention described in the preceding paragraphs, any one or more of the following can be applied as appropriate. The CA Regions can be determined in at least two, five, ten, or 21 pairs of human chromosomes. The cancer cell can be an ovarian, breast, lung, bladder or esophageal cancer cell. The reference can be 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 20 or greater. The at least one pair of human chromosomes

can exclude human chromosome 17. The DNA damaging agent can be cisplatin, carboplatin, oxalaplatin, nedaplatin, iproplatin or picoplatin, the anthracycline can be epirubicin or doxorubicin, the topoisomerase I inhibitor can be camptothecin, topotecan, or irinotecan, or the PARP inhibitor can be iniparib, olaparib or velapirib. The patient can be a treatment naïve patient.

[0057] As described herein, a sample (*e.g.*, bladder cancer cell sample or a sample containing DNA derived from one or more cancer cells) can be identified as having an “HRD signature” (or alternatively called “HDR-deficiency signature”) if the genome of the cells being assessed contains (a) any of an LOH Region Score, a TAI Region Score or an LST Region Score exceeding a reference or (b) a Combined CA Region Score exceeding a reference. Conversely, a sample (*e.g.*, cancer cell sample or a sample containing DNA derived from one or more cancer cells) can be identified as lacking an “HRD signature” (or alternatively called “HDR-deficiency signature”) if the genome of the cells being assessed contains (a) an LOH Region Score, a TAI Region Score and an LST Region Score each not exceeding a reference or (b) a Combined CA Region Score not exceeding a reference.

[0058] Cells (*e.g.*, bladder cancer cells) identified as having an HRD signature can be classified as having an increased likelihood of having an HDR deficiency and/or as having an increased likelihood of having a deficient status in one or more genes in the HDR pathway. For example, bladder cancer cells identified as having an HRD signature can be classified as having an increased likelihood of having an HDR deficient status. In some cases, bladder cancer cells identified as having an HRD signature can be classified as having an increased likelihood of having a deficient status for one or more genes in the HDR pathway. As used herein, deficient status for a gene means the sequence, structure, expression and/or activity of the gene or its product is/are deficient as compared to normal. Examples include, but are not limited to, low or no mRNA or protein expression, deleterious mutations, hypermethylation, attenuated activity (*e.g.*, enzymatic activity, ability to bind to another biomolecule), etc. As used herein, deficient status for a pathway (*e.g.*, HDR pathway) means at least one gene in that pathway (*e.g.*, BRCA1) is deficient. Examples of highly deleterious mutations include frameshift mutations, stop codon mutations, and mutations that lead to altered RNA splicing. Deficient status in a gene in the HDR pathway may result in deficiency or reduced activity in homology directed repair in the cancer cells. Examples of genes in the HDR pathway include, without limitation, the genes listed in Table 1.

Table 1. Selected HDR Pathway Genes

Gene Name	Entrez Gene Symbol (if assigned)	Entrez Gene Id	Gene Name	Entrez Gene Symbol (if assigned)	Entrez Gene Id
<i>BLM</i>	<i>BLM</i>	641	<i>RA</i>	<i>RAD50</i>	10111
<i>BRC</i>	<i>BRCA1</i>	672	<i>RA</i>	<i>RAD51</i>	5888
<i>BRC</i>	<i>BRCA2</i>	675	<i>RA</i>	<i>RAD51AP1</i>	10635
<i>CtIP</i>	<i>RBBP8</i>	5932	<i>RA</i>	<i>RAD51L1</i>	5890
A DN polymerase delta	<i>POLD1</i>	5424	<i>RA</i>	<i>RAD51C</i>	5889
	<i>POLD2</i>	5424	<i>RA</i>	<i>RAD51L3</i>	5892
	<i>POLD3</i>	10714	<i>RA</i>	<i>ATR</i>	546
	<i>POLD4</i>	57804	<i>RA</i>	<i>RAD54B</i>	25788
A DN polymerase eta	<i>POLH</i>	5429	<i>RM</i>	<i>RMI1</i>	80010
<i>DN</i>	<i>DNA2</i>	1763	<i>RM</i>	<i>C16orf75</i>	116028

<i>EM</i> <i>E1</i>	<i>EME1</i>	1 46956	<i>RP</i> <i>A</i>	<i>RPA1</i>	61 17
<i>ERC</i> <i>C1</i>	<i>ERCC</i> <i>I</i>	2 067	<i>RT</i> <i>EL1</i>	<i>RTEL</i> <i>I</i>	51 750
<i>EX</i> <i>O1</i>	<i>EXO1</i>	9 156	<i>SL</i> <i>X1</i>		
<i>FAN</i> <i>CM</i>	<i>FANC</i> <i>M</i>	5 7697	<i>SL</i> <i>X2</i>		
<i>GE</i> <i>N1</i>	<i>GEN1</i>	3 48654	<i>SL</i> <i>X4</i>	<i>SLX4</i>	84 464
<i>MR</i> <i>E11</i>	<i>MRE1</i> <i>IA</i>	4 361	<i>TO</i> <i>P2A</i>	<i>TOP2</i> <i>A</i>	71 53
<i>MU</i> <i>S81</i>	<i>MUS8</i> <i>I</i>	8 0198	<i>XP</i> <i>F</i>	<i>ERC</i> <i>C4</i>	20 72
<i>NBS</i> <i>I</i>	<i>NBN</i>	4 683	<i>XR</i> <i>CC2</i>	<i>XRC</i> <i>C2</i>	75 16
<i>PAL</i> <i>B2</i>	<i>PALB</i> <i>2</i>	7 9728	<i>XR</i> <i>CC3</i>	<i>XRC</i> <i>C3</i>	75 17
<i>PC</i> <i>NA</i>	<i>PCNA</i>	5 111			

[0059] As described herein, identifying CA loci (as well as the size and number of CA Regions) can include, first, determining the genotype of a sample at various genomic loci (e.g., SNP loci, individual bases in large-scale sequencing) and, second, determining whether the loci exhibit any of LOH, TAI or LST. Any appropriate technique can be used to determine genotypes at loci of interest within the genome of a cell. For example, single nucleotide polymorphism (SNP) arrays (e.g., human genome-wide SNP arrays), targeted sequencing of loci of interest (e.g., sequencing SNP loci and their surrounding sequences), and even large-scale sequencing (e.g., whole exome,

transcriptome, or genome sequencing) can be used to identify loci as being homozygous or heterozygous. Typically, an analysis of the homozygous or heterozygous nature of loci over a length of a chromosome can be performed to determine the length of CA Regions. For example, a stretch of SNP locations that are spaced apart (*e.g.*, spaced about 25 kb to about 100 kb apart) along a chromosome can be evaluated using SNP array results to determine not only the presence of a region of homozygosity (*e.g.*, LOH) along a chromosome but also the length of that region. Results from a SNP array can be used to generate a graph that plots allele dosages along a chromosome. Allele dosage d_i for SNP i can be calculated from adjusted signal intensities of two alleles (A_i and B_i): $d_i = A_i / (A_i + B_i)$. Numerous variations on nucleic acid arrays useful in the invention are known in the art. These include the arrays, *e.g.*, Affymetrix 500K GeneChip array and Affymetrix OncoScan™ FFPE Express 2.0 Services (Formerly MIP CN Services).

[0060] Once a sample's genotype has been determined for a plurality of loci (*e.g.*, SNPs), common techniques can be used to identify loci and regions of LOH, TAI and LST (including those described in International Application no. PCT/US2011/040953 (published as WO/2011/160063); International Application no. PCT/US2011/048427 (published as WO/2012/027224); Popova *et al.*, *Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation*, CANCER RES. (2012) 72:5454-5462). In some embodiments determining whether chromosomal imbalance or large scale transitions includes determining whether these are somatic or germline aberrations. One way to determine to do this is to compare the somatic genotype to the germline. For example, the genotype for a plurality of loci (*e.g.*, SNPs) can be determined in both a germline (*e.g.*, blood) sample and a somatic (*e.g.*, tumor) sample. The genotypes for each sample can be compared (typically computationally) to determine where the genome of the germline cell was heterozygous and the genome of the somatic cell is homozygous. Such loci are LOH loci and regions of such loci are LOH Regions.

[0061] Computational techniques can also be used to determine whether an aberration is germline or somatic. Such techniques are particularly useful when a germline sample is not available for analysis and comparison. For example, algorithms such as those described elsewhere can be used to detect LOH regions using information from SNP arrays (Nannya *et al.*, Cancer Res. (2005) 65:6071-6079 (2005)). Typically these algorithms do not explicitly take into account contamination of tumor samples with benign tissue. *Cf.* International Application No. PCT/US2011/026098 to Abkevich *et al.*; Goransson *et al.*, PLoS One (2009) 4(6):e6057. This

contamination is often high enough to make the detection of LOH regions challenging. Improved analytical methods according to the present invention for identifying LOH, TAI and LST, even in spite of contamination, include those embodied in computer software products as described below.

[0062] The following is one example. If the observed ratio of the signals of two alleles, A and B, is two to one, there are two possibilities. The first possibility is that cancer cells (*e.g.*, bladder cancer cells) have LOH with deletion of allele B in a sample with 50% contamination with normal cells. The second possibility is that there is no LOH but allele A is duplicated in a sample with no contamination with normal cells. An algorithm can be implemented as a computer program as described herein to reconstruct LOH regions based on genotype (*e.g.*, SNP genotype) data. One point of the algorithm is to first reconstruct allele specific copy numbers (ASCN) at each locus (*e.g.*, SNP). ASCNs are the numbers of copies of both paternal and maternal alleles. An LOH region is then determined as a stretch of SNPs with one of the ASCNs (paternal or maternal) being zero. The algorithm can be based on maximizing a likelihood function and can be conceptually akin to a previously described algorithm designed to reconstruct total copy number (rather than ASCN) at each locus (*e.g.*, SNP). *See* International Application No. PCT/US2011/026098 to Abkevich *et al.* The likelihood function can be maximized over ASCN of all loci, level of contamination with benign tissue, total copy number averaged over the whole genome, and sample specific noise level. The input data for the algorithm can include or consist of (1) sample-specific normalized signal intensities for both allele of each locus and (2) assay-specific (specific for different SNP arrays and for sequence based approach) set of parameters defined based on analysis of large number of samples with known ASCN profiles.

[0063] In some cases, nucleic acid sequencing techniques can be used to genotype loci. For example, genomic DNA from a cell sample (*e.g.*, a cancer cell sample) can be extracted and fragmented. Any appropriate method can be used to extract and fragment genomic nucleic acid including, without limitation, commercial kits such as QIAampTM DNA Mini Kit (QiagenTM), MagNATM Pure DNA Isolation Kit (Roche Applied ScienceTM) and GenEluteTM Mammalian Genomic DNA Miniprep Kit (Sigma-AldrichTM). Once extracted and fragmented, either targeted or untargeted sequencing can be done to determine the sample's genotypes at loci. For example, whole genome, whole transcriptome, or whole exome sequencing can be done to determine genotypes at millions or even billions of base pairs (*i.e.*, base pairs can be "loci" to be evaluated).

[0064] In some cases, targeted sequencing of known polymorphic loci (*e.g.*, SNPs and surrounding sequences) can be done as an alternative to microarray analysis. For example, the genomic DNA can be enriched for those fragments containing a locus (*e.g.*, SNP location) to be analyzed using kits designed for this purpose (*e.g.*, Agilent SureSelect™, Illumina TruSeq Capture™, and Nimblegen SeqCap EZ Choice™). For example, genomic DNA containing the loci to be analyzed can be hybridized to biotinylated capture RNA fragments to form biotinylated RNA/genomic DNA complexes. Alternatively, DNA capture probes may be utilized resulting in the formation of biotinylated DNA/genomic DNA hybrids. Streptavidin coated magnetic beads and a magnetic force can be used to separate the biotinylated RNA/genomic DNA complexes from those genomic DNA fragments not present within a biotinylated RNA/genomic DNA complex. The obtained biotinylated RNA/genomic DNA complexes can be treated to remove the captured RNA from the magnetic beads, thereby leaving intact genomic DNA fragments containing a locus to be analyzed. These intact genomic DNA fragments containing the loci to be analyzed can be amplified using, for example, PCR techniques. The amplified genomic DNA fragments can be sequenced using a high-throughput sequencing technology or a next-generation sequencing technology such as Illumina HiSeq™, Illumina MiSeq™, Life Technologies SOLiD™ or Ion Torrent™, or Roche 454™.

[0065] The sequencing results from the genomic DNA fragments can be used to identify loci as exhibiting or not exhibiting a CA, analogous to the microarray analysis described herein. In some cases, an analysis of the genotype of loci over a length of a chromosome can be performed to determine the length of CA Regions. For example, a stretch of SNP locations that are spaced apart (*e.g.*, spaced about 25 kb to about 100 kb apart) along a chromosome can be evaluated by sequencing, and the sequencing results used to determine not only the presence of a CA Region but also the length of that CA Region. Obtained sequencing results can be used to generate a graph that plots allele dosages along a chromosome. Allele dosage d_i for SNP i can be calculated from adjusted number of captured probes for two alleles (A_i and B_i): $d_i = A_i / (A_i + B_i)$. Determining whether an aberration is germline or somatic can be performed as described herein.

[0066] In some cases, a selection process can be used to select loci (*e.g.*, SNP loci) to be evaluated using an assay configured to genotype loci (*e.g.*, SNP array-based assays and sequencing-based assays). For example, any human SNP location can be selected for inclusion in a SNP array-based assay or a sequencing-based assay configured to genotype loci. In some cases, 0.5, 1.0, 1.5,

2.0, 2.5 million or more SNP locations present within the human genome can be evaluated to identify those SNPs that (a) are not present on the Y chromosome, (b) are not mitochondrial SNPs, (c) have a minor allele frequency of at least about five percent in Caucasians, (d) have a minor allele frequency of at least about one percent in three races other than Caucasians (*e.g.*, Chinese, Japanese, and Yoruba), and/or (e) do not have a significant deviation from Hardy Weinberg equilibrium in any of the four races. In some cases, more than 100,000, 150,000, or 200,000 human SNPs can be selected that meet criteria (a) through (e). Of the human SNPs meeting criteria (a) through (e), a group of SNPs (*e.g.*, top 110,000 SNPs) can be selected such that the SNPs have a high degree of allele frequency in Caucasians, cover the human genome in a somewhat evenly spaced manner (*e.g.*, at least one SNP every about 25 kb to about 500 kb), and are not in linkage disequilibrium with another selected SNP for in any of the four races. In some cases, about 40, 50, 60, 70, 80, 90, 100, 110, 120, 130 thousand or more SNPs can be selected as meeting each of these criteria and included in an assay configured to identify CA Regions across a human genome. For example, between about 70,000 and about 90,000 (*e.g.*, about 80,000) SNPs can be selected for analysis with a SNP array-based assay, and between about 45,000 and about 55,000 (*e.g.*, about 54,000) SNPs can be selected for analysis with a sequencing-based assay.

[0067] As described herein, any appropriate type of sample can be assessed. For example, a sample containing cancer cells (*e.g.*, bladder cancer cells) can be assessed to determine if the genome of the cancer cells contains an HRD signature, lacks an HRD signature, has an increased number of Indicator CA Regions or has an increased CA Region Score. Examples of samples containing cancer cells (*e.g.*, bladder cancer cells) that can be assessed as described herein include, without limitation, tumor biopsy samples (*e.g.*, bladder tumor biopsy samples), formalin-fixed, paraffin-embedded tissue samples containing cancer cells, core needle biopsies, fine needle aspirates, and samples containing cancer cells shed from a tumor (*e.g.*, blood, urine or other bodily fluids). For formalin-fixed, paraffin-embedded tissue samples, the sample can be prepared by DNA extraction using a genomic DNA extraction kit optimized for FFPE tissue, including but not limited to those described above (*e.g.*, QuickExtractTM FFPE DNA Extraction Kit (EpicentreTM), and QIAampTM DNA FFPE Tissue Kit (QiagenTM)).

[0068] In some cases, laser dissection techniques can be performed on a tissue sample to minimize the number of non-cancer cells within a cancer cell sample to be assessed. In some cases, antibody based purification methods can be used to enrich for cancer cells and/or deplete non-cancer

cells. Examples of antibodies that could be used for cancer cell enrichment include, without limitation, anti-EpCAM, anti-TROP-2, anti-c-Met, anti-Folate binding protein, anti-N-Cadherin, anti-CD318, anti-antimesencymal stem cell antigen, anti-Her2, anti-MUC1, anti-EGFR, anti-cytokeratins (*e.g.*, cytokeratin 7, cytokeratin 20, etc.), anti-Caveolin-1, anti-PSA, anti-CA125, and anti-surfactant protein antibodies.

[0069] Any type of cancer cell can be assessed using the methods and materials described herein. For example, bladder cancer cells, breast cancer cells, ovarian cancer cells, liver cancer cells, esophageal cancer cells, lung cancer cells, head and neck cancer cells, prostate cancer cells, colon, rectal, or colorectal cancer cells, and pancreatic cancer cells can be assessed to determine if the genome of the cancer cells contains an HRD signature, lacks an HRD signature, has an increased number of Indicator CA Regions or has an increased CA Region Score. In some embodiments, the cancer cells are primary or metastatic cancer cells of bladder cancer.

[0070] In some embodiments bladder cancer is assessed using the methods and materials described herein. The term “bladder cancer,” as used herein, refers to all forms of malignancy of urinary bladder tissues, but particularly includes malignancies arising from the epithelial lining (*e.g.*, the urothelium) of the urinary bladder. The most common type of bladder cancer, which recapitulates the normal histology of the urothelium, is known as transitional cell carcinoma, or urothelial cell carcinoma. Bladder cancers are often staged according to the following criteria:

[0071] Stage I. Cancer at this stage occurs in the bladder’s inner lining but has not invaded the muscular bladder wall. This stage is commonly referred to as T1.

[0072] Stage II. At this stage, cancer has invaded the bladder wall but is still confined to the bladder. This stage is commonly referred to as T2.

[0073] Stage III. At this stage the cancer cells have spread through the bladder wall to surrounding tissue, and may also have spread to the prostate in men or the uterus or vagina in women. This stage is commonly referred to as T3.

[0074] Stage IV. Cancer cells, by this stage may have spread to the lymph nodes and other organs, such as the lungs, bones or liver. This stage is commonly referred to as T4

[0075] The phrase “recurrent bladder cancer,” as used herein, refers to those forms of bladder cancer that have reoccurred following an intervention (initial or subsequent), including

surgical interventions, intended to remove any existing bladder cancer. Often the surgical intervention utilized for early stage bladder cancers is transurethral resection, in which superficial tumors (those which have not entered the surrounding muscle layer) are removed by electrocautery. The removed material can then be used for pathological examination and subsequent staging of the cancer. Recurrent bladder cancers may also arise after any combination of treatment by surgical intervention, chemotherapy, immunotherapy, and radiation treatment, with the latter methods typically being used in stage II, III and IV bladder cancers. Recurrent bladder cancers may also arise after surgical removal of all or part of the urinary bladder (*e.g.*, cystectomy), particularly with metastatic forms of bladder cancer.

[0076] When assessing the genome of cancer cells (*e.g.*, bladder cancer cells) for the presence or absence of an HRD signature, one or more (*e.g.*, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23) pairs of chromosomes can be assessed. In some cases, the genome of cancer cells (*e.g.*, bladder cancer cell) is assessed for the presence or absence of an HRD signature using one or more (*e.g.*, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23) pairs of chromosomes.

[0077] In some cases, it can be helpful to exclude certain chromosomes from this analysis. For example, in the case of females, a pair to be assessed can include the pair of X sex chromosomes; whereas, in the case of males, a pair of any autosomal chromosomes (*i.e.*, any pair other than the pair of X and Y sex chromosomes) can be assessed. As another example, in some cases the chromosome number 17 pair may be excluded from the analysis. It has been determined that certain chromosomes carry unusually high levels of CA in certain cancers and, thus, it can be helpful to exclude such chromosomes when analyzing samples as described herein from patients having these cancers. In some cases, the sample is from a patient having ovarian cancer, and the chromosome to be excluded is chromosome 17.

[0078] Thus, a predefined number of chromosomes may be analyzed to determine the number of Indicator CA Regions (or the CA Region Score or Combined CA Region Score), preferably the number of CA Regions of a length of greater than 9 megabases, 10 megabases, 12 megabases, 14 megabases, more preferably greater than 15 megabases. Alternatively or in addition,

the sizes of all identified Indicator CA Regions may be summed up to obtain a total length of Indicator CA Regions.

[0079] As described herein, patients having cancer cells such as bladder cancer cells (or samples derived therefrom) identified as having an HRD signature status can be classified, based at least in part on such HRD signature, as being likely to respond to a particular cancer treatment regimen. For example, patients having cancer cells (*e.g.*, bladder cancer cells) with an HRD signature can be classified, based at least in part on such HRD signature, as being likely to respond to a cancer treatment regimen that includes the use of a DNA damaging agent, a synthetic lethality agent (*e.g.*, a PARP inhibitor), radiation, or a combination thereof. In some embodiments the patients are treatment naïve patients. Examples of DNA damaging agents include, without limitation, platinum-based chemotherapy drugs (*e.g.*, cisplatin, carboplatin, oxaliplatin, nedaplatin, iproplatin, and picoplatin), anthracyclines (*e.g.*, epirubicin and doxorubicin), topoisomerase I inhibitors (*e.g.*, camptothecin, topotecan, and irinotecan), DNA crosslinkers such as mitomycin C, and triazene compounds (*e.g.*, dacarbazine and temozolomide). Synthetic lethality therapeutic approaches typically involve administering an agent that inhibits at least one critical component of a biological pathway that is especially important to a particular tumor cell's survival. For example, when a tumor cell such as a bladder cancer cell has a deficient homologous repair pathway (*e.g.*, as determined according to the present invention), inhibitors of poly ADP ribose polymerase (or platinum drugs, double strand break repair inhibitors, etc.) can be especially potent against such tumors because two pathways critical to survival become obstructed (one biologically, *e.g.*, by BRCA1 mutation, and the other synthetically, *e.g.*, by administration of a pathway drug). Synthetic lethality approaches to cancer therapy are described in, *e.g.*, O'Brien *et al.*, *Converting cancer mutations into therapeutic opportunities*, EMBO MOL. MED. (2009) 1:297-299. Examples of synthetic lethality agents include, without limitation, PARP inhibitors or double strand break repair inhibitors in homologous repair-deficient tumor cells, PARP inhibitors in PTEN-deficient tumor cells, methotrexate in MSH2-deficient tumor cells, etc. Examples of PARP inhibitors include, without limitation, olaparib, iniparib, and veliparib. Examples of double strand break repair inhibitors include, without limitation, KU55933 (ATM inhibitor) and NU7441 (DNA-PKcs inhibitor). Examples of information that can be used in addition to the presence of an HRD signature to base a classification of being likely to respond to a particular cancer treatment regimen include, without limitation, previous treatment results, germline or somatic DNA mutations, gene or

protein expression profiling (*e.g.*, ER/PR/HER2 status, PSA levels), tumor histology (*e.g.*, adenocarcinoma, squamous cell carcinoma, papillary serous carcinoma, mucinous carcinoma, invasive ductal carcinoma, ductal carcinoma *in situ* (non-invasive), etc.), disease stage, tumor or cancer grade (*e.g.*, well, moderately, or poorly differentiated (*e.g.*, Gleason, modified Bloom Richardson), etc.), number of previous courses of treatment, etc.

[0080] Once classified as being likely to respond to a particular cancer treatment regimen (*e.g.*, a cancer treatment regimen that includes the use of a DNA damaging agent, a PARP inhibitor, radiation, or a combination thereof), the cancer patient can be treated with such a cancer treatment regimen. In some embodiments, the patients are treatment naïve patients. The invention thus provides a method of treating a patient comprising detecting an HRD signature as described herein and administering (or recommending or prescribing) a treatment regimen comprising the use of a DNA damaging agent, a PARP inhibitor, radiation, or a combination thereof. Any appropriate method for treating the cancer at issue can be used to treat a cancer patient identified as having cancer cells having an HRD signature. For example, platinum-based chemotherapy drugs or a combination of platinum-based chemotherapy drugs can be used to treat cancer as described elsewhere (*see, e.g.*, U.S. Patent Nos. 3,892,790, 3,904,663, 7,759,510, 7,759,488 and 7,754,684. In some cases, anthracyclines or a combination of anthracyclines can be used to treat cancer as described elsewhere (*see, e.g.*, U.S. Patent Nos. 3,590,028, 4,138,480, 4,950,738, 6,087,340, 7,868,040, and 7,485,707). In some cases, topoisomerase I inhibitors or a combination of topoisomerase I inhibitors can be used to treat cancer as described elsewhere (*see, e.g.*, U.S. Patent Nos. 5,633,016 and 6,403,563. In some cases, PARP inhibitors or a combination of PARP inhibitors can be used to treat cancer as described elsewhere (*see, e.g.*, U.S. Patent Nos. 5,177,075, 7,915,280, and 7,351,701. In some cases, radiation can be used to treat cancer as described elsewhere (*see, e.g.*, U.S. Patent No. 5,295,944). In some cases, a combination comprising different agents (*e.g.*, a combination comprising any of platinum-based chemotherapy drugs, anthracyclines, topoisomerase I inhibitors, and/or PARP inhibitors) with or without radiation treatments can be used to treat cancer. In some cases, a combination treatment may comprise any of the above agents or treatments (*e.g.*, a DNA damaging agent, a PARP inhibitor, radiation, or a combination thereof) together with another agent or treatment—*e.g.*, a taxane agent (*e.g.*, doxorubicin, paclitaxel, abiraterone), a growth factor or growth factor receptor inhibitor (*e.g.*, erlotinib, gefitinib, lapatinib, sunitinib, bevacizumab, cetuximab, trastuzumab, panitumumab), and/or an antimetabolite (*e.g.*, 5-fluorouracil, methotrexate).

[0081] In some cases, patients identified as having cancer cells (*e.g.*, bladder cancer cells) lacking an HRD signature can be classified, based at least in part on a sample lacking an HRD signature, as being less likely to respond to a treatment regimen that includes a DNA damaging agent, a PARP inhibitor, radiation, or a combination thereof. In turn, such a patient can be classified as likely to respond to a cancer treatment regimen, for example a bladder cancer treatment regimen, that includes the use of one or more cancer treatment agents not associated with HDR, such as a taxane agent (*e.g.*, doxorubicin, paclitaxel, abiraterone), a growth factor or growth factor receptor inhibitor (*e.g.*, erlotinib, gefitinib, lapatinib, sunitinib, bevacizumab, cetuximab, trastuzumab, panitumumab), and/or an antimetabolite agent (*e.g.*, 5-fluorouracil, methotrexate). In some embodiments, the patients are treatment naïve patients. Once classified as being likely to respond to a particular cancer treatment regimen (*e.g.*, a cancer treatment regimen that includes the use of a cancer treatment agent not associated with HDR), the cancer patient can be treated with such a cancer treatment regimen. The invention thus provides a method of treating a patient comprising detecting the absence of an HRD signature as described herein and administering (or recommending or prescribing) a treatment regimen not comprising the use of a DNA damaging agent, a PARP inhibitor, radiation, or a combination thereof. In some embodiments the treatment regimen comprises one or more of a taxane agent (*e.g.*, doxorubicin, paclitaxel, abiraterone), a growth factor or growth factor receptor inhibitor (*e.g.*, erlotinib, gefitinib, lapatinib, sunitinib, bevacizumab, cetuximab, trastuzumab, panitumumab), and/or an antimetabolite agent (*e.g.*, 5-fluorouracil, methotrexate). Any appropriate method for the cancer being treated (*e.g.*, a bladder cancer) can be used to treat a cancer patient identified as having cancer cells lacking an HRD signature. Examples of information that can be used in addition to the absence of an HRD signature to base a classification of being likely to respond to a particular cancer treatment regimen include, without limitation, previous treatment results, germline or somatic DNA mutations, gene or protein expression profiling (*e.g.*, ER/PR/HER2 status, PSA levels), tumor histology (*e.g.*, adenocarcinoma, squamous cell carcinoma, papillary serous carcinoma, mucinous carcinoma, invasive ductal carcinoma, ductal carcinoma *in situ* (non-invasive), etc.), disease stage, tumor or cancer grade (*e.g.*, well, moderately, or poorly differentiated (*e.g.*, Gleason, modified Bloom Richardson), etc.), number of previous courses of treatment, etc.

[0082] Once treated for a particular period of time (*e.g.*, between one to six months), the patient can be assessed to determine whether or not the treatment regimen has an effect. If a

beneficial effect is detected, the patient can continue with the same or a similar cancer treatment regimen. If a minimal or no beneficial effect is detected, then adjustments to the cancer treatment regimen can be made. For example, the dose, frequency of administration, or duration of treatment can be increased. In some cases, additional anti-cancer agents can be added to the treatment regimen or a particular anti-cancer agent can be replaced with one or more different anti-cancer agents. The patient being treated can continue to be monitored as appropriate, and changes can be made to the cancer treatment regimen as appropriate.

[0083] In addition to predicting likely treatment response or selecting desirable treatment regimens, an HRD signature can be used to determine a patient's prognosis. Thus, in one aspect, this document features a method for determining a patient's prognosis based at least in part of detecting the presence or absence of an HRD signature in a sample from the patient. The method comprises, or consists essentially of, (a) determining whether a sample from the patient comprises cancer cells such as bladder cancer cells (or whether a sample comprises DNA derived from such cells) having an HRD signature (sometimes referred to herein as having high HRD) as described herein (*e.g.*, wherein the presence of more Indicator CA Regions or a higher CA Region Score or Combined CA Region Score than a reference), and (b)(1) determining, based at least in part on the presence of the HRD signature or having high HRD, that the patient has a relatively good prognosis, or (b)(2) determining, based at least in part on the absence of the HRD signature, that the patient has a relatively poor prognosis. Prognosis may include the patient's likelihood of survival (*e.g.*, progression-free survival, overall survival), wherein a relatively good prognosis would include an increased likelihood of survival as compared to some reference population (*e.g.*, average patient with this patient's cancer type/subtype, average patient not having an HRD signature, etc.). Conversely, a relatively poor prognosis in terms of survival would include a decreased likelihood of survival as compared to some reference population (*e.g.*, average patient with this patient's cancer type/subtype, average patient having an HRD signature, etc.).

[0084] As described herein, this document provides methods for assessing patients for cells (*e.g.*, bladder cancer cells) having an HRD signature. In some embodiments, one or more clinicians or medical professionals can determine whether a sample from the patient comprises cancer cells (or whether a sample comprises DNA derived from such cells) having an HRD signature. In some cases, one or more clinicians or medical professionals can determine if a patient contains cancer cells (*e.g.*, bladder cancer cells) having an HRD signature by obtaining a cancer cell sample from the

patient (*e.g.*, the bladder cancer patient) and assessing the DNA of cancer cells of the cancer cell sample to determine the presence or absence of an HRD signature as described herein.

[0085] In some cases, one or more clinicians or medical professionals can obtain a cancer cell sample from a patient (*e.g.*, a bladder cancer patient) and provide that sample to a testing laboratory having the ability to assess DNA of cancer cells of the cancer cell sample to provide an indication about the presence or absence of an HRD signature as described herein. In some embodiments, the patients are treatment naïve patients. In such cases, the one or more clinicians or medical professionals can determine if a sample from the patient comprises cancer cells (or whether a sample comprises DNA derived from such cells) having an HRD signature by receiving information about the presence or absence of an HRD signature as described herein directly or indirectly from the testing laboratory. For example, a testing laboratory, after assessing DNA of cancer cells for presence or absence of an HRD signature as described herein, can provide a clinician or medical professional with, or access to, a written, electronic, or oral report or medical record that provides an indication about the presence or absence of an HRD signature for a particular patient (or patient sample) being assessed. Such a written, electronic, or oral report or medical record can allow the one or more clinicians or medical professionals to determine if a particular patient being assessed contains cancer cells having an HRD signature.

[0086] Once a clinician or medical professional or group of clinicians or medical professionals determines that a particular patient being assessed contains cancer cells (*e.g.*, bladder cancer cells) having an HRD signature, the clinician or medical professional (or group) can classify that patient as having cancer cells whose genome contains the presence of an HRD signature. In some embodiments, the patients are treatment naïve patients. In some cases, a clinician or medical professional or group of clinicians or medical professionals can diagnose a patient determined to have cancer cells whose genome contains the presence of an HRD signature as having cancer cells deficient in (or likely to be deficient in) HDR. Such a diagnosis can be based solely on a determination that a sample from the patient comprises cancer cells such as bladder cancer cells (or whether a sample comprises DNA derived from such cells) having an HRD signature or can be based at least in part on a determination that a sample from the patient comprises cancer cells (or whether a sample comprises DNA derived from such cells) having an HRD signature. For example, a patient determined to have cancer cells having an HRD signature can be diagnosed as likely to be deficient in HDR based on the combination of the presence of an HRD signature and deficient status

in one or more tumor suppressor genes (*e.g.*, BRCA1/2, RAD51C), a family history of cancer, or the presence of behavioral risk factors (*e.g.*, smoking).

[0087] In some cases, a clinician or medical professional or group of clinicians or medical professionals can diagnose a patient determined to have cancer cells (*e.g.*, bladder cancer cells) whose genome contains the presence of an HRD signature as having cancer cells likely to contain genetic mutations in one or more genes in the HDR pathway. In some embodiments, the patients are treatment naïve patients. Such a diagnosis can be based solely on a determination that a particular patient being assessed contains cancer cells having a genome containing an HRD signature or can be based at least in part on a determination that a particular patient being assessed contains cancer cells having a genome containing an HRD signature. For example, a patient determined to have cancer cells (*e.g.*, bladder cancer cells) whose genome contains the presence of an HRD signature can be diagnosed as having cancer cells likely to contain genetic mutations in one or more genes in the HDR pathway based on the combination of the presence of an HRD signature and a family history of cancer, or the presence of behavioral risk factors (*e.g.*, smoking).

[0088] In some cases, a clinician or medical professional or group of clinicians or medical professionals can diagnose a patient determined to have cancer cells (*e.g.*, bladder cancer cells) having an HRD signature as having cancer cells likely to respond to a particular cancer treatment regimen. In some embodiments, the patients are treatment naïve patients. Such a diagnosis can be based solely on a determination that a sample from the patient comprises cancer cells (or whether a sample comprises DNA derived from such cells) having an HRD signature or can be based at least in part on a determination that a sample from the patient comprises cancer cells (or whether a sample comprises DNA derived from such cells) having an HRD signature. For example, a patient determined to have cancer cells (*e.g.*, bladder cancer cells) having an HRD signature can be diagnosed as being likely to respond to a particular cancer treatment regimen based on the combination of the presence of an HRD signature and deficient status in one or more tumor suppressor genes (*e.g.*, BRCA1/2, RAD51), a family history of cancer, or the presence of behavioral risk factors (*e.g.*, smoking). As described herein, a patient determined to have cancer cells (*e.g.*, bladder cancer cells) having an HRD signature can be diagnosed as likely to respond to a cancer treatment regimen that includes the use of a platinum-based chemotherapy drug such as cisplatin, carboplatin, oxaliplatin, nedaplatin, iproplatin, or picoplatin, an anthracycline such as epirubicin or doxorubicin, a topoisomerase I inhibitor such as camptothecin, topotecan, or irinotecan, a PARP

inhibitor, radiation, a combination thereof, or a combination of any of the preceding with another anti-cancer agent. In some embodiments, the patients are treatment naïve patients.

[0089] Once a clinician or medical professional or group of clinicians or medical professionals determines that a sample from the patient comprises cancer cells such as bladder cancer cells (or whether a sample comprises DNA derived from such cells) having a genome lacking an HRD signature, the clinician or medical professional (or group) can classify that patient as having cancer cells whose genome lacks an HRD signature. In some embodiments, the patients are treatment naïve patients. In some cases, a clinician or medical professional or group of clinicians or medical professionals can diagnose a patient determined to have cancer cells containing a genome lacking an HRD signature as having cancer cells likely to have functional HDR. In some cases, a clinician or medical professional or group of clinicians or medical professionals can diagnose a patient determined to have cancer cells containing a genome lacking an HRD signature as having cancer cells that do not likely contain genetic mutations in one or more genes in the HDR pathway. In some cases, a clinician or medical professional or group of clinicians or medical professionals can diagnose a patient determined to have cancer cells containing a genome lacking an HRD signature or containing an increased number of CA Regions that cover the whole chromosome as having cancer cells that are less likely to respond to a platinum-based chemotherapy drug such as cisplatin, carboplatin, oxaloplatin, nedaplatin, iproplatin, or picoplatin, an anthracycline such as epirubicin or doxorubicin, a topoisomerase I inhibitor such as camptothecin, topotecan, or irinotecan, a PARP inhibitor, or radiation and/or more likely to respond to a cancer treatment regimen that includes the use of a cancer treatment agent not associated with HDR such as one or more taxane agents, growth factor or growth factor receptor inhibitors, anti-metabolite agents, etc. In some embodiments, the patients are treatment naïve patients.

[0090] As described herein, this document also provides methods for performing a diagnostic analysis of a nucleic acid sample (*e.g.*, a genomic nucleic acid sample or nucleic acids amplified therefrom) of a cancer patient to determine if a sample from the patient comprises cancer cells such as bladder cancer cells (or whether a sample comprises DNA derived from such cells) containing an HRD signature and/or an increased number of CA Regions that cover the whole chromosome. In some embodiments, the patients are treatment naïve patients. For example, one or more laboratory technicians or laboratory professionals can detect the presence or absence of an HRD signature in the genome of cancer cells (or DNA derived therefrom) of the patient or the presence or absence of

an increased number of CA Regions that cover the whole chromosome in the genome of cancer cells of the patient. In some cases, one or more laboratory technicians or laboratory professionals can detect the presence or absence of an HRD signature or the presence or absence of an increased number of CA Regions that cover the whole chromosome in the genome of cancer cells (*e.g.*, bladder cancer cells) of the patient by (a) receiving a cancer cell sample obtained from the patient, receiving a genomic nucleic acid sample obtained from cancer cells obtained from the patient, or receiving a sample containing nucleic acids enriched and/or amplified from such a genomic nucleic acid sample obtained from cancer cells obtained from the patient and (b) performing an analysis (*e.g.*, a SNP array-based assay or a sequencing-based assay) using the received material to detect the presence or absence of an HRD signature or the presence or absence of an increased number of CA Regions that cover the whole chromosome as described herein. In some cases, one or more laboratory technicians or laboratory professionals can receive a sample to be analyzed (*e.g.*, a bladder cancer cell sample obtained from the patient, a genomic nucleic acid sample obtained from cancer cells obtained from the patient, or a sample containing nucleic acids enriched and/or amplified from such a genomic nucleic acid sample obtained from cancer cells obtained from the patient) directly or indirectly from a clinician or medical professional. In some embodiments, the patients are treatment naïve patients.

[0091] Once a laboratory technician or laboratory professional or group of laboratory technicians or laboratory professionals detects the presence of an HRD signature as described herein, the laboratory technician or laboratory professional (or group) can associate that HRD signature or the result (or results or a summary of results) of the performed diagnostic analysis with the corresponding patient's name, medical record, symbolic/numerical identifier, or a combination thereof. Such identification can be based solely on detecting the presence of an HRD signature or can be based at least in part on detecting the presence of an HRD signature. For example, a laboratory technician or laboratory professional can identify a patient having cancer cells (*e.g.*, bladder cancer cells) that were detected to have an HRD signature as having cancer cells potentially deficient in HDR (or as having an increased likelihood of responding to a particular treatment as described at length herein) based on a combination of the presence of an HRD signature and the results of other genetic and biochemical tests performed at the testing laboratory. In some embodiments, the patients are treatment naïve patients.

[0092] The converse of the preceding is also true. Namely, once a laboratory technician or laboratory professional or group of laboratory technicians or laboratory professionals detects the absence of an HRD signature, the laboratory technician or laboratory professional (or group) can associate the absence of an HRD signature or the result (or results or a summary of results) of the performed diagnostic analysis with the corresponding patient's name, medical record, symbolic/numerical identifier, or a combination thereof. In some cases, a laboratory technician or laboratory professional or group of laboratory technicians or laboratory professionals can identify a patient having cancer cells that were detected to lack an HRD signature as having cancer cells (*e.g.*, bladder cancer cells) with potentially intact HDR (or having a decreased likelihood of responding to a particular treatment as described at length herein) either based solely on the absence of an HRD signature or based on a combination of the presence of an HRD signature and the results of other genetic and biochemical tests performed at the testing laboratory. In some embodiments, the patients are treatment naïve patients.

[0093] The results of any analyses according to the invention will often be communicated to physicians, genetic counselors and/or patients (or other interested parties such as researchers) in a transmittable form that can be communicated or transmitted to any of the above parties. Such a form can vary and can be tangible or intangible. The results can be embodied in descriptive statements, diagrams, photographs, charts, images or any other visual forms. For example, graphs or diagrams showing genotype or LOH (or HRD status) information can be used in explaining the results. The statements and visual forms can be recorded on a tangible medium such as papers, computer readable media such as floppy disks, compact disks, flash memory, etc., or in an intangible medium, *e.g.*, an electronic medium in the form of email or website on internet or intranet. In addition, results can also be recorded in a sound form and transmitted through any suitable medium, *e.g.*, analog or digital cable lines, fiber optic cables, etc., via telephone, facsimile, wireless mobile phone, internet phone and the like.

[0094] Thus, the information and data on a test result can be produced anywhere in the world and transmitted to a different location. As an illustrative example, when an assay is conducted outside the United States, the information and data on a test result may be generated, cast in a transmittable form as described above, and then imported into the United States. Accordingly, the present invention also encompasses a method for producing a transmittable form of information on an HRD signature for at least one patient sample. The method comprises the steps of (1)

determining an HRD signature according to methods of the present invention; and (2) embodying the result of the determining step in a transmittable form. The transmittable form is a product of such a method.

[0095] Several embodiments of the invention described herein involve a step of correlating the presence of an HRD signature according to the present invention (*e.g.*, the total number of Indicator CA Regions or a CA Region Score or Combined CA Region Score greater than a reference) to a particular clinical feature (*e.g.*, an increased likelihood of a deficiency in the BRCA1 or BRCA2 gene; an increased likelihood of HDR deficiency; an increased likelihood of response to a treatment regimen comprising a DNA damaging agent, an anthracycline, a topoisomerase I inhibitor, radiation, and/or a PARP inhibitor; etc.) and optionally correlating the absence of a HRD signature to one or more other clinical features. Throughout this document, wherever such an embodiment is described, another embodiment of the invention may involve, in addition to or instead of a correlating step, one or both of the following steps: (a) concluding that the patient has the clinical feature based at least in part on the presence or absence of the HRD signature; or (b) communicating that the patient has the clinical feature based at least in part on the presence or absence of the HRD signature.

[0096] By way of illustration, but not limitation, one embodiment described in this document is a method of predicting a cancer patient's response to a cancer treatment regimen comprising a DNA damaging agent, an anthracycline, a topoisomerase I inhibitor, radiation, and/or a PARP inhibitor, said method comprising: (1) determining in a sample two or more of (a) an LOH Region Score for the sample; (b) a TAI Region Score for the sample; or (c) an LST Region Score for the sample; and (2)(a) correlating a combination of two or more of the LOH Region Score, the TAI Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score) exceeding a reference to an increased likelihood of responding to the treatment regimen; or optionally (2)(b) correlating a combination of two or more of the LOH Region Score, the TAI Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score) not exceeding a reference to a not increased likelihood of responding to the treatment regimen; or optionally (2)(c) correlating an average (*e.g.*, arithmetic mean) of the LOH Region Score, the TAI Region Score, and the LST Region Score. According to the preceding paragraph, this description of this embodiment is understood to include a description of two alternative related embodiments. One such embodiment provides a method of predicting a

cancer patient's response to a cancer treatment regimen comprising a DNA damaging agent, an anthracycline, a topoisomerase I inhibitor, radiation, and/or a PARP inhibitor, said method comprising: (1) determining in a sample two or more of (a) an LOH Region Score for the sample; (b) a TAI Region Score for the sample; or (c) an LST Region Score for the sample; or (d) an average (*e.g.*, arithmetic mean) of the LOH Region Score, the TAI Region Score, and the LST Region Score; and (2)(a) concluding that said patient has an increased likelihood of responding to said cancer treatment regimen based at least in part on a combination of two or more of the LOH Region Score, the TAI Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score) exceeding a reference; or optionally (2)(b) concluding that said patient has a not increased likelihood of responding to said cancer treatment regimen based at least in part on a combination of two or more of the LOH Region Score, the TAI Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score), or an average (*e.g.*, arithmetic mean) of the LOH Region Score, the TAI Region Score, and the LST Region Score, not exceeding a reference. Another such embodiment provides a method of predicting a cancer patient's response to a cancer treatment regimen comprising a DNA damaging agent, an anthracycline, a topoisomerase I inhibitor, radiation, and/or a PARP inhibitor, said method comprising: (1) determining in a sample two or more of (a) an LOH Region Score for the sample; (b) a TAI Region Score for the sample; or (c) an LST Region Score for the sample; or (d) an average (*e.g.*, arithmetic mean) of the LOH Region Score, the TAI Region Score, and the LST Region Score; and (2)(a) communicating that said patient has an increased likelihood of responding to said cancer treatment regimen based at least in part on a combination of two or more of the LOH Region Score, the TAI Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score); or an average (*e.g.*, arithmetic mean) of the LOH Region Score, the TAI Region Score, and the LST Region Score, exceeding a reference; or optionally (2)(b) communicating that said patient has a not increased likelihood of responding to said cancer treatment regimen based at least in part on a combination of two or more of the LOH Region Score, the TAI Region Score and the LST Region Score (*e.g.*, a Combined CA Region Score); or an average (*e.g.*, arithmetic mean) of the LOH Region Score, the TAI Region Score, and the LST Region Score, not exceeding a reference.

[0097] In each embodiment described in this document involving correlating a particular assay or analysis output (*e.g.*, total number of Indicator CA Regions greater than a reference number, presence of an HRD signature etc.) to some likelihood (*e.g.*, increased, not increased, decreased, etc.) of some clinical feature (*e.g.*, response to a particular treatment, cancer-specific death, etc.), or

additionally or alternatively concluding or communicating such clinical feature based at least in part on such particular assay or analysis output, such correlating, concluding or communicating may comprise assigning a risk or likelihood of the clinical feature occurring based at least in part on the particular assay or analysis output. In some embodiments, such risk is a percentage probability of the event or outcome occurring. In some embodiments, the patient is assigned to a risk group (*e.g.*, low risk, intermediate risk, high risk, etc.). In some embodiments “low risk” is any percentage probability below 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50%. In some embodiments “intermediate risk” is any percentage probability above 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% and below 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%. In some embodiments “high risk” is any percentage probability above 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%.

[0098] As used herein, “communicating” a particular piece of information means to make such information known to another person or transfer such information to a thing (*e.g.*, a computer). In some methods of the invention, a patient’s prognosis or likelihood of response to a particular treatment is communicated. In some embodiments, the information used to arrive at such a prognosis or response prediction (*e.g.*, HRD signature according to the present invention, etc.) is communicated. This communication may be auditory (*e.g.*, verbal), visual (*e.g.*, written), electronic (*e.g.*, data transferred from one computer system to another), etc. In some embodiments, communicating a cancer classification (*e.g.*, prognosis, likelihood of response, appropriate treatment, etc.) comprises generating a report that communicates the cancer classification. In some embodiments the report is a paper report, an auditory report, or an electronic record. In some embodiments the report is displayed and/or stored on a computing device (*e.g.*, handheld device, desktop computer, smart device, website, etc.). In some embodiments the cancer classification is communicated to a physician (*e.g.*, a report communicating the classification is provided to the physician). In some embodiments the cancer classification is communicated to a patient (*e.g.*, a report communicating the classification is provided to the patient). Communicating a cancer classification (*e.g.*, a bladder cancer classification) can also be accomplished by transferring information (*e.g.*, data) embodying the classification to a server computer and allowing an intermediary or end-user to access such information (*e.g.*, by viewing the information as displayed from the server, by downloading the information in the form of one or more files transferred from the server to the intermediary or end-user’s device, etc.).

[0099] Wherever an embodiment of the invention comprises concluding some fact (*e.g.*, a patient's prognosis or a patient's likelihood of response to a particular treatment regimen), this may include in some embodiments a computer program concluding such fact, typically after performing an algorithm that applies information on CA Regions according to the present invention.

[00100] In each embodiment described herein involving a number of CA Regions (*e.g.*, Indicator CA Regions), or a total combined length of such CA Regions, or an average (*e.g.*, arithmetic mean) of the combined CAR Region scores, the present invention encompasses a related embodiment involving a test value or score (*e.g.*, CA Region Score, LOH Region Score, etc.) derived from, incorporating, and/or, at least to some degree, reflecting such number or length. In other words, the bare CA Region numbers or lengths need not be used in the various methods, systems, etc. of the invention; a test value or score derived from such numbers or lengths may be used. For example, one embodiment of the invention provides a method of treating cancer in a patient, comprising: (1) determining in a sample from said patient two or more of, or an average (*e.g.*, arithmetic mean) of, (a) the number of Indicator LOH Regions, (b) the number of Indicator TAI Regions, or (c) the number of Indicator LST Regions; (2) providing one or more test values derived from said number of Indicator LOH Regions, Indicator TAI Regions, and/or Indicator LST Regions; (3) comparing said test value(s) to one or more reference values (*e.g.*, reference values derived from the number of Indicator LOH regions, Indicator TAI Regions, and/or Indicator LST Regions in a reference population (*e.g.*, mean, median, terciles, quartiles, quintiles, etc.)); and (4)(a) administering to said patient an anti-cancer drug, or recommending or prescribing or initiating a treatment regimen comprising chemotherapy and/or a synthetic lethality agent based at least in part on said comparing step revealing that one or more of the test values is greater (*e.g.*, at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10-fold greater; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 standard deviations greater) than at least one said reference value; or optionally (4)(b) recommending or prescribing or initiating a treatment regimen not comprising chemotherapy and/or a synthetic lethality agent based at least in part on said comparing step revealing that one or more of the test values is not greater (*e.g.*, not more than 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10-fold greater; not more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 standard deviations greater) than at least one said reference value. The invention encompasses, *mutatis mutandis*, corresponding embodiments where the test value or score is used to determine the patient's prognosis, the patient's likelihood of response to a particular treatment regimen, the

patient's or patient's sample's likelihood of having a BRCA1, BRCA2, RAD51C or HDR deficiency, etc.

[00101] Figure 2 shows an exemplary process by which a computing system (or a computer program (*e.g.*, software) containing computer-executable instructions) can identify LOH loci or regions from genotype data as described herein. This process may be adapted to use in determining TAI and LST as will be apparent to those skilled in the art. If the observed ratio of the signals of two alleles, A and B, is two to one, there are two possibilities. The first possibility is that cancer cells (*e.g.*, bladder cancer cells) have LOH with deletion of allele B in a sample with 50% contamination with normal cells. The second possibility is that there is no LOH but allele A is duplicated in a sample with no contamination with normal cells. The process begins at box 1500, where the following data are collected by the computing system; (1) sample-specific normalized signal intensities for both alleles of each locus and (2) assay-specific (specific for different SNP arrays and for sequence based approach) set of parameters defined based on analysis of large number of samples with known ASCN profiles. As described herein, any appropriate assay such as a SNP array-based assay or sequencing-based assay can be used to assess loci along a chromosome for homozygosity or heterozygosity. In some cases, a system including a signal detector and a computer can be used to collect data (*e.g.*, fluorescent signals or sequencing results) regarding the homozygous or heterozygous nature of the plurality of loci (*e.g.*, sample-specific normalized signal intensities for both alleles of each locus). At box 1510, allele specific copy numbers (ASCN) are reconstructed at each locus (*e.g.*, each SNP). ASCNs are the numbers of copies of both paternal and maternal alleles. At box 1530, a likelihood function is used to determine whether a homozygous locus or region of homozygous loci is due to LOH. This can be conceptually analogous to a previously described algorithm designed to reconstruct total copy number (rather than ASCN) at each locus (*e.g.*, SNP). *See* International Application No. PCT/US2011/026098 to Abkevich *et al.* The likelihood function can be maximized over ASCN of all loci, level of contamination with benign tissue, total copy number averaged over the whole genome, and sample specific noise level. At box 1540, an LOH region is determined as a stretch of SNPs with one of the ASCNs (paternal or maternal) being zero. In some embodiments, the computer process further comprises a step of inquiring or determining whether a patient is treatment naïve.

[00102] Figure 3 shows an exemplary process by which a computing system can determine the presence or absence of an LOH signature and is included to illustrate how this process

can, as will be apparent to those skilled in the art, be applied to TAI and LST. The process begins at box 300, where data regarding the homozygous or heterozygous nature of a plurality of loci along a chromosome is collected by the computing system. As described herein, any appropriate assay such as a SNP array-based assay or sequencing-based assay can be used to assess loci along a chromosome for homozygosity or heterozygosity. In some cases, a system including a signal detector and a computer can be used to collect data (*e.g.*, fluorescent signals or sequencing results) regarding the homozygous or heterozygous nature of the plurality of loci. At box 310, data regarding the homozygous or heterozygous nature of a plurality of loci as well as the location or spatial relationship of each locus is assessed by the computing system to determine the length of any LOH regions present along a chromosome. At box 320, data regarding the number of LOH regions detected and the length of each detected LOH region is assessed by the computing system to determine the number of LOH regions that have a length (a) greater than or equal to a preset number of Mb (*e.g.*, 15 Mb) and (b) less than the entire length of the chromosome containing that LOH region. Alternatively the computing system can determine the total or combined LOH length as described above. At box 330, the computing system formats an output providing an indication of the presence or absence of an HRD signature. Once formatted, the computing system can present the output to a user (*e.g.*, a laboratory technician, clinician, or medical professional). As described herein, the presence or absence of an HRD signature can be used to provide an indication about a patient's likely HDR status, an indication about the likely presence or absence of genetic mutations in genes of the HDR pathway, and/or an indication about possible cancer treatment regimens.

[00103] Figure 4 is a diagram of an example of a computer device 1400 and a mobile computer device 1450, which may be used with the techniques described herein. Computing device 1400 is intended to represent various forms of digital computers, such as laptops, desktops, workstations, personal digital assistants, servers, blade servers, mainframes, and other appropriate computers. Computing device 1450 is intended to represent various forms of mobile devices, such as personal digital assistants, cellular telephones, smart phones, and other similar computing devices. The components shown here, their connections and relationships, and their functions, are meant to be exemplary only, and are not meant to limit implementations of the inventions described and/or claimed in this document.

[00104] Computing device 1400 includes a processor 1402, memory 1404, a storage device 1406, a high-speed interface 1408 connecting to memory 1404 and high-speed expansion

ports 1410, and a low speed interface 1415 connecting to low speed bus 1414 and storage device 1406. Each of the components 1402, 1404, 1406, 1408, 1410, and 1415, are interconnected using various busses, and may be mounted on a common motherboard or in other manners as appropriate. The processor 1402 can process instructions for execution within the computing device 1400, including instructions stored in the memory 1404 or on the storage device 1406 to display graphical information for a GUI on an external input/output device, such as display 1416 coupled to high speed interface 1408. In other implementations, multiple processors and/or multiple buses may be used, as appropriate, along with multiple memories and types of memory. Also, multiple computing devices 1400 may be connected, with each device providing portions of the necessary operations (*e.g.*, as a server bank, a group of blade servers, or a multi-processor system).

[00105] The memory 1404 stores information within the computing device 1400. In one implementation, the memory 1404 is a volatile memory unit or units. In another implementation, the memory 1404 is a non-volatile memory unit or units. The memory 1404 may also be another form of computer-readable medium, such as a magnetic or optical disk.

[00106] The storage device 1406 is capable of providing mass storage for the computing device 1400. In one implementation, the storage device 1406 may be or contain a computer-readable medium, such as a floppy disk device, a hard disk device, an optical disk device, or a tape device, a flash memory or other similar solid state memory device, or an array of devices, including devices in a storage area network or other configurations. A computer program product can be tangibly embodied in an information carrier. The computer program product may also contain instructions that, when executed, perform one or more methods, such as those described herein. The information carrier is a computer- or machine-readable medium, such as the memory 1404, the storage device 1406, memory on processor 1402, or a propagated signal.

[00107] The high speed controller 1408 manages bandwidth-intensive operations for the computing device 1400, while the low speed controller 1415 manages lower bandwidth-intensive operations. Such allocation of functions is exemplary only. In one implementation, the high-speed controller 1408 is coupled to memory 1404, display 1416 (*e.g.*, through a graphics processor or accelerator), and to high-speed expansion ports 1410, which may accept various expansion cards (not shown). In the implementation, low-speed controller 1415 is coupled to storage device 1406 and low-speed expansion port 1414. The low-speed expansion port, which may include various

communication ports (*e.g.*, USB, Bluetooth, Ethernet, or wireless Ethernet) may be coupled to one or more input/output devices, such as a keyboard, a pointing device, a scanner, an optical reader, a fluorescent signal detector, or a networking device such as a switch or router, *e.g.*, through a network adapter.

[00108] The computing device 1400 may be implemented in a number of different forms, as shown in the figure. For example, it may be implemented as a standard server 1420, or multiple times in a group of such servers. It may also be implemented as part of a rack server system 1424. In addition, it may be implemented in a personal computer such as a laptop computer 1422. Alternatively, components from computing device 1400 may be combined with other components in a mobile device (not shown), such as device 1450. Each of such devices may contain one or more of computing device 1400, 1450, and an entire system may be made up of multiple computing devices 1400, 1450 communicating with each other.

[00109] Computing device 1450 includes a processor 1452, memory 1464, an input/output device such as a display 1454, a communication interface 1466, and a transceiver 1468, among other components (*e.g.*, a scanner, an optical reader, a fluorescent signal detector). The device 1450 may also be provided with a storage device, such as a microdrive or other device, to provide additional storage. Each of the components 1450, 1452, 1464, 1454, 1466, and 1468, are interconnected using various buses, and several of the components may be mounted on a common motherboard or in other manners as appropriate.

[00110] The processor 1452 can execute instructions within the computing device 1450, including instructions stored in the memory 1464. The processor may be implemented as a chipset of chips that include separate and multiple analog and digital processors. The processor may provide, for example, for coordination of the other components of the device 1450, such as control of user interfaces, applications run by device 1450, and wireless communication by device 1450.

[00111] Processor 1452 may communicate with a user through control interface 1458 and display interface 1456 coupled to a display 1454. The display 1454 may be, for example, a TFT LCD (Thin-Film-Transistor Liquid Crystal Display) or an OLED (Organic Light Emitting Diode) display, or other appropriate display technology. The display interface 1456 may comprise appropriate circuitry for driving the display 1454 to present graphical and other information to a user. The control interface 1458 may receive commands from a user and convert them for

submission to the processor 1452. In addition, an external interface 1462 may be provide in communication with processor 1452, so as to enable near area communication of device 1450 with other devices. External interface 1462 may provide, for example, for wired communication in some implementations, or for wireless communication in other implementations, and multiple interfaces may also be used.

[00112] The memory 1464 stores information within the computing device 1450. The memory 1464 can be implemented as one or more of a computer-readable medium or media, a volatile memory unit or units, or a non-volatile memory unit or units. Expansion memory 1474 may also be provided and connected to device 1450 through expansion interface 1472, which may include, for example, a SIMM (Single In Line Memory Module) card interface. Such expansion memory 1474 may provide extra storage space for device 1450, or may also store applications or other information for device 1450. For example, expansion memory 1474 may include instructions to carry out or supplement the processes described herein, and may include secure information also. Thus, for example, expansion memory 1474 may be provide as a security module for device 1450, and may be programmed with instructions that permit secure use of device 1450. In addition, secure applications may be provided via the SIMM cards, along with additional information, such as placing identifying information on the SIMM card in a non-hackable manner.

[00113] The memory may include, for example, flash memory and/or NVRAM memory, as discussed below. In one implementation, a computer program product is tangibly embodied in an information carrier. The computer program product contains instructions that, when executed, perform one or more methods, such as those described herein. The information carrier is a computer- or machine-readable medium, such as the memory 1464, expansion memory 1474, memory on processor 1452, or a propagated signal that may be received, for example, over transceiver 1468 or external interface 1462.

[00114] Device 1450 may communicate wirelessly through communication interface 1466, which may include digital signal processing circuitry where necessary. Communication interface 1466 may provide for communications under various modes or protocols, such as GSM voice calls, SMS, EMS, or MMS messaging, CDMA, TDMA, PDC, WCDMA, CDMA2000, or GPRS, among others. Such communication may occur, for example, through radio-frequency transceiver 1468. In addition, short-range communication may occur, such as using a Bluetooth,

WiFi, or other such transceiver (not shown). In addition, GPS (Global Positioning System) receiver module 1470 may provide additional navigation- and location-related wireless data to device 1450, which may be used as appropriate by applications running on device 1450.

[00115] Device 1450 may also communicate audibly using audio codec 1460, which may receive spoken information from a user and convert it to usable digital information. Audio codec 1460 may likewise generate audible sound for a user, such as through a speaker, *e.g.*, in a handset of device 1450. Such sound may include sound from voice telephone calls, may include recorded sound (*e.g.*, voice messages, music files, etc.) and may also include sound generated by applications operating on device 1450.

[00116] The computing device 1450 may be implemented in a number of different forms, as shown in the figure. For example, it may be implemented as a cellular telephone 1480. It may also be implemented as part of a smartphone 1482, personal digital assistant, or other similar mobile device.

[00117] Various implementations of the systems and techniques described herein can be realized in digital electronic circuitry, integrated circuitry, specially designed ASICs (application specific integrated circuits), computer hardware, firmware, software, and/or combinations thereof. These various implementations can include implementation in one or more computer programs that are executable and/or interpretable on a programmable system including at least one programmable processor, which may be special or general purpose, coupled to receive data and instructions from, and to transmit data and instructions to, a storage system, at least one input device, and at least one output device.

[00118] These computer programs (also known as programs, software, software applications or code) include machine instructions for a programmable processor, and can be implemented in a high-level procedural and/or object-oriented programming language, and/or in assembly/machine language. As used herein, the terms “machine-readable medium” and “computer-readable medium” refer to any computer program product, apparatus and/or device (*e.g.*, magnetic discs, optical disks, memory, and Programmable Logic Devices (PLDs)) used to provide machine instructions and/or data to a programmable processor, including a machine-readable medium that receives machine instructions as a machine-readable signal. The term “machine-readable signal” refers to any signal used to provide machine instructions and/or data to a programmable processor.

[00119] To provide for interaction with a user, the systems and techniques described herein can be implemented on a computer having a display device (*e.g.*, a CRT (cathode ray tube) or LCD (liquid crystal display) monitor) for displaying information to the user and a keyboard and a pointing device (*e.g.*, a mouse or a trackball) by which the user can provide input to the computer. Other kinds of devices can be used to provide for interaction with a user as well; for example, feedback provided to the user can be any form of sensory feedback (*e.g.*, visual feedback, auditory feedback, or tactile feedback); and input from the user can be received in any form, including acoustic, speech, or tactile input.

[00120] The systems and techniques described herein can be implemented in a computing system that includes a back end component (*e.g.*, as a data server), or that includes a middleware component (*e.g.*, an application server), or that includes a front end component (*e.g.*, a client computer having a graphical user interface or a Web browser through which a user can interact with an implementation of the systems and techniques described herein), or any combination of such back end, middleware, or front end components. The components of the system can be interconnected by any form or medium of digital data communication (*e.g.*, a communication network). Examples of communication networks include a local area network (“LAN”), a wide area network (“WAN”), and the Internet.

[00121] The computing system can include clients and servers. A client and server are generally remote from each other and typically interact through a communication network. The relationship of client and server arises by virtue of computer programs running on the respective computers and having a client-server relationship to each other.

[00122] In some cases, a computing system provided herein can be configured to include one or more sample analyzers. A sample analyzer can be configured to produce a plurality of signals about genomic DNA of at least one pair of human chromosomes of a cancer cell. For example, a sample analyzer can produce signals that are capable of being interpreted in a manner that identifies the genotype of loci along a chromosome. In some cases, a sample analyzer can be configured to carry out one or more steps of a SNP array-based assay or sequencing-based assay and can be configured to produce and/or capture signals from such assays. In some cases, a computing system provided herein can be configured to include a computing device. In such cases, the computing device can be configured to receive signals from a sample analyzer. The computing

device can include computer-executable instructions or a computer program (*e.g.*, software) containing computer-executable instructions for carrying out one or more of the methods or steps described herein. In some cases, such computer-executable instructions can instruct a computing device to analyze signals from a sample analyzer, from another computing device, from a SNP array-based assay, or from a sequencing-based assay. The analysis of such signals can be carried out to determine genotypes, homozygosity or other chromosomal aberrations at certain loci, regions of CA, the number of CA Regions, to determine the size of CA Regions, to determine the number of CA Regions having a particular size or range of sizes, to determine whether or not a sample is positive for an HRD signature, to determine the number of Indicator CA Regions in at least one pair of human chromosomes, to determine a likelihood of a deficiency in BRCA1 and/or BRCA2 genes, to determine a likelihood of a deficiency in HDR, to determine a likelihood that a cancer patient will respond to a particular cancer treatment regimen (*e.g.*, a regimen that includes a DNA damaging agent, an anthracycline, a topoisomerase I inhibitor, radiation, a PARP inhibitor, or a combination thereof), or to determine a combination of these items.

[00123] In some cases, a computing system provided herein can include computer-executable instructions or a computer program (*e.g.*, software) containing computer-executable instructions for formatting an output providing an indication about the number of CA Regions, the size of CA Regions, the number of CA Regions having a particular size or range of sizes, whether or not a sample is positive for an HRD signature, the number of Indicator CA Regions in at least one pair of human chromosomes, a likelihood of a deficiency in BRCA1 and/or BRCA2 genes, to determine a likelihood of a deficiency in HDR, a likelihood that a cancer patient will respond to a particular cancer treatment regimen (*e.g.*, a regimen that includes a DNA damaging agent, an anthracycline, a topoisomerase I inhibitor, radiation, a PARP inhibitor, or a combination thereof), or a combination of these items. In some cases, a computing system provided herein can include computer-executable instructions or a computer program (*e.g.*, software) containing computer-executable instructions for determining a desired cancer treatment regimen for a particular patient based at least in part on the presence or absence of an HRD signature or on the number of Indicator CA Regions.

[00124] In some cases, a computing system provided herein can include a pre-processing device configured to process a sample (*e.g.*, bladder cancer cells) such that a SNP array-based assay or sequencing-based assay can be performed. Examples of pre-processing devices

include, without limitation, devices configured to enrich cell populations for cancer cells as opposed to non-cancer cells, devices configured to lyse cells and/or extract genomic nucleic acid, and devices configured to enrich a sample for particular genomic DNA fragments.

[00125] This document also provides kits for assessing samples (*e.g.*, bladder cancer cells) as described herein. For example, this document provides kits for assessing cancer cells (*e.g.*, bladder cancer cells) for the presence of an HRD signature or to determine the number of Indicator CA Regions in at least one pair of human chromosomes. A kit provided herein can include either SNP probes (*e.g.*, an array of SNP probes for carrying out a SNP array-based assay described herein) or primers (*e.g.*, primers designed for sequencing SNP regions via a sequencing-based assay) in combination with a computer program product containing computer-executable instructions for carrying out one or more of the methods or steps described herein (*e.g.*, computer-executable instructions for determining the number of Indicator CA Regions). In some cases, a kit provided herein can include at least 500, 1000, 10,000, 25,000, or 50,000 SNP probes capable of hybridizing to polymorphic regions of human genomic DNA. In some cases, a kit provided herein can include at least 500, 1000, 10,000, 25,000, or 50,000 primers capable of sequencing polymorphic regions of human genomic DNA. In some cases, a kit provided herein can include one or more other ingredients for performing a SNP array-based assay or a sequencing-based assay. Examples of such other ingredients include, without limitation, buffers, sequencing nucleotides, enzymes (*e.g.*, polymerases), etc. This document also provides the use of any appropriate number of the materials provided herein in the manufacture of a kit for carrying out one or more of the methods or steps described herein. For example, this document provides the use of a collection of SNP probes (*e.g.*, a collection of 10,000 to 100,000 SNP probes) and a computer program product provided herein in the manufacture of a kit for assessing cancer cells for the presence of an HRD signature. As another example, this document provides the use of a collection of primers (*e.g.*, a collection of 10,000 to 100,000 primers for sequencing SNP regions) and a computer program product provided herein in the manufacture of a kit for assessing cancer cells (*e.g.*, bladder cancer cells) for the presence of an HRD signature.

SPECIFIC EMBODIMENTS

[00126] As follows are specific embodiments of the present disclosure, that is, exemplary but non-limiting details of methods and systems according to the more general description above.

[00127] In some embodiments, the sample used is a frozen tumor sample. In some embodiments, the sample is from a particular bladder cancer. In some embodiments, the laboratory assay portion of the method, system, etc. comprises assaying the sample to determine the allele dosage (*e.g.*, genotype, copy number, etc.) for at least 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000 or more selected SNPs across the complete genome. In some embodiments the SNP analysis is done using an oligonucleotide microarray as discussed above. In some embodiments the SNP analysis is performed using a probe capture (*e.g.*, probes to each SNP to be analyzed) with subsequent PCR enrichment technique (*e.g.*, AgilentTM SureSelect XT). In some embodiments the SNP analysis is performed by processing the output from the enrichment technique using a “next-generation” sequencing platform (*e.g.*, IlluminaTM HiSeq2500). In some embodiments the sample is analyzed for germline mutations, which may include large rearrangements. In some embodiments, the sample is analyzed for BRCA1 promoter methylation (*e.g.*, by a qPCR assay (*e.g.*, SA Biosciences)). In some embodiments, a sample is determined to have high methylation (or are “methylated”) if the sample has greater than 10% (or 5%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%) methylation (*e.g.*, % of BRCA1 or BRCA2 promoter CpGs methylated).

[00128] In some embodiments, LOH Region Score can be calculated by counting the number of LOH regions that are >15 Mb in length, but shorter than the length of a complete chromosome. In some embodiments, TAI Region Score can be calculated by counting the number of telomeric regions > 11 Mb in length with allelic imbalance that extends to one of the subtelomeres, but does not cross the centromere. In some embodiments, LST Region Score can be calculated by counting the number of breakpoints between regions longer than 10 megabases having stable copy number after filtering out regions shorter than 3 megabases. In some embodiments the LST Region Score can be modified by adjusting it by ploidy: $LST_m = LST - kP$, where P is ploidy and k is a constant (in some embodiments, $k=15.5$). In some embodiments response to treatment can

be partial complete response (“pCR”), which in some embodiments can be defined as Miller-Payne 5 status following treatment (e.g., neoadjuvant).

[00129] In some embodiments, the claimed method predicts BRCA deficiency with a p-value of at least 8×10^{-12} , 6×10^{-6} , 0.0009, 0.01, 0.03, 2×10^{-16} , 3×10^{-6} , 10^{-6} , 0.0009, 8×10^{-12} , 2×10^{-16} , 8×10^{-8} , 6×10^{-6} , 3×10^{-6} , or 0.0002 (e.g., each CA Region Score is predefined and optionally multiple scores are combined in such a way as to yield these p-values). In some embodiments p-values are calculated according to Kolmogorov-Smirnov test. In some embodiments HRD scores and age at diagnosis can be coded as a numeric (e.g., integer) variable, bladder cancer stage and subtype can be coded as categorical variables, and grade can be analyzed as either a numeric or categorical variable, or both.

[00130] In some embodiments p-values are two-sided. In some embodiments, logistic regression analysis can be used to predict BRCA1/2 deficiency based on an HRD score as disclosed herein, including the HRD-combined score). In some embodiments, the various CA Region Scores are correlated according to (e.g., defined in order to achieve) the following correlation coefficients: LOH Region Score and TAI Region Score = 0.69 ($p = 10^{-39}$), between LOH and LST = 0.55 ($p = 2 \times 10^{-19}$), and between TAI and LST = 0.39 ($p = 10^{-9}$).

[00131] In some embodiments the method combines the LOH Region Score and TAI Region Score as follows to detect BRCA1/2 deficiency and/or predict therapy response (e.g., platinum therapy response, e.g., cisplatin): Combined CA Region Score = $0.32 \times \text{LOH Region Score} + 0.68 \times \text{TAI Region Score}$. In some embodiments the method combines the LOH Region Score, TAI Region Score, and LST Region Score as follows to detect BRCA1/2 deficiency and/or predict therapy response (e.g., platinum therapy response, e.g., cisplatin): Combined CA Region Score = $0.21 \times \text{LOH Region Score} + 0.67 \times \text{TAI Region Score} + 0.12 \times \text{LST Region Score}$. In some embodiments the method combines the LOH Region Score, TAI Region Score, and LST Region Score as follows to detect BRCA1/2 deficiency and/or predict therapy response (e.g., platinum therapy response, e.g., cisplatin): Combined CA Region Score = $0.11 \times \text{LOH Region Score} + 0.25 \times \text{TAI Region Score} + 0.12 \times \text{LST Region Score}$. In some embodiments the method combines the LOH Region Score, TAI Region Score, and LST Region Score as follows to detect BRCA1/2 deficiency and/or predict therapy response (e.g., platinum therapy response, e.g., cisplatin):

Combined CA Region Score = Arithmetic Mean of LOH Region Score, TAI Region Score and LST Region Score.

[00132] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 – HRD predicts response to neoadjuvant chemotherapy in bladder cancer

[00133] This example evaluates the ability of HRD to predict response to neoadjuvant chemotherapy in bladder cancer.

Methods

[00134] HRD scores were generated from tissue derived from trans urethral resection of the bladder (TURB) immediately prior to cystectomy. The eligible study population included any patient diagnosed with cT2-4aNx transitional cell carcinoma of the bladder from 1990 to 2013 and was treated by radical cystectomy at the University of Indiana Hospitals and Clinics. In addition, patients were treated with neo-adjuvant Cisplatin combination chemotherapy. Patients had available tissue from TURB prior to any therapeutic intervention or at least 6 months after last treatment with intravesicle therapy (*e.g.*, BCG). Patients with prior history of systemic therapies were also excluded. The study cohort included 72 patients with passing HRD scores. Outcomes were complete response (pT0N0), and complete response or pathologic downstaging (<pT2N0). By definition these outcomes were largely nested.

Results

[00135] A total of 19 patients had complete response and an additional 17 had pathologic downstaging. Only one patient with complete response progressed to recurrence and death. The HRD score was predictive of complete response (OR = 2.39 (95% CI 1.15, 4.95), p-value = 0.011). The HRD score was also predictive of disease recurrence (HR = 0.49 (95% CI 0.28, 0.86), p-value = 0.0074), even when adjusted for pCR (HR = 0.57 (95% CI 0.33, 0.99), p-value = 0.034). (Figure 1).

Conclusion

[00136] This study demonstrates that the HRD score could be used to identify men who are likely to respond to neoadjuvant chemotherapy, or who are likely to have disease recurrence.

Example 2 – Evaluation of the prognostic and predictive utility of HRD and genetic sequencing in patients undergoing neoadjuvant chemotherapy in bladder cancer

[00137] This example describes an effort to identify potential biomarkers in urothelial bladder cancer (UBC) that will aid in characterizing disease aggressiveness and/or predict cisplatin chemo-sensitivity using a combination of nucleic acid-based assays.

Methods

[00138] Between 2003 and 2014 formalin-fixed paraffin-embedded bladder tumor from time of TURBT was obtained from patients before treatment with neoadjuvant chemotherapy (NACT). All patients had had muscle invasive UBC and underwent radical cystectomy. Molecular assays included the homologous recombination deficiency (HRD) score and genetic sequencing of a set of genes recently implicated as potential drivers in UBC. HRD scores and gene mutations were correlated to complete pathologic response (pCR) and recurrence-free survival (RFS). The cohort demographics are illustrated in Table 2.

Table 2

Variable		n (%)
Number of patients		84
Median Age		Mean=64.4
Race (Caucasian)		98%
Neoadjuvant Chemotherapy	Gemcitabine/cisplatin	70 (83)
	Cisplatin/Etoposide	9 (11)
	MVAC	5 (6)
Follow-up (years)		2.5
Variant Histology type of TURBT	None	49 (58)
	Squamous	11 (13)
	Small cell	11 (13)
	Micropapillary	5 (6)
	Glandular	4 (5)
	Plasmacytoid	2 (2)
	Sarcomoid	1 (1)
	Nested	1 (1)
CIS at TURBT		23 (27)
Lymph-Vascular Invasion at TURBT		10 (12)
Variant Histology Type at Cx	None	60 (71)
	Squamous	10 (12)
	Small cell	6 (7)

	Micropapillary	3 (4)
	Plasmacytoid	3 (4)
	Sarcomoid	1 (1)
	Nested	1 (1)
CIS at Cx		36 (43)
Positive Lymph Nodes		28 (33)
T Stage	pT0	21 (25)
	pTis	13 (15)
	pT1	8 (10)
	pT2a	7 (8)
	pT2b	8 (10)
	pT3a	4 (5)
	pT3b	13 (15)
	pT4a	10 (12)
Pathologic complete Response (pT0N0)		19 (23%)
Pathologic Downstaging (<pT2N0)		36 (43%)
2-year Risk of recurrence		40%
2-year Risk of dead of disease		30%

Results

[00139] A total of 90 samples were obtained from patients who underwent NACT followed by cystectomy, of which 70 patient samples were able to yield HRD scores. Age, Gender, Race, Variant histology at TURBT, Lymph-Vascular Invasion at TURBT, CIS at TURBT, and neoadjuvant chemotherapy were not significant for univariate analyses of pCR. The distribution of HRD scores is shown in Figure 5. Higher HRD scores were associated with pCR on univariate analysis (OR 2.10, CI 1.04-4.22, $p=0.033$). Among 82 genes sequenced, mutations identified in RB1 and TP53 genes were associated with pCR ($p=0.0040$ and $p=0.030$, respectively) (Figure 6). Best multivariable models for pCR were RB1 and HRD (OR 4.24, CI 1.38-14.1, $p=0.011$ and OR 1.95, CI 0.91-4.21, $p=0.081$, respectively) (Figures 7 and 8).

[00140] Regarding analysis of RFS, age at surgery, gender, race, and CIS at Cx were no significant for univariate analyses. An HRD score greater than 30 (4th quartile) was associated with a decreased risk of recurrence, and remained predictive of decreased recurrence when accounting for pCR ($p=0.019$) (Figure 9). Other significant variables for recurrence-free survival are shown in Table 3.

Table 3

Variable		Univariate		Best Multivariable Model (chosen by backward selection, n=72)	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Lymph Nodes Positive		9.25 (4.16, 22.74)	1.9 $\times 10^{-9}$	3.67 (1.39, 10.5)	0.0083
Stage (coded as continuous)		1.47 (1.26, 1.74)	8.4 $\times 10^{-7}$	1.33 (1.07, 1.68)	0.0093
Variant Histology at Cx	Other	-	0.00053	-	-
	Squamous	3.69 (1.32, 8.96)		-	
	Small cell	8.04 (2.58, 21.12)		-	
HRD score		0.96 (0.92, 0.99)	0.0074	0.95 (0.92, 0.99)	0.014
pCR		0.18 (0.03, 0.61)	0.0032	-	-
Neoadjuvant Chemotherapy	MVA C	-	0.0043	-	0.034
	Gemcitabine/Cisplatin	1.47 (0.31, 26.4)		0.68 (0.13, 12.5)	
	Cisplatin/Etoposide	5.37 (0.91, 101.74)		3.09 (0.5, 59.7)	

Conclusion

[00141] RB1 mutations are associated with response to cisplatin NACT in UBC patients. The predictive ability appears to be improved by the addition of HRD scores, which is useful to identify chemo-responsive patients. In addition, HRD can be used to predict risk of recurrence in patients after NACT followed by cystectomy.

[00142] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

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WHAT IS CLAIMED IS:

1. An *in vitro* method of predicting patient response to a bladder cancer treatment regimen comprising a DNA damaging agent, anthracycline, topoisomerase I inhibitor, or PARP inhibitor, the method comprising:

(1) determining, in a sample comprising a bladder cancer cell, the number of Indicator CA Regions comprising at least two types chosen from Indicator LOH Regions, Indicator TAI Regions, or Indicator LST Regions in at least one pair of human chromosomes of a bladder cancer cell of said cancer patient; and

(2) diagnosing a patient in whose sample said number of Indicator LOH Regions, Indicator TAI Regions, and/or Indicator LST Regions is greater than a reference number as having an increased likelihood of responding to said bladder cancer treatment regimen.

2. The method of Claim 1, said at least one pair of human chromosomes is representative of the entire genome.

3. The method of Claim 1 or Claim 2, wherein said Indicator CA Regions are determined in at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 pairs of human chromosomes.

4. The method of any one of Claims 1-3, wherein the reference number of Indicator LOH Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, the reference number of Indicator TAI Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, and the reference number of Indicator LST Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more.

5. The method of any one of Claims 1-4, wherein said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but less than a either a complete chromosome or a complete chromosome arm, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15,

16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but not extending across a centromere, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length.

6. The method of any one of Claims 1-5, wherein said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci.

7. The method of any one of Claims 1-6, wherein said DNA damaging agent is cisplatin, carboplatin, oxaloplatin, nedaplatin, iproplatin, or picoplatin, said anthracycline is epirubicin or doxorubicin, said topoisomerase I inhibitor is camptothecin, topotecan, or irinotecan, or said PARP inhibitor is iniparib, olaparib or velapirib.

8. The method of any one of Claims 1-7, further comprising administering said bladder cancer treatment regimen to said patient diagnosed as having an increased likelihood of responding to said bladder cancer treatment regimen.

9. The method of any one of Claims 1-8, further comprising identifying mutations in RB1 and/or TP53.

10. An *in vitro* method of predicting patient response to a bladder cancer treatment regimen comprising a platinum agent, the method comprising:

(1) determining, in a sample comprising a bladder cancer cell, the number of Indicator CA Regions comprising at least two types chosen from Indicator LOH Regions, Indicator TAI Regions, and/or Indicator LST Regions in at least one pair of human chromosomes of a bladder cancer cell of said cancer patient; and

(2) diagnosing a patient in whose sample said number of Indicator LOH Regions, Indicator TAI Regions, or Indicator LST Regions is greater than a reference number or as having an increased likelihood of responding to said bladder cancer treatment regimen.

11. The method of Claim 10, said at least one pair of human chromosomes is representative of the entire genome.

12. The method of Claim 10 or Claim 11, wherein said Indicator CA Regions are determined in at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 pairs of human chromosomes.

13. The method of any one of Claims 10-12, wherein the reference number of Indicator LOH Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, the reference number of Indicator TAI Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, and the reference number of Indicator LST Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more.

14. The method of any one of Claims 10-13, wherein said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but less than a either a complete chromosome or a complete chromosome arm, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but not extending across a centromere, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length.

15. The method of any one of Claims 10-14, wherein said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci.

16. The method of any one of Claims 10-15, wherein said DNA damaging agent is cisplatin, carboplatin, oxaloplatin, nedaplatin, iproplatin, or picoplatin, said anthracycline is

epirubicin or doxorubicin, said topoisomerase I inhibitor is camptothecin, topotecan, or irinotecan, or said PARP inhibitor is iniparib, olaparib or velaparib.

17. The method of any one of Claims 10-16, further comprising identifying mutations in RB1 and/or TP53.

18. An *in vitro* method of predicting patient response to a bladder cancer treatment regimen comprising a DNA damaging agent, anthracycline, topoisomerase I inhibitor, or PARP inhibitor, the method comprising:

(1) determining, in a sample comprising a bladder cancer cell, the number of Indicator CA Regions comprising at least two types chosen from Indicator LOH Regions, Indicator TAI Regions, or Indicator LST Regions in at least one pair of human chromosomes of a bladder cancer cell of said cancer patient;

(2) providing a test value derived from the number of said Indicator CA Regions;

(3) comparing said test value to one or more reference values derived from the number of said Indicator CA Regions in a reference population; and

(4) diagnosing a patient in whose sample said test value is greater than said one or more reference numbers as having an increased likelihood of responding to said bladder cancer treatment regimen.

19. The method of Claim 18, said at least one pair of human chromosomes is representative of the entire genome.

20. The method of Claim 18 or Claim 19, wherein said Indicator CA Regions are determined in at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 pairs of human chromosomes.

21. The method of any one of Claims 18-20, wherein the reference number of Indicator LOH Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, the reference number of Indicator TAI Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, and the reference number of Indicator LST Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more.

22. The method of any one of Claims 18-21, wherein said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but less than a either a complete chromosome or a complete chromosome arm, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but not extending across a centromere, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length.

23. The method of any one of Claims 18-22, wherein said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci.

24. The method of any one of Claims 18-23, wherein said DNA damaging agent is cisplatin, carboplatin, oxaloplatin, nedaplatin, iproplatin, or picoplatin, said anthracycline is epirubicin or doxorubicin, said topoisomerase I inhibitor is camptothecin, topotecan, or irinotecan, or said PARP inhibitor is iniparib, olaparib or velaparib.

25. The method of any one of Claims 18-24, further comprising diagnosing a patient in whose sample said test value is not greater than said one or more reference numbers as not having an increased likelihood of responding to said bladder cancer treatment regimen and recommending, prescribing, initiating or continuing a treatment regimen not comprising a DNA damaging agent, anthracycline, topoisomerase I inhibitor, or PARP inhibitor in said patient diagnosed as not having an increased likelihood of responding to said bladder cancer treatment regimen.

26. The method of any one of Claims 18-25, wherein said test value is derived by calculating the arithmetic mean of the numbers of Indicator LOH Regions, Indicator TAI Regions and Indicator LST Regions in said sample as follows:

$$\text{Test Value} = \frac{(\# \text{ of Indicator LOH Regions}) + (\# \text{ of Indicator TAI Regions}) + (\# \text{ of Indicator LST Regions})}{3}$$

3

and said one or more reference values were derived by calculating the arithmetic mean of the numbers of Indicator LOH Regions, Indicator TAI Regions and Indicator LST Regions in samples from said reference population as follows:

$$\text{Test Value} = \frac{(\# \text{ of Indicator LOH Regions}) + (\# \text{ of Indicator TAI Regions}) + (\# \text{ of Indicator LST Regions})}{3}$$

3.

27. The method of any one of Claims 18-26, comprising diagnosing a patient in whose sample said test value is at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10-fold greater, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 standard deviations greater, or at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% greater than said one or more reference numbers as having an increased likelihood of responding to said bladder cancer treatment regimen.

28. The method of any one of Claims 18-27, further comprising identifying mutations in RB1 and/or TP53.

29. A method of treating bladder cancer patients, comprising:

(1) determining, in a sample comprising a bladder cancer cell, the number of Indicator CA Regions comprising Indicator LOH Regions, Indicator TAI Regions, and Indicator LST Regions in at least one pair of human chromosomes of a bladder cancer cell of said cancer patient;

(2) providing a test value derived from the number of said Indicator CA Regions;

(3) comparing said test value to one or more reference values derived from the number of said Indicator CA Regions in a reference population; and either

(4)(a) recommending, prescribing, initiating or continuing a treatment regimen comprising a DNA damaging agent, anthracycline, topoisomerase I inhibitor, or PARP inhibitor in a patient in whose sample the test value is greater than at least one said reference value; or

(4)(b) recommending, prescribing, initiating or continuing a treatment regimen comprising a DNA damaging agent, anthracycline, topoisomerase I inhibitor, or PARP

inhibitor in a patient in whose sample the test value is not greater than at least one said reference value.

30. The method of Claim 29, said at least one pair of human chromosomes is representative of the entire genome.

31. The method of Claim 29 or Claim 30, wherein said Indicator CA Regions are determined in at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 pairs of human chromosomes.

32. The method of any one of Claims 29-31, wherein the reference number of Indicator LOH Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, the reference number of Indicator TAI Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more, and the reference number of Indicator LST Regions is two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more.

33. The method of any one of Claims 29-32, wherein said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but less than either a complete chromosome or a complete chromosome arm, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length but not extending across a centromere, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more megabases in length.

34. The method of any one of Claims 29-33, wherein said Indicator LOH Regions are defined as LOH Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, said Indicator TAI Regions are defined as TAI Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci, and said Indicator LST Regions are defined as LST Regions at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50 or more loci.

35. The method of any one of Claims 29-34, wherein said DNA damaging agent is cisplatin, carboplatin, oxaloplatin, nedaplatin, iproplatin, or picoplatin, said anthracycline is epirubicin or doxorubicin, said topoisomerase I inhibitor is camptothecin, topotecan, or irinotecan, or said PARP inhibitor is iniparib, olaparib or velaparib.

36. The method of any one of Claims 29-35, wherein said test value is derived by calculating the arithmetic mean of the numbers of Indicator LOH Regions, Indicator TAI Regions and Indicator LST Regions in said sample as follows:

$$\text{Test Value} = \frac{(\# \text{ of Indicator LOH Regions}) + (\# \text{ of Indicator TAI Regions}) + (\# \text{ of Indicator LST Regions})}{3}$$

and said one or more reference values were derived by calculating the arithmetic mean of the numbers of Indicator LOH Regions, Indicator TAI Regions and Indicator LST Regions in samples from said reference population as follows:

$$\text{Test Value} = \frac{(\# \text{ of Indicator LOH Regions}) + (\# \text{ of Indicator TAI Regions}) + (\# \text{ of Indicator LST Regions})}{3}.$$

37. The method of any one of Claims 29-36, comprising diagnosing a patient in whose sample said test value is at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10-fold greater, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 standard deviations greater, or at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% greater than said one or more reference numbers as having an increased likelihood of responding to said bladder cancer treatment regimen.

38. The method of any one of Claims 29-37, further comprising identifying mutations in RB1 and/or TP53.

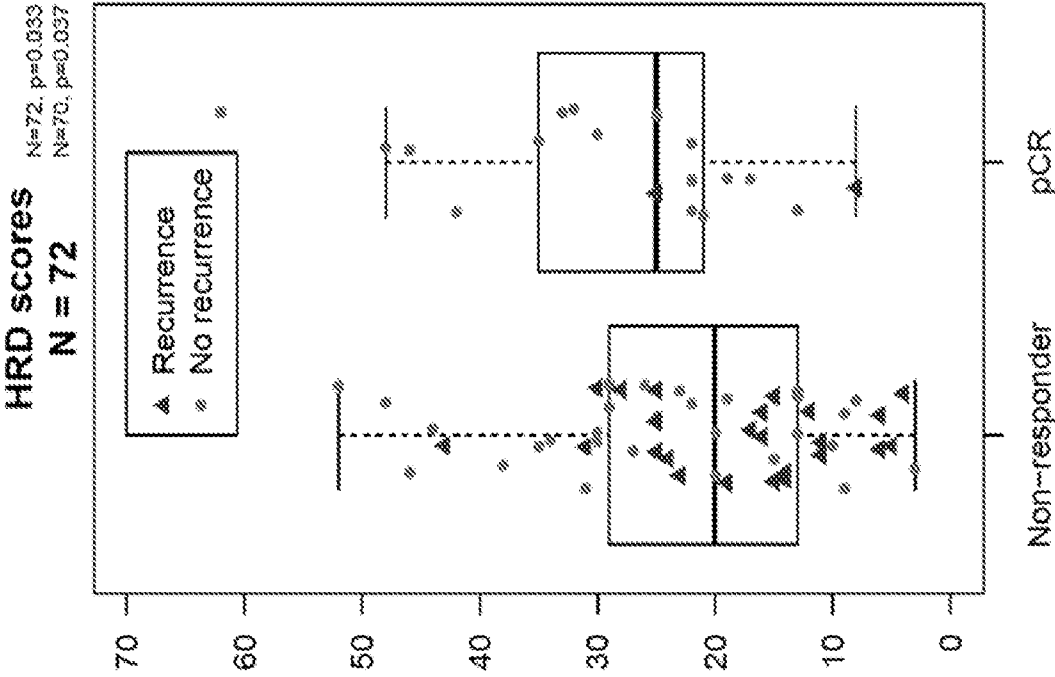


Figure 1

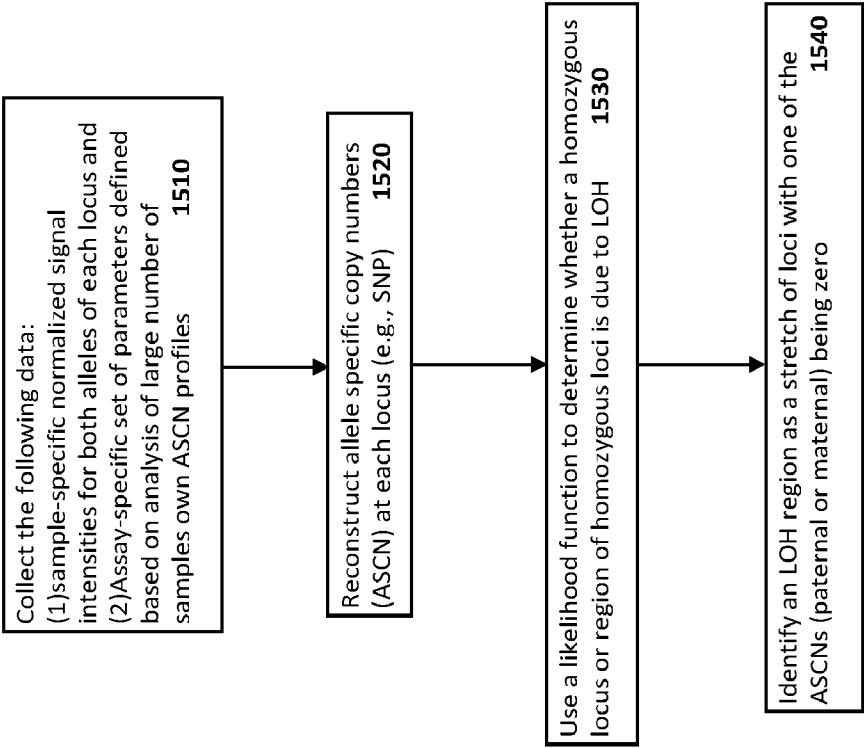


Figure 2

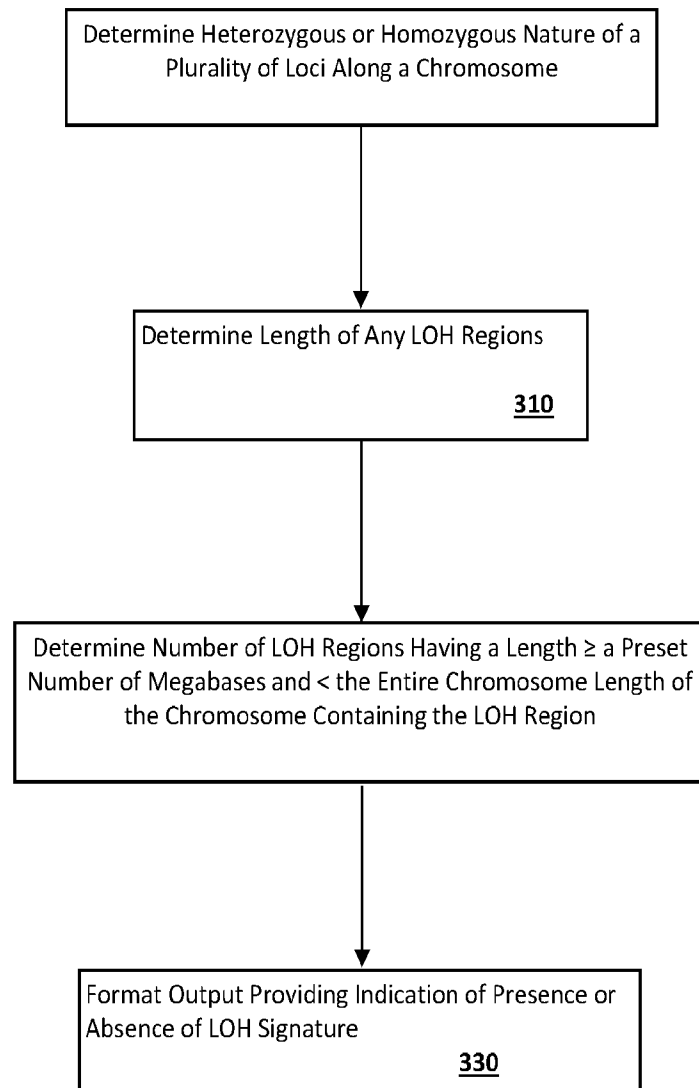


Figure 3

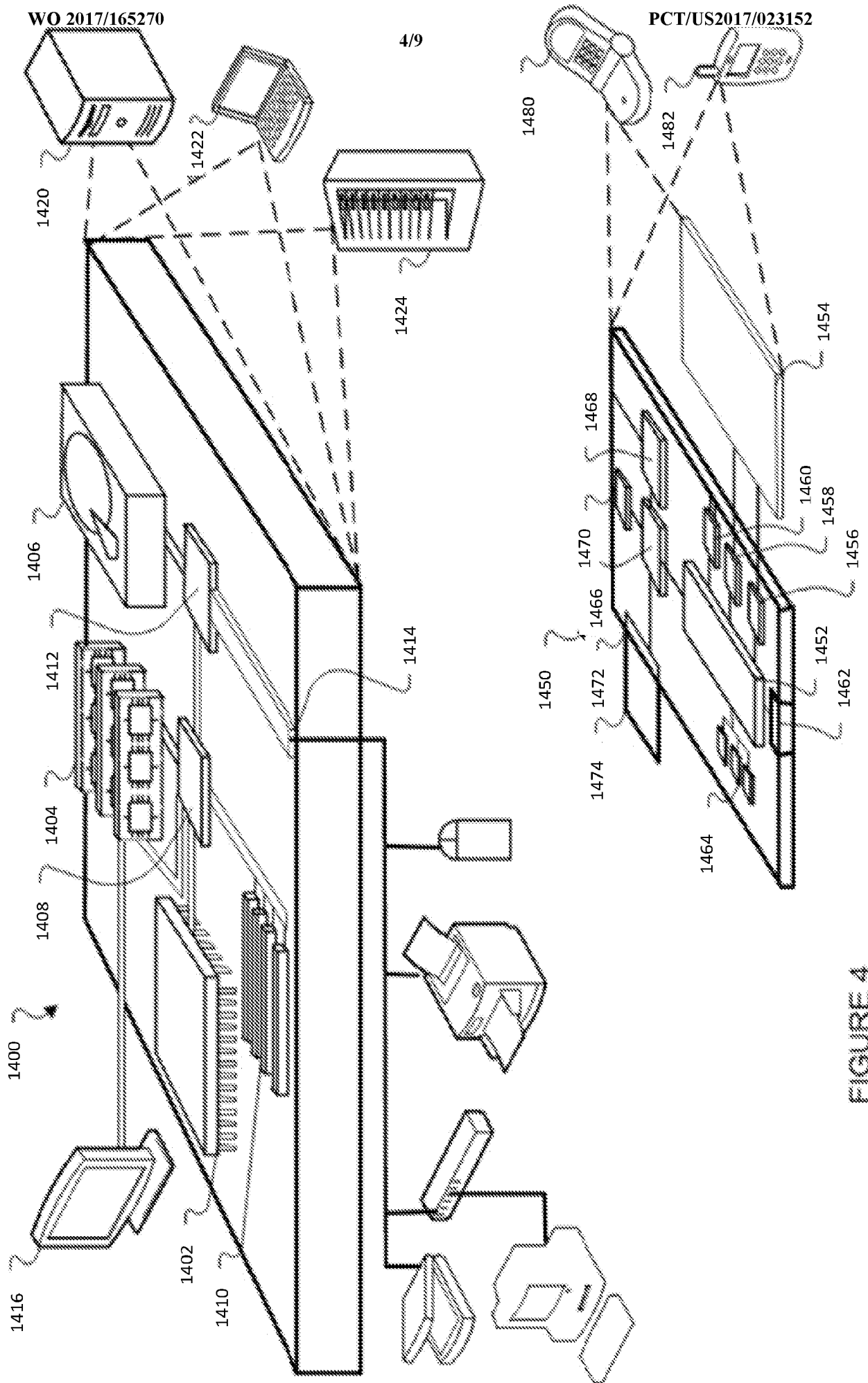


FIGURE 4

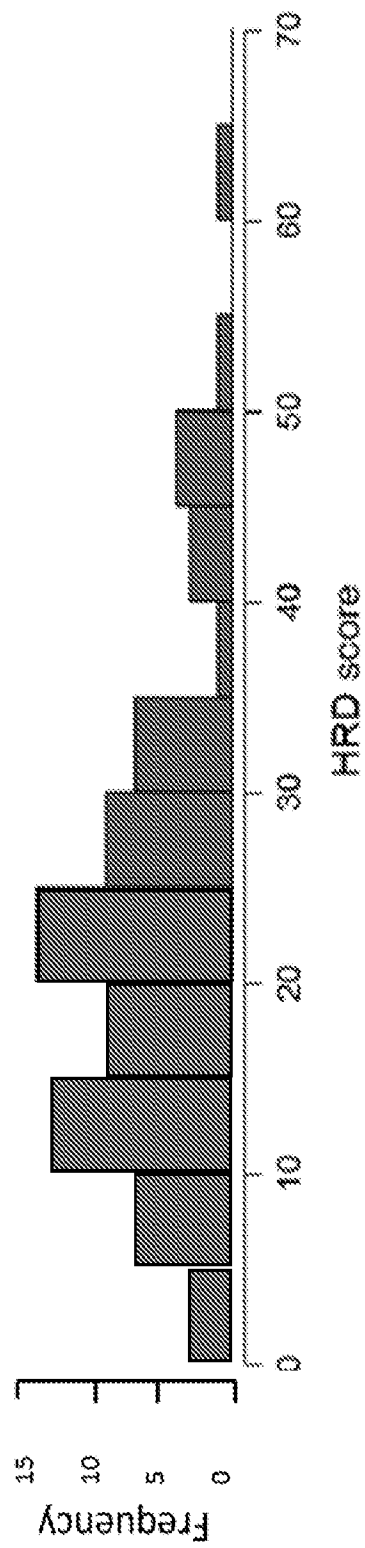
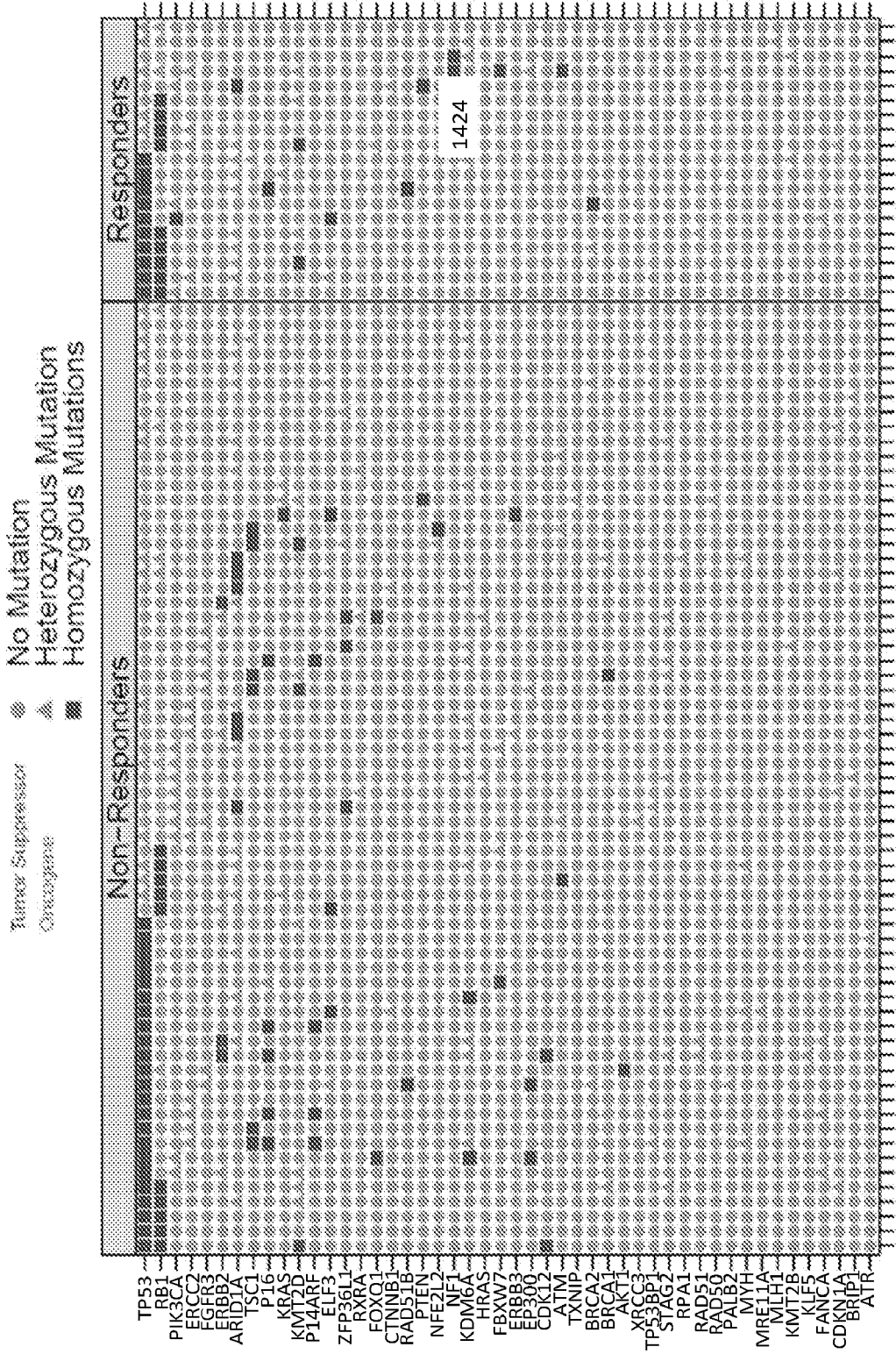


Figure 5



Patient Tumor

Figure 6

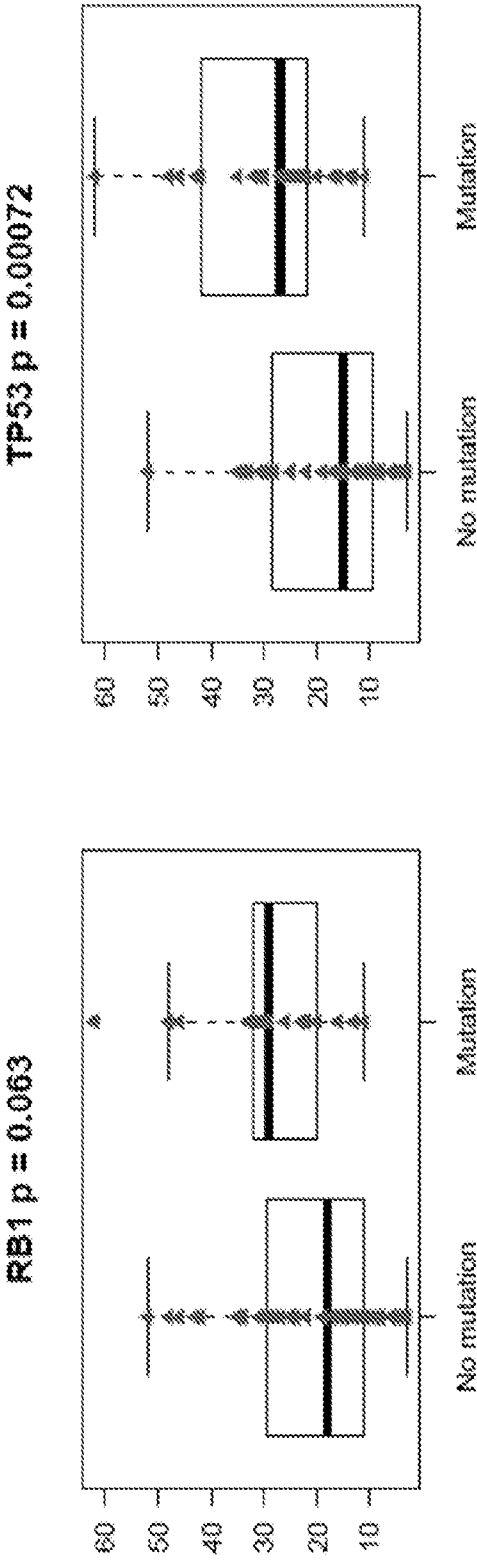


Figure 7

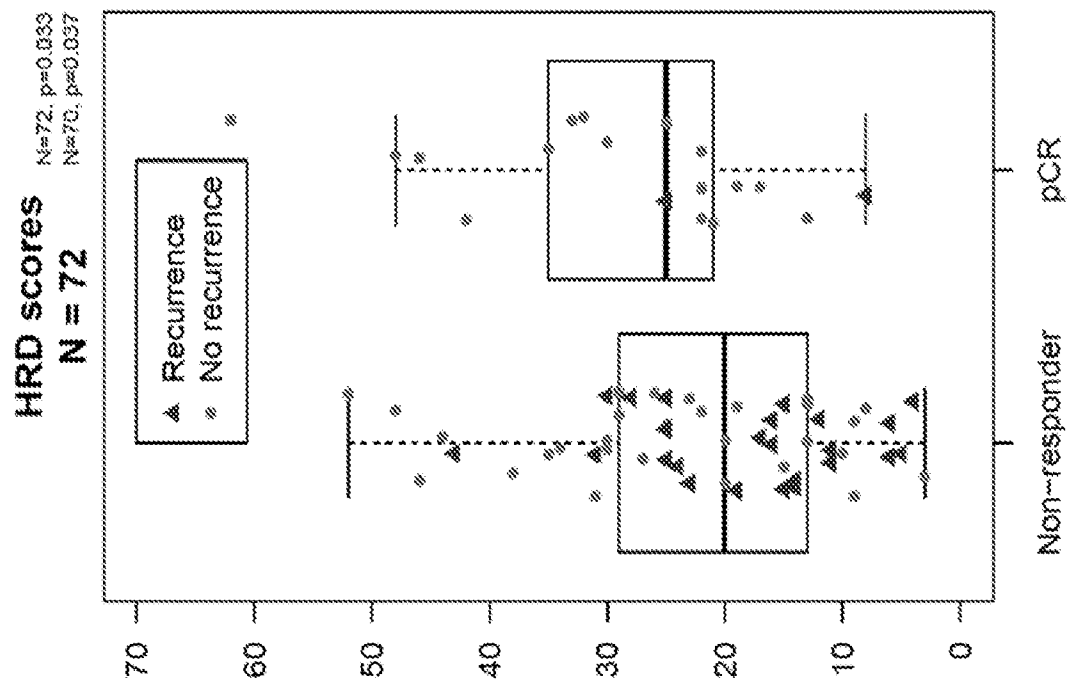


Figure 8

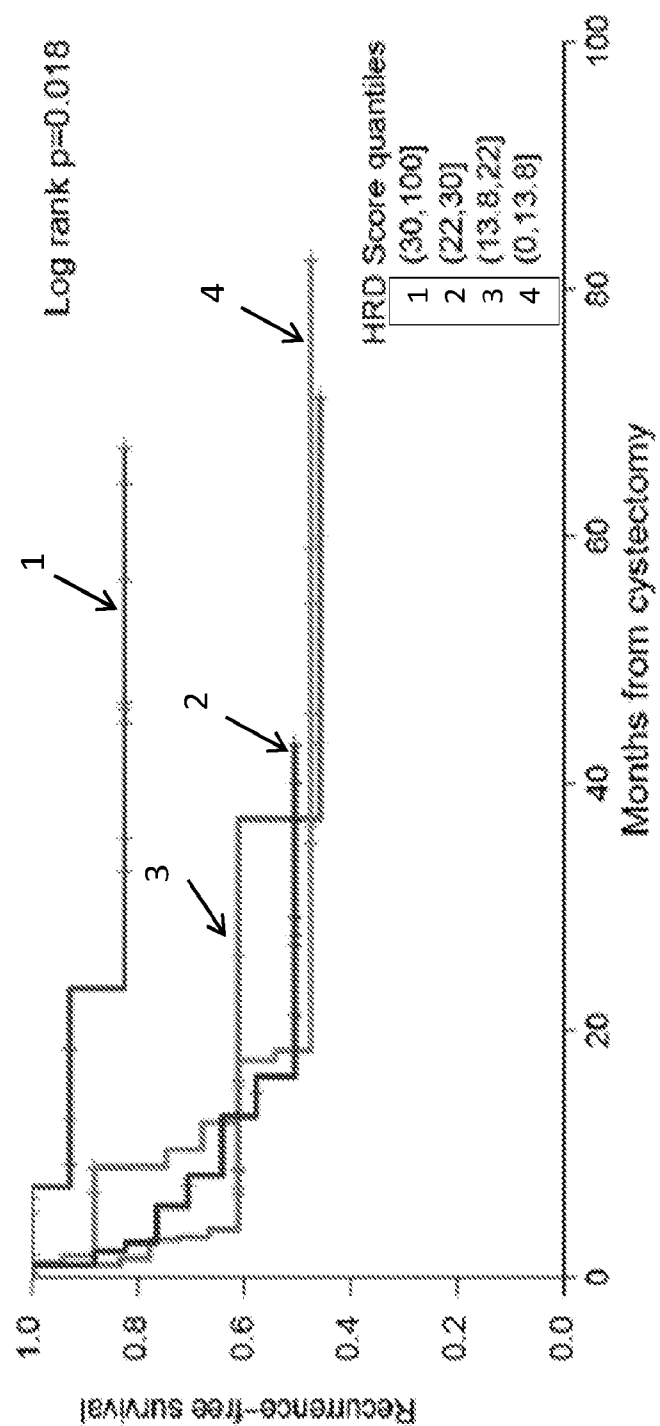


Figure 9

A. CLASSIFICATION OF SUBJECT MATTER**C12Q 1/68(2006.01)i, G06F 19/00(2011.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q 1/68; A61K 33/24; G06F 19/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & keywords: bladder cancer treatment, DNA damaging agent, anthracycline, topoisomerase I inhibitor, PARP inhibitor, CA region, LOH region, TAI region, LST region

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014-0363521 A1 (MYRIAD GENETICS, INC.) 11 December 2014 See claims 1-3, 9-11, 18-20.	1-3, 10-12, 18-20
Y	WO 2012-027224 A1 (DANA-FARBER CANCER INSTITUTE, INC. et al.) 01 March 2012 See claims 1, 5.	1-3, 10-12, 18-20
A	WO 2015-086473 A1 (INSTITUT CURIE et al.) 18 June 2015 See paragraphs [00186]-[00192], [00217] and claims 1, 3.	1-3, 10-12, 18-20
A	KAMAT et al., "Chemotherapy induced microsatellite instability and loss of heterozygosity in chromosomes 2, 5, 10, and 17 in solid tumor patients" Cancer Cell International, Vol. 14, Article No. 118, Internal pages 1-9 (2014) See the whole document.	1-3, 10-12, 18-20
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Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

05 June 2017 (05.06.2017)

Date of mailing of the international search report

07 June 2017 (07.06.2017)

Name and mailing address of the ISA/KR

International Application Division

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 8, 25, 27, 29-38
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 8, 25, 27, 29-38 pertain to methods for treatment of the human body by therapy, as well as diagnostic methods, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-9, 13-17, 21-28, 32-38
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2017/023152

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