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(54) **Titre : MARQUEURS D'INTERACTION CHROMOSOMIQUE**
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(57) **Abrégé/Abstract:**

A process for analysing chromosome interactions relating to immunotherapy of cancer.

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Abstract:

A process for analysing chromosome interactions relating to immunotherapy of cancer.

Chromosome Interaction Markers

Field of the Invention

The invention relates to immunotherapy.

Background of the Invention

5 Cancer is a major burden of disease worldwide. Each year, tens of millions of people are diagnosed with cancer around the world, and more than half of the patients eventually die from it. In many countries, cancer ranks the second most common cause of death following cardiovascular diseases. With significant improvement in treatment and prevention of cardiovascular diseases, cancer has or will soon become the number one killer in many parts of the world. As elderly people are most susceptible to
10 cancer and population aging continues in many countries, cancer will remain a major health problem around the globe.

Whilst the primary purpose of the immune system is to fight infections caused by external foreign agents such as pathogens, it also has the important function of attacking and eliminating cancer cells. Immunotherapy of cancer usually works by assisting the immune system in some way to fight cancer
15 cells.

Summary of the Invention

The inventors have identified chromosome conformation signatures that define states of the immune system that are relevant to therapy of cancer. This elucidates the role of this modality in regulation of the immune system and allows a 'readout' of in respect of how a patient's immune system will respond
20 to immunotherapy. It has also allowed identification of certain types of responder population for which immunotherapy is not appropriate, and in fact be very harmful. This analysis at the level of the 3D architecture of the genome defined by chromosome interactions offers very early readouts of patient response to immunotherapy allowing decisions to be made at early disease stages as to the most appropriate therapies. Detection of the relevant chromosome interactions has according to the
25 invention has been found to be robust, working across different immunotherapies and cancers.

The identified markers are consistent with deregulations in T cells, NK (natural killer) cells, macrophages, B cells and dendritic cells (DC) showing the role played by the specific set up at cellular level of the adaptive and innate immune system in individual patients as part of the cancer-host interaction which defines disease progression (hyper-progressors) and responsiveness to immunotherapy.

Accordingly, the invention provides a method of determining how an individual responds to immunotherapy for cancer comprising detecting the presence or absence in the individual of:

- all of the chromosome interactions shown in Table 8 to thereby determine whether the individual will be responsive to immunotherapy; and/or
- all of the chromosome interactions shown in Table 2 to thereby determine whether the individual is a hyper-progressor in whom immunotherapy will accelerate disease.

The method of determining how an individual responds to immunotherapy for cancer may comprise detecting the presence or absence in the individual of all of the chromosome interactions shown in Table 1 to thereby determine whether the individual will be responsive to immunotherapy.

Brief Description of the Drawings

Figure 1 shows a preferred method for typing chromosome interactions, essentially based on the EpiSwitch method.

Figure 2 shows predictive and prognostic base-line patient profiling and shows data for response to PD-L1 (Avelumab in second-line (2L) non-small cell lung cancer (NSCLC). For the top graph in each of figures 2a, 2b and 2c the vertical axis shows survival probability from 0.00 to 1.00 and the horizontal axis shows time from 0 to 1200, with the p values for the dotted lines being <0.0001, 0.76 and <0.0001 on figures 2a, 2b and 2c respectively. The bottom table on each figure shows the corresponding number at risk for each line of the graphs across time from 0 to 1200.

Figure 3 relates to validation of EpiSwitch anti-PD-L1 response markers in an independent cohort for 21 patients across cancer types and checkpoint inhibitor therapies.

Figure 4 shows the high concordance between baseline EpiSwitch calls, PD-L1 expression and observed clinical response.

Figure 5 shows data for a training set for an 11 marker model based on 80 NSCLC patients who are a mixture of 1L, 2L Avelumab (54) and 2L Pembrolizumab (36).

Figure 6 shows data for a test set of the 11 marker model based on 38 NSCLC patients who are a mixture of 1L, 2L Avelumab (27) and 2L Pembrolizumab (11).

Figures 7 and 8 show data for a second test set for the Malaysian Observational Study looking a mixture of checkpoint inhibitors and tumours.

Figure 9 shows longitudinal calls in blind Asian samples.

Figure 10 shows the actual EpiSwitch calls for the patients sampled over multiple time points.

- 5 Figures 11 and 12 shows sample selection and patient details for the work relating to hyper-progressors.

Figure 13 shows EpiSwitch chromosome conformation marker selection with associated genetic locations.

Figures 14 and 15 shows the pathway analysis of the genetic locations.

Figure 16 shows the training set for hyper-progressors.

- 10 Figure 17 shows the test set for hyper-progressors.

Figure 18 shows the logistic PCA (principle component analysis) of the training set for hyper-progressors. The squares show H and the circles show S.

Figure 19 shows the logistic PCA of the training set with predicted test samples for hyper-progressors. The squares show H and the dark circles show S.

- 15 Figure 20 shows the logistic PCA of the training set with PFS as label for hyper-progressors. The squares show H and the circles show S.

Figure 21 shows the logistic PCA of the training set with OS as label for hyper-progressors. The squares show H and the circles show S.

- 20 Figure 22 shows a confusion matrix and statistics. The training model is 78 patients. 30 were NR (non-responders). 39 were R (responders). 9 were SD (stable disease).

Figure 23 shows a confusion matrix and statistics. Test set is 24 patients. 8 were NR. 12 were R. 4 were SD.

Figure 24 shows a confusion matrix and statistics. Test set is 128 patients.

- 25 Figure 25 shows the global variable importance for different markers. From the top the bottom the results are shows for (i) obd189_q65-q67_p65, (ii) obd189_q53_q55_p53, (iii) obd189_q81_q83_p81, (iv) obd148_q893_q895_p893, (v) obd189_q49_q51_p49, (vi) obd189_q29_q31_p31, (vii)

obd189_q57_q59_p57, (viii) obd189_q05_q07_p05. The horizontal axis show top model features. The vertical axis shows value going from 0 to 20.

Figure 26 shows the genetic locations of markers.

Figure 27 shows pathways associated with the genes of Figure 26.

5 Description of the Tables

Table 1 shows the universal marker set and how each marker relates to responsiveness (R) or non-responsiveness (NR) to immunotherapy.

Table 2 shows the marker set for detection of hyper-progressors and how each marker relates to hyper-progression (HS) or being stable (S).

10 Table 3 shows immune checkpoint molecules that can be targeted and/or modulated by the immunotherapy.

Table 4 to 6 provides examples of cancer immunotherapies for which responder status can be determined by the invention and are also the therapies that can be given to the individual based on the outcome of determination of the responder status according to the invention. These tables also show
15 preferred cancers.

Table 7 shows markers relevant to the screens carried out in Example 2 to develop the set of markers shown in Table 8.

Table 8 shows a further universal marker set and how each marker relates to responsiveness (R) or non-responsiveness (NR) to immunotherapy.

20 Table 9 gives patient data for Example 2. The patients shown with an asterisk (*) were studied in the second screen described in Example 2.

Detailed Description of the Invention

Terms Used Herein

The method of the invention may be referred to as the 'process' of the invention herein.

25 The chromosome interactions which are typed may be referred to as 'markers', 'CCS', 'chromosome conformation signature', 'epigenetic interaction' or 'EpiSwitch markers' herein.

The word 'type' will be interpreted as per the context, but will usually refer to detection of whether a specific chromosome interaction is present or absent. The typing will generally be by physical determination of whether the chromosome interaction is present.

The word 'responder' is used to refer to response to immunotherapy, and covers both aspects relating to responsiveness to immunotherapy (the universal marker set) and detection of hyper-progressors. The term 'responder group' covers all four of the different groups discussed herein:

- responder to immunotherapy
- non-responder to immunotherapy
- hyper-progressor when given immunotherapy
- stable disease (non-hyper-progressor) when given immunotherapy.

The chromosome interactions which are typed in the method of the invention are defined in Tables 2 and 8. The chromosome interactions which are typed in the method of the invention are further defined in Table 1. They are defined by means of the probe sequences which detect the ligated product made by an EpiSwitch method (see Figure 1). They are also defined by the position numbers of the interaction which are within the probe name and they are also defined by the primer sequences which allow detection of the ligated sequence.

The Epigenetic Interactions Relevant to the Invention

The chromosome interactions which are typed in the invention are typically interactions between distal regions of a chromosome, said interactions being dynamic and altering, forming or breaking depending upon the state of the region of the chromosome. That state will reflect how the immune system interacts with immunotherapy which is given which responder group the individual falls into.

The chromosome interaction may, for example, reflect if it is being transcribed or repressed. Chromosome interactions which are specific to responder 'groups' as defined herein have been found to be stable, thus providing a reliable means of measuring the differences between groups (for example reflecting different responses to immunotherapy).

Chromosome interactions specific to responder groups will normally be present before or in the early stages of a disease process, for example compared to other epigenetic markers such as methylation or changes to binding of histone proteins. Thus the process of the invention is able to provide valuable information about the way the immune system will react at an early stage. This allows early intervention

(for example treatment) which as a consequence will be more effective and also allows early choices to be made of the type of treatment which is appropriate for the patient, and which treatments should not be used. Chromosome interactions also reflect the current state of the individual and therefore can be used to assess changes to disease status. Furthermore there is little variation in the relevant chromosome interactions between individuals within the same group.

The chromosome interactions which are detected in the invention could be impacted by changes to the underlying DNA sequence, by environmental factors, DNA methylation, non-coding antisense RNA transcripts, non-mutagenic carcinogens, histone modifications, chromatin remodelling and specific local DNA interactions. However it must be borne in mind that chromosome interactions as defined herein are a regulatory modality in their own right and do not have a one to one correspondence with any genetic marker (DNA sequence change) or any other epigenetic marker.

The changes which lead to the chromosome interactions may be impacted by changes to the underlying nucleic acid sequence which themselves do not directly affect a gene product or the mode of gene expression. Such changes may be for example, SNPs within and/or outside of the genes, gene fusions and/or deletions of intergenic DNA, microRNA, and non-coding RNA. For example, it is known that roughly 20% of SNPs are in non-coding regions, and therefore the process as described is also informative in non-coding situation. Typically regions of the chromosome which come together to form the interaction are less than 5 kb, 3 kb, 1 kb, 500 base pairs or 200 base pairs apart on the same chromosome.

The Process of the Invention

The process of the invention comprises a typing system for detecting chromosome interactions relating to responder status. Any suitable typing method can be used, for example a method in which the proximity of the chromosomes in the interaction is detected and/or in which a marker that reflects chromosome interaction status is detected. The typing method may be performed using the *EpiSwitch*[™] system mentioned herein, which for example may be carried out by a method comprising the following steps (for example on DNA and/or a sample from the subject):

- (i) cross-linking regions of chromosome which have come together in a chromosome interaction;
- (ii) optionally isolating the cross-linked DNA from said chromosomal locus;
- (iii) subjecting the cross-linked DNA to cleavage; and

(iv) ligating the nucleic acids present in the cross-linked entity to derive ligated nucleic acids with sequence from both the regions which formed a chromosomal interaction.

Detection of this ligated nucleic acid allows determination of the presence or absence of a particular chromosome interaction. The ligated nucleic acid therefore acts as a marker for the presence of the chromosome interaction. Preferably the ligated nucleic acid is detected by PCR or a probe based method, including a qPCR method.

In the method the chromosomes can be cross-linked by any suitable means, for example by a cross-linking agent, which is typically a chemical compound. In a preferred aspect, the interactions are cross-linked using formaldehyde, but may also be cross-linked by any aldehyde, or D-Biotinoyl-e-aminocaproic acid-N-hydroxysuccinimide ester or Digoxigenin-3-O-methylcarbonyl-e-aminocaproic acid-N-hydroxysuccinimide ester. Para-formaldehyde can cross link DNA chains which are 4 Angstroms apart. Preferably the chromosome interactions are on the same chromosome. Typically the chromosome interactions are 2 to 10 Angstroms apart.

The cross-linking is preferably *in vitro*. The cleaving is preferably by restriction digestion with an enzyme, such as TaqI. The ligating may form DNA loops.

Where PCR (polymerase chain reaction) is used to detect or identify the ligated nucleic acid, the size of the PCR product produced may be indicative of the specific chromosome interaction which is present, and may therefore be used to identify the status of the locus. In preferred aspects the primers shown in any table herein are used, for example the primer pairs shown in Table 2 or 8 are used (corresponding to the chromosome interaction which is being detected). The primers shown in Table 1 may be used. Homologues of such primers or primer pairs may also be used, which can have at least 70% identity to the original sequence.

Where a probe is used to detect or identify the ligated nucleic acid, this is generally by Watson-Crick based base-pairing between the probe and ligated nucleic acid. Probe sequences as shown in any table herein may be used, for example the probe sequences shown in Table 2 or 8 (corresponding to the chromosome interaction which is being detected). Probe sequences as shown in Table 1 may be used. Homologues of such probe sequences may also be used, which can have at least 70% identity to the original sequence.

Typing according to the process of the invention may be carried out at multiple time points, for example to monitor the progression of the disease. This may be at one or more defined time points, for example at at least 1, 2, 5, 8 or 10 different time points. The durations between at least 1, 2, 5 or 8 of the time

points may be at least 5, 10, 20, 50, 80 or 100 days. Typically there are 3 time points at least 50 days apart.

The Individual to Tested and/or Treated

The individual who is tested in the method of the invention is preferably a eukaryote, animal, bird or mammal. Most preferably the individual is a human. The individual may be male or female. In the case of a human individual they are typically aged 65 or above.

The invention includes detecting and treating particular groups in a population, typically differing in their responder status, for example their response to immunotherapy. The inventors have discovered that chromosome interactions differ between these groups, and identifying these differences will allow physicians to categorize their patients as a part of a particular group of the population. The invention therefore provides physicians with a process of personalizing medicine for an individual based on their epigenetic chromosome interactions. Such testing may be used to select how to subsequently treat the patient, for example the type of drug that will be administered. The process of the invention may be carried out to select treatment for an individual, for example whether or not to give any specific treatment mentioned herein is administered to the individual.

The individual that is tested in the process of the invention may have been selected in some way, for example based on a risk factor, symptom or physical characteristic. The individual may have been selected based on having a symptom of cancer and/or or being in the early stages of cancer.

The individual may be susceptible to any cancer mentioned herein and/or may be in need of any therapy mentioned in. The individual may be receiving any therapy mentioned herein. In particular, the individual may have, or be suspected of having, cancer, for example any specific cancer mentioned herein.

Types of Cancer

The cancer which is relevant to the invention can include any cancer mentioned herein, and preferably is melanoma, lung cancer, non-small cell lung carcinoma (NSCLC), diffuse large B-cell lymphoma, liver cancer, hepatocellular carcinoma, prostate cancer, breast cancer, leukaemia, acute myeloid leukaemia, pancreatic cancer, thyroid cancer, nasal cancer, brain cancer, bladder cancer, cervical cancer, non-Hodgkin lymphoma, ovarian cancer, colorectal cancer or kidney cancer. The cancer may be one which can be treated by immunotherapy, for example any specific immunotherapy mentioned herein.

Types of Immunotherapy

The invention relates to determining response to immunotherapy, and in particular whether the individual is responsive to immunotherapy and/or whether they are hyper-progressors in whom immunotherapy will cause acceleration of disease.

- 5 Preferably the response which is determined is to a therapy which comprises a molecule or cell that is relevant to the immune system, such as a composition that comprises an antibody or immune cell (for example a T cell or dendritic cell) or any therapeutic substance mentioned herein. It may be response to a substance that modulates or stimulates the immune system, such as a vaccine therapy. The immunotherapy may modulate, block or stimulate an immune checkpoint, and thus may target or
- 10 modulate PD-L1, PD-L2 or CTLA4 or any other immune checkpoint molecule disclosed herein, and thus is preferably immunocheckpoint therapy. Preferably the response is responsiveness to an antibody therapy, or to any specific therapy disclosed herein. The therapy may be a combination therapy, for example any specific combination therapy disclosed herein.

In one embodiment the response is to a PD-1 inhibitor or PD-L1 inhibitor, including an antibody specific

15 for PD-1 or PD-L1. PD-1 is 'programmed cell death protein' and PD-L1 is 'programmed death-ligand 1'.

The term 'antibody' includes all fragments and derivatives of an antibody that retain the ability to bind the antigen target, for example single chain scFV's or Fab's.

The therapy may be mono or combination therapy, for example with immunocheckpoint modulators (preferably inhibitors) for PD-1 and or its ligand, PD-L1. The therapy could comprise administering at

20 least one immunocheckpoint modulator, for example as disclosed herein, such as in any table, figure or example. The therapy could be a combination of an anti-PD-1 or anti-PD-L1 combined with another drug that targets a checkpoint like CTLA4 (Ipilimumab/Yervoy) or small molecules. The PD-1 inhibitors could be pembrolizumab (Keytruda) or nivolumab (Opdivo). The modulator of PD-L1 or therapeutic agent could be Atezolizumab (Tecentriq), Avelumab (Bavencio), Durvalumab (Imfinzi), CA-170, Ipilimumab,

25 Tremelimumab, Nivolumab, Pembrolizumab, Pidilizumab, BMS935559, GVAXMPDL3280A, MEDI4736, MSB0010718C, MDX-1105/BMS-936559, AMP-224, MEDI0680.

The therapy may comprise administering agents that target and/or modulate interferon gamma or the JAK-STAT pathway.

The therapeutic agent may be any such agent disclosed in any table herein or may target any 'target' disclosed herein, including any protein disclosed herein. It is understood that any agent that is disclosed in a combination should be seen as also disclosed for administration individually.

Hyper-Progressors

- 5 Hyper-progression in cancer can be recognised in a straightforward manner by the skilled person, and it is preferably an increase in disease progression in cancer upon administration of immunotherapy and/or an adverse response to immunotherapy in an individual with cancer. It be measured using any suitable parameter for disease, such as a 2-fold increase in tumour size. It is typically more than a 50% increase in tumour burden within 60 days of administration of immunotherapy. In one aspect hyper-progressors
- 10 can be defined as having less than 60 days progression-free after administration of immunotherapy and/or overall survival of less than 150 days after immunotherapy.

Choice of Treatment

Based on the results of testing by the method of the invention decisions can be made as to what treatments will be administered or not administered to the individual.

- 15 If a person is found to be responsive to immunotherapy they could be given any immunotherapy mentioned herein. In one aspect if an individual is found to be a non-responsiveness then can be given a combination therapy, such as any combination therapy listed herein. Typically a combination therapy comprises an antibody and a small molecule.

The Data in the Tables Provided Herein

- 20 Tables 1, 2 and 8 show specific markers which can be used to detect responder status. Their presence or absence can be used in such a detection (i.e. they are 'disseminating' markers). Tables 1 and 8 show markers which detect responsiveness to immunotherapy, and the table shows which are linked to responsiveness and which are linked to non-responsiveness. Table 2 shows markers which detect hyper-progressors, and the tables shows which are linked to being a hyper-progressor and which are linked to
- 25 stable disease.

The markers are defined using probe sequences (which detect a ligated product as defined herein). The first two sets of Start-End positions show probe positions, and the second two sets of Start-End positions show the relevant 4kb region.

The following information is provided in the probe data table:

RP - Rsum the Rank Product statistics evaluated per each chromosome interaction.

FC - Interaction frequency (positive or negative).

Pfp - estimated percentage of false positive predictions (pfp), both considering positive and negative chromosome interactions.

5 Pval - estimated pvalues per each CCSs being positive and negative.

Adj.P.value (FDR) - False discovery rate adjusted p.value.

Type - which state the loop is found in.

Simple permutation-based estimation is used to determine how likely a given RP value or better is observed in a random experiment. This has the following steps:

- 10 1. Generate p permutations of k rank lists of length n .
2. Calculate the rank products of the n CCS in the p permutations.
3. Count (c) how many times the rank products of the CCS in the permutations are smaller or equal to the observed rank product. Set c to this value.
4. Calculate the average expected value for the rank product by: $Erp(g)=c/p$.
- 15 5. Calculate the percentage of false positives as: $pfp(g)=Erp(g)/rank(g)$ where $rank(g)$ is the rank of CCS g in a list of all n CCSs sorted by increasing RP.

The rank product statistic ranks chromosome interactions according to intensities within each microarray and calculates the product of these ranks across multiple microarrays. This technique can identify chromosome interactions that are consistently detected among the most differential

- 20 chromosome interactions in a number of replicated microarrays. Where the p-value is 0 this indicates that there is very little variation in the Rank Product of the CCS across the samples, this is a good example of the signal to noise and effect size of CCS. Where p value is 0 and pfp is 0 this means that permuted Rank Product doesn't differ from the actual observed Rank Product. These methods are described Breitling R and Herzyk P (2005) Rank-based methods as a non-parametric alternative of the t-
- 25 test for the analysis of biological microarray data. J Bioinf Comp Biol 3, 1171–1189.

The FC indicates prevalence of marker in each comparison, 2 means twice over average test, 1.5 means 1.5 over the average test, etc., and so FC indicates the weight of a marker to phenotype/group. The FC value can be used to give an indication of how many markers are needed for a highly effective test.

The probes are designed to be 30bp away from the Taq1 site. In case of PCR, PCR primers are typically designed to detect ligated product but their locations from the Taq1 site vary. Probe locations:

Start 1 - 30 bases upstream of TaqI site on fragment 1

End 1 - TaqI restriction site on fragment 1

5 Start 2 - TaqI restriction site on fragment 2

End 2 - 30 bases downstream of TaqI site on fragment 2

4kb Sequence Location:

Start 1 - 4000 bases upstream of TaqI site on fragment 1

End 1 - TaqI restriction site on fragment 1

10 Start 2 - TaqI restriction site on fragment 2

End 2 - 4000 bases downstream of TaqI site on fragment 2

Types of Detection

When detection is performed using a probe, typically sequence from both regions of the probe (i.e. from both sites of the chromosome interaction) could be detected. In preferred aspects probes are used in the process which comprise or consist of the same or complementary sequence to a probe shown in any table. In some aspects probes are used which comprise sequence which is homologous to any of the probe sequences shown in the tables.

The Approach Taken to Identify Markers and Panels of Markers

20 The invention described herein relates to chromosome conformation profile and 3D architecture as a regulatory modality in its own right, closely linked to the phenotype. The discovery of biomarkers was based on annotations through pattern recognition and screening on representative cohorts of clinical samples representing the differences in phenotypes. We annotated and screened significant parts of the genome, across coding and non-coding parts and over large sways of non-coding 5' and 3' of known genes for identification of statistically disseminating consistent conditional disseminating chromosome conformations, which for example anchor in the non-coding sites within (intronic) or outside of open reading frames.

In selection of the best markers we are driven by statistical data and p values for the marker leads. Selected and validated chromosome conformations within the signature are disseminating stratifying entities in their own right, irrespective of the expression profiles of the genes used in the reference.

Further work may be done on relevant regulatory modalities, such as SNPs at the anchoring sites, changes in gene transcription profiles, changes at the level of H3K27ac.

We are taking the question of clinical phenotype differences and their stratification from the basis of fundamental biology and epigenetic controls over phenotype - including for example from the
5 framework of network of regulation. As such, to assist stratification, one can capture changes in the network and it is preferably done through signatures of several biomarkers, for example through following a machine learning algorithm for marker reduction which includes evaluating the optimal number of markers to stratify the testing cohort with minimal noise. This may end with 3-20 markers.

Selection of markers for panels may be done by cross-validation statistical performance (and not for
10 example by the functional relevance of the neighbouring genes, used for the reference name).

A panel of markers (with names of adjacent genes) is a product of clustered selection from the screening across significant parts of the genome, in non-biased way analysing statistical disseminating powers over 14,000-60,000 annotated EpiSwitch sites across significant parts of the genome. It should not be
15 perceived as a tailored capture of a chromosome conformation on the gene of know functional value for the question of stratification. The total number of sites for chromosome interaction are 1.2 million, and so the potential number of combinations is 1.2 million to the power 1.2 million. The approach that we have followed nevertheless allows the identifying of the relevant chromosome interactions.

The specific markers that are provided by this application have passed selection, being statistically (significantly) associated with the condition or subgroup. This is what the data in the relevant table
20 demonstrates. Each marker can be seen as representing an event of biological epigenetic as part of network deregulation that is manifested in the relevant condition. In practical terms it means that these markers are prevalent across groups of patients when compared to controls. On average, as an example, an individual marker may typically be present in 80% of the relevant responder group and in 10% of controls, and therefore the results of the testing by the method of the invention is straightforward to
25 interpret and essentially amounts to a 'binary readout'.

Simple addition of all markers would not directly represent the network interrelationships between some of the deregulations. This is where the standard multivariate biomarker analysis GLMNET (R package) can be brought in. GLMNET package helps to identify interdependence between some of the markers, that reflect their joint role in achieving deregulations leading to disease phenotype. Modelling
30 and then testing markers with highest GLMNET scores offers not only identify the minimal number of markers that accurately identifies the patient cohort, but also the minimal number that offers the least

false positive results in the control group of patients, due to background statistical noise of low prevalence in the control group. Typically a group (combination) of selected markers (such as 3 to 11) offers the best balance between both sensitivity and specificity of detection, emerging in the context of multivariate analysis from individual properties of all the selected statistical significant markers for the condition.

The tables herein show the reference names for the array probes (60-mer) for array analysis that overlaps the juncture between the long range interaction sites, the chromosome number and the start and end of two chromosomal fragments that come into juxtaposition.

In a preferred aspect all 11 of the markers of Table 1 are typed. In another preferred aspect all 11 of the markers of Table 2 are typed. In another preferred aspect all 8 of the markers of Table 8 are typed.

Samples and Sample Treatment

The process of the invention will normally be carried out on a sample. The sample may be obtained at a defined time point, for example at any time point defined herein. The sample will normally contain DNA from the individual. It will normally contain cells. In one aspect a sample is obtained by minimally invasive means, and may for example be a blood sample. DNA may be extracted and cut up with a standard restriction enzyme. This can pre-determine which chromosome conformations are retained and will be detected with the EpiSwitch™ platforms. Due to the synchronisation of chromosome interactions between tissues and blood, including horizontal transfer, a blood sample can be used to detect the chromosome interactions in tissues, such as tissues relevant to disease.

Preferred Aspects for Sample Preparation and Chromosome Interaction Detection

Methods of preparing samples and detecting chromosome conformations are described herein. Optimised (non-conventional) versions of these processes can be used, for example as described in this section.

Typically the sample will contain at least 2×10^5 cells. The sample may contain up to 5×10^5 cells. In one aspect, the sample will contain 2×10^5 to 5.5×10^5 cells.

Crosslinking of epigenetic chromosomal interactions present at the chromosomal locus is described herein. This may be performed before cell lysis takes place. Cell lysis may be performed for 3 to 7 minutes, such as 4 to 6 or about 5 minutes. In some aspects, cell lysis is performed for at least 5 minutes and for less than 10 minutes.

Digesting DNA with a restriction enzyme is described herein. Typically, DNA restriction is performed at about 55°C to about 70°C, such as for about 65°C, for a period of about 10 to 30 minutes, such as about 20 minutes.

Preferably a frequent cutter restriction enzyme is used which results in fragments of ligated DNA with an average fragment size up to 4000 base pair. Optionally the restriction enzyme results in fragments of ligated DNA have an average fragment size of about 200 to 300 base pairs, such as about 256 base pairs. In one aspect, the typical fragment size is from 200 base pairs to 4,000 base pairs, such as 400 to 2,000 or 500 to 1,000 base pairs.

In one aspect of the EpiSwitch process a DNA precipitation step is not performed between the DNA restriction digest step and the DNA ligation step.

DNA ligation is described herein. Typically the DNA ligation is performed for 5 to 30 minutes, such as about 10 minutes.

The protein in the sample may be digested enzymatically, for example using a proteinase, optionally Proteinase K. The protein may be enzymatically digested for a period of about 30 minutes to 1 hour, for example for about 45 minutes. In one aspect after digestion of the protein, for example Proteinase K digestion, there is no cross-link reversal or phenol DNA extraction step.

In one aspect PCR detection is capable of detecting a single copy of the ligated nucleic acid, preferably with a binary read-out for presence/absence of the ligated nucleic acid.

Figure 1 shows a preferred process of detecting chromosome interactions.

Processes and Uses of the Invention

The process of the invention can be described in different ways. It can be described as a process of making one or more ligated nucleic acids comprising (i) *in vitro* cross-linking of chromosome regions which have come together in a chromosome interaction; (ii) subjecting said cross-linked DNA to cutting or restriction digestion cleavage; and (iii) ligating said cross-linked cleaved DNA ends to form one or more ligated nucleic acids, wherein optionally detection of the ligated nucleic acid may be used to determine the chromosome state at a locus, and wherein preferably the chromosomal interactions may be 1, 3, 5, 8 or all the chromosome interactions of Table 1 or 2. In this process the chromosomal interactions may be 1, 3, 5 or 8 of the chromosome interactions of Table 8.

Homologues

Homologues of polynucleotide / nucleic acid (e.g. DNA) sequences are referred to herein. Such homologues typically have at least 70% homology, preferably at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98% or at least 99% homology, for example over a region of at least 10, 15, 20, 30, 100 or more contiguous nucleotides, or across the portion of the nucleic acid which is from the region of the chromosome involved in the chromosome interaction. The homology may be calculated on the basis of nucleotide identity (sometimes referred to as "hard homology").

Therefore, in a particular aspect, homologues of polynucleotide / nucleic acid (e.g. DNA) sequences are referred to herein by reference to percentage sequence identity. Typically such homologues have at least 70% sequence identity, preferably at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98% or at least 99% sequence identity, for example over a region of at least 10, 15, 20, 30, 100 or more contiguous nucleotides, or across the portion of the nucleic acid which is from the region of the chromosome involved in the chromosome interaction. The homologues may have at least 70% sequence identity, preferably at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98% or at least 99% sequence identity across the entire probe, primer or primer pair.

For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology and/or % sequence identity (for example used on its default settings) (Devereux *et al* (1984) Nucleic Acids Research 12, p387-395). The PILEUP and BLAST algorithms can be used to calculate homology and/or % sequence identity and/or line up sequences (such as identifying equivalent or corresponding sequences (typically on their default settings)), for example as described in Altschul S. F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10.

Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W5 T and X determine the sensitivity and

speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) Proc. Natl. Acad. Sci. USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g.,
 5 Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two polynucleotide sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about
 10 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

The homologous sequence typically differs by 1, 2, 3, 4 or more bases, such as less than 10, 15 or 20 bases (which may be substitutions, deletions or insertions of nucleotides). These changes may be measured across any of the regions mentioned above in relation to calculating homology and/or % percentage sequence identity.

15 Homology of a 'pair of primers' can be calculated, for example, by considering the two sequences as a single sequence (as if the two sequences are joined together) for the purpose of then comparing against the another primer pair which again is considered as a single sequence.

The Threshold of Detection

The markers which are disclosed herein have been found to be 'disseminating markers' capable of
 20 determining responder status and tables 1 and 2 show which responder group each marker is present in (responder/non-responder to immunotherapy, or hyper-progressor/stable disease).

In practical terms it means that these markers are prevalent across the relevant responder group when compared to controls (as is shown by the FC value, for example). On average, as an example, an individual marker may typically be present in 80% of the relevant responder group and in 10% of
 25 controls. When testing an individual the result will be a combination of 'present' and 'absent' chromosome interactions for each of the markers shown in Table 1 or 2 allowing determination of the responder group for the individual. Typically presence/absence of at least 8 markers out of 11 compared to the 'ideal' result shown in the table can be used to assign the individual to a responder group.

Therapeutic Agents and Treatments

This section is relevant both to immunotherapies which define the responder group of the individual and also to therapy which may be given to individuals based on the results of the testing method of the invention.

5 The invention provides therapeutic agents for use in preventing or treating any condition mentioned herein. This may comprise administering to an individual in need a therapeutically effective amount of the agent. The invention provides use of the agent in the manufacture of a medicament to prevent or treat the condition, for example in individuals tested by the method of the invention.

10 The formulation of the agent will depend upon the nature of the agent. The agent will be provided in the form of a pharmaceutical composition containing the agent and a pharmaceutically acceptable carrier or diluent. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. Typical oral dosage compositions include tablets, capsules, liquid solutions and liquid suspensions. The agent may be formulated for parenteral, intravenous, intramuscular, subcutaneous, transdermal or oral administration.

15 The dose of an agent may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the individual to be treated; the route of administration; and the required regimen. A physician will be able to determine the required route of administration and dosage for any particular agent. A suitable dose may however be from 0.1 to 100 mg/kg body weight such as 1 to 40 mg/kg body weight, for example, to be taken from 1 to 3 times daily.

20 The invention provides an immunotherapeutic agent, preferably selected from any of tables 4 to 6, for use in a method of treating an individual identified as being responsive to immunotherapy, optionally said method comprising:

- identifying whether an individual is responsive to immunotherapy by the method of the invention, and
- administering to any individual identified as responsive to immunotherapy said agent.

25 The invention provides (i) a combination immunotherapy or (ii) a therapeutic agent which is not an immunotherapy for use in a method of treating an individual identified as being non-responsive to immunotherapy, optionally said method comprising:

- identifying whether an individual is responsive to immunotherapy by the method of the invention, and

- administering to any individual identified as non-responsive (i) and/or (ii), wherein optionally the combination therapy of (i) is any combination therapy shown in table 4 or which comprises at least one agent chosen from tables 4 and 5.

Screening for Therapeutic Agents

- 5 The invention provides a screening method to identify therapeutic agents for cancer comprising determining whether a candidate agent is able to cause a change to all of the chromosome interactions shown in Table 1 and/or Table 2. This screening method may comprise determining whether a candidate agent is able to cause a change to all of the chromosome interactions shown in Table 8.

Nucleic Acids of the Inventions

- 10 The invention provides certain nucleic acids, including probes and primers. Preferably the nucleic acids are DNA. It is understood that where a specific sequence is provided the invention may use the complementary sequence as required in the particular aspect.

The primers or probes shown in Table 1 or 2 may be used in the invention. In one aspect probes or primers are used which comprise any of: the sequences shown in Table 1 or 2; or fragments and/or

- 15 homologues of any sequence shown in Table 1 or 2. The primers or probes shown in Table 8 may be used in the invention. In one aspect probes or primers are used which comprise any of: the sequences shown in Table 8; or fragments and/or homologues of any sequence shown in Table 8.

Labelled Nucleic Acids and Pattern of Hybridisation

- The nucleic acids mentioned herein may be labelled, preferably using an independent label such as a
20 fluorophore (fluorescent molecule) or radioactive label which assists detection of successful hybridisation. Certain labels can be detected under UV light.

Forms of the Substance Mentioned Herein

- Any of the substances, such as nucleic acids or therapeutic agents, mentioned herein may be in purified or isolated form. They may be in a form which is different from that found in nature, for example they
25 may be present in combination with other substance with which they do not occur in nature. The nucleic acids (including portions of sequences defined herein) may have sequences which are different to those found in nature, for example having at least 1, 2, 3, 4 or more nucleotide changes in the sequence as described in the section on homology. The nucleic acids may have heterologous sequence at the 5' or 3' end. The nucleic acids may be chemically different from those found in nature, for

example they may be modified in some way, but preferably are still capable of Watson-Crick base pairing. Where appropriate the nucleic acids will be provided in double stranded or single stranded form. The invention provides all of the specific nucleic acid sequences mentioned herein in single or double stranded form, and thus includes the complementary strand to any sequence which is disclosed.

- 5 The invention provides a kit for carrying out any process of the invention, including detection of a chromosomal interaction relating to prognosis. Such a kit can include a specific binding agent capable of detecting the relevant chromosomal interaction, such as agents capable of detecting a ligated nucleic acid generated by processes of the invention. Preferred agents present in the kit include probes capable of hybridising to the ligated nucleic acid or primer pairs, for example as described herein, capable of
10 amplifying the ligated nucleic acid in a PCR reaction. Preferred agents include any of the specific primers and probes disclosed herein and/or homologues of such primers and probes.

The invention provides a device that is capable of detecting the relevant chromosome interactions. The device preferably comprises any specific binding agents, probe or primer pair capable of detecting the chromosome interaction, such as any such agent, probe or primer pair described herein.

15 *Detection Process*

- In one aspect quantitative detection of the ligated sequence which is relevant to a chromosome interaction is carried out using a probe which is detectable upon activation during a PCR reaction, wherein said ligated sequence comprises sequences from two chromosome regions that come together in an epigenetic chromosome interaction, wherein said process comprises contacting the ligated
20 sequence with the probe during a PCR reaction, and detecting the extent of activation of the probe, and wherein said probe binds the ligation site. The process typically allows particular interactions to be detected in a MIQE compliant manner using a dual labelled fluorescent hydrolysis probe.

- The probe is generally labelled with a detectable label which has an inactive and active state, so that it is only detected when activated. The extent of activation will be related to the extent of template (ligation
25 product) present in the PCR reaction. Detection may be carried out during all or some of the PCR, for example for at least 50% or 80% of the cycles of the PCR.

- The probe can comprise a fluorophore covalently attached to one end of the oligonucleotide, and a quencher attached to the other end of the nucleotide, so that the fluorescence of the fluorophore is quenched by the quencher. In one aspect the fluorophore is attached to the 5' end of the
30 oligonucleotide, and the quencher is covalently attached to the 3' end of the oligonucleotide.

Fluorophores that can be used in the process of the invention include FAM, TET, JOE, Yakima Yellow,

HEX, Cyanine3, ATTO 550, TAMRA, ROX, Texas Red, Cyanine 3.5, LC610, LC 640, ATTO 647N, Cyanine 5, Cyanine 5.5 and ATTO 680. Quenchers that can be used with the appropriate fluorophore include TAM, BHQ1, DAB, Eclip, BHQ2 and BBQ650, optionally wherein said fluorophore is selected from HEX, Texas Red and FAM. Preferred combinations of fluorophore and quencher include FAM with BHQ1 and Texas Red with BHQ2.

Use of the Probe in a qPCR Assay

Hydrolysis probes of the invention are typically temperature gradient optimised with concentration matched negative controls. Preferably single-step PCR reactions are optimized. More preferably a standard curve is calculated. An advantage of using a specific probe that binds across the junction of the ligated sequence is that specificity for the ligated sequence can be achieved without using a nested PCR approach. The processes described herein allow accurate and precise quantification of low copy number targets. The target ligated sequence can be purified, for example gel-purified, prior to temperature gradient optimization. The target ligated sequence can be sequenced. Preferably PCR reactions are performed using about 10ng, or 5 to 15 ng, or 10 to 20ng, or 10 to 50ng, or 10 to 200ng template DNA.

Forward and reverse primers are designed such that one primer binds to the sequence of one of the chromosome regions represented in the ligated DNA sequence, and the other primer binds to other chromosome region represented in the ligated DNA sequence, for example, by being complementary to the sequence.

Choice of Ligated DNA Target

The invention includes selecting primers and a probe for use in a PCR process as defined herein comprising selecting primers based on their ability to bind and amplify the ligated sequence and selecting the probe sequence based properties of the target sequence to which it will bind, in particular the curvature of the target sequence.

Probes are typically designed/chosen to bind to ligated sequences which are juxtaposed restriction fragments spanning the restriction site. In one aspect of the invention, the predicted curvature of possible ligated sequences relevant to a particular chromosome interaction is calculated, for example using a specific algorithm referenced herein. The curvature can be expressed as degrees per helical turn, e.g. 10.5° per helical turn. Ligated sequences are selected for targeting where the ligated sequence has a curvature propensity peak score of at least 5° per helical turn, typically at least 10°, 15° or 20° per helical turn, for example 5° to 20° per helical turn. Preferably the curvature propensity score per helical turn is calculated for at least 20, 50, 100, 200 or 400 bases, such as for 20 to 400 bases upstream and/or

downstream of the ligation site. Thus in one aspect the target sequence in the ligated product has any of these levels of curvature. Target sequences can also be chosen based on lowest thermodynamic structure free energy.

Particular Aspects

- 5 In particular aspects certain chromosome interactions are not typed, for example any specific interaction mentioned not mentioned herein. In some aspects only the markers of Table 1 or Table 2 are typed and no other markers are typed. In some aspects only the markers of Table 2 or Table 8 are typed and no other markers are typed. In some aspect only the markers of Table 1 and Table 2 are typed and no other markers are typed. In some aspect only the markers of Table 2 and Table 8 are typed and no
10 other markers are typed.

Paragraphs Describing the Invention

The invention includes aspects described in the following numbered paragraphs:

1. A method of determining how an individual responds to immunotherapy for cancer comprising detecting the presence or absence in the individual of:
 - 15 - all of the chromosome interactions shown in Table 1 to thereby determine whether the individual will be responsive to immunotherapy; and/or
 - all of the chromosome interactions shown in Table 2 to thereby determine whether the individual is a hyper-progressor in whom immunotherapy will accelerate disease.
2. A method according to paragraph 1 wherein the presence or absence of the chromosome interactions
20 is determined:
 - in a sample from the individual, and/or
 - in DNA from the individual, and/or
 - by detecting the presence or absence of a DNA loop at the site of the chromosome interactions, and/or
 - detecting the presence or absence of distal regions of a chromosome being brought together in a
25 chromosome conformation, and/or
 - by detecting the presence of a ligated nucleic acid which is generated during said typing and whose sequence comprises two regions each corresponding to the regions of the chromosome which come together in the chromosome interaction, and/or

- by a process which detects the proximity of the chromosome regions which have come together in the chromosome interaction.

3. A method according to paragraph 1 or 2 wherein said detecting of the presence or absence of the chromosome interactions is by a process comprising:

- 5 (i) in vitro crosslinking of epigenetic chromosomal interactions which are present;
 - (ii) optionally isolating the cross-linked DNA;
 - (iii) subjecting said cross-linked DNA to cleaving;
 - (iv) ligating said cross-linked cleaved DNA ends to form ligated DNA; and
 - (v) identifying the presence or absence in said ligated DNA of a DNA sequence that corresponds to each
 - 10 chromosome interaction;
- to thereby determine the presence or absence of each chromosome interaction.

4. A method according to paragraph 2 or 3 wherein said ligated DNA is detected by PCR or by use of a probe.

5. A method according to paragraph 4 wherein:

- 15 (i) detection is by use of a probe, wherein said probe preferably has at least 70% identity to any of the probes shown in Table 1 or 2, or
- (ii) detection is by use of PCR, wherein the PCR preferably uses a primer pair that has at least 70% identity to any of the primer pairs shown in Table 1 or 2.

6. A method according to any one of the preceding paragraphs wherein:

- 20 (i) the method is carried out prior to the individual receiving immunotherapy and/or is carried out to select which therapy the individual should receive for cancer, and/or
- (ii) the method is carried out on an individual that has cancer or is suspected of having cancer, and/or
- (iii) the method is carried out on individual that has been preselected based on a physical characteristic, risk factor or the presence of a symptom for cancer.

25 7. A method according to any one of the preceding paragraphs in which the individual:

- is at an early stage of cancer; and/or

- is undergoing, or is about to undergo, cancer therapy, for example cancer immunotherapy.

8. A method according to any one of the preceding paragraphs wherein the cancer is:

(i) one in which immune-checkpoint inhibitors PD-1/PD-L1 are used for therapy; and/or

(ii) melanoma, lung cancer, hepatocellular carcinoma (liver cancer), bladder, prostate, nasal cancer, parotid gland cancer (salivary gland cancer), alveolar soft part sarcoma (soft tissue cancer); and/or

(iii) breast cancer, cervical cancer, colon cancer, head and neck cancer, Hodgkin lymphoma, kidney cancer, stomach cancer, rectal cancer or a solid tumour.

9. A method according to any one of the preceding paragraphs in which the immunotherapy:

(i) comprises an antibody or immune cell, preferably a T cell or dendritic cell; and/or

(ii) comprises a vaccine, preferably against the cancer; and/or

(iii) modulates, blocks or stimulates an immune checkpoint, and preferably targets or modulates PD-L1, PD-L2 or CTLA4 or any other immune checkpoint molecule disclosed in Table 3; and/or

(iv) comprises a therapy shown in any one of tables 4 to 6; and/or

(v) increases the killing of cancer cells by the immune system, preferably wherein such killing is by a T cell.

10. A method according to any one of the preceding paragraphs wherein the immunotherapy is:

(i) a PD-1 inhibitor or PD-L1 inhibitor, and is preferably an antibody specific for PD-1 or PD-L1; and/or

(ii) a PD-2 inhibitor or PD-L2 inhibitor, and is preferably an antibody specific for PD-2 or PD-L2.

11. A method according to any one of the preceding paragraphs, wherein the typing of chromosome interactions comprises specific detection of the ligated product by quantitative PCR (qPCR) which uses primers capable of amplifying the ligated product and a probe which binds the ligation site during the PCR reaction, wherein said probe comprises sequence which is complementary to sequence from each of the chromosome regions that have come together in the chromosome interaction, wherein preferably said probe comprises:

- an oligonucleotide which specifically binds to said ligated product, and/or

- a fluorophore covalently attached to the 5' end of the oligonucleotide, and/or

- a quencher covalently attached to the 3' end of the oligonucleotide, and
optionally

- said fluorophore is selected from HEX, Texas Red and FAM; and/or

- said probe comprises a nucleic acid sequence of length 10 to 40 nucleotide bases, preferably a length
5 of 20 to 30 nucleotide bases.

12. An immunotherapy for cancer for use in a method of treating a cancer in an individual, wherein said method of treating comprises:

- identifying whether the individual is responsive to immunotherapy by the method of any one of the preceding paragraphs, and

10 - administering to an individual that has been identified responsive to immunotherapy said immunotherapy.

13. A combination therapy for cancer for use in a method of treating a cancer in an individual, wherein said method of treating comprises:

15 - identifying whether the individual is responsive to immunotherapy by the method of any one of the preceding paragraphs, and

- administering to an individual that has been identified non-responsive to immunotherapy said combination therapy, wherein said combination therapy comprises a therapeutic agent disclosed in any of tables 4 to 6 or a combination therapy disclosed in any of tables 4 to 6.

20 14. An anti-cancer therapy which is not an immunotherapy for use in a method of treating a cancer in an individual, wherein said method of treating comprises:

- identifying whether the individual is a hyper-progressor for immunotherapy by the method of any one of the preceding paragraphs, and

- administering to an individual that has been identified as being a hyper-progressor for immunotherapy said anti-cancer therapy.

25 *Disclosure in Publications and Priority Applications*

The contents of all publications mentioned herein are incorporated by reference into the present specification and may be used to further define the features relevant to the invention. The contents of

all priority applications are incorporated by reference into the present specification and may be used to define the features relevant to the invention.

Techniques Used to Identify the Specific Relevant Chromosome Interactions

The EpiSwitch™ platform technology detects epigenetic regulatory signatures of regulatory changes between normal and abnormal conditions at loci. The EpiSwitch™ platform identifies and monitors the fundamental epigenetic level of gene regulation associated with regulatory high order structures of human chromosomes also known as chromosome conformation signatures. Chromosome signatures are a distinct primary step in a cascade of gene deregulation. They are high order biomarkers with a unique set of advantages against biomarker platforms that utilize late epigenetic and gene expression biomarkers, such as DNA methylation and RNA profiling.

EpiSwitch™ Array Assay

The custom EpiSwitch™ array-screening platforms come in 4 densities of, 15K, 45K, 100K, and 250K unique chromosome conformations, each chimeric fragment is repeated on the arrays 4 times, making the effective densities 60K, 180K, 400K and 1 million respectively.

Custom Designed EpiSwitch™ Arrays

The 15K EpiSwitch™ array can screen the whole genome including around 300 loci interrogated with the EpiSwitch™ Biomarker discovery technology. The EpiSwitch™ array is built on the Agilent SurePrint G3 Custom CGH microarray platform; this technology offers 4 densities, 60K, 180K, 400K and 1 million probes. The density per array is reduced to 15K, 45K, 100K and 250K as each EpiSwitch™ probe is presented as a quadruplicate, thus allowing for statistical evaluation of the reproducibility. The average number of potential EpiSwitch™ markers interrogated per genetic loci is 50, as such the numbers of loci that can be investigated are 300, 900, 2000, and 5000.

EpiSwitch™ Custom Array Pipeline

The EpiSwitch™ array is a dual colour system with one set of samples, after EpiSwitch™ library generation, labelled in Cy5 and the other of sample (controls) to be compared/ analyzed labelled in Cy3. The arrays are scanned using the Agilent SureScan Scanner and the resultant features extracted using the Agilent Feature Extraction software. The data is then processed using the EpiSwitch™ array processing scripts in R. The arrays are processed using standard dual colour packages in Bioconductor in R: Limma*. The normalisation of the arrays is done using the normalisedWithinArrays function in Limma* and this is done to the on chip Agilent positive controls and EpiSwitch™ positive controls. The

data is filtered based on the Agilent Flag calls, the Agilent control probes are removed and the technical replicate probes are averaged, in order for them to be analysed using Limma*. The probes are modelled based on their difference between the 2 scenarios being compared and then corrected by using False Discovery Rate. Probes with Coefficient of Variation (CV) $\leq 30\%$ that are ≤ -1.1 or ≥ 1.1 and pass the

5 p ≤ 0.1 FDR p-value are used for further screening. To reduce the probe set further Multiple Factor Analysis is performed using the FactorMineR package in R.

* Note: LIMMA is Linear Models and Empirical Bayes Processes for Assessing Differential Expression in Microarray Experiments. Limma is an R package for the analysis of gene expression data arising from microarray or RNA-Seq.

- 10 The pool of probes is initially selected based on adjusted p-value, FC and CV $< 30\%$ (arbitrary cut off point) parameters for final picking. Further analyses and the final list are drawn based only on the first two parameters (adj. p-value; FC).

Statistical Pipeline

- 15 EpiSwitch™ screening arrays are processed using the EpiSwitch™ Analytical Package in R in order to select high value EpiSwitch™ markers for translation on to the EpiSwitch™ PCR platform.

Step 1

- Probes are selected based on their corrected p-value (False Discovery Rate, FDR), which is the product of a modified linear regression model. Probes below p-value ≤ 0.1 are selected and then further reduced by their Epigenetic ratio (ER), probes ER have to be ≤ -1.1 or ≥ 1.1 in order to be selected for further
- 20 analysis. The last filter is a coefficient of variation (CV), probes have to be below ≤ 0.3 .

Step 2

The top 40 markers from the statistical lists are selected based on their ER for selection as markers for PCR translation. The top 20 markers with the highest negative ER load and the top 20 markers with the highest positive ER load form the list.

- 25 *Step 3*

The resultant markers from step 1, the statistically significant probes form the bases of enrichment analysis using hypergeometric enrichment (HE). This analysis enables marker reduction from the significant probe list, and along with the markers from step 2 forms the list of probes translated on to the EpiSwitch™ PCR platform.

The statistical probes are processed by HE to determine which genetic locations have an enrichment of statistically significant probes, indicating which genetic locations are hubs of epigenetic difference.

The most significant enriched loci based on a corrected p-value are selected for probe list generation.

Genetic locations below p-value of 0.3 or 0.2 are selected. The statistical probes mapping to these

- 5 genetic locations, with the markers from step 2, form the high value markers for EpiSwitch™ PCR translation.

Array design and processing

Array Design

Genetic loci are processed using the SII software (currently v3.2) to:

- 10 - Pull out the sequence of the genome at these specific genetic loci (gene sequence with 50kb upstream and 20kb downstream)
- Define the probability that a sequence within this region is involved in CCs
- Cut the sequence using a specific RE
- Determine which restriction fragments are likely to interact in a certain orientation
- 15 - Rank the likelihood of different CCs interacting together.
- Determine array size and therefore number of probe positions available (x)
- Pull out x/4 interactions.
- For each interaction define sequence of 30bp to restriction site from part 1 and 30bp to restriction site of part 2. Check those regions are not repeats, if so exclude and take next interaction down on the list.
- 20 Join both 30bp to define probe.
- Create list of x/4 probes plus defined control probes and replicate 4 times to create list to be created on array
- Upload list of probes onto Agilent Sure design website for custom CGH array.
- Use probe group to design Agilent custom CGH array.

25 *Array Processing*

- Process samples using EpiSwitch™ Standard Operating Procedure (SOP) for template production.

- Clean up with ethanol precipitation by array processing laboratory.
- Process samples as per Agilent SureTag complete DNA labelling kit - Agilent Oligonucleotide Array-based CGH for Genomic DNA Analysis Enzymatic labelling for Blood, Cells or Tissues
- Scan using Agilent C Scanner using Agilent feature extraction software.

5 *EpiSwitch*[™] biomarker signatures demonstrate high robustness, sensitivity and specificity in the stratification of complex disease phenotypes. This technology takes advantage of the latest breakthroughs in the science of epigenetics, monitoring and evaluation of chromosome conformation signatures as a highly informative class of epigenetic biomarkers. Current research methods deployed in academic environment require from 3 to 7 days for biochemical processing of cellular material in order
10 to detect CCSs. Those procedures have limited sensitivity, and reproducibility; and furthermore, do not have the benefit of the targeted insight provided by the *EpiSwitch*[™] Analytical Package at the design stage.

EpiSwitch[™] Array *in silico* marker identification

CCS sites across the genome are directly evaluated by the *EpiSwitch*[™] Array on clinical samples from
15 testing cohorts for identification of all relevant stratifying lead biomarkers. The *EpiSwitch*[™] Array platform is used for marker identification due to its high-throughput capacity, and its ability to screen large numbers of loci rapidly. The array used was the Agilent custom-CGH array, which allows markers identified through the *in silico* software to be interrogated.

EpiSwitch[™] PCR

20 Potential markers identified by *EpiSwitch*[™] Array are then validated either by *EpiSwitch*[™] PCR or DNA sequencers (i.e. Roche 454, Nanopore MinION, etc.). The top PCR markers which are statistically significant and display the best reproducibility are selected for further reduction into the final *EpiSwitch*[™] Signature Set, and validated on an independent cohort of samples. *EpiSwitch*[™] PCR can be performed by a trained technician following a standardised operating procedure protocol established.
25 All protocols and manufacture of reagents are performed under ISO 13485 and 9001 accreditation to ensure the quality of the work and the ability to transfer the protocols. *EpiSwitch*[™] PCR and *EpiSwitch*[™] Array biomarker platforms are compatible with analysis of both whole blood and cell lines. The tests are sensitive enough to detect abnormalities in very low copy numbers using small volumes of blood.

Use of a Classifier

The method of the invention may include analysis of the chromosome interactions identified in the individual, for example using a classifier, which may increase performance, such as sensitivity or specificity. The classifier is typically one that has been 'trained' on samples from the population and such training may assist the classifier to detect any responder group mentioned herein.

The invention is illustrated by the following:

ExamplesExample 1. Development of a Universal Marker Set and a Marker Set for Detecting Hyper-progressors

In working on populations of patients undergoing immunotherapy for cancer two distinct marker sets were developed: one is a universal marker set that allows responsiveness to therapy to be detected across a range of cancers and specific therapies, and the second is a marker set that detects hyper-progressors who should never be treated with particular types of immunotherapy.

We have now defined a specialised distinct and optimised panel of 11 biomarkers (the universal set), from a few hundreds identified in an original array screen and later tested on specific cohorts of patients. The unique feature of each of these 11 markers is that each of them is statistically significant (as part of the discovered core) across all PD-1/PD-L1 cases in all tested oncological indications, defining a universal core of response/non-response to treatment by PD-1/PD-L1. A classifier using these 11 markers works very robustly as a distinct performance entity across all tested patient cohorts.

As background to the present work, Figure 1 shows performance of baseline prediction of response /non-response for avelumab (PD-L1) in NSCLC for based on a large set of markers tested.

In contrast, for the universal 11 marker set the list of all treatments by various therapeutic assets PD-1/PD-L1 and various oncological indications we have worked with is shown in List A below: - PD-1 and PD-L1 assets, such as pembrolizumab, durvalumab, avelumab, atezolizumab, in melanoma, NSCLC, Lung, HCC, Bladder, Prostate, NPC, Parotid Gland, Alveolar Soft Part Sarcoma. The universal 11 marker classifier works well across all those cohorts and identifies universal profile that delivers robust baseline classification for response/non-response, irrespective of which exactly PD-1 or PD-L1 treatment and what exactly type of cancer was tested. We capture with 11 markers a very specific conducive/non-conductive epigenetic systemic network set up of features which define outcomes in immune-checkpoint therapies.

Turning to the second set of markers, a very serious issue in cancer immunotherapy is the consistent presence of a subgroup of patients who should never be treated with PD-1/PD-L1 therapies. They are called hyper-progressors (or super-progressors), where progressor means progression into disease. These patients upon treatment react very differently - their rate of tumour growth shoots up and they die very quickly, essentially in a matter of weeks.

Hyper-progressors can be defined as patients who respond adversely to immuno-checkpoint immuno-oncology treatment by demonstrating significant reduction in either progression-free survival (as a measure of survival in response to drug treatment) (PFS<60 days) or overall survival (OS<150 days).

Average trials demonstrate between 8-15% of their patients as showing a super-progressor profile.

Currently, there are no means to identify and exclude these patients to prevent serious adverse effect of the immunotherapy. In most studies hyper-progressors are categorised with the bigger group of non-responders, also termed as progressors/progressive disease (PD). Most of non-responders are patients who do not benefit from immunotherapy. Today, the use of checkpoint inhibitors is justified by the overall benefits among the percentage of patients who respond to immunotherapy (10-70%).

Here we utilized patients from immunotherapy cohorts, focusing on those with a PFS/OS within the range of hyper-progressors and those beyond those survival time limits (marked S as "Standard" in the slides and tables). The hyper-progressor markers identify patient profile that is predicted to demonstrate short PFS/OS upon the treatment. On a group of 32 patients (equal arms of H and S) tested before treatment, when compared to PFS and OS after the treatment 11 super-progressor markers predicted correctly 15 out of 17 H (sensitivity 0.88), 14/15 (specificity 0.93), 15/16 (PPV 0.94), 14/16 (0.875).

These markers are specifically selected to identify and exclude patients prior to treatment on the basis of a predicted severe reduction in their survival as a consequence of immunotherapy. This could be seen as a subgroup of the bigger cohort of non-responders that could be identified and predicted by the universal 11 marker set.

Whilst the present work has been carried out on patients with melanoma, lung cancer, hepatocellular carcinoma (liver cancer), bladder, prostate, nasal cancer, parotid gland (salivary gland cancer), alveolar soft part sarcoma (soft tissue cancer), it also applies to other cancers where immune-checkpoint inhibitors PD-1/PD-L1 are used for therapy, such as breast cancer, cervical cancer, colon cancer, head and neck cancer, Hodgkin lymphoma, kidney cancer, stomach cancer, rectal cancer, and any solid tumour.

Mechanism of Detection

The marker sets that have been identified capture a network of deregulations at the level of cellular 3D genomics, which reflects a network of deregulated cell types acting in conjunction to sustain and advance the pathological or physiological phenotype of cancer. So far, observing statistically significant chromosome conformations as evidence of deregulation in conjunction with cell sub-typing CD loci, we can state that observed universal signatures contain and represent deregulations in T cells, NK (natural killer) cells, macrophages, B cells and dendritic cells (DC). This emphasizes the role played by the specific set up at cellular level of the adaptive and innate immune system in individual patients as part of the cancer-host interaction, which defines disease progression (hyper-progressors) and responsiveness to immuno-checkpoint inhibitors PD-1/PD-L1.

Methods

Initial studies of chromosome interactions were carried out on the following populations (work described in Figure 1):

List A – Initial work

- 16 anti-PD-1 (Pembrolizumab) Melanoma cohort
- 16 anti-PD-L1 NSCLC cohort
- 99 anti-PD-L1 NSCLC cohort
- 49 anti-PD-1 NSCLC cohort
- 50 anti-PD-1 and combined therapy NSCLC cohort
- 48 anti-PD-1 (pembrolizumab) Melanoma cohort, including hyper-progressors
- 550 anti-PD-L1 Urethral Cancer

Observational Longitudinal:

- anti-PD-L1 (Durvalumab, Atezolizumab) and anti-PD1(Pembrolizumab)
- Lung, HCC, Bladder, Prostate, NPC, Parotid Gland, Alveolar Soft Part Sarcoma.

The marker sets were developed using the following patients:

Training 80 patients all NSCLC

Mixture of 1L, 2L Avelumab (54) and 2L Pembrolizumab (36)

Test: 38 Patients all NSCLC

Mixture of 1L, 2L Avelumab (27) and 2L Pembrolizumab (11)

5 Test: 20 Samples Mixture

Mixture 2L patients with different Check point inhibitors and different solid tumours

Atezolizumab (7), Durvalumab (3), and Pembrolizumab (10)

Lung (13), NPC (2), HCC (2), Bladder (1), Alveolar Sarcoma (1), and Parotid Gland (1)

Total 138 samples.

- 10 For testing the universal markers a blind cohort was collected to test the feature of Non-Response. This cohort was collected in Malaysia and consisted of 21 patients who all provided blood samples at baseline prior to immunotherapy. All patients had previous lines of therapy. 3 check-points were used: Atezolizumab (anti-PD-L1), Durvalumab (anti-PD-L1) and Pembrolizumab (anti-PD-1). There were 7 disease indications: Lung, HCC, Bladder, Prostate, NPC, Parotid Gland and Alveolar soft part sarcoma. 11
- 15 of the patients had multiple collections: 2-4. 3 patients had up to 4 collections. The ethnicity of the Patients was either Han Chinese or Indonesian.

Figure 4 shows the high concordance between baseline EpiSwitch calls, PD-L1 expression and observed clinical response.

- 20 Figure 5 shows data for a training set for an 11 marker model based on 80 NSCLC patients who are a mixture of 1L, 2L Avelumab (54) and 2L Pembrolizumab (36).

Figure 6 shows data for a test set of the 11 marker model based on 38 NSCLC patients who are a mixture of 1L, 2L Avelumab (27) and 2L Pembrolizumab (11).

Figures 7 and 8 show data for a second test set for the Malaysian Observational Study looking a mixture of CPI and tumours.

- 25 Figure 9 shows the calls for the patients who had multiple collections. The calls over the time points are largely consistent and concordant:

Patient 12 on Durvalumab shows the profile of Responder over 4 collections, and a score over the second collection entering into grey zone R/NR. Overall profile of probabilities actually get stronger over time for Responder.

Patient 1 on Atezolizumab, shows an interesting initial non-response, but becomes a late responder.

- 5 Patient 17 on Pembrolizumab is an NPC patient, and shows Response profile over both samplings.

This emphasizes the ability of EpiSwitch™ Markers to find Non-Responders and Responders to capture common features of host response profile for multi check-point inhibitors under diverse oncological conditions.

- 10 Figure 10 shows EpiSwitch calls for patients sampled over multiple time points, with OBD.261.263 and OBD.301.303 representing universal response markers and OBD.029.031, OBD.045.047, OBD.645.647, OBD.753.755 and OBD.8.69.871 representing universal non-response markers.

For the work relating to hyper-progressors, Figure 11 shows sample selection with hyper-progressors selected based on having a PFS≤60 days (2months) and OS ≤ 150 days (4.5months), the survival group (S) were selected as they mostly all had over 1 year PFS.

- 15 Figure 13 shows 11 EpiSwitch CCSs selected from a total of 60, markers selected based on binary difference, OS and PFS rank log analysis. The associated genetic locations are also shown. Figures 10 and 11 show a pathway analysis of the genetic locations.

- 20 Figure 16 shows data for a training set for hyper-progressors: an XGBoost 11 marker model. Figure 17 shows data for a test test for hyper-progressors with 6 samples excluded from marker selection. Figure 18 shows a logistic principal component analysis (PCA) of a training set. Figure 19 shows a logistic PCA of the training set with predicted test samples. This is a second classification approach and is in concordance with XGBoost. Figure 20 shows the logistic PCA of the training set with PFS as label. Figure 21 shows the logistic PCA of the training set with OS as label.

Approach to Analysing the Patients

- 25 A specific approach was taken to the way the patient population was analysed. The relevant patient cohorts had either been given prior therapy (one round of cisplatin-based chemotherapy) or had been treated with check-point inhibitors only. We also only looked at patients with a defined response, so either complete response, partial response or no response. We removed from the analysis patients who experienced stable disease.

The EpiSwitch™ nested PCR platform data output was analysed with multiple statistical techniques, including, but not limited to, established univariate (Fishers Exact test) and multivariant (permuted GLMNET, Random Forest with SHapley Additive exPlanations values (SHAP)), procedures.

- For development of the diagnostic and prognostic EpiSwitch™ classifiers, the following statistical analysis were used: (i) XGBoost: A gradient boosted decision tree algorithm. An ensemble of weak decision tree models is generated and combined to produce one strong classification model (level wise tree growth); (ii) Logistic Principal Component Analysis (PCA): Principal component Analysis optimized to use binary data; and (iii) GLMNET: Generalized linear model fitted via penalized maximum likelihood technique (iv) LightGBM: gradient boosting framework that uses tree based learning algorithms (vertical leaf tree growth).

A SHAP analysis is shown below for universal marker set.

Marker	Shap1	Rank_1	Shap2	Rank_2	Shap3	Rank_3	Average
OBD117_029.031_2	0.939792	3	0.898573	5	1.210319	2	3
OBD148_045.047_1	0.516578	6	1.335644	1	1.457146	1	3
OBD148_869.871_2	0.989211	1	1.034225	3	0.942668	4	3
OBD148_929.931_2	0.971228	2	0.998071	4	1.128059	3	3
OBD148_261.263_1	0.400625	8	1.172577	2	0.681067	5	5
OBD148_753.755_2	0.798995	4	0.549536	6	0.679744	6	5
OBD148_397.399_1	0.428492	7	0.254456	11	0.567525	7	8
OBD148_645.647_4	0.675223	5	0.407179	9	0.48619	9	8
OBD148_301.303_2	0.329259	10	0.541	7	0.263039	11	9
OBD148_777.779_2	0.396967	9	0.311894	10	0.520779	8	9
OBD148_821.823_2	0.180874	11	0.461653	8	0.335826	10	10

Markers ranked by their SHAP scores, with the best marker being OBD117_029_0.31. The SHAP (SHapley Additive exPlanations) value is a united approach to explaining the output of any machine learning model. There are three important benefits.

The first one is global interpretability — the collective SHAP values can show how much each predictor contributes, either positively or negatively, to the target variable. This is like the variable importance plot but it is able to show the positive or negative relationship for each variable with the target.

The second benefit is local interpretability — each observation gets its own set of SHAP values This greatly increases its transparency. We can explain why a case receives its prediction and the contributions of the predictors. Traditional variable importance algorithms only show the results across the entire population but not on each individual case. The local interpretability enables us to pinpoint and contrast the impacts of the factors.

Third, the SHAP values can be calculated for any tree-based model (our model is an XGboost, boosted tree based model), while other methods use linear regression or logistic regression models as the surrogate models.

This represents the analytical pipeline for marker selection. The high performance of the two markers sets is shown in the figures and tables. In particular for the universal marker set all 7 types of cancer shown in Figure 3 were represented in 21 patients observed longitudinally, where the performance was 100% on specificity and positive predictive value across the cohort.

Example 2. Further Work Leading to Development of the Set of Markers Shown in Table 8

Immune checkpoint inhibitors are a class of drugs targeting a narrow set of proteins in a specific regulatory network present in immune cells, like T cells, and some cancer cells of the patients. The checkpoint protein targets and the network they control help keep immune responses from being too strong and provide additional protection from the autoimmune conditions, but in the case of cancer can keep T cells from killing cancer cells. Use of immune checkpoint inhibitors helps to reactivate the immune response in cancer patients, with efficacious outcome and improved survival in patients.

Immune checkpoint inhibitors act by resetting and activating immune response by targeting either: 1) PD-1 (Nivolumab, Pembrolizumab, Cemiplimab, Camrelizumab, Tislelizumab, Sasanlimab); or 2) PD-1 ligand, called PD-L1 (Avelumab, Atezolizumab and Durvalumab).

The present work has asked several questions, including if, taking into account the role played by immune system of the patient in successful response to immune checkpoint inhibitors and the limited

number of targets for the therapy, particularly PD-1 receptor and its ligand PD-L1, one can discover and validate EpiSwitch biomarkers in a qPCR format of detection for baseline patients that would universally predict response/non-response to treatment in advance, irrespective of the type of the checkpoint inhibitor used and across the spectrum on oncological conditions.

- 5 MIQE compliant qPCR format is the standard for clinical PCR based tests. This format is very different from nested PCR or array format, due to its limitations on primer and probes sequence designs and continuous range of detection, traditionally measured through Cq cycle numbers.

The following steps were undertaken as part of discovery and validation of these biomarkers (table 9 shows patient data):

- 10 Stage 1. From previous markers identified by arrays, the top 24 markers were identified that satisfied initial theoretical restrictions and requirements for sequence designs on qPCR primers and probes, i.e. unique sequences for detection, correct annealing temperature of annealing for primers and probes, overlapping 3C juncture, for the detection of the chromosome conformations. At the experimental stage, the designs for 20 markers passed quality control and satisfactory optimization on temperature
15 gradient.

- Stage 2. qPCR formatted marker leads were used to identify if there is a minimal number of biomarkers which as a signature will have strong stratification power for predicting response and non-response across an extensive cohort for patients treated with immune checkpoint inhibitors - Pembrolizumab, Avelumab, Atezolizumab, Durvalumab against each of three targets (PD-1, PD-L1) from a broad selection
20 of oncological conditions: melanoma, non-small cell lung cancer, urethral, HCC, Bladder, Prostate, NPC, Parotid Gland, Alveolar Soft Part Sarcoma, Nasopharyngeal carcinoma, vulval carcinoma, colon cancer, Breast Cancer, Bone Cancer, Brain Cancer, sagittal sinus Carcinoma, lymphoma, larynx cancer, Cervix Cancer, Oral Cavity Carcinoma.

- Stage 3: In screen 1 all 20 markers were first evaluated on pooled DNA templates from 3 patients clinical
25 outcome categories: responders, non-responders and stable diseases. The sample cohorts represented patients with various type of cancers (see annotations attached) who received various immuno-checkpoints inhibitors as monotherapies: Avelumab, Pembrolizumab, Atezolizumab and Durvalumab.

- Stage 4: In screen 2, the top 13 markers, shortlisted from screen 1 were evaluated on individual samples from the same patients used in screen 1: 24 patients/samples in total (see Table 9, patients shown with
30 an asterisk).

The selection of best markers at stage 2 and 3 was carried out using a linear model. The linear model is fitted to the Cq values for each marker, comparing PR v PD, PR v SD and PD v SD. The coefficients of the fitted models describe the differences between the CCSs in each of the comparisons. The linear models are then used to compute moderated t-statistics log-odds of differential CCSs by empirical Bayes
 5 moderation of the standard errors towards a global value (0 log or 1 linear). The markers are then ranked by the adjusted p value and their CCSs abundance difference between the groups. Markers between PR v PD are given more weight.

Stage 5: In screen 3 the top 8 markers (shortlisted from screen 2) were validated on IO samples consisting of patients with the following clinical outcomes: 55% NR (non-responders), 24% SD (stable
 10 disease, according to the regulatory ruling and clinical practice, SD should be subject to IO therapy just as the group of potential responders), 20% R (responders, also referred as PR for partial responders) and 1% CR (complete responders). The efficacy of stratification by the model based on 8 markers is shown in Figures 22 to 24. Predictive Value (PPV) = $100 \times TP / (TP + FP)$. Negative Predictive Value (NPV) = $100 \times TN / (FN + TN)$.

15 The immunotherapy checkpoint classifier is built using CatBoost. Catboost is a member of the Gradient Boosted Decision Trees (GBDT's) machine learning ensemble techniques (see Hancock and Khoshgoftaar; J. Big Data (2020) 7:94).

Types of Format for the Test

Both nested and qPCR formats impose stringent limitations on which of the array-based marker leads
 20 could be successfully translated into one or the other format. This is particularly true in case of qPCR format where we have to determine if we can use two primers and a fluorescent probe over the 3C juncture (this one is similar to the array probe), which 1) have unique sequence across the whole genome for specific detection, 2) have very similar annealing temperatures, 3) show how efficacy in amplification in single PCR procedure, and not in two sequential reactions as required by nested PCR
 25 (first with one pair of primers, second one with another pair of primers). Requirements for qPCR are much more stringent and selective.

Conclusions

Based on qPCR evaluation of 8 conditional chromatin-conformations as blood-based regulatory biomarkers, individuals could be evaluated for likelihood of response to Immune Checkpoint Inhibitor
 30 (ICI) monotherapies. In the cross talk between patient tumour microenvironment and patient immune system, the PD-1 pathway, comprising receptor Programmed Death 1 (Pd-1) and its ligand PD-L1,

mediates local immunosuppression in the tumour microenvironment. The present work directly relates to ICI that were antagonists targeting PD-1 (Pembrolizumab) or its ligand, PD-L1 (Atezolizumab, Avelumab & Durvalumab). Stratification based on 8 marker classifier places patients into groups of likely responders or non-responders to ICI monotherapy, prior to the application of the therapy. The classification is applied across all ICI monotherapies against PD-L1 and its ligand PD-L1, in the context of all oncological indications used in ICI monotherapy treatments.

Figures 25 to 27 describe characteristics of the identified chromosome interactions markers. Figure 25 shows the importance of markers in terms of their power in the model. Figure 26 shows the genetic locations. Figure 27 shows the pathways the genes are associated with. These are pathways implicated in checkpoint response. This indicates the model predictive, and it is noteworthy the markers within the model have biological relevance. In fact, one of the markers is located between PD-L1 and PD-L2 (q057_q059).

	Probe	RP/Rsum	FC
1	PDCD1LG2_9_5495992_5498009_5563479_5572986_RR	6499	-1.13
2	MYC_8_127691489_127694045_127738939_127740424_FR	6220	1.15
3	IKBKB_8_42264241_42271203_42331044_42332799_FR	1585	1.3
4	ORF712_9_120888366_120893320_120913546_120919710_RR	8471	1.11
5	ITK_5_157178319_157181048_157266725_157271762_RR	4605	1.15
6	IL17D_13_20664875_20671757_20688261_20691044_FF	6848	-1.1
7	IKBKB_8_42264241_42271203_42290979_42292124_FR	2465	1.25
8	IGF1R_15_98731539_98737034_98785670_98790114_FF	5450	1.15
9	CASP6_4_109703339_109705583_109735036_109741090_RF	1727	1.29
10	TRAF2_9_136904007_136906211_136939587_136941363_RF	5090	1.16
11	ORF313_13_20664875_20671757_20695143_20698635_FF	4910	-1.16

Table 1.a1

	PFP	P.value	FDR
1	0.3857	0.01934	0.188603463
2	0.3231	0.01577	0.169755579
3	0.001699	0.00000779	0.00118933
4	0.6186	0.06146	0.308076103
5	0.1332	0.003607	0.076416861
6	0.4249	0.02454	0.210154857
7	0.01154	0.0001149	0.00763801
8	0.2259	0.008388	0.12237213

9	0.002472	0.0000133	0.001705674
10	0.1832	0.005986	0.101918497
11	0.2061	0.004999	0.091747389

Table 1.a2

	Probe sequence
	60 mer
1	ACAGTTATTAGAAAAATAAAACATTTGGTCGAACAGCAAAGAGAAGATATTCAACTGCGA
2	AGGGAGAACAAAAGAAGTTCCATCCATCTCGACGGAGTCCTCCCCGCAGGGCAGCCCCGA
3	CCACCCCCGCCCCGGGGGAGTCGCCCCGGTCGACCCCTGACATGGGGCTGCCTGGAGCAG
4	AGTGCTGGGTTCCACACCTCTCAGCTCTTCGACCTCCAGGTCCCCGCCACTTCCACGGC
5	CAAAATCAACACAAATCTAATCAAACCTTCGATGTTTGGGGGCGGAGGGCTTTGATGAGA
6	TTAAAGAAGCTAATTTTAAAAATAAATGTCGAAGAGATTGTCACGTTAGAGTTATGTAAA
7	CCACCCCCGCCCCGGGGGAGTCGCCCCGGTCGATTTCCAAAAGCTCACACATGGGTGCACA
8	CGTAGAACTAAGATGTATTCAAAGTCAGTCGAAATCACCTGTCCCGGCCTCTTTCCAAAC
9	GGGGCCTCCAGAGTCCCCTTTACAGGCATCGACGCCCCCTGCCTACCTGCCGGGTGCCCC
10	CCGCCTCACCTCCCGCATGGTCTTGAGGTCGAGCATGCAGCGCATCTGAGCAGTGAGGCT
11	TTAAAGAAGCTAATTTTAAAAATAAATGTCGAGGAGCATCTGGATTTAATGATAGTTCAA

Table 1.a3

	Probe Location				
	Chr	Start1	End1	Start2	End2
1	9	5495994	5496023	5563481	5563510
2	8	127694014	127694043	127738941	127738970
3	8	42271172	42271201	42331046	42331075
4	9	120888368	120888397	120913548	120913577
5	5	157178321	157178350	157266727	157266756
6	13	20671726	20671755	20691013	20691042
7	8	42271172	42271201	42290981	42291010
8	15	98737003	98737032	98790083	98790112
9	4	109703341	109703370	109741059	109741088
10	9	136904009	136904038	136941332	136941361
11	13	20671726	20671755	20698604	20698633

5 Table 1.a4

4 kb Sequence Location					
	Chr	Start1	End1	Start2	End2
1	9	5495994	5499993	5563481	5567480
2	8	127690044	127694043	127738941	127742940
3	8	42267202	42271201	42331046	42335045
4	9	120888368	120892367	120913548	120917547
5	5	157178321	157182320	157266727	157270726
6	13	20667756	20671755	20687043	20691042
7	8	42267202	42271201	42290981	42294980
8	15	98733033	98737032	98786113	98790112
9	4	109703341	109707340	109737089	109741088
10	9	136904009	136908008	136937362	136941361
11	13	20667756	20671755	20694634	20698633

Table 1.a5

	Primer ID	Primer Sequence	Primer ID
1	OBD117_029	CTCACTGCCCAACAGGCTAGAA	OBD117_031
2	OBD148_045	CCCTAAGCAACCACCTTGACTG	OBD148_047
3	OBD148_261	CGGTGAGCACGGTCTGTCTACTT	OBD148_263
4	OBD148_301	CCCAGTTGTCCAGGTTGCTGCCT	OBD148_303
5	OBD148_397	GTCTCCTGAGGTGAAGCAAGAGG	OBD148_399
6	OBD148_645	TCTCTACTTCAGGCAGGCAGTGTAAG	OBD148_647
7	OBD148_753	GGTGTAACGGGGGTCATTTC	OBD148_755
8	OBD148_777	AATTCACCACACCCCAACAT	OBD148_779
9	OBD148_821	CAGACTAAGGGGCCTCCAGA	OBD148_823
10	OBD148_869	TGAAGAAGCACTCGTCGTTG	OBD148_871
11	OBD148_929	AGTTTTCCACCCCTTCTCC	OBD148_931

Table 1.a6

	Primer Sequence	Marker	Type
1	TCTTGACTCAGAGCCCACAACAA	OBD117_029.031	NR
2	GCTTCGCTTACCAGAGTCGCTGC	OBD148_045.047	NR
3	GTCCTGGGTCCTGGGTGAAAGTC	OBD148_261.263	R
4	TGGAGCAGAACCTGTCAGACCTG	OBD148_301.303	R
5	AGTCAGCCCACTCATCCCCTTCC	OBD148_397.399	NR
6	GGGAGACCATTCTGTCTACTCTGAG	OBD148_645.647	NR
7	TGTGGGAACCATACCTGTGC	OBD148_753.755	NR
8	CTCCGGAGGATTCTGTGAA	OBD148_777.779	NR
9	CGCAATCAGAACCAACTGGC	OBD148_821.823	NR
10	AGCGGCACACCTCTACTCTC	OBD148_869.871	NR

11	GGGCTGTGTCCTGATAAACC	OBD148_929.931	R
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Table 1.a7

	probe	RP/Rsum	FC
1	ORF479_8_81007411_81018107_81095100_81099880_FR	5655	1.14
2	ORF482_5_168579937_168582137_168614429_168620163_RR	8291	-1.11
3	PDCD1LG2_9_5495992_5498009_5563479_5572986_RR	6499	-1.13
4	IL17D_13_20664875_20671757_20688261_20691044_FF	6848	-1.1
5	CASP6_4_109703339_109705583_109735036_109741090_RF	1727	1.29
6	ORF102_17_34316073_34325822_34367538_34373948_RF	1958	-1.26
7	TNFSF8_9_114957908_114962933_114975258_114977746_RF	8214	-1.11
8	ORF712_9_120888366_120893320_120913546_120919710_RR	8471	1.11
9	ORF698_18_62296384_62304812_62385139_62386748_FF	7473	1.12
10	ORF197_8_26561792_26565691_26638318_26644530_FR	3611	-1.2
11	IKBKB_8_42264241_42271203_42331044_42332799_FR	1585	1.3

Table 2.a1

5

	PFP	P.value	FDR
1	0.252	0.01003	0.13564257
2	0.5777	0.05624	0.298153549
3	0.3857	0.01934	0.188603463
4	0.4249	0.02454	0.210154857
5	0.002472	0.0000133	0.001705674
6	0.009056	0.0000289	0.002854389
7	0.5681	0.0541	0.293528769
8	0.6186	0.06146	0.308076103
9	0.4918	0.03615	0.248717729
10	0.09085	0.0009981	0.034849498
11	0.001699	0.00000779	0.00118933

Table 2.a2

	Probe sequence
	60 mer
1	TCAGATAAGTAACTTCCTGATAATTAACGCGATGCCAATCCACGTCATTAGATGAGGACC
2	GAATGGCCGAACAGCCATGACAGTCCTCTCGAGGCTACTGGAGTCATTGAAAAGAGGAAT
3	ACAGTTATTAGAAAAATAAAACATTTGGTCGAACAGCAAAGAGAAGATATTCAACTGCGA
4	TTAAAGAAGCTAATTTTAAAAATAAATGTGCGAAGAGATTGTCACGTTAGAGTTATGTAA

5	GGGGCCTCCAGAGTCCCCTTTACAGGCATCGACGCCCCCTGCCTACCTGCCGGGTGCCCC
6	ATATAAATCTACTTTATAAATAAGGAAATCGAAGTATAATTCAATATACTGTCCAGTAAA
7	AGTAGTGCAATCATAGCTCACTGAAACCTCGAAAGCTAATGAGGTATGAGGGGAGAATAC
8	AGTGCTGGGTTCACACCTCTCAGCTCTTCGACCTCCAGGTCCCCCGCCACTTCCACGGC
9	GTTGGTGAAAAAGAAAGAAGAAATGGACTCGACCGCTACCACCCCAGCATTTCAGCAGG
10	ATAAATAGACTCCACTATGTATAATGACTCGAAATTTTGCTATAAATGTGAGCTTTGAAA
11	CCACCCCGCCCCGGGGGAGTCGCCCCGGTCGACCCCTGACATGGGGCTGCCTGGAGCAG

Table 2.a3

	Probe Location				
	Chr	Start1	End1	Start2	End2
1	8	81018076	81018105	81095102	81095131
2	5	168579939	168579968	168614431	168614460
3	9	5495994	5496023	5563481	5563510
4	13	20671726	20671755	20691013	20691042
5	4	109703341	109703370	109741059	109741088
6	17	34316075	34316104	34373917	34373946
7	9	114957910	114957939	114977715	114977744
8	9	120888368	120888397	120913548	120913577
9	18	62304781	62304810	62386717	62386746
10	8	26565660	26565689	26638320	26638349
11	8	42271172	42271201	42331046	42331075

Table 2.a4

	4 kb Sequence Location				
	Chr	Start1	End1	Start2	End2
1	8	81014106	81018105	81095102	81099101
2	5	168579939	168583938	168614431	168618430
3	9	5495994	5499993	5563481	5567480
4	13	20667756	20671755	20687043	20691042
5	4	109703341	109707340	109737089	109741088
6	17	34316075	34320074	34369947	34373946
7	9	114957910	114961909	114973745	114977744
8	9	120888368	120892367	120913548	120917547
9	18	62300811	62304810	62382747	62386746
10	8	26561690	26565689	26638320	26642319
11	8	42267202	42271201	42331046	42335045

5 Table 2.a5

	Primer ID	Primer Sequence	Primer ID	Primer Sequence
1	OBD148_105	GGACAGCCACTACTCAACCTTTTCCT	OBD148_107	AAATGCTGGGCTCCTCTTTTGTCTC
2	OBD148_669	CCGACCCTAACATTCAAGGTGTCTCT	OBD148_671	CCACTTCATTTTCATCCCTACTGCCAC
3	OBD117_029	CTCACTGCCCAACAGGCTAGAA	OBD117_031	TCTTGACTCAGAGCCCACAACAA
4	OBD148_645	TCTCTACTTCAGGCAGGCAGTGTAAAG	OBD148_647	GGGAGACCATTCTGTTCCTCTGAG
5	OBD148_821	CAGACTAAGGGGCTCCAGA	OBD148_823	CGCAATCAGAACCAACTGGC
6	OBD148_893	ACTTGTGGCTTCCTTAGCCC	OBD148_895	TCCTTTGCAGGTATGGACATC
7	OBD148_917	TTGCTTGTGAGTTTGATGCAG	OBD148_919	AAGCCAAATGGGCCTAGCCA
8	OBD148_301	CCCAGTTGTCCAGTTGCTGCCT	OBD148_303	TGGAGCAGAACCTGTCAGACCTG
9	OBD148_505	TGTGTTTATTCCTACAGAGCAGGTT	OBD148_507	TGAGCACTGGTTCCCCGCAAACTAGT
10	OBD148_661	GATGCTGCTGGTGAGAGTAGTCC	OBD148_663	CATTACTACTCTCCAGGGCAGG
11	OBD148_261	CGGTGAGCACGGTCTGTCTACTT	OBD148_263	GTCCTGGGTCTGGGTGAAAGTC

Table 2.a6

	Probe	Marker	Type
1	ORF479_8_81007411_81018107_81095100_81099880_FR	OBD148_105.107	S
2	ORF482_5_168579937_168582137_168614429_168620163_RR	OBD148_669.671	H
3	PDCD1LG2_9_5495992_5498009_5563479_5572986_RR	OBD117_029.031	H
4	IL17D_13_20664875_20671757_20688261_20691044_FF	OBD148_645.647	H
5	CASP6_4_109703339_109705583_109735036_109741090_RF	OBD148_821.823	S
6	ORF102_17_34316073_34325822_34367538_34373948_RF	OBD148_893.895	S
7	TNFSF8_9_114957908_114962933_114975258_114977746_RF	OBD148_917.919	S
8	ORF712_9_120888366_120893320_120913546_120919710_RR	OBD148_301.303	S
9	ORF698_18_62296384_62304812_62385139_62386748_FF	OBD148_505.507	S
10	ORF197_8_26561792_26565691_26638318_26644530_FR	OBD148_661.663	S
11	IKBKB_8_42264241_42271203_42331044_42332799_FR	OBD148_261.263	S

Table 2.a7

Stimulatory checkpoint molecules	Inhibitory checkpoint molecules
CD27	A2AR
CD28	B7-H3
CD40	B7-H4
CD122	CTLA-4
CD137	IDO
OX40	KIR
GITR	LAG3
ICOS	PD-1
	TIM-3
	VISTA

Table 3

Drug	targets	Preferred Disease
Ipilimumab & Nivolumab	PD-1 and CTLA-4	metastatic melanoma
Paclitaxel, ipilimumab & carboplatin	CTLA4	non-small-cell lung cancer
Ipilimumab & GVAX	CTLA-4	pancreatic cancer
Pdilizumab & rituximab	PD-1	hematologic malignancies
L19-IL2 & L19-TNF	STAT	Melanoma
MEDI0680 & Durvalumab	PD1/PDL1	Advanced solid malignancies

Table 4. Combinations in cancer immunotherapy (biologics, immunocytokines (L19-IL2 and L19-TNF), cytotoxics (Paxlitaxel)

Drug	targets	Preferred Cancer
CA-170 (small molecule)	PD1-PDL1 and VISTA	Advanced solid tumour and lymphoma
Ruxolitinib (small molecule)	JAK	myelofibrosis and multiple myeloma
Tofacitinib (small molecule)	JAK	autoimmune disease
Galiellelactone (small molecule)	STAT3	prostate cancer
Ipilimumab (monoclonal antibody)	CTLA4	melanoma, prostate
L19-IL2 (immunocytokine)	STAT	melanoma, pancreatic cancer, RCC
L19-TNF (immunocytokine)	STAT	Melanoma
Tremelimumab (monoclonal antibody)	CTLA4	Mesothelioma
Nivolumab (ditto)	PD1	melanoma, non-small-cell lung cancer, renal cell carcinoma, and other solid tumors
Pembrolizumab	PD1	melanoma, non-small-cell lung cancer, renal cell carcinoma, and other solid tumors
Pdilizumab	PD1	hematologic malignancies
BMS935559	PD-L1	variety of solid tumors
GVAXMPDL3280A	PD-L1	bladder cancer, head and neck cancer, and GI malignancies
MEDI4736	PD-L1	bladder cancer, head and neck cancer, and GI malignancies
MSB0010718C	PD-L1	bladder cancer, head and neck cancer, and GI malignancies
MDX-1105/BMS-936559	PD-L1	Cancer
AMP-224	PD1	colorectal cancer
MEDI0680	PD1	advanced solid tumors
Durvalumab	PDL1	non-small –cell lung cancer
Atezolizumab	PDL1	advanced or metastatic urothelial carcinoma

Avelumab	PDL1	metastatic Merkel cell carcinoma
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Table 5. Other single molecules, immunocytokines and biologics for cancer therapy

Drug	Targets
Alemtuzumab (monoclonal antibody).	CD52
Ofatumumab (Second generation human IgG1 antibody).	CD20
Pegylated liposomal doxorubicin (PLD) plus motolimod (VTX2337).	
Sipuleucel-T (Approved Cancer Vaccine).	
Rituximab (monoclonal antibody).	CD20
Interferon gamma	
Combinatorial ablation and immunotherapy.	
Polysaccharide-K	
Adoptive cell therapy	
Anti-CD47 antibodies.	CD47
Polypurine reverse Hoogsteen oligonucleotides (PPRHs).	
Anti-GD2 antibodies.	GD2
BGB-A317 (monoclonal antibody).	PD-1 inhibitor
Affimer biotherapeutic.	PD-L1 inhibitor
Polysaccharides	
Neoantigens	

Table 6

	Probe	RP/Rsum	FC
1	PDCD1LG2_9_5495992_5498009_5563479_5572986_RR	6499	-1.13
2	MYC_8_127691489_127694045_127738939_127740424_FR	6220	1.15
3	IKBKB_8_42264241_42271203_42331044_42332799_FR	1585	1.3
4	ORF712_9_120888366_120893320_120913546_120919710_RR	8471	1.11
5	ITK_5_157178319_157181048_157266725_157271762_RR	4605	1.15
6	IL17D_13_20664875_20671757_20688261_20691044_FF	6848	-1.1
7	IKBKB_8_42264241_42271203_42290979_42292124_FR	2465	1.25
8	IGF1R_15_98731539_98737034_98785670_98790114_FF	5450	1.15
9	CASP6_4_109703339_109705583_109735036_109741090_RF	1727	1.29
10	TRAF2_9_136904007_136906211_136939587_136941363_RF	5090	1.16
11	ORF313_13_20664875_20671757_20695143_20698635_FF	4910	-1.16
12	ORF479_8_81007411_81018107_81095100_81099880_FR	5655	1.14
13	ORF482_5_168579937_168582137_168614429_168620163_RR	8291	-1.11
14	ORF102_17_34316073_34325822_34367538_34373948_RF	1958	-1.26

15	TNFSF8_9_114957908_114962933_114975258_114977746_RF	8214	-1.11
16	ORF698_18_62296384_62304812_62385139_62386748_FF	7473	1.12
17	ORF197_8_26561792_26565691_26638318_26644530_FR	3611	-1.2
18	ORF243_1_161633494_161637462_161657362_161661864_RF	1345	1.16
19	ORF313_13_20664875_20671757_20737979_20744490_FR	2891	1.2
20	ORF369_13_46087370_46090583_46186579_46193039_RF	2719	1.11
21	ORF480_11_77430379_77437843_77514783_77519103_RF	3101	-1.1
22	ORF698_18_62330039_62332469_62356961_62362521_FR	6988	-1.28
23	ORF703_1_6461604_6466207_6514024_6515315_FR	1109	-1.25
24	ORF705_9_114855753_114859111_114920994_114929419_FR	898	-1.16

Table 7a

	PFP	P.value	FDR
1	0.3857	0.01934	0.188603463
2	0.3231	0.01577	0.169755579
3	0.001699	0.00000779	0.00118933
4	0.6186	0.06146	0.308076103
5	0.1332	0.003607	0.076416861
6	0.4249	0.02454	0.210154857
7	0.01154	0.0001149	0.00763801
8	0.2259	0.008388	0.12237213
9	0.002472	0.0000133	0.001705674
10	0.1832	0.005986	0.101918497
11	0.2061	0.004999	0.091747389
12	0.252	0.01003	0.13564257
13	0.5777	0.05624	0.298153549
14	0.009056	0.0000289	0.002854389
15	0.5681	0.0541	0.293528769
16	0.4918	0.03615	0.248717729
17	0.09085	0.0009981	0.034849498
18	0.001212	6.11E-06	0.000216321
19	0.0315	0.000344332	0.002847073
20	0.031	0.000300988	0.002596993
21	0.04015	0.000446091	0.003407902
22	0.4301	0.026579755	0.067536623
23	0.000988	5.35E-07	6.03E-05
24	0.000547	2.59E-07	4.28E-05

Table 7b

	Probe sequence
	60 mer
1	ACAGTTATTAGAAAAATAAAACATTTGGTCGAACAGCAAAGAGAAGATATTCAACTGCGA
2	AGGGAGAACAAAAGAAGTTCCATCCATCTCGACGGAGTCCTCCCCGCAGGGCAGCCCCGA
3	CCACCCCCGCCCCGGGGGAGTCGCCCCGGTCGACCCCCTGACATGGGGCTGCCTGGAGCAG
4	AGTGCTGGGTTCACACCTCTCAGCTCTTCGACCTCCAGGTCCCCGCCACTTCCACGGC
5	CAAAATCAAACACAAATCTAATCAAACCTTCGATGTTTGGGGGCGGAGGGCTTTGATGAGA
6	TTAAAGAAGCTAATTTTAAAAATAAATGTCGAAGAGATTGTCACGTTAGAGTTATGTAAA
7	CCACCCCCGCCCCGGGGGAGTCGCCCCGGTCGATTTCCAAAAGCTCACACATGGGTGCACA
8	CGTAGAACTAAGATGTATTCAAAGTCAGTCGAAATCACCTGTCCCGGCCTCTTTCCAAAC
9	GGGGCCTCCAGAGTCCCCTTTACAGGCATCGACGCCCCCTGCCTACCTGCCGGGTGCCCC
10	CCGCCTCACCTCCCGCATGGTCTTGAGGTCGAGCATGCAGCGCATCTGAGCAGTGAGGCT
11	TTAAAGAAGCTAATTTTAAAAATAAATGTCGAGGAGCATCTGGATTTAATGATAGTTCAA
12	TCAGATAAGTAACTTCCTGATAATTAACCTCGATGCCAATCCACGTCATTAGATGAGGACC
13	GAATGGCCGAACAGCCATGACAGTCCTCTCGAGGCTACTGGAGTCATTGAAAAGAGGAAT
14	ATATAAATCTACTTTATAAATAAGGAAATCGAAGTATAATTCAATATACTGTCCAGTAAA
15	AGTAGTGCAATCATAGCTCACTGAAACCTCGAAAGCTAATGAGGTATGAGGGGAGAATAC
16	GTTGGTGAAAAAGAAAGAAGAAATGGACTCGACCGCTACCACCCCAGCATTTCAGCAGG
17	ATAAATAGACTCCACTATGTATAATGACTCGAAATTTTGCTATAAATGTGAGCTTTGAAA
18	AAAGCACGCGTCAGAGTGGGTGGGGCTGTCGATTGTCATCCTCTAGGACTTACAGTTTCT
19	TTAAAGAAGCTAATTTTAAAAATAAATGTCGAAATTACTTTAAATTAATACAAGCCCCTA
20	AGGAGGGAGAAAAGTGATGAAGGCCATTTTCGAGATGGGTGCCTGGGTGAGAATTTTAATA
21	TAACAAAAGTAACACCTCTTTGGTATCATCGAAGAGTCCTTGTTCCCATTTTGGCCCAGT
22	GAGAATCAATTCCATTTTAAAGCTTAGTCGATTTTGAGGGCTTCTACAACCTCTAGATT
23	CCGCGCCCGCAGGGCCCCGCCCCGCGCCGTCGAGAAGCATAAAGCAGGGACAGGTATGGAG
24	TTCACTGTTGCCTTTTGTGTGATTATATCGAGTAATACTGACACTCCTGCCCCACAGAA

Table 7c

	Probe Location				
	Chr	Start1	End1	Start2	End2
1	9	5495994	5496023	5563481	5563510
2	8	127694014	127694043	127738941	127738970
3	8	42271172	42271201	42331046	42331075
4	9	120888368	120888397	120913548	120913577
5	5	157178321	157178350	157266727	157266756
6	13	20671726	20671755	20691013	20691042
7	8	42271172	42271201	42290981	42291010
8	15	98737003	98737032	98790083	98790112
9	4	109703341	109703370	109741059	109741088

10	9	136904009	136904038	136941332	136941361
11	13	20671726	20671755	20698604	20698633
12	8	81018076	81018105	81095102	81095131
13	5	168579939	168579968	168614431	168614460
14	17	34316075	34316104	34373917	34373946
15	9	114957910	114957939	114977715	114977744
16	18	62304781	62304810	62386717	62386746
17	8	26565660	26565689	26638320	26638349
18	1	161633496	161633525	161661833	161661862
19	13	20671726	20671755	20737981	20738010
20	13	46087372	46087401	46193008	46193037
21	11	77430381	77430410	77519072	77519101
22	18	62332438	62332467	62356963	62356992
23	1	6466176	6466205	6514026	6514055
24	9	114859080	114859109	114920996	114921025

Table 7d

	4 kb Sequence Location				
	Chr	Start1	End1	Start2	End2
1	9	5495994	5499993	5563481	5567480
2	8	127690044	127694043	127738941	127742940
3	8	42267202	42271201	42331046	42335045
4	9	120888368	120892367	120913548	120917547
5	5	157178321	157182320	157266727	157270726
6	13	20667756	20671755	20687043	20691042
7	8	42267202	42271201	42290981	42294980
8	15	98733033	98737032	98786113	98790112
9	4	109703341	109707340	109737089	109741088
10	9	136904009	136908008	136937362	136941361
11	13	20667756	20671755	20694634	20698633
12	8	81014106	81018105	81095102	81099101
13	5	168579939	168583938	168614431	168618430
14	17	34316075	34320074	34369947	34373946
15	9	114957910	114961909	114973745	114977744
16	18	62300811	62304810	62382747	62386746
17	8	26561690	26565689	26638320	26642319
18	1	161633496	161637495	161657863	161661862
19	13	20667756	20671755	20737981	20741980
20	13	46087372	46091371	46189038	46193037

21	11	77430381	77434380	77515102	77519101
22	18	62328468	62332467	62356963	62360962
23	1	6462206	6466205	6514026	6518025
24	9	114855110	114859109	114920996	114924995

Table 7e

	Probe	Primer ID
1	PDCD1LG2_9_5495992_5498009_5563479_5572986_RR	OBD189-q057
2	MYC_8_127691489_127694045_127738939_127740424_FR	OBD189-q013
3	IKBKB_8_42264241_42271203_42331044_42332799_FR	OBD148-q261
4	ORF712_9_120888366_120893320_120913546_120919710_RR	OBD189-q017
5	ITK_5_157178319_157181048_157266725_157271762_RR	OBD189-q065
6	IL17D_13_20664875_20671757_20688261_20691044_FF	OBD189-q077
7	IKBKB_8_42264241_42271203_42290979_42292124_FR	OBD189-q025
8	IGF1R_15_98731539_98737034_98785670_98790114_FF	OBD189-q001
9	CASP6_4_109703339_109705583_109735036_109741090_RF	OBD189-q005
10	TRAF2_9_136904007_136906211_136939587_136941363_RF	OBD189-q009
11	ORF313_13_20664875_20671757_20695143_20698635_FF	OBD189-q081
12	ORF479_8_81007411_81018107_81095100_81099880_FR	OBD189-q061
13	ORF482_5_168579937_168582137_168614429_168620163_RR	OBD189-q021
14	ORF102_17_34316073_34325822_34367538_34373948_RF	OBD148-q893
15	TNFSF8_9_114957908_114962933_114975258_114977746_RF	OBD148-q917
16	ORF698_18_62296384_62304812_62385139_62386748_FF	OBD189-q045
17	ORF197_8_26561792_26565691_26638318_26644530_FR	OBD189-q069
18	ORF243_1_161633494_161637462_161657362_161661864_RF	OBD189-q041
19	ORF313_13_20664875_20671757_20737979_20744490_FR	OBD189-q073
20	ORF369_13_46087370_46090583_46186579_46193039_RF	OBD189-q053
21	ORF480_11_77430379_77437843_77514783_77519103_RF	OBD189-q033
22	ORF698_18_62330039_62332469_62356961_62362521_FR	OBD189-q037
23	ORF703_1_6461604_6466207_6514024_6515315_FR	OBD189-q029
24	ORF705_9_114855753_114859111_114920994_114929419_FR	OBD189-q049

Table 7f

	Primer Sequence	Primer ID
1	GAGGGTCACTCACTGCCCAACAGGC	OBD189-q059
2	GTCACCTTCATCTCCTTCTCACAGCAG	OBD189-q015
3	CGGTGAGCACGGTCTGTCTACTT	OBD148-q263
4	CCCAGTTGTCCAGGTTGCTGCCT	OBD189-q019

5	TGTATGTCTCCTGAGGTGAAGCAAGAGG	OBD189-q067
6	GGAAGTGCCACGAGAAGGAGGATGGTCC	OBD189-q079
7	GGTGAGCACGGTCTGTCTACTTTCCC	OBD189-q027
8	GGCTGGTGGGAGTATTTCAAAGAGAAC	OBD189-q003
9	CCCCAACTCACAACCCCCAGAC	OBD189-q007
10	AGCACTCGTCGTTGGGCGTGTAG	OBD189-q011
11	GAAGTGCCACGAGAAGGAGGATGGTCC	OBD189-q083
12	TGGACAGCCACTACTCAACCTTTTCCTA	OBD189-q063
13	CCGACCCCTAACATTCAAGGTGTCTCTAT	OBD189-q023
14	ACTTGTGGCTTCCTTAGCCC	OBD148-q895
15	TTGCTTGTGAGTTTGATGCAG	OBD148-q919
16	CATAGACCCAGGTGTGCTCCGTGGCAGC	OBD189-q047
17	CAGTATGAGTGTTCTGTGGCTGCTCCCA	OBD189-q071
18	TTGCCACCTGTCTCAGATACCCTTGTT	OBD189-q043
19	GGAAGTGCCACGAGAAGGAGGATGGTCC	OBD189-q075
20	TAGAAGCAGGGAGTAGTTGAGCAATGGG	OBD189-q055
21	CATAACCACACTGCTACCAACACACCTA	OBD189-q035
22	CCTACTGGCACCCTGTGTTGGCTGG	OBD189-q039
23	TGCCCCTCGTGGTTCCGCCTTCA	OBD189-q031
24	CCATTGTTGCTCAGGCTGCCCTCTTGC	OBD189-q051

Table 7g

	Primer Sequence	Probe ID
1	GACTGTAAGGTAGAAATCCTGCCTGGGT	OBD189-p057
2	GCTTCGCTTACCAGAGTCGCTGC	OBD189-p013
3	GTCCTGGGTCCTGGGTGAAAGTC	OBD148-p261
4	CCTGGAGCAGAACCTGTCAGACC	OBD189-p017
5	CTTCCACCGTGCCCGCAGCCAGC	OBD189-p065
6	CCACCCAGTTCTCCAGGCATAGCAGG	OBD189-p077
7	GGACCCAGGCTCTGCTGCTACAG	OBD189-p025
8	GCTCTGTTCAAGTGGCTCTGTTCCA	OBD189-p001
9	AGAGGAGGGCAAGGTGTCTGGCT	OBD189-p005
10	CGGCACACCTCTACTCTCAGCCT	OBD189-p009
11	GGGCTGTGTCCTGATAAACCATTGTTA	OBD189-p081
12	CAAACCCAGATTGGACCTCACAGCCCC	OBD189-p061
13	GAGTCAGCGTGTAGTGCTCCAC	OBD189-p021
14	TCCTTTGCAGGTATGGACATC	OBD148-p893
15	AAGCCAAATGGGCCTAGCCA	OBD148-p917
16	GAGCACTGGTTCCTCGCAAATACTGGG	OBD189-p045

17	GCGTGTCTCTCAGGGAAGGCAGGATGC	OBD189-p069
18	GCTGCTCCTCTTGCCTGGAATGCCTATT	OBD189-p041
19	GGTAAGATGAGGCTGTGGGCAAGGAGC	OBD189-p073
20	TCTTCACTTGTGCTATTGGCTTTCCAGC	OBD189-p053
21	CTGGTTATTCGGACACTCATAGGACTGG	OBD189-p033
22	TATCATAATCAGGCAACTGGCTGGTGC	OBD189-p037
23	AGAGACCCACCCAGCCTCCTGA	OBD189-p029
24	GCATTCAAGTGACAGAGAGAAAAGAGGC	OBD189-p049

Table 7h

	Probe Sequence	Probe ID
1	ACATTTGGTCGAACAGCAAAGAGAAGATATTCAAC	OBD189-p059
2	AGAAGTTCCATCCATCTCGACGGAGTCCTCCC	OBD189-p015
3	TCGCCCCGGTCGACCCCCCTGACATGG	OBD148-p263
4	TTCCACACCTCTCAGCTCTTCGACCTCCAGGT	OBD189-p019
5	AACACAAATCTAATCAAACCTTCGATGTTTGGG	OBD189-p067
6	TAAATGTCTGAAGAGATTGTCACGTTAGAGTTATG	OBD189-p079
7	TCGCCCCGGTCGATTTCCAAAAGCTCACACATGG	OBD189-p027
8	TCAAAGTCAGTCGAAATCACCTGTCCCGGCCTC	OBD189-p003
9	TCCAGAGTCCCCTTTACAGGCATCGACGCCC	OBD189-p007
10	ATGGTCTTGAGGTCGAGCATGCAGCGCATCTG	OBD189-p011
11	ATAAATGTCTGAGGAGCATCTGGATTTAATGATAG	OBD189-p083
12	ACTTCCTGATAATTAACCTCGATGCCAATCCACGTC	OBD189-p063
13	AACAGCCATGACAGTCCTCTCGAGGCTACTGG	OBD189-p023
14	TAAGGAAATCGAAGTATAATTCAATATACTGTCCA	OBD148-p895
15	TGAAACCTCGAAAGCTAATGAGGTATGA	OBD148-p919
16	AGAAGAAATGGACTCGACCGCTACCACCCCAG	OBD189-p047
17	AGACTCCACTATGTATAATGACTCGAAATTTTGC	OBD189-p071
18	TCAGAGTGGGTGGGGCTGTGATTGTCATCCT	OBD189-p043
19	TAAATGTCTGAAATTACTTTAAATTAATACAAGCCC	OBD189-p075
20	AGTGATGAAGGCCATTTTCGAGATGGGTGCCTGG	OBD189-p055
21	ACCTCTTTGGTATCATCGAAGAGTCCTTGTTCCC	OBD189-p035
22	AAGCTTAGTCGATTTTGAGGGCTTCTCACAACCTC	OBD189-p039
23	CGCGCCGTCGAGAAGCATAAAGCAGGGACA	OBD189-p031
24	TCATTATATCGAGTAATACTGACACTCCTGGCCC	OBD189-p051

Table 7i

	Probe Sequence	probe
1	TGAATATCTTCTTTGCTGTTGACCAAATGTT	PDCD1LG2_9_5495992_5498009_5563479_5572986_RR
2	TCCGTCGAGATGGATGGAACCTCTTTGTTCTCCC	MYC_8_127691489_127694045_127738939_127740424_FR

3	CCATGTCAGGGGGTCGACCGGGCGA	IKBKB_8_42264241_42271203_42331044_42332799_FR
4	ACCTGGAGGTCTGAAGAGCTGAGAGGTGTGGAA	ORF712_9_120888366_120893320_120913516_120919710_RR
5	AACATCGAAGTTTGATTAGATTTGTGTTTGATT	ITK_5_157178319_157181048_157266725_157271762_RR
6	ACTCTAACGTGACAATCTCTTCGACATTTATTTT	IL17D_13_20664875_20671757_20688261_20691044_FF
7	TGTGAGCTTTTGGAAATCGACCGGGCGACTCC	IKBKB_8_42264241_42271203_42290979_42292124_FR
8	AGAGGCCGGGACAGGTGATTTCGACTGACTTTG	IGF1R_15_98731539_98737034_98785670_98790114_FF
9	GGGCGTCGATGCTGTAAAGGGGACTCTGGA	CASP6_4_109703339_109705583_109735036_109741090_RF
10	AGATGCGCTGCATGCTCGACCTCAAGACCATG	TRAF2_9_136904007_136906211_136939587_136941363_RF
11	ACTATCATTAAATCCAGATGCTCCTCGACATTTA	ORF313_13_20664875_20671757_20695143_20698635_FF
12	TGACGTGGATTGGCATCGAGTTAATTATCAGGAAG	ORF479_8_81007411_81018107_81095100_81099880_FR
13	AGTAGCCTCGAGAGGACTGTCATGGCTGTTTCG	ORF482_5_168579937_168582137_168614429_168620163_RR
14	TGGACAGTATATTGAATTATACTTCGATTTCTTA	ORF102_17_34316073_34325822_34367538_34373948_RF
15	TCATACCTCATTAGCTTTTCGAGGTTTCA	TNFSF8_9_114957908_114962933_114975258_114977746_RF
16	TGGTAGCGGTGCGAGTCCATTTCTTCTTTCTT	ORF698_18_62296384_62304812_62385139_62386748_FF
17	AGCAAAATTTTCGAGTCATTATACATAGTGGAGTC	ORF197_8_26561792_26565691_26638318_26644530_FR
18	AGGATGACAATCGACAGCCCCACCACTCTGA	ORF243_1_161633494_161637462_161657362_161661864_RF
19	GGGCTTGTATTAATTTAAAGTAATTCGACATTTA	ORF313_13_20664875_20671757_20737979_20744490_FR
20	ACCCAGGCACCCATCTCGAAATGGCCTTCATCA	ORF369_13_46087370_46090583_46186579_46193039_RF
21	TGGGAACAAGGACTCTTCGATGATACCAAAGAGG	ORF480_11_77430379_77437843_77514783_77519103_RF
22	AGAGTTGTGAGAAGCCCTCAAAATCGACTAAGC	ORF698_18_62330039_62332469_62356961_62362521_FR
23	TGTCCTGCTTTATGCTTCTCGACGGCGCG	ORF703_1_6461604_6466207_6514024_6515315_FR
24	TGGGCCAGGAGTGTCTAGTATTACTCGATATAAT	ORF705_9_114855753_114859111_114920994_114929419_FR

Table 7j

	Marker	Type
1	OBD189-q057.q059.p057	Checkpoint Inhibitor Non-responder
2	OBD189-q013.q015.p013	Checkpoint Inhibitor Responder
3	OBD148-q261.q263.p261	Checkpoint Inhibitor Responder
4	OBD189-q017.q019.p017	Checkpoint Inhibitor Responder
5	OBD189-q065.q067.p065	Checkpoint Inhibitor Responder
6	OBD189-q077.q079.p077	Checkpoint Inhibitor Non-responder
7	OBD189-q025.q027.p025	Checkpoint Inhibitor Responder
8	OBD189-q001.q003.p003	Checkpoint Inhibitor Responder
9	OBD189-q005.q007.p005	Checkpoint Inhibitor Responder
10	OBD189-q009.q011.p009	Checkpoint Inhibitor Responder
11	OBD189-q081.q083.p081	Checkpoint Inhibitor Non-responder
12	OBD189-q061.q063.p061	Checkpoint Inhibitor Responder
13	OBD189-q021.q023.p021	Checkpoint Inhibitor Non-responder
14	OBD148-q0893.q0895.p0893	Checkpoint Inhibitor Non-responder

15	OBD148-q917.q919.p917	Checkpoint Inhibitor Non-responder
16	OBD189-q045.q047.p045	Checkpoint Inhibitor Responder
17	OBD189-q069.q071.p069	Checkpoint Inhibitor Non-responder
18	OBD189-q041.q043.p043	Checkpoint Inhibitor Non-responder
19	OBD189-q073.q075.p073	Checkpoint Inhibitor Non-responder
20	OBD189-q053.q055.p053	Checkpoint Inhibitor Non-responder
21	OBD189-q033.q035.p033	Checkpoint Inhibitor Responder
22	OBD189-q037.q039.p037	Checkpoint Inhibitor Responder
23	OBD189-q029.q031.p031	Checkpoint Inhibitor Responder
24	OBD189-q049.q051.p049	Checkpoint Inhibitor Responder

Table 7k

	probe	Marker
1	PDCD1LG2_9_5495992_5498009_5563479_5572986_RR	OBD189-q057.q059.p057
2	ITK_5_157178319_157181048_157266725_157271762_RR	OBD189-q065.q067.p065
3	CASP6_4_109703339_109705583_109735036_109741090_RF	OBD189-q005.q007.p005
4	ORF313_13_20664875_20671757_20695143_20698635_FF	OBD189-q081.q083.p081
5	ORF102_17_34316073_34325822_34367538_34373948_RF	OBD148-q0893.q0895.p0893
6	ORF369_13_46087370_46090583_46186579_46193039_RF	OBD189-q053.q055.p053
7	ORF703_1_6461604_6466207_6514024_6515315_FR	OBD189-q029.q031.p031
8	ORF705_9_114855753_114859111_114920994_114929419_FR	OBD189-q049.q051.p049

Table 8a

	Primer ID	Primer Sequence	Primer ID
1	OBD189-q057	GAGGGTCACTCACTGCCCAACAGGC	OBD189-q059
2	OBD189-q065	TGTATGTCTCCTGAGGTGAAGCAAGAGG	OBD189-q067
3	OBD189-q005	CCCCAACTCACAAACCCCCAGAC	OBD189-q007
4	OBD189-q081	GAAGTGCCACGAGAAGGAGGATGGTCC	OBD189-q083
5	OBD148-q893	ACTTGTGGCTTCCTTAGCCC	OBD148-q895
6	OBD189-q053	TAGAAGCAGGGAGTAGTTGAGCAATGGG	OBD189-q055
7	OBD189-q029	TGCCCCGTCGTGGTTCCGCCTTCA	OBD189-q031
8	OBD189-q049	CCATTGTTGCTCAGGCTGCCCTCTTGC	OBD189-q051

5 Table 8b

	Primer Sequence	Probe ID	Probe Sequence
1	GACTGTAAGGTAGAAATCCTGCCTGGGT	OBD189-p057	ACATTTGGTCGAACAGCAAAGAGAAGATATTCAAC
2	CTTCCACCGTGCCCGCAGCCAGC	OBD189-p065	AACACAAATCTAATCAAACCTCGATGTTTGGG
3	AGAGGAGGGCAAGGTGTCTGGCT	OBD189-p005	TCCAGAGTCCCCTTTACAGGCATCGACGCCC
4	GGGCTGTGCTCTGATAAACCCATTGTTA	OBD189-p081	ATAAATGTCGAGGAGCATCTGGATTTAATGATAG
5	TCCTTTGCAGGTATGGACATC	OBD148-p893	TAAGGAAATCGAAGTATAATTCAATATACTGTCCA
6	TCTTCACTTGTGCTATTGGCTTTCCAGC	OBD189-p053	AGTGATGAAGGCCATTTGAGATGGGTGCCTGG
7	AGAGACCCACCCAGCCTCCTGA	OBD189-p031	TGTCCCTGCTTTATGCTTCTCGACGGCGCG
8	GCATTCAAGTGACAGAGAGAAAAGAGGC	OBD189-p049	TCATTATATCGAGTAATACTGACACTCCTGGCCC

Table 8c

		Baseline
	Patient Sample ID	Clinical Diagnosis
1	IOMA1002 *	Hepatocellular carcinoma (liver) (HCC)
2	IOMA1004	CA Parotid Gland
3	IOMA1007	Alveolar soft part sarcoma of gluteal region
4	IOMA1008-B	Ca Liver - Hepatocellular carcinoma
5	IOMA1009-B	Ca NPC
6	IOMP1002	NPC, Gastric Cancer
7	IOMP1005	T3N3M0 Nasopharyngeal carcinoma
8	IOMP1006	Nasopharyngeal carcinoma - metastatic
9	IOMP1007	CA Bladder
10	IOMP1009	
11	IOMP1010	Metastatic carcinoma of prostate
12	IOMP1011	NPC
13	NIOA1001-B	Ca larynx
14	NIOA1003 (OBDM-0770) *	Ca lung
15	NIOA1004	NPC
16	NIOA1005	Small cell neuroendocrine tumour of stomach
17	NIOA1006 (OBDM-094)	Small cell lung carcinoma
18	NIOA1007	Lymphoepithelial carcinoma of lacrimal gland
19	NIOA1008	HCC of liver
20	NIOA1009 (OBDM-082)	Metastatic lung carcinoma
21	NIOA1010	HCC
22	NIOA1011	NPC
23	NIOA1012	Lung carcinoma
24	NIOA1013	High-grade metastatic, invasive breast carcinoma
25	NIOA1014	Metastatic high-grade neuroendocrine tumour

26	NIOA1015	Small cell lung cancer with TTF-1/CD56+
27	NIOA1016	Non-small cell lung carcinoma – metastatic
28	NIOA1017	Hepatocellular carcinoma
29	NIOA1018	Multi-focal hepatoma
30	NIOA1019	Metastatic mucoepidermoid carcinoma of salivary gland (parotid gland)
31	NIOP1001-B	Nasopharyngeal carcinoma
32	NIOP1003	Ca cervix
33	NIOP1004 (OBDM-079)	Lung carcinoma - metastatic (bone)
34	NIOP1005	Metastatic NPC
35	NIOP1006	NPC – metastatic
36	NIOP1007 (OBDM-096)	Metastatic lung carcinoma
37	NIOP1008	Oral cavity - (1) Right lateral tongue; (2) Right gingival sulcus
38	NIOP1009	Carcinoma of transverse colon
39	NIOP1010	Metastatic malignant melanoma
40	NIOP1011	Metastatic malignant melanoma
41	NIOP1012	NPC
42	NIOP1013	Metastatic NPC
43	NIOP1014	NPC
44	NIOP1015	Right temporal brain tumour
45	NIOP1016 *	Ca kidney – metastatic
46	NIOP1017	Poorly differentiated adenocarcinoma of lung
47	NIOP1018 *	Adenocarcinoma - head of pancreas
48	NIOP1019	Stage 4 adenocarcinoma of descending colon
49	NIOP1020	Metastatic NPC
50	NIOP1021	Recurrent vulval carcinoma
51	NIOP1022	Ca kidney (RCC)
52	NIOP1023	Metastatic NPC
53	NIOP1024	Metastatic lung carcinoma (NSCLC)
54	NIOP1025	Left lung squamous cell carcinoma - metastatic stage 4
55	NIOP1026	Metastatic sagittal sinus recurrence with right parotid node recurrence
56	NIOP1002	Metastatic nasopharyngeal carcinoma
57	OBDM-001 (IOMP1003)	Lung
58	OBDM-002 (IOMP1008)	Lung
59	OBDM-008 (IOMD1003)	Lung
60	OBDM-014 (IOMP1004)	Lung
61	OBDM-019 (IOMA1003)	Lung
62	OBDM-033 (IOMA1001)	Lung
63	OBDM-034 (IOMA1005)	Lung
64	OBDM-035 (IOMA1006)	Lung
65	OBDM-042 (IOMP1001)	Lung
66	OBDM-044 (IOMD1001)	Lung
67	OBDM-046 (IOMD1004)	Lung
68	OBDM-015 (IOMD1002)	Lung
69	OBDM-074 (NIOA1002)	Metastatic, anaplastic small cell carcinoma of lung

70	NIOP1027	Metastatic, poorly differentiated squamous cell carcinoma of lung
71	NIOP1028	Metastatic carcinoma of lung
72	NIOP1029	Peri-hilar cholangis carcinoma (Klatskin's Tumour)
73	NIOP1030	Hepatocellular carcinoma
74	NIOP1031	Metastatic carcinoma of stomach
75	NIOP1032	Bile duct (intrahepatic) carcinoma
76	NIOP1033	Squamous cell carcinoma of left maxilla
77	NIOP1034	Lung carcinoma
78	NIOP1035	NPC
79	NIOP1036	Metastatic lung carcinoma
80	NIOP1037	NPC
81	NIOP1038	NPC
82	NIOP1039	Pancreatic carcinoma – metastatic
83	NIOA1020	Metastatic lung carcinoma
84	NIOA1021	Hepatocellular carcinoma – liver
85	NIOA1022	Small cell carcinoma of lung

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Table 9.1

	Therapy	Response to therapy	SEX	Follow Up 1	
1	Atezolizumab	PD (progressive disease)	M	IOMA2002	PD (progressive disease)
2	Atezolizumab	N/A	F	IOMA2004	N/A
3	Atezolizumab	N/A	M		
4	Atezolizumab	PD (progressive disease)	M		
5	Atezolizumab	PD (progressive disease)	M	IOMA2009*	PD (progressive disease)
6	Pembrolizumab	PD (progressive disease)	M		
7	Pembrolizumab	SD (stable disease)	M	IOMP2005	SD (stable disease)
8	Pembrolizumab	N/A	M	IOMP2006	N/A
9	Pembrolizumab	N/A	M	IOMP2007	N/A
10		N/A	M		
11	Pembrolizumab	N/A	M		
12	Pembrolizumab	N/A	F	IOMP2011	N/A
13	Atezolizumab	PD (progressive disease)	M	NIOA2001*	PD (progressive disease)
14	Atezolizumab	PD (progressive disease)	M	NIOA2003*	PD (progressive disease)
15	Atezolizumab	SD (progressive disease)	M	NIOA2004*	N/A
16	Atezolizumab	N/A	M	NIOA2005	N/A
17	Atezolizumab	N/A	M	NIOA2006	N/A
18	Atezolizumab	N/A	M		
19	Atezolizumab	N/A	M		
20	Atezolizumab	PD (progressive disease)	M		
21	Atezolizumab	PD (progressive disease)	F		

22	Atezolizumab	SD	M	NIOA2011	SD (stable disease)
23	Atezolizumab	PD (progressive disease)	M	NIOA2012	PD (progressive disease)
24	Atezolizumab	PD (progressive disease)	F		
25	Atezolizumab	PR (partial response)	M	NIOA2014*	PR (partial response)
26	Atezolizumab	N/A	M	NIOA2015	N/A
27	Atezolizumab	PD (progressive disease)	F	NIOA2016	PD (progressive disease)
28	Atezolizumab	PD (progressive disease)	M	NIOA2017	PD (progressive disease)
29	Atezolizumab	PD (progressive disease)	M	NIOA2018	PD (progressive disease)
30	Atezolizumab	PD (progressive disease)	F		
31	Pembrolizumab	PD (progressive disease)	M	NIOP2001*	PD (progressive disease)
32	Pembrolizumab	PD (progressive disease)	F		
33	Pembrolizumab	SD	M	NIOP2004	SD (stable disease)
34	Pembrolizumab	PD (progressive disease)	M		
35	Pembrolizumab	PD (progressive disease)	F		
36	Pembrolizumab	PD (progressive disease)	M	NIOP2007	PD (progressive disease)
37	Pembrolizumab	N/A	F		
38	Pembrolizumab	N/A	M	NIOP2009	N/A
39	Pembrolizumab	N/A	M		
40	Pembrolizumab	N/A	F		
41	Pembrolizumab	PD (progressive disease)	M	NIOP2012	PD (progressive disease)
42	Pembrolizumab	SD (stable disease)	F	NIOP2013	SD (stable disease)
43	Pembrolizumab	PD (progressive disease)	M	NIOP2014	PD (progressive disease)
44	Pembrolizumab	N/A	M		
45	Pembrolizumab	PR (partial response)	M	NIOP2016	PR (partial response)
46	Pembrolizumab	PD (progressive disease)	F	NIOP2017	PD (progressive disease)
47	Pembrolizumab	PD (progressive disease)	M	NIOP2018*	PD (progressive disease)
48	Pembrolizumab	PD (progressive disease)	M	NIOP2019	PD (progressive disease)
49	Pembrolizumab	PD (progressive disease)	F	NIOP2020	PD (progressive disease)
50	Pembrolizumab	PR (partial response)	F	NIOP2021	PR (partial response)
51	Pembrolizumab	SD (stable disease)	M	NIOP2022*	SD (stable disease)
52	Pembrolizumab	SD (stable disease)	F	NIOP2023	SD (stable disease)
53	Pembrolizumab	PD (progressive disease)	M		
54	Pembrolizumab	PD (progressive disease)	F		
55	Pembrolizumab	CR (complete response)	M	NIOP2026	CR (complete response)
56	Pembrolizumab	PD (progressive disease)	M	NIOP2002	PD (progressive disease)
57	Pembrolizumab	SD (stable disease)	M	IOMP2003	SD (stable disease)
58	Pembrolizumab	PD (progressive disease)	M		
59	durvalumab		F	IOMD2003	PR (partial response)
60	Pembrolizumab	PR (partial response)	M	IOMP2004	PR (partial response)
61	Atezolizumab	PD (progressive disease)	M		
62	Atezolizumab	PD (progressive disease)	M	IOMA2001*	PD (progressive disease)
63	Atezolizumab	SD	M	IOMA2005	SD (stable disease)
64	Atezolizumab	N/A	M	IOMA2006	N/A
65	Pembrolizumab	N/A	F		

66	Durvalumab	SD	M	IOMD2001	SD (stable disease)
67	Durvalumab	N/A	M	IOMD2004	N/A
68	Durvalumab	PR (partial response)	M	IOMD2002	PR (partial response)
69	Atezolizumab	PD (progressive disease)	F		
70	Pembrolizumab	PD (progressive disease)	F	NIOP2027	PD (progressive disease)
71	Pembrolizumab	PD (progressive disease)	F		
72	Pembrolizumab	PD (progressive disease)	F		
73	Pembrolizumab	PD (progressive disease)	F	NIOP2030	PD (progressive disease)
74	Pembrolizumab	N/A	F		
75	Pembrolizumab	PD (progressive disease)	M		
76	Pembrolizumab	N/A	M		
77	Pembrolizumab	PR (partial response)	M		
78	Pembrolizumab	N/A	M		
79	Pembrolizumab	N/A	F		
80	Pembrolizumab	N/A	F		
81	Pembrolizumab	N/A	M		
82	Pembrolizumab	N/A	M		
83	Atezolizumab	N/A	M	NIOA2020	N/A
84	Atezolizumab	N/A	M		
85	Atezolizumab	N/A	M		

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Table 9.2

	Follow Up 2		Follow Up 3	
1	IOMA3002	PD (progressive disease)	IOMA4002	PD (progressive disease)
2	IOMA3004	N/A		
3				
4				
5				
6				
7	IOMP3005	SD (stable disease)	IOMP4005	SD (stable disease)
8	IOMP3006	N/A	IOMP4006	N/A
9	IOMP3007	N/A		
10				
11				
12				
13	NIOA3001	PD (progressive disease)		
14	NIOA3003*	PD (progressive disease)		
15	NIOA3004	SD (stable disease)		
16				
17				

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18				
19				
20				
21				
22	NIOA3011*	SD (stable disease)	NIOA4011	SD (stable disease)
23				
24				
25	NIOA3014*	PR (partial response)	NIOA4014	PR (partial response)
26				
27				
28				
29				
30				
31				
32				
33	NIOP3004	N/A	NIOP4004	SD (stable disease)
34				
35				
36				
37				
38	NIOP3009	N/A	NIOP4009	N/A
39				
40				
41				
42				
43				
44				
45	NIOP3016	PR (partial response)	NIOP4016	PR (partial response)
46				
47				
48	NIOP3019	PD (progressive disease)		
49	NIOP3020	PD (progressive disease)	NIOP4020	PD (progressive disease)
50	NIOP3021	PR (partial response)	NIOP4021	PR (partial response)
51	NIOP3022	SD (stable disease)		
52	NIOP3023	SD (stable disease)		
53				
54				
55	NIOP3026	CR (complete response)		
56				
57	IOMP3003	SD (stable disease)	IOMP4003	SD (stable disease)
58				
59	IOMD3003	PR (partial response)	IOMD4003*	PR (partial response)
60	IOMP3004	PR (partial response)		
61				

62	IOMA3001	PD (progressive disease)		
63				
64				
65				
66	IOMD3001	SD (stable disease)	IOMD4001*	SD (stable disease)
67				
68	IOMD3002*	PR (partial response)	IOMD4002	PR (partial response)
69				
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Table 9.3

	Follow Up 4		Follow Up 5	
1	IOMA5002	PD (progressive disease)		
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25	NIOA5014	PR (partial response)		
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36				
37				
38				
39				
40				
41				
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44				
45				
46				
47				
48				
49				
50	NIOP5021	PR (partial response)		
51				
52				
53				
54				
55				
56				
57	IOMP5003	SD (stable disease)		

58				
59				
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62				
63				
64				
65				
66	IOMD5001	SD (stable disease)	IOMD6001	SD (stable disease)
67				
68	IOMD5002*	PR (partial response)	IOMD6002	PR (partial response)
69				
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Table 9.4

	Follow Up 6	
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63		
64		
65		
66	IOMD7001*	SD (stable disease)
67		
68	IOMD7002	PR (partial response)
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Table 9.5

CLAIMS

1. A method of determining how an individual responds to immunotherapy for cancer comprising detecting the presence or absence in the individual of:

- all of the chromosome interactions shown in Table 8 to thereby determine whether the individual will be responsive to immunotherapy; and/or
- all of the chromosome interactions shown in Table 2 to thereby determine whether the individual is a hyper-progressor in whom immunotherapy will accelerate disease.

2. A method according to claim 1 further comprising detecting the presence or absence in the individual of all of the chromosome interactions shown in Table 1 to thereby determine whether the individual will be responsive to immunotherapy.

3. A method according to claim 1 or 2 wherein the presence or absence of the chromosome interactions is determined:

- in a sample from the individual, and/or
- in DNA from the individual, and/or

- by detecting the presence or absence of a DNA loop at the site of the chromosome interactions, and/or

- detecting the presence or absence of distal regions of a chromosome being brought together in a chromosome conformation, and/or

- by detecting the presence of a ligated nucleic acid which is generated during said typing and whose sequence comprises two regions each corresponding to the regions of the chromosome which come together in the chromosome interaction, and/or

- by a process which detects the proximity of the chromosome regions which have come together in the chromosome interaction.

4. A method according to any one of the preceding claims wherein said detecting of the presence or absence of the chromosome interactions is by a process comprising:

- (i) in vitro crosslinking of epigenetic chromosomal interactions which are present;
- (ii) optionally isolating the cross-linked DNA;
- (iii) subjecting said cross-linked DNA to cleaving;

(iv) ligating said cross-linked cleaved DNA ends to form ligated DNA; and

(v) identifying the presence or absence in said ligated DNA of a DNA sequence that corresponds to each chromosome interaction;

to thereby determine the presence or absence of each chromosome interaction.

5 5. A method according to claim 3 or 4 wherein said ligated DNA is detected by PCR or by use of a probe.

6. A method according to claim 5 wherein:

(i) detection is by use of a probe, wherein said probe preferably has at least 70% identity to any of the probes shown in Table 1, 2, or 8; or

10 (ii) detection is by use of PCR, wherein the PCR preferably uses a primer pair that has at least 70% identity to any of the primer pairs shown in Table 1, 2 or 8.

7. A method according to any one of the preceding claims wherein:

(i) the method is carried out prior to the individual receiving immunotherapy and/or is carried out to select which therapy the individual should receive for cancer, and/or

(ii) the method is carried out on an individual that has cancer or is suspected of having cancer, and/or

15 (iii) the method is carried out on individual that has been preselected based on a physical characteristic, risk factor or the presence of a symptom for cancer.

8. A method according to any one of the preceding claims in which the individual:

- is at an early stage of cancer; and/or

- is undergoing, or is about to undergo, cancer therapy, for example cancer immunotherapy.

20 9. A method according to any one of the preceding claims wherein the cancer is:

(i) one in which immune-checkpoint inhibitors PD-1/PD-L1 are used for therapy; and/or

(ii) melanoma, lung cancer, hepatocellular carcinoma (liver cancer), bladder, prostate, nasal cancer, parotid gland cancer (salivary gland cancer), alveolar soft part sarcoma (soft tissue cancer); and/or

25 (iii) breast cancer, cervical cancer, colon cancer, head and neck cancer, Hodgkin lymphoma, kidney cancer, stomach cancer, rectal cancer or a solid tumour.

10. A method according to any one of the preceding claims in which the immunotherapy:

- (i) comprises an antibody or immune cell, preferably a T cell or dendritic cell; and/or
- (ii) comprises a vaccine, preferably against the cancer; and/or
- (iii) modulates, blocks or stimulates an immune checkpoint, and preferably targets or modulates PD-L1, PD-L2 or CTLA4 or any other immune checkpoint molecule disclosed in Table 3; and/or

5 (iv) comprises a therapy shown in any one of tables 4 to 6; and/or

(v) increases the killing of cancer cells by the immune system, preferably wherein such killing is by a T cell.

11. A method according to any one of the preceding claims wherein the immunotherapy is:

(i) a PD-1 inhibitor or PD-L1 inhibitor, and is preferably an antibody specific for PD-1 or PD-L1; and/or

10 (ii) a PD-2 inhibitor or PD-L2 inhibitor, and is preferably an antibody specific for PD-2 or PD-L2.

12. A method according to any one of the preceding claims, wherein the typing of chromosome interactions comprises specific detection of the ligated product by quantitative PCR (qPCR) which uses primers capable of amplifying the ligated product and a probe which binds the ligation site during the PCR reaction, wherein said probe comprises sequence which is complementary to sequence from each
15 of the chromosome regions that have come together in the chromosome interaction, wherein preferably said probe comprises:

- an oligonucleotide which specifically binds to said ligated product, and/or
- a fluorophore covalently attached to the 5' end of the oligonucleotide, and/or
- a quencher covalently attached to the 3' end of the oligonucleotide, and

20 optionally

- said fluorophore is selected from HEX, Texas Red and FAM; and/or
- said probe comprises a nucleic acid sequence of length 10 to 40 nucleotide bases, preferably a length of 20 to 30 nucleotide bases.

13. An immunotherapy for cancer for use in a method of treating a cancer in an individual, wherein said
25 method of treating comprises:

- identifying whether the individual is responsive to immunotherapy by the method of any one of the preceding claims, and

- administering to an individual that has been identified responsive to immunotherapy said immunotherapy.

14. A combination therapy for cancer for use in a method of treating a cancer in an individual, wherein said method of treating comprises:

5 - identifying whether the individual is responsive to immunotherapy by the method of any one of the preceding claims, and

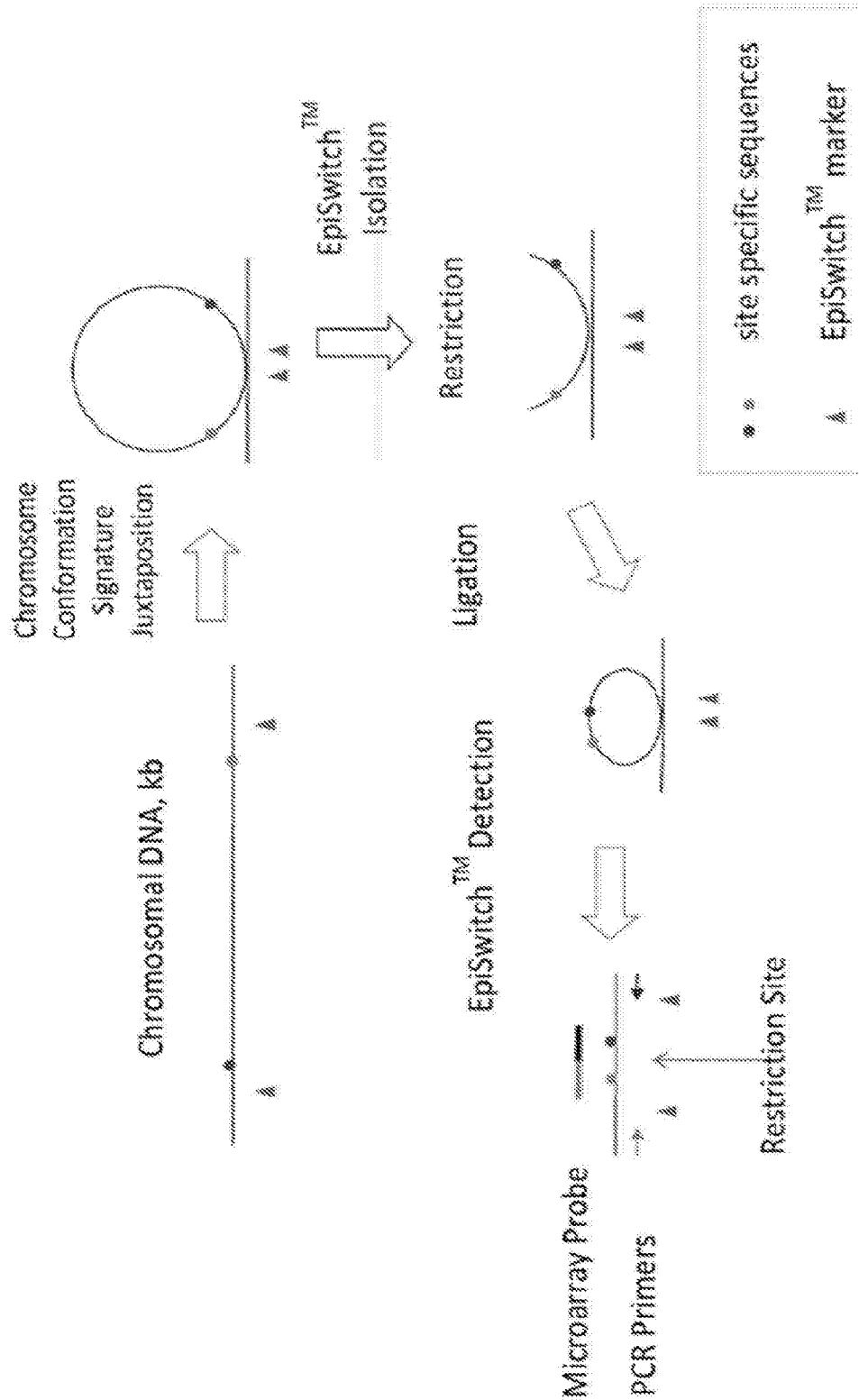
- administering to an individual that has been identified non-responsive to immunotherapy said combination therapy, wherein said combination therapy comprises a therapeutic agent disclosed in any of tables 4 to 6 or a combination therapy disclosed in any of tables 4 to 6.

10 15. An anti-cancer therapy which is not an immunotherapy for use in a method of treating a cancer in an individual, wherein said method of treating comprises:

- identifying whether the individual is a hyper-progressor for immunotherapy by the method of any one of the preceding claims, and

15 - administering to an individual that has been identified as being a hyper-progressor for immunotherapy said anti-cancer therapy.

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**Figure 1**

PFS: Clinical Outcome RECIST 1.1

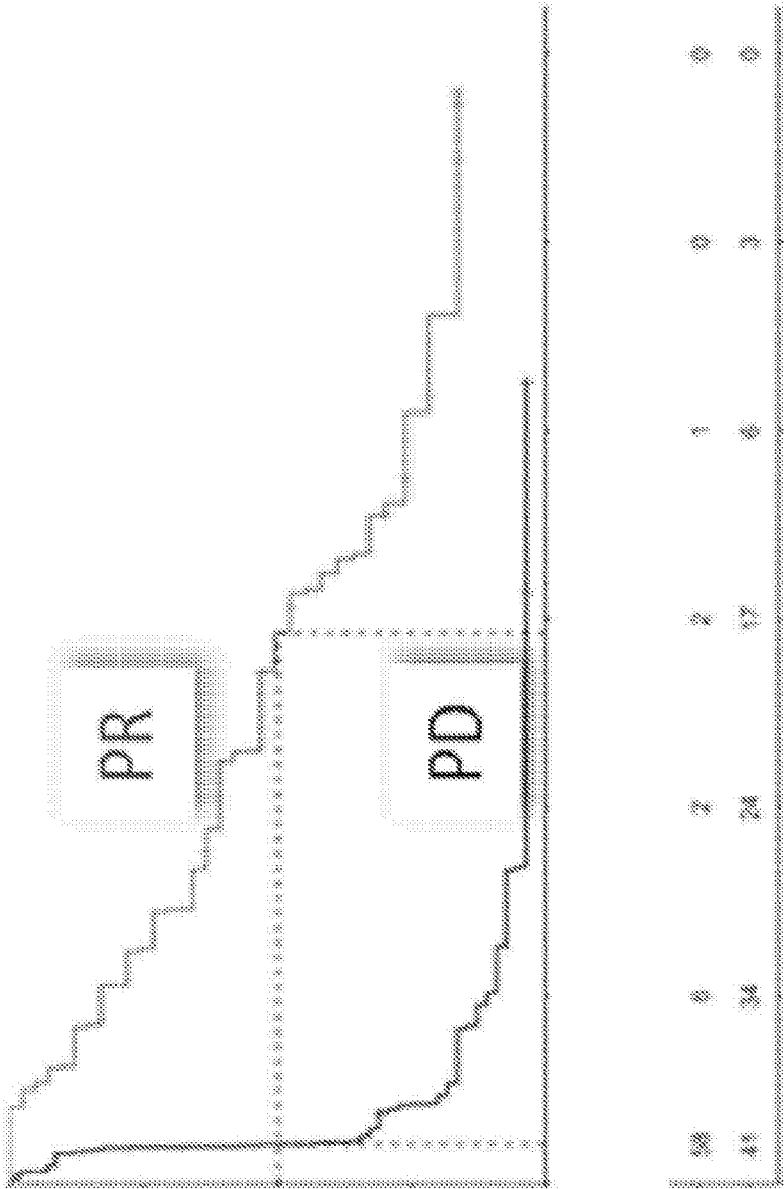


Figure 2a

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PFS: PD-L1 IHC % at Baseline

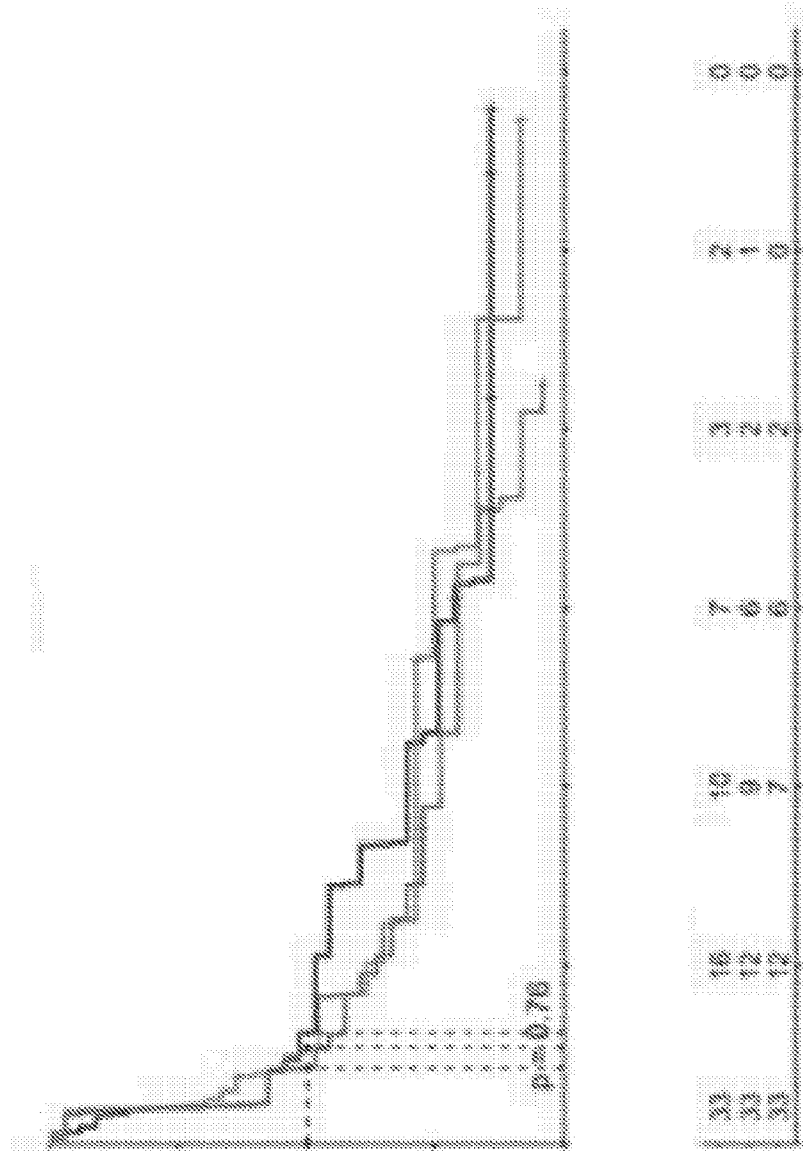


Figure 2b

PFS: EpiSwitch™ Predicted at Baseline

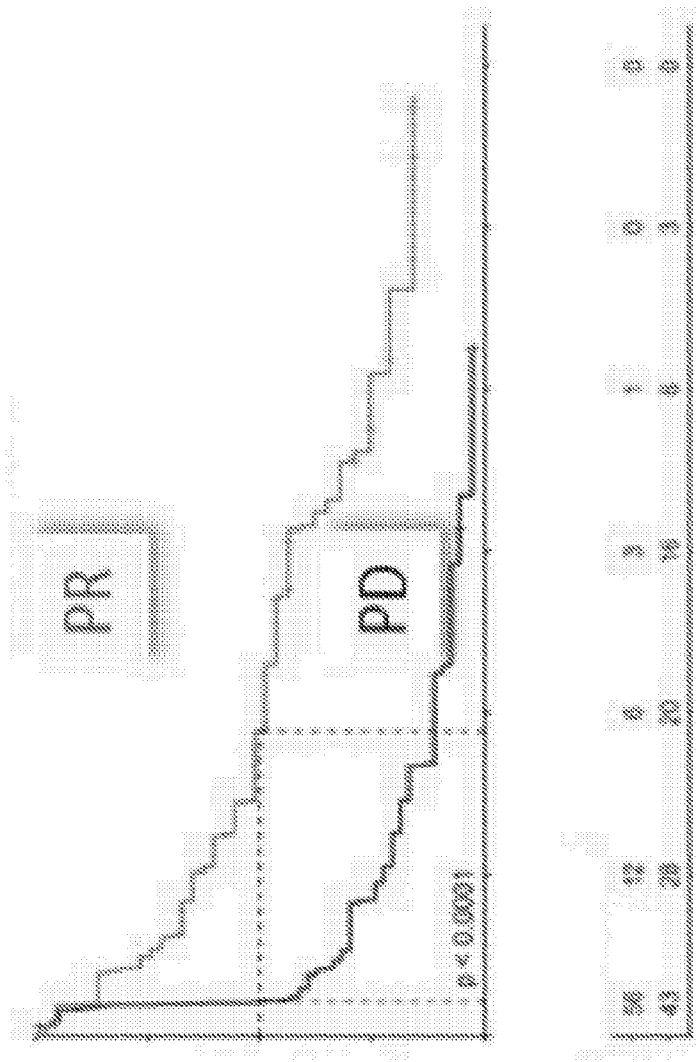


Figure 2c

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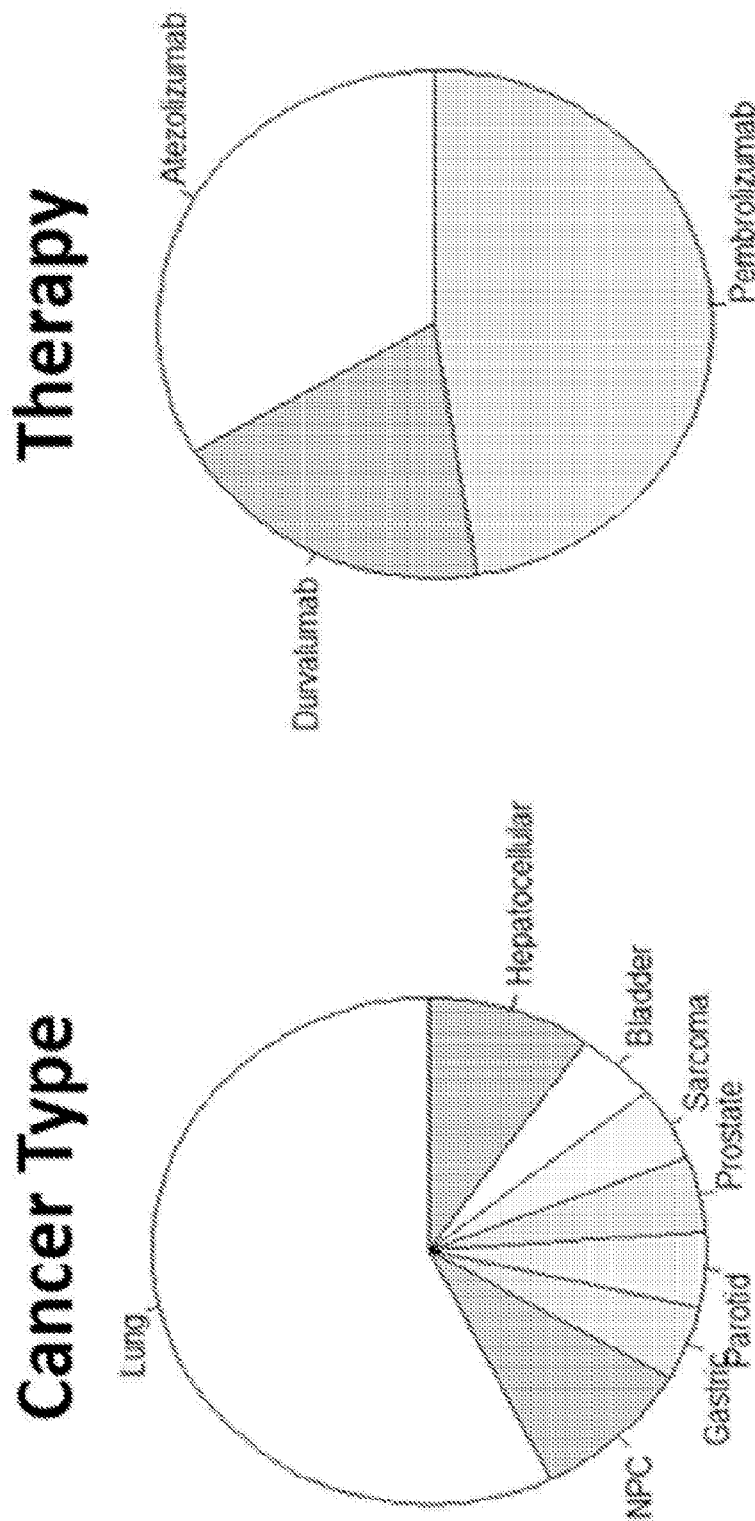


Figure 3

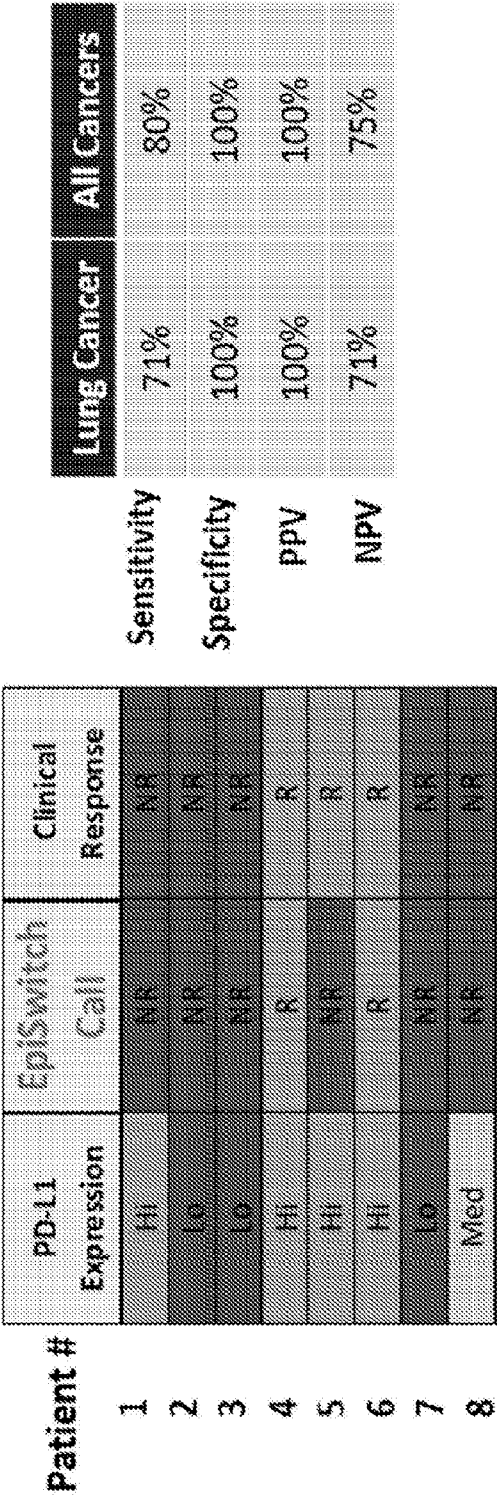
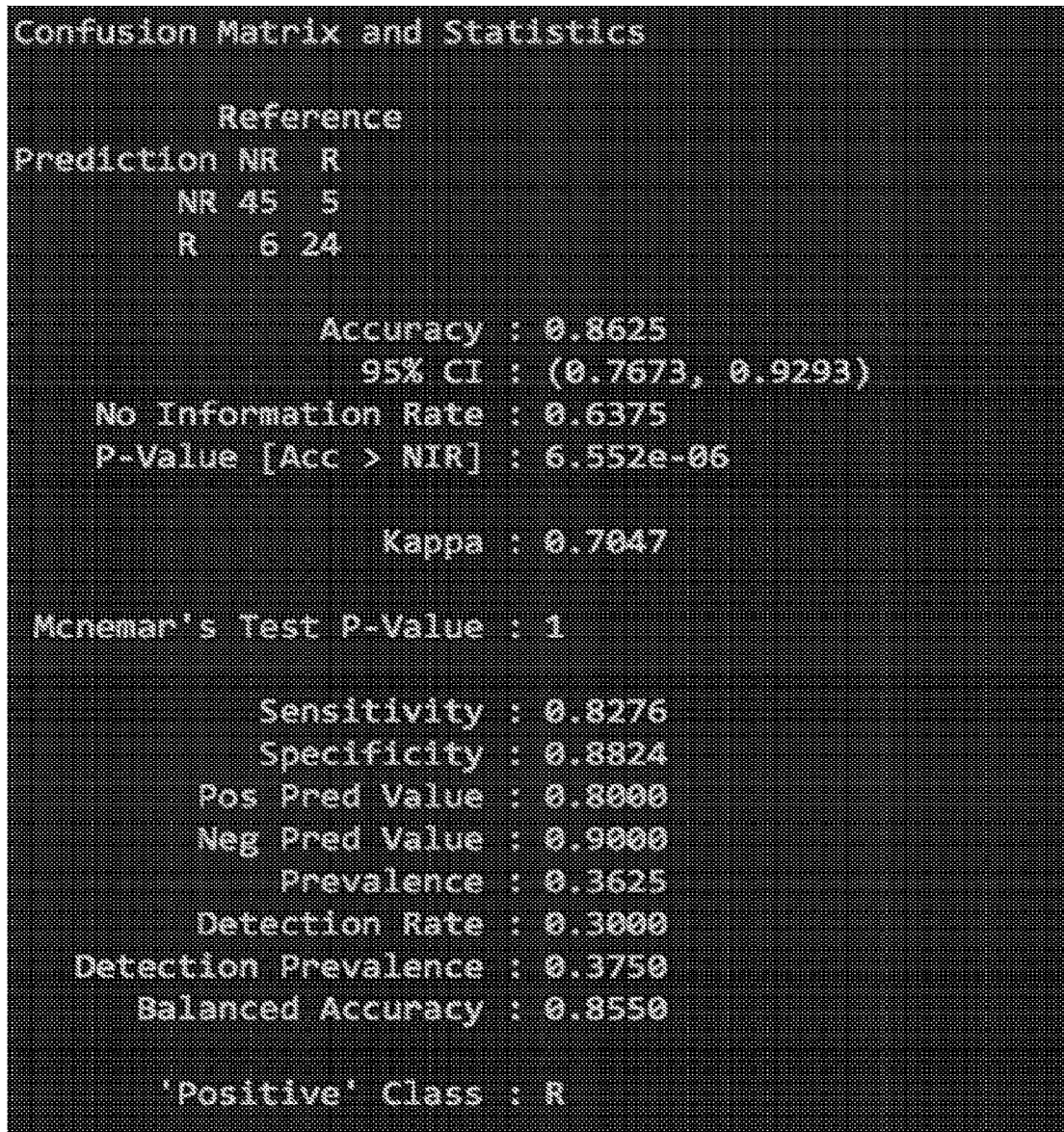
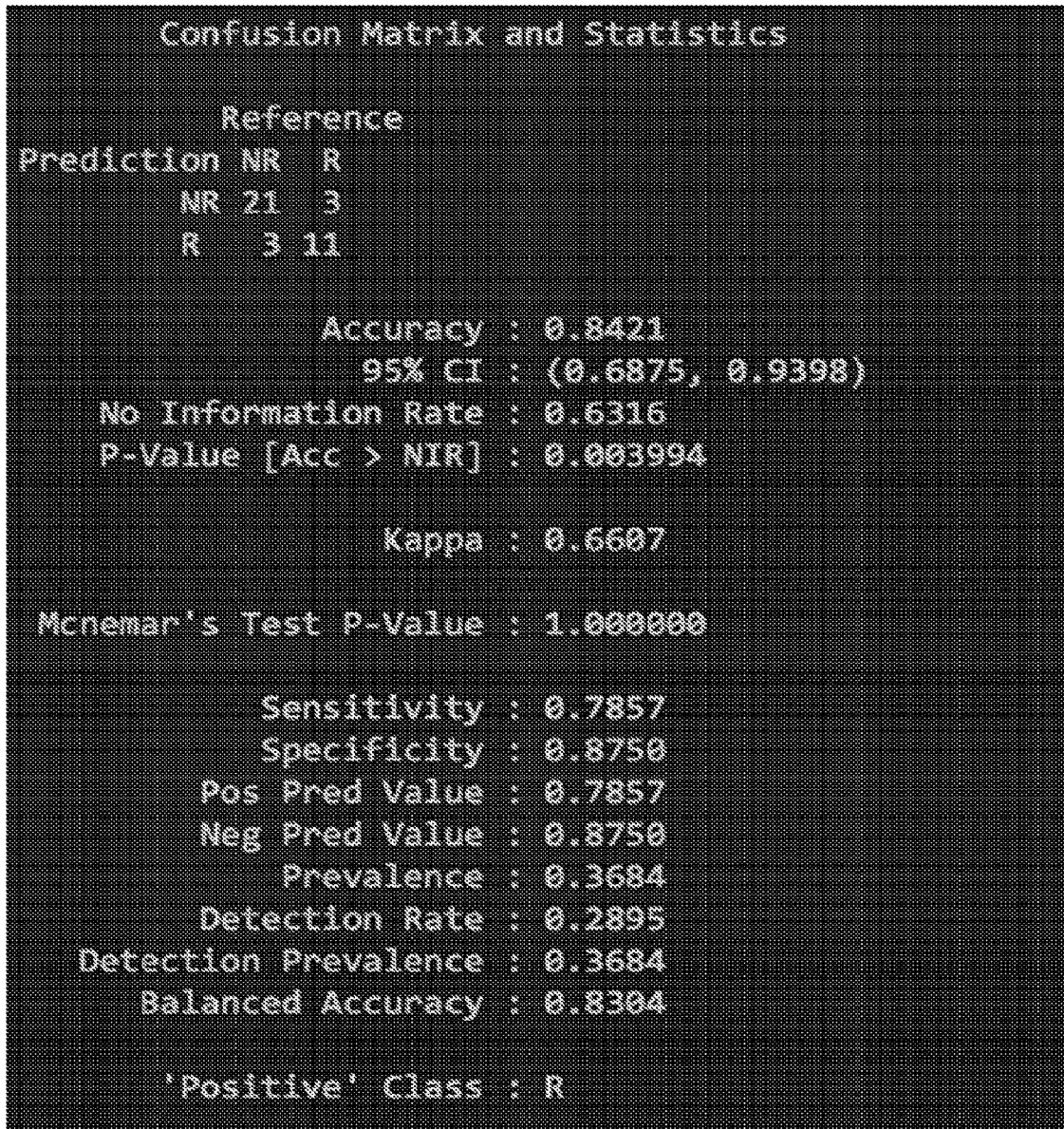


Figure 4

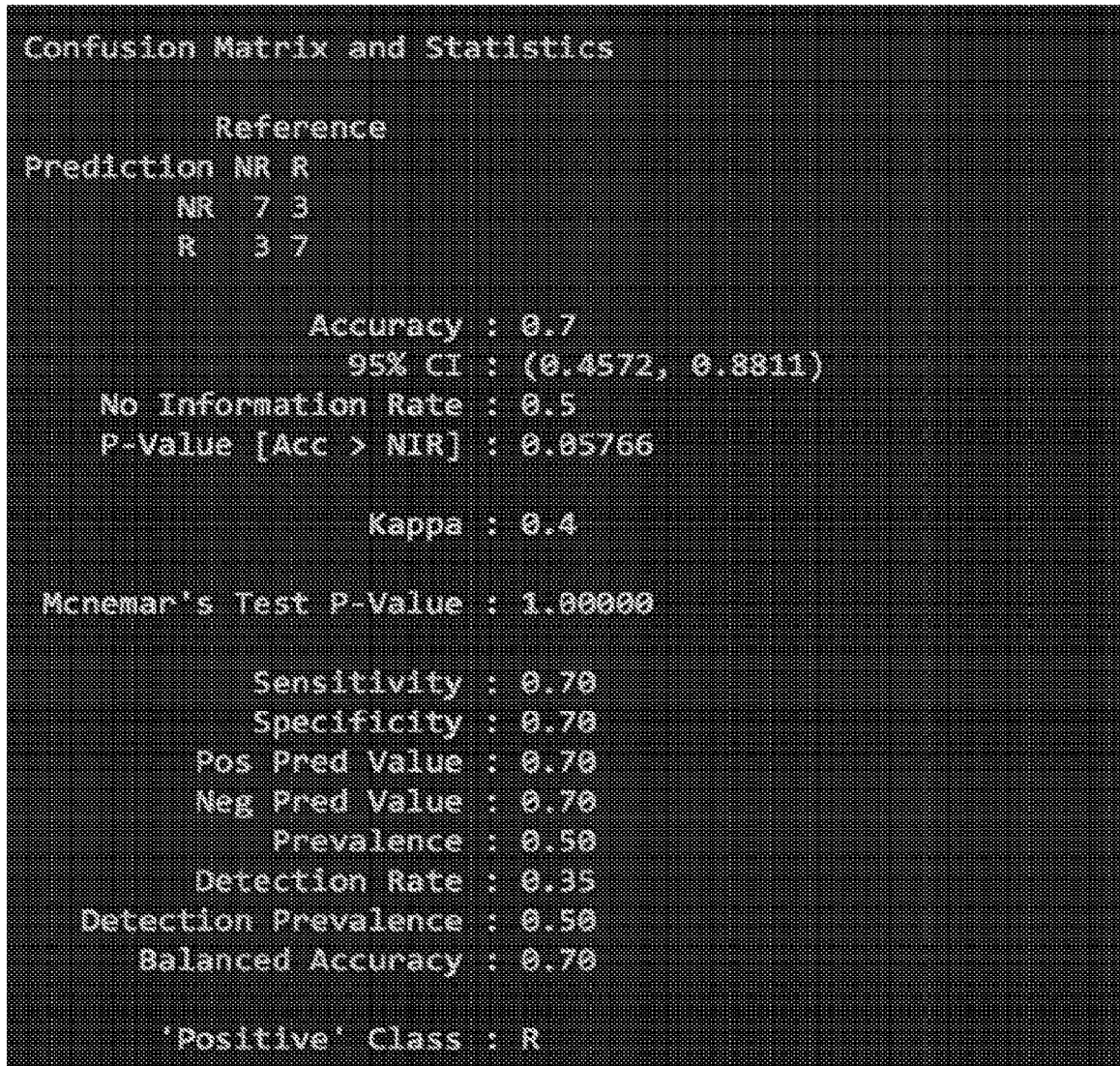
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**Figure 5**

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**Figure 6**

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**Figure 7**

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Sample	call	Resp	Time	CPI	Disease	Status
IOA1007	NR	NR		7.1	Atezolizumab	Alveolar soft part sarcoma
IOA1007	R	R		18.1	Pembrolizumab	Bladder
IOA1002	R	R		2.1	Atezolizumab	HCC
IOA1009	NR	NR		20.1	Pembrolizumab	HCC
OBDM-033	R	NR		1.1	Atezolizumab	Lung
OBDM-019	NR	NR		3.1	Atezolizumab	Lung
OBDM-034	R	R		5.1	Atezolizumab	Lung
OBDM-035	NR	R		6.1	Atezolizumab	Lung
OBDM-044	R	R		8.1	Durvalumab	Lung
OBDM-015	NR	R		9.1	Durvalumab	Lung
OBDM-008	R	R		10.1	Durvalumab	Lung
OBDM-042	R	NR		12.1	Pembrolizumab	Lung
OBDM-001	NR	NR		14.1	Pembrolizumab	Lung
OBDM-014	R	R		15.1	Pembrolizumab	Lung
IOA1006	NR	R		17.1	Pembrolizumab	Lung
OBDM-002	NR	NR		19.1	Pembrolizumab	Lung
IOA1010	R	NR		21.1	Pembrolizumab	Lung
IOA1005	NR	NR		16.1	Pembrolizumab	NPC
IOA1002	NR	NR		13.1	Pembrolizumab	NPC_Gastric Cancer
IOA1004	R	R		4.1	Atezolizumab	Parotid Gland

Figure 8

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1	ORD_ID	Patient	Clinical path	EpSwitch	Disease	Drug	National	Time line	Clinical diagnostics	MR	R
2	ICM01001	8 PR	R		Lung	Durvalumab	Chinese	06 Jun 18	BL Stage 3A T3N2M0 Carcinoma of lung	0.445274	0.554726
3	ICM02001	8 PR	NR		Lung	Durvalumab	Chinese	03 Aug 18	2m Stage 3A T3N2M0 Carcinoma of lung	0.533819	0.466181
4	ICM03001	8 PR	R		Lung	Durvalumab	Chinese	29 Sep 18	4m Stage 3A T3N2M0 Carcinoma of lung	0.45912	0.54088
5	ICM04001	8 PR	R		Lung	Durvalumab	Chinese	23 Nov 18	6m Stage 3A T3N2M0 Carcinoma of lung	0.115234	0.884766
6	ICM05001	8 PR	R		Lung	Durvalumab	Chinese	08 Jan 19	8m Stage 3A T3N2M0 Carcinoma of lung	0.339278	0.660722
7											
8	ICM01004	15 PR	R		Lung	Pembrolizumab	Chinese	28 Aug 18	BL Ca Lung (left)	0.306813	0.693187
9	ICM02004	15 PR	R		Lung	Pembrolizumab	Chinese	03 Nov 18	2m Ca Lung (left)	0.288984	0.711016
10	ICM03004	15 PR	NR		Lung	Pembrolizumab	Chinese	01 Feb 19	5m Ca Lung (left)	0.531593	0.468407
11											
12	ICM04001	1 PD	NR		Lung	Atezolizumab	Chinese	07 Aug 18	BL Poorly differentiated adenocarcinoma of lung	0.597943	0.402057
13	ICM05001	1 SD	R		Lung	Atezolizumab	Chinese	19 Sep 18	1m Poorly differentiated adenocarcinoma of lung	0.226769	0.773231
14	ICM06001	1 PD	R		Lung	Atezolizumab	Chinese	10 Oct 18	2m Poorly differentiated adenocarcinoma of lung	0.306813	0.693187
15											
16	ICM01006	17 PR	R		MPC	Pembrolizumab	Chinese	08 Sep 18	BL Nasopharyngeal carcinoma - metastatic	0.35016	0.64984
17	ICM02006	17 PR	R		MPC	Pembrolizumab	Chinese	12 Dec 18	3m Nasopharyngeal carcinoma - metastatic	0.049696	0.950304

Figure 9

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Patient	Drug	Time	Clinical	Episodic Cells	Q8D.829.031	Q8D.865.047	Q8D.261.263	Q8D.301.303	Q8D.645.649	Q8D.753.755	Q8D.868.871
COM01001	Durvalumab	BL	PR	R	1	1	1	1	0	1	1
COM02001	Durvalumab	2m	PR	NR	1	1	1	0	0	1	1
COM03001	Durvalumab	4m	PR	R	0	0	1	1	0	1	1
COM04001	Durvalumab	6m	PR	R	0	0	1	1	0	1	1
COM05001	Durvalumab	8m	PR	R	1	1	1	1	0	1	0
COM06001	Pembrolizumab	BL	PR	R	0	0	0	0	0	0	0
COM07001	Pembrolizumab	2M	PR	R	0	0	0	0	0	1	1
COM08001	Pembrolizumab	5M	PR	NR	0	1	1	0	0	1	1
COM09001	Atezolizumab	BL	PD	NR	1	0	0	0	0	0	0
COM10001	Atezolizumab	1M	SD	R	0	0	0	0	0	0	1
COM11001	Atezolizumab	2M	PD	R	0	0	0	0	0	0	0
COM12001	Pembrolizumab	BL	PR	R	0	1	1	0	0	1	0
COM13001	Pembrolizumab	3M	PR	R	0	1	1	0	0	1	0
COM14001	Atezolizumab	1M	PD	NR	1	0	0	0	0	0	0
COM15001	Atezolizumab	BL	PD	R	0	0	0	0	0	0	0
COM16001	Atezolizumab	3M	PD	R	0	0	0	0	0	0	0

Figure 10

SUBSTITUTE SHEET (RULE 26)

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	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
	Patient ID	OS	PPS	IRFS	OS_C	PPS_C	HP	Subject ID	Specimen ID	bor_1	class_1	bor_2	class_2	Cohort_Type	Cohort
1	509175_A1	1387	1160	1160	0	0	0.5	1070025	AF023355-4	PR	R	PR	R	2L	2
2	509259_A1	1149	1147	1147	0	0	0.5	1580011	AF485796-4	CR	R	CR	R	2L	2
3	521939_A1	855	836	836	0	0	0.5	1820014	AF4466127-4	PR	R	SD	R	1L	2
4	521728_A1	771	751	751	0	0	0.5	1930050	AF020021-4	PR	R	PR	R	1L	2
5	517408_A1	719	715	715	0	0	0.5	4030014	EE515570-4	PR	R	PR	R	1L	1
6	509211_A1	1479	710	710	0	0	0.5	1280003	AF756710-4	CR	R	CR	R	2L	2
7	509237_A1	625	554	554	0	0	0.5	1420008	AE774158-4	PR	R	PR	R	2L	2
8	521683_A1	885	542	542	0	0	0.5	2040009	AF021334-3	PR	R	PR	R	1L	2
9	509218_A1	1401	723	723	0	0	1.5	1220011	AF756715-4	PR	R	PR	R	2L	2
10	509274_A1	1312	631	631	0	0	1.5	1670004	AF187296-1	PR	R	PR	R	2L	2
11	509289_A1	1128	627	627	0	0	1.5	2760002	AF615475-4	PR	R	PR	R	2L	2
12	509194_A1	1247	585	585	0	0	1.5	1110004	AF515133-4	PR	R	PR	R	2L	2
13	521705_A1	771	460	460	0	0	1.5	1220039	AF049795-4	PR	R	PR	R	1L	1
14	517257_A1	6	6	6	1	1	1.H	4030013	EE515663-4	NE	NR	NE	NR	1L	2
15	521772_A1	13	13	13	1	1	1.H	3100025	AF859494-4	NE	NR	NE	NR	1L	2
16	509166_A1	28	19	19	1	1	1.H	1030001	AE757094-4	PD	NR	PD	NR	2L	2
17	529813_A1	32	32	32	1	1	1.H	1050007	AF4439175-4	NE	NR	NE	NR	1L	2
18	521892_A1	35	35	35	1	1	1.H	1570026	AF329215-6	NE	NR	NE	NR	1L	2
19	509295_A1	52	39	39	1	1	1.H	1940003	AF386993-4	PD	NR	PD	NR	2L	2
20	517042_A1	53	40	40	1	1	1.H	1880019	AGE76167-4	PD	NR	PD	NR	1L	2
21	509221_A1	57	13	13	1	1	1.H	1220022	AF838757-4	PD	NR	PD	NR	2L	2
22	509300_A1	81	50	50	1	1	1.H	2080002	AF277894-4	PD	NR	PD	NR	2L	2
23	509233_A1	84	32	32	1	1	1.H	1410002	AF077263-4	PD	NR	PD	NR	2L	1
24	509280_A1	102	45	45	1	1	1.H	1690005	AF058025-4	PD	NR	PD	NR	2L	2
25	521933_A1	127	42	42	1	1	1.H	1350016	AF5204913-4	PD	NR	PD	NR	2L	2
26	521838_A1	132	42	42	1	1	1.H	1370031	AF050807-4	PD	NR	PD	NR	1L	2

Figure 11

SUBSTITUTE SHEET (RULE 26)

1	Patient ID	OS	PFS	IRPFS	DS_C	PFS_C	HP	Subject ID	Specimen ID	bot_1	class_1	bot_2	class_2	Cohort_Type	Cohort
2	521913_A1	759	612	612	0	0	0.5	1350013	AH336622-4	PR	R	PR	R	1L	2
3	521709_A1	813	710	710	0	0	1.5	1240012	AH323360-4	PR	R	PR	R	1L	2
4	509283_A1	1343	1087	1087	0	0	0.5	1710002	AFC06432-4	PR	R	PR	R	2L	2
5	521755_A1	22	12	12	22	1	1 H	1950027	AH471940-4	PD	NR	PD	NR	1L	2
6	509245_A1	119	40	40	40	1	1 H	1510008	AF237674-4	PD	NR	PD	NR	2L	2
7	521919_A1	42	42	42	42	1	1 H	1930019	AH327970-4	NE	NR	NE	NR	1L	2

Figure 12

SUBSTITUTE SHEET (RULE 26)

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1	markerName	OS.pval	PFS.pval	Diff	Gene
2	OB0148_105.107_2	0.02101101	0.02260266	5	PAG1
3	OB0148_669.671_2	0.17777781	0.04222486	-3	PANK3
4	OB0117_029.031_2	0.28452726	0.08518713	-3	CD274_PDCD1LG2
5	OB0148_645.647_4	0.05317131	0.1075997	-5	IFT88_IL17D
6	OB0148_821.823_1	0.05384985	0.12730792	4	CASP6_PLA2G12A_CFI
7	OB0148_893.895_2	0.0667315	0.14221077	5	CCL8_CCL13_CCL1
8	OB0148_917.919_4	0.03030755	0.14565984	6	TNFSF8_TNC
9	OB0148_301.303_2	0.04261089	0.14989053	5	TRAF1_PHF19
10	OB0148_505.507_2	0.07939328	0.2284663	5	TNFRSF11A
11	OB0148_661.663_1	0.05941606	0.31868899	5	DPYSL2
12	OB0148_261.263_1	0.06613001	0.44550018	5	IKBKB

Figure 13

SUBSTITUTE SHEET (RULE 26)

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DETAILED RESULTS

Pathways: 36, Matching Gene(s): 14

Enter filter text

	Score	Name	# Matched Genes (Total Genes)	Sources
+	27.05	SuperPath: PAX Pathway	8 (683)	
+	26.10	SuperPath: PEDF Induced Signaling	8 (743)	
+	22.49	SuperPath: TRAF Pathway	5 (188)	
+	22.18	SuperPath: Akt Signaling	7 (682)	
+	20.32	SuperPath: NF-kappa B Signaling Pathway	4 (102)	
+	18.71	SuperPath: CCR5 Pathway in Macrophages	5 (321)	
+	18.58	SuperPath: NF-kappaB Signaling	5 (327)	
+	17.65	SuperPath: Translocation of ZAP-70 to Immunological Synapse	3 (46)	
+	17.29	SuperPath: Apoptosis and Survival Anti-apoptotic TNFs/NF-kB/Iso-2 Pathway	3 (50)	
+	17.00	SuperPath: Apoptosis and Autophagy	4 (183)	
+	16.66	SuperPath: T Cell Co-Signaling Pathway: Ligand-Receptor Interactions	3 (58)	
+	16.30	SuperPath: TNFR1 Pathway	4 (207)	
+	14.12	SuperPath: RANK Signaling in Osteoclasts	3 (105)	
+	14.04	SuperPath: Chemokine Superfamily Pathway: Human/Mouse Ligand-Receptor Interactions	3 (107)	
+	13.18	SuperPath: ERK Signaling	6 (1179)	
+	12.81	SuperPath: EBV LMP1 Signaling	2 (24)	
+	10.47	SuperPath: Apoptosis Modulation and Signaling	3 (249)	
+	10.14	SuperPath: P75(NTR)-mediated Signaling	2 (61)	
+	9.95	SuperPath: TGF-Beta Pathway	4 (653)	
+	9.92	SuperPath: TCR Signaling in Naive CD4+ T Cells	2 (66)	

Figure 14

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DETAILED RESULTS

Pathways: 36, Matching Gene(s): 14

Enter filter text

	Score	Name	# Matched Genes (Total Genes)	Sources
	9.71	SuperPath: Microglia Activation During Neuroinflammation: Microglia Polarization	2 (71)	BD
	9.67	SuperPath: TNF Signaling (REACTOME)	2 (72)	
	9.17	SuperPath: CD28 Co-stimulation	2 (86)	
	8.98	SuperPath: Small Cell Lung Cancer	2 (92)	
	8.72	SuperPath: Class I MHC Mediated Antigen Processing and Presentation	4 (823)	
	8.62	SuperPath: Innate Immune System	6 (2124)	
	8.59	SuperPath: P70S6K Signaling	3 (392)	
	8.42	SuperPath: TNF Signaling Pathway	2 (112)	
	8.37	SuperPath: CDK-mediated Phosphorylation and Removal of Cdc6	4 (879)	
	8.32	SuperPath: IL-17 Family Signaling Pathways	2 (116)	BD
	8.18	SuperPath: TCR Signaling (REACTOME)	2 (122)	
	8.07	SuperPath: C-type Lectin Receptor Signaling Pathway	2 (127)	BD
	8.04	SuperPath: Osteoclast Differentiation	2 (128)	
	7.71	SuperPath: Brain-Derived Neurotrophic Factor (BDNF) Signaling Pathway	2 (144)	
	7.68	SuperPath: Immune Response CCR3 Signaling in Eosinophils	2 (146)	
	7.66	SuperPath: Cell Adhesion Molecules (CAMs)	2 (147)	

Items per page: 20

Figure 15

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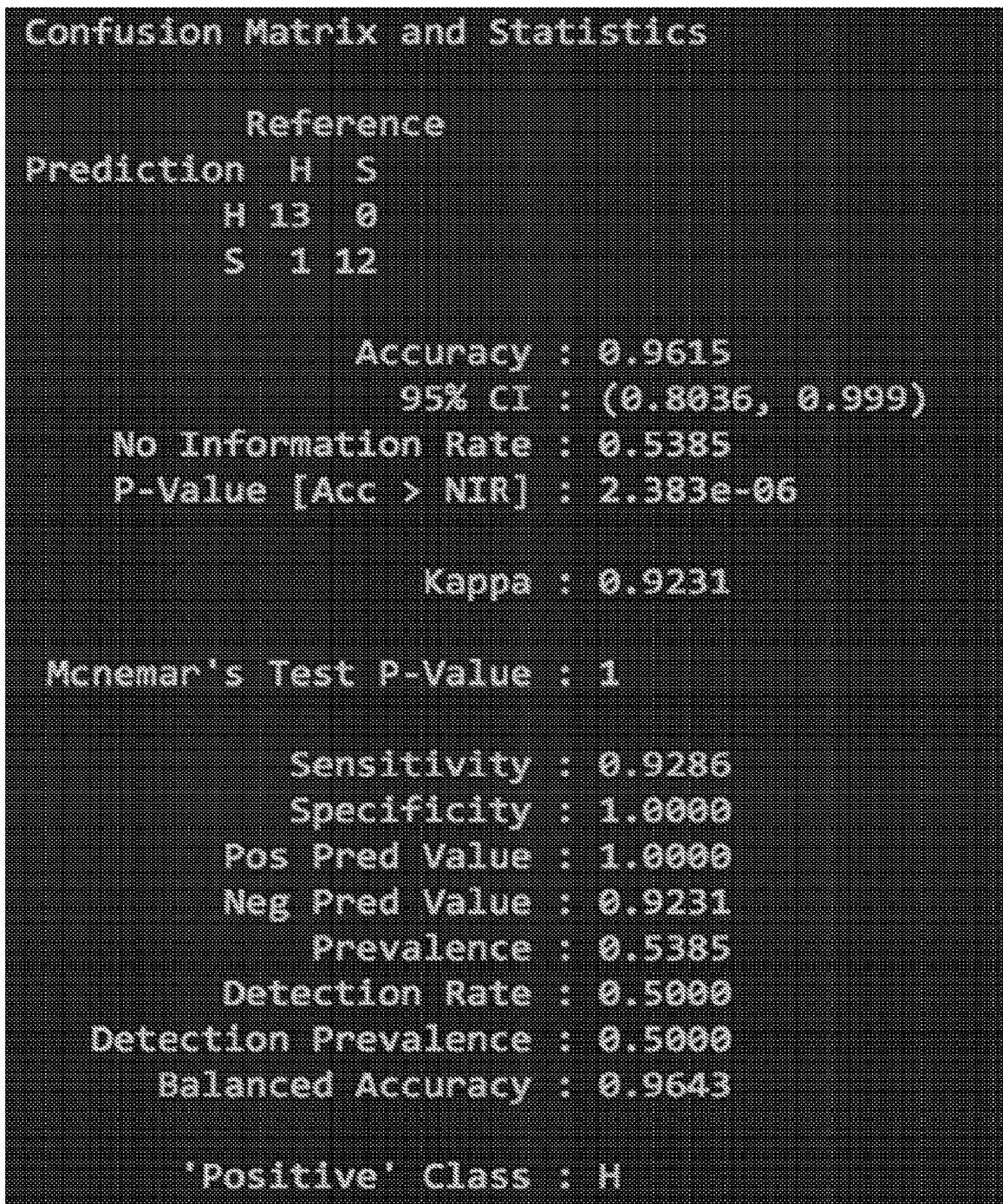


Figure 16

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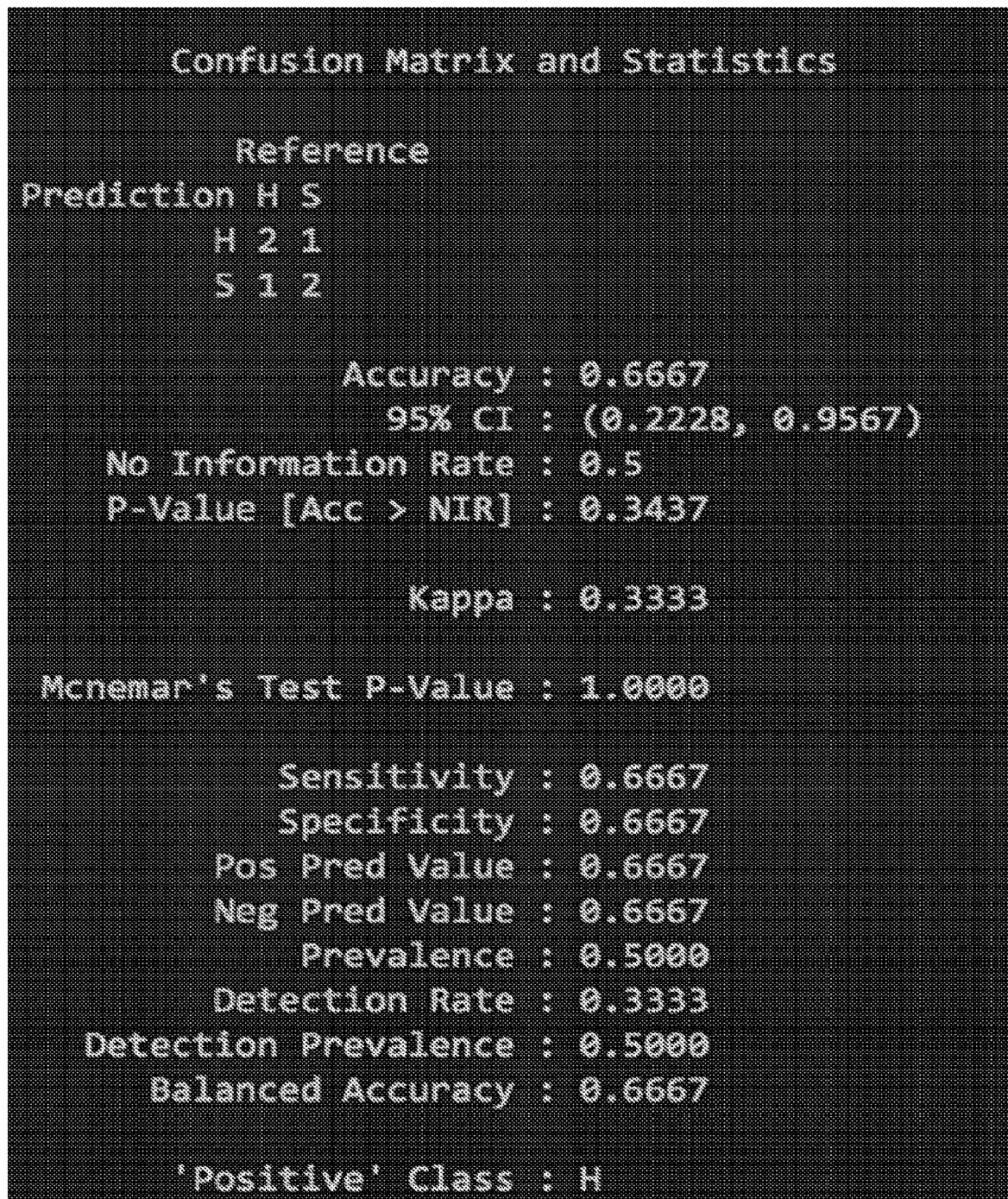
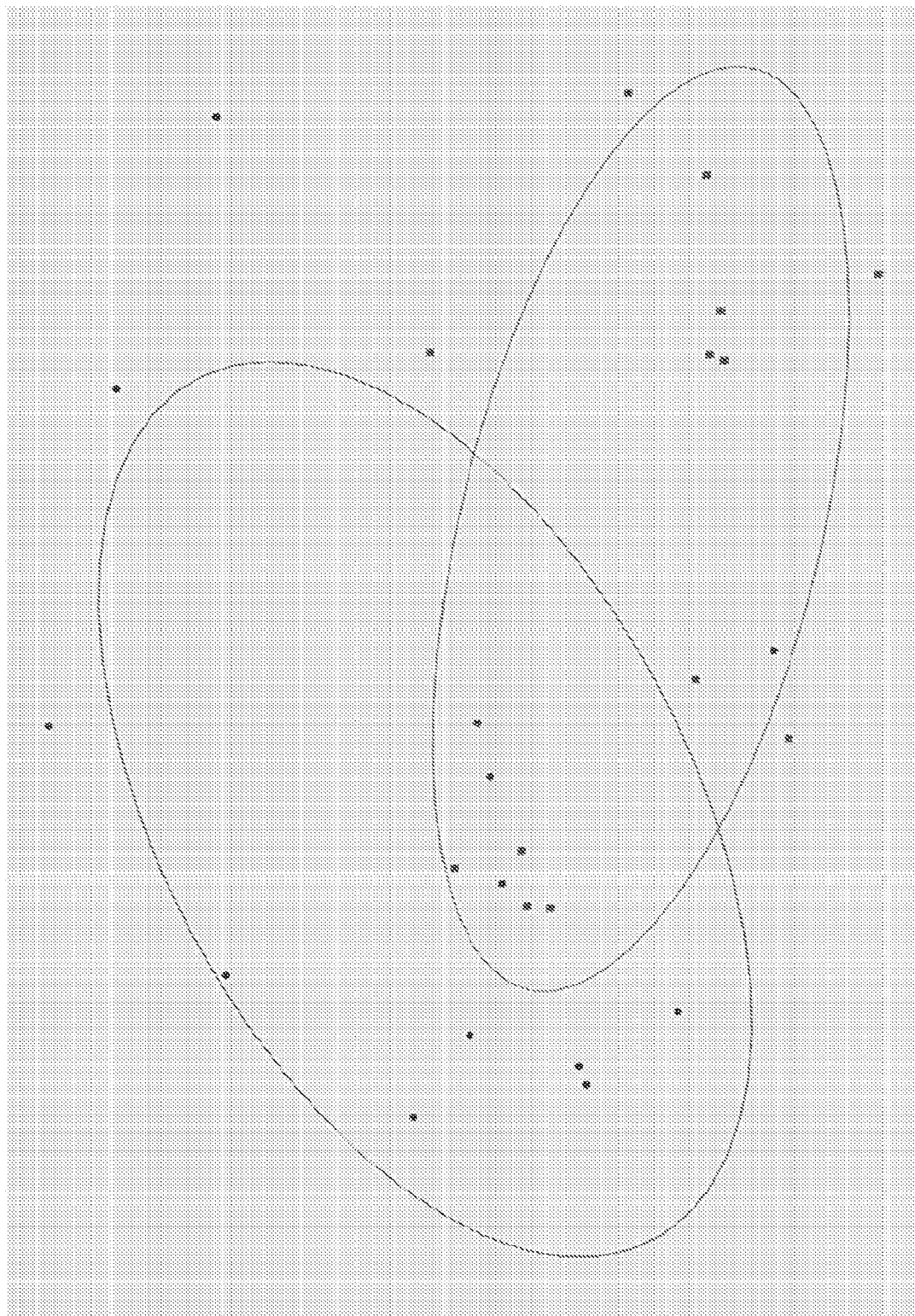


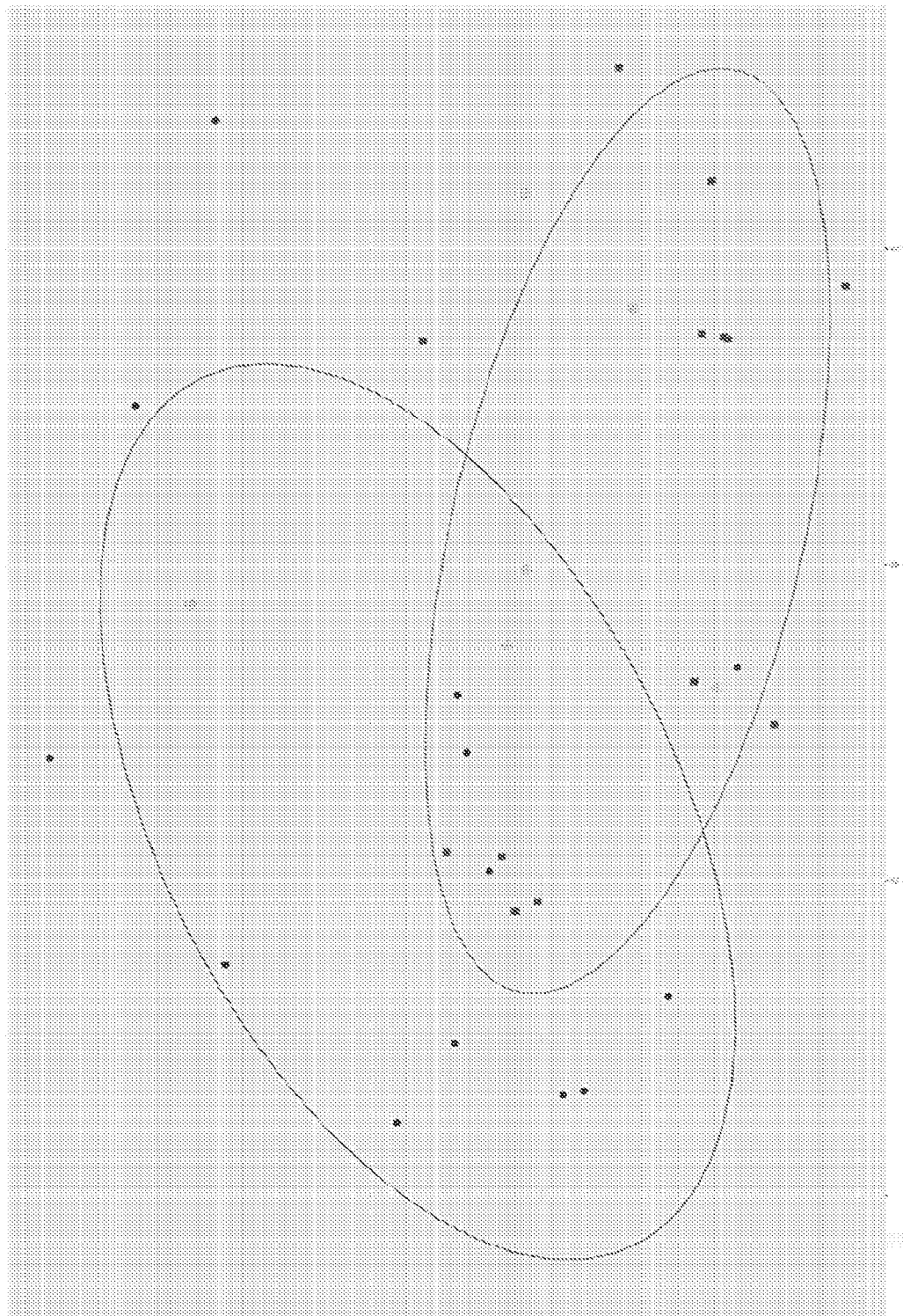
Figure 17

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**Figure 18**

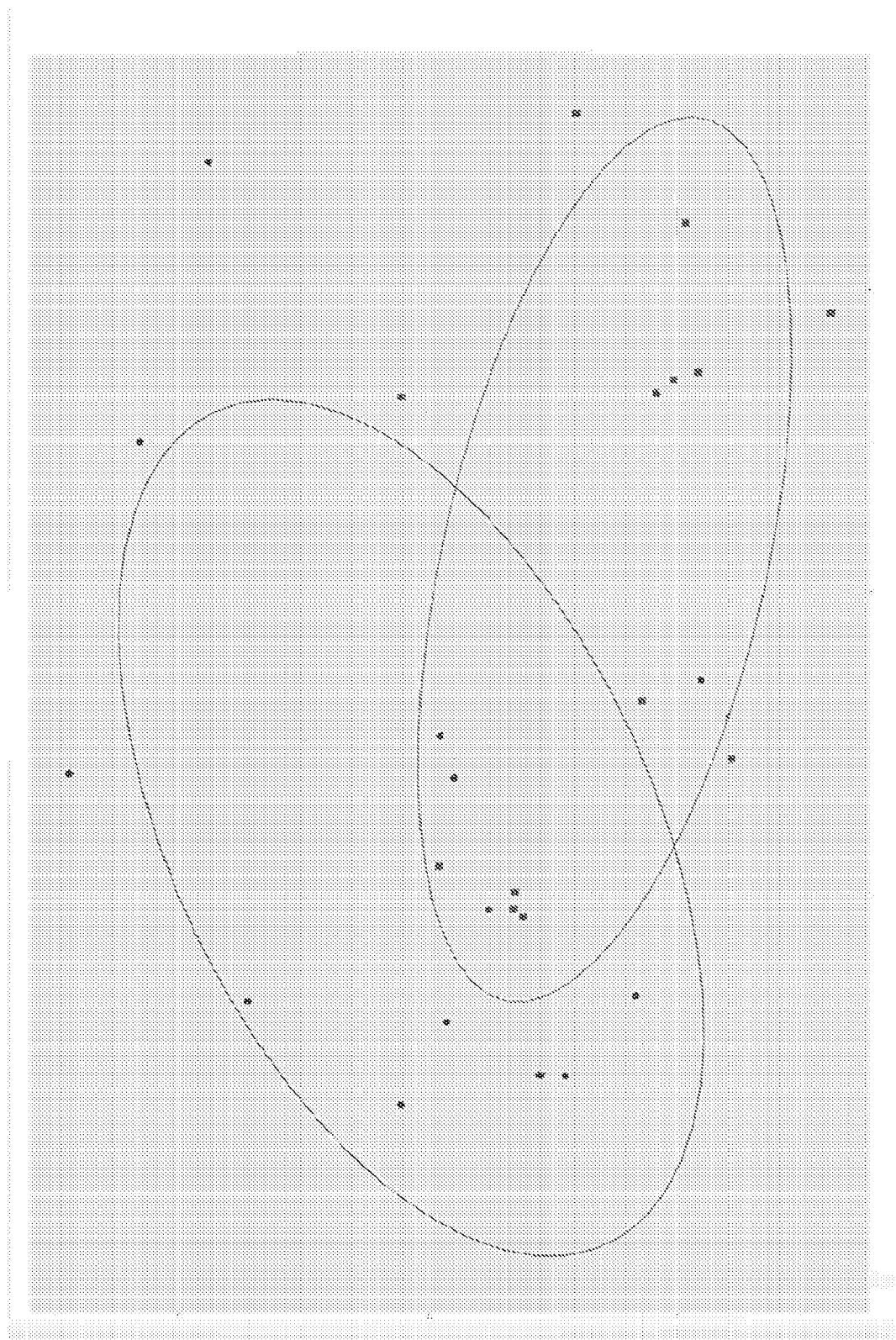
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**Figure 19**

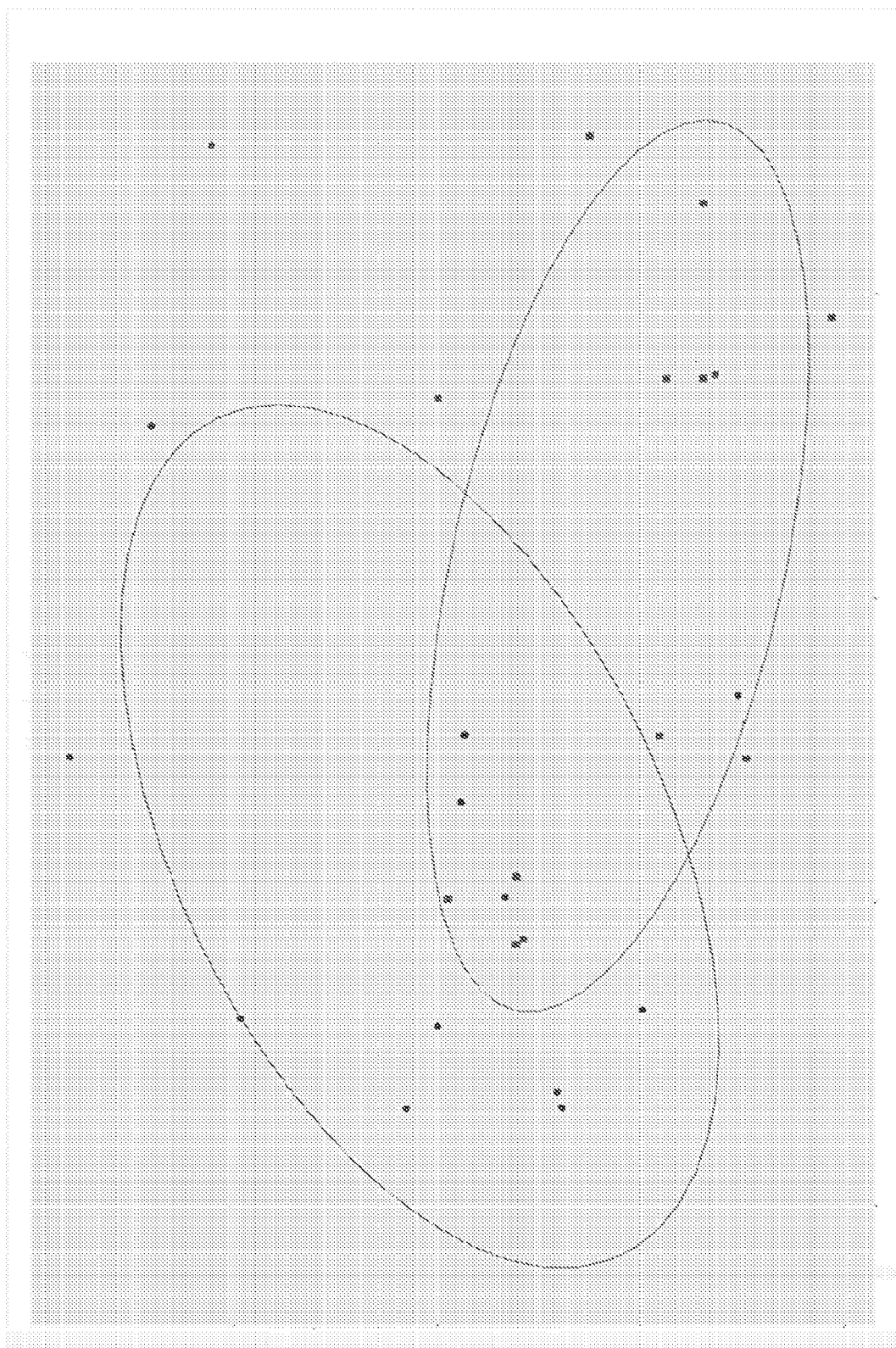
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**Figure 20**

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**Figure 21**

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24/29**Confusion Matrix and Statistics**

	Reference	
Prediction	NR	R
NR	39	0
R	0	39

Accuracy : 1
 95% CI : (0.9538, 1)
 No Information Rate : 0.5
 P-Value [Acc > NIR] : < 2.2e-16

Kappa : 1

McNemar's Test P-Value : NA

Sensitivity : 1.0
 Specificity : 1.0
 Pos Pred Value : 1.0
 Neg Pred Value : 1.0
 Prevalence : 0.5
 Detection Rate : 0.5
 Detection Prevalence : 0.5
 Balanced Accuracy : 1.0

'Positive' Class : R

~~~~~

**Figure 22**

**25/29****Confusion Matrix and Statistics**

|               |           |  |
|---------------|-----------|--|
|               | Reference |  |
| Prediction NR | R         |  |
| NR 10         | 2         |  |
| R 2           | 10        |  |

Accuracy : 0.8333  
 95% CI : (0.6262, 0.9526)  
 No Information Rate : 0.5  
 P-Value [Acc > NIR] : 0.0007719

Kappa : 0.6667

McNemar's Test P-Value : 1.0000000

Sensitivity : 0.8333  
 Specificity : 0.8333  
 Pos Pred Value : 0.8333  
 Neg Pred Value : 0.8333  
 Prevalence : 0.5000  
 Detection Rate : 0.4167  
 Detection Prevalence : 0.5000  
 Balanced Accuracy : 0.8333

'Positive' Class : R

**Figure 23**

**26/29****Confusion Matrix and Statistics**

|            |    |           |   |
|------------|----|-----------|---|
|            |    | Reference |   |
| Prediction | NR | R         |   |
|            | NR | 92        | 2 |
|            | R  | 27        | 7 |

Accuracy : 0.7734

95% CI : (0.6911, 0.8427)

No Information Rate : 0.9297

P-Value [Acc &gt; NIR] : 1

Kappa : 0.2412

McNemar's Test P-Value : 8.324e-06

Sensitivity : 0.7731

Specificity : 0.7778

Pos Pred Value : 0.9787

Neg Pred Value : 0.2059

Prevalence : 0.9297

Detection Rate : 0.7188

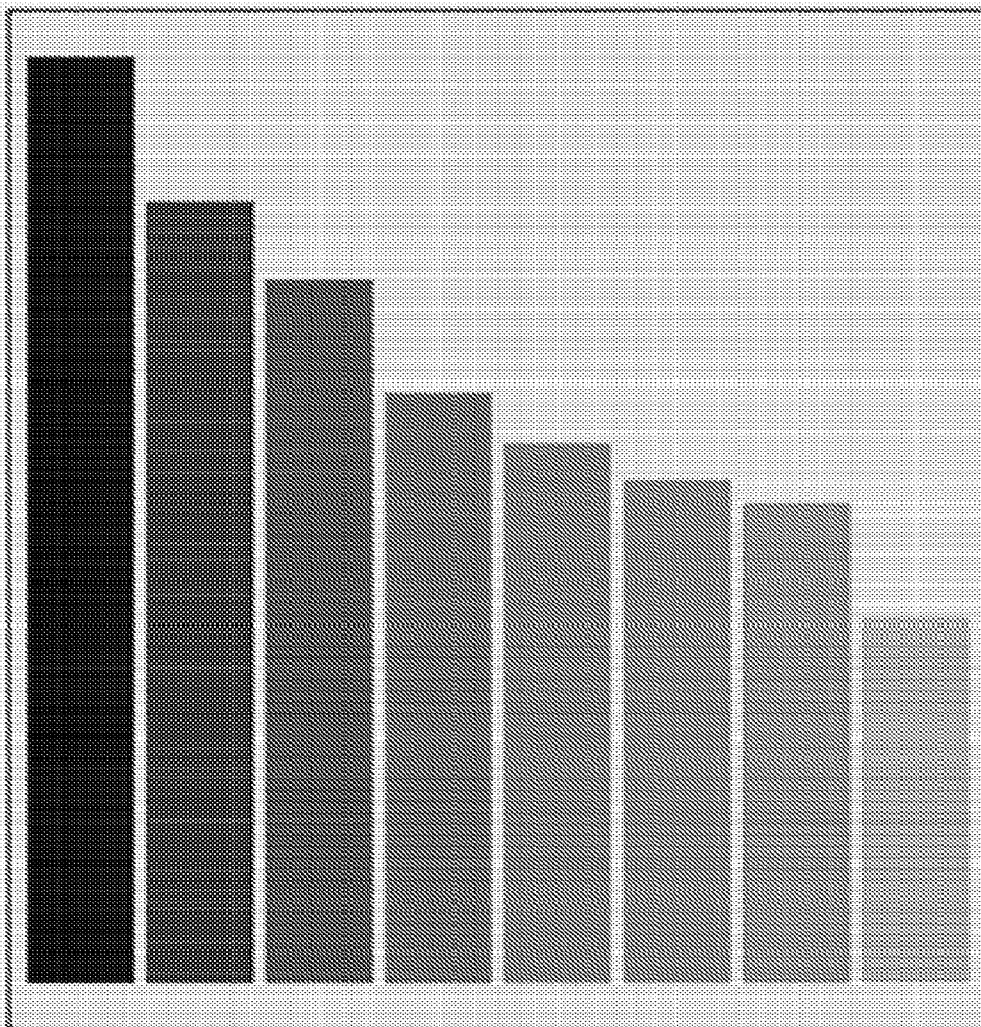
Detection Prevalence : 0.7344

Balanced Accuracy : 0.7754

'Positive' Class : NR

**Figure 24**

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**Figure 25**

SUBSTITUTE SHEET (RULE 26)

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| 1  | Marker           | Gene     | Distance | Rank_Model |
|----|------------------|----------|----------|------------|
| 2  | OBD189_q065_q067 | ITK      | 0        | 1          |
| 3  | OBD189_q065_q067 | CYFIP2   | 0        | 1          |
| 4  | OBD189_q053_q055 | CPB2     | 0        | 2          |
| 5  | OBD189_q053_q055 | LCP1     | 0        | 2          |
| 6  | OBD189_q081_q083 | IFT88    | 0        | 3          |
| 7  | OBD189_q081_q083 | IL17D    | 3493     | 3          |
| 8  | OBD189_q893_q895 | CCL8     | 0        | 4          |
| 9  | OBD189_q893_q895 | CCL13    | 0        | 4          |
| 10 | OBD189_q893_q895 | CCL13    | 0        | 4          |
| 11 | OBD189_q049_q051 | TNFSF8   | 0        | 5          |
| 12 | OBD189_q049_q051 | TNFSF15  | 49628    | 5          |
| 13 | OBD189_q049_q051 | TNC      | 90160    | 5          |
| 14 | OBD189_q029_q031 | TNFRSF25 | 0        | 6          |
| 15 | OBD189_q029_q031 | PLEKHG5  | 0        | 6          |
| 16 | OBD189_q057_q059 | PDCD1LG2 | 0        | 7          |
| 17 | OBD189_q057_q059 | CD274    | 25427    | 7          |
| 18 | OBD189_q005_q007 | CASP6    | 0        | 8          |
| 19 | OBD189_q005_q007 | PLA2G12A | 0        | 8          |
| 20 | OBD189_q005_q007 | CFI      | 0        | 8          |

Figure 26

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|    | Score | Name                                                                                           | # Matched<br>Genes (Total<br>Genes) | Sources                                                                               |
|-------------------------------------------------------------------------------------|-------|------------------------------------------------------------------------------------------------|-------------------------------------|---------------------------------------------------------------------------------------|
|    | 23.30 | SuperPath: PEDF Induced Signaling                                                              | 8 (745)                             |    |
|    | 23.90 | SuperPath: NF-kappaB Signaling                                                                 | 6 (327)                             |    |
|    | 19.89 | SuperPath: Akt Signaling                                                                       | 7 (632)                             |    |
|    | 19.83 | SuperPath: PAK Pathway                                                                         | 7 (686)                             |    |
|    | 17.18 | SuperPath: CCR5 Pathway in Macrophages                                                         | 5 (322)                             |    |
|    | 16.80 | SuperPath: Translocation of ZAP-70 to Immunological Synapse                                    | 3 (46)                              |    |
|    | 16.44 | SuperPath: Apoptosis and Survival Anti-apoptotic TNFs/NF-kB/Iccl-2 Pathway                     | 3 (50)                              |    |
|    | 16.11 | SuperPath: TNF Superfamily - Human Ligand-Receptor Interactions and Their Associated Functions | 3 (54)                              |    |
|    | 14.67 | SuperPath: ERK Signaling                                                                       | 7 (1185)                            |    |
|    | 13.25 | SuperPath: Integrin Pathway                                                                    | 5 (570)                             |    |
|    | 13.19 | SuperPath: Chemokine Superfamily: Human/Mouse Ligand-Receptor Interactions                     | 3 (107)                             |    |
|   | 12.74 | SuperPath: Death Receptor Signaling                                                            | 3 (119)                             |   |
|  | 12.14 | SuperPath: Innate Immune System                                                                | 8 (2124)                            |  |
|  | 10.88 | SuperPath: Cell Adhesion_Cell-matrix Glycoconjugates                                           | 2 (39)                              |  |
|  | 10.82 | SuperPath: TRAF Pathway                                                                        | 3 (188)                             |  |
|  | 10.42 | SuperPath: TNFR1 Pathway                                                                       | 3 (207)                             |  |
|  | 9.56  | SuperPath: IL 10 Signaling Pathway                                                             | 2 (62)                              |  |
|  | 8.87  | SuperPath: TGF-Beta Pathway                                                                    | 4 (653)                             |  |
|  | 8.63  | SuperPath: CD28 Co-stimulation                                                                 | 2 (86)                              |  |
|  | 8.57  | SuperPath: Complement and Coagulation Cascades                                                 | 2 (88)                              |  |

Figure 27