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(54) Title: GENETIC BRAIN TUMOR MARKERS

(57) Abstract: The present invention relates to methods of genetic analysis for the prediction of treatment sensitivity and survival prognosis of patients with brain tumors, especially oligodendroglial tumors. The invention provides a method for producing a classification scheme for oligodendroglial tumors comprising the steps of a) providing a plurality of reference samples, said reference samples comprising cell samples from a plurality of reference subjects suffering from oligodendroglial tumors; b) providing reference profiles by establishing a gene expression profile for each of said reference samples individually; c) clustering said individual reference profiles according to similarity, and d) assigning an oligodendroglial tumor class to each cluster.

Title: Genetic brain tumor markers

The invention relates to the field of diagnosis of tumors, especially brain tumors, more especially oligodendroglial tumors, more particular to the prediction of susceptibility to treatment for patients with brain tumor.

Diffuse gliomas are the most common primary central nervous system tumors in adults (Legler, J.M. et al., (1999) J. Natl. Cancer Inst. 91: 1382-1390; Macdonald, D.R. (2003) Semin. Oncol. 30: 72-76) and it is estimated that approximately 18,000 new patients per annum are diagnosed with a primary brain tumor in the USA (CBTRUS 2004-2005 statistical report). The worldwide standard for grading and classification of these tumors is at present the WHO classification (Kleihues, P and Cavenee, W.K., World Health Organization Classification of Tumours of the Nervous System, Lyon: WHO/IARC, 2000). Based on their histological appearance gliomas can be divided into astrocytic tumors, pure oligodendroglial tumors and mixed oligoastrocytic tumors. The latter two are grouped together as oligodendroglial tumors. The oligodendrogliomas comprise approximately 20% of all gliomas, and in comparison to most other gliomas, have a relatively long average survival time (5-12 years) after diagnosis (Okamoto, Y. et al., (2004) Acta Neuropathol. 28:28; Johannesen, T.B. et al. (2003) J. Neurosurg. 99: 854-862). Two malignancy grades are recognized in oligodendrocytic tumors, Grades II (low-grade) and III (anaplastic) (Collins, V.P. (2004) J. Neurol. Neurosurg. Psych. 75 Suppl. 2: ii2-ii11).

One of the striking differences between oligodendroglial tumors and other glioma subtypes is their sensitivity to therapy, especially radiotherapy and chemotherapy. The majority of oligodendroglial tumors respond favourably to chemotherapy with alkylating agents (either temolozomide or PCV, a combination therapy of procarbazine, CCNU, and vincristine), whereas other gliomas are often chemoresistant (Van den Bent, M.J. et al. (1998) Neurology 51: 1140-1145; Van den Bent, M.J. et al. (2003) J. Clin. Oncol. 21:

2525-2528). The most favourable clinical behaviour of oligodendral tumors renders it therefore important to correctly identify this subtype of gliomas. Unfortunately, histological classification and grading of gliomas has a significant subjective component. However, malignant gliomas can also be classified according to their gene expression profile (Nutt, C.L. et al. (2003) Cancer Res. 63: 1602-1607).

In oligodendroglial tumors, there is a strong correlation between chromosomal aberrations and response to treatment (chemotherapy and/or radiotherapy). For example, a common genomic aberration is a combined loss of the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q) (Okamoto, Y et al.,2004; Cairncross J.G. et al., (1998) J. Natl. Cancer Inst.90:1473-1479; Kros J.M. et al., (1999) J. Pathol. 188:282-288; Smith J.S. et al., (1999) Oncogene 18:4144-4152; Thiessen B. et al., (2003) J. Neurooncol. 64:271-278; van den Bent, M.J. et al, (2003) Cancer 97:1276-1284.). Loss of heterozygosity (LOH) on both chromosomal arms is correlated with a favourable response to therapy: A response to treatment is observed in 80-90% of oligodendroglial tumors with 1p LOH and in 25-30% without 1p LOH (Cairncross, J.G. et al, 1998; Thiessen, B. et al, 2003; van den Bent, M.J. et al., 2003). Other chromosomal aberrations observed at lower frequency include LOH on 10q and amplification of 7p11 (Kitange G. et al. (2004) Genes Chromosomes Cancer). These aberrations are correlated with poor prognosis and are negatively correlated with LOH on 1p and 19q. This correlation between response to treatment and chromosomal aberrations can therefore help identify chemosensitive oligodendroglial tumors. However, predicting the tumors' response to treatment by its chromosomal status also incorrectly classifies a significant percentage of tumors.

Thus, there still is a need for a more accurate prediction whether a patient with oligodendroglial tumors will be responsive to treatment and/or to predict the survival of a brain tumor patient. Expression profiling can be an alternative approach to identify oligodendroglial tumors that will benefit from therapeutic treatment. Although expression profiling has been performed on oligodendroglial tumors, mRNA expression has thus far not been correlated to treatment response.

The current inventors have now surprisingly shown that gene expression can be used to be correlated with susceptibility to treatment and increased survival,independant of the (1p and 19q) chromosomal status of the tumor. Further, also correlations have been found between gene expression and loss of 1p and 19q.

Summary of the Invention

The invention now comprise a method for producing a classification scheme for oligodendroglial tumors comprising the steps of:

a) providing a plurality of reference samples, said reference samples comprising cell samples from a plurality of reference subjects suffering from oligodendroglial tumors, with known responsiveness to therapy and survival;

b) providing reference profiles by establishing a gene expression profile, matched with parameters for sensitivity to treatment and survival for each of said reference samples individually;

c) clustering said individual reference profiles according to a statistical procedure, comprising:

    (i) K-means clustering;
    (ii) hierarchical clustering; and
    (iii) Pearson correlation coefficient analysis; and

d) assigning an oligodendroglial tumor class according to sensitivity to treatment and/or survival to each cluster.

Specifically in such a method the clustering of said gene expression profiles is performed based on the information of differentially-expressed genes and the sensitivity to treatment and/or survival of the subject, wherein, preferably, the clustering of said gene expression profiles with respect to treatment response is performed based on the information of the genes of Table 3, whereas the clustering of said gene expression profiles with respect to survival is performed based on the information of the genes of Table 4. Another embodiment of the invention is a method for classifying an oligodendroglial tumor of a subject suffering from an glial tumor, comprising the steps of:

a) providing a classification scheme for oligodendroglial tumors according to the above described method;
b) providing a subject profile by establishing a gene expression profile for said subject;
c) clustering the subject profile together with reference profiles;
d) determining in said scheme the clustered position of said subject profile among the reference profiles, and
e) assigning to said glial tumor the oligodendroglial tumor class that corresponds to said clustered position.

Preferably herein the gene expression profile with respect to treatment response comprises the expression parameters of a set of genes according to table 3, still more preferably 1 to 50 genes of the genes of table 3, whereas the gene expression profile with respect to survival comprises the expression parameters of a set of genes according to Table 4, more preferably 1 tot 50 genes of the genes of Table 4. A further embodiment of the invention is a method of determining the prognosis for a subject suffering from an oligodendroglial tumor, said method comprising the steps of:

a) providing a classification scheme for oligodendroglial tumors by producing such a scheme according to the above described method;
b) determining the prognosis for each olidendroglial tumor class in said scheme based on clinical records for the subjects comprised in said class;
c) establishing the oligodendroglial class of a subject suffering from an oligodendroglial tumor by classifying the oligodendroglial tumor in said subject according to a method according to the invention, and

d) assigning to said subject the prognosis corresponding to the established oligodendroglial tumor class of said subject.

Alternatively, the invention provides for a method of determining the prognosis for a subject suffering from an oligodendroglial tumor, said method comprising the steps of:

a) isolation of RNA from tumor cells of said subject;

b) preparation of antisense, biotinylated RNA to the RNA of step a);

c) hybridisation of said antisense, biotinylated DNA on Affymetrix U133A or U133 Plus2.0 GeneChips®;

d) normalising the measured values for the gene set of Table 3;

e) clustering the obtained data together with reference data, obtained from a reference set of patients with known prognoses; and

f) determining the prognosis on basis of the subgroup/cluster to which the data of the subject are clustering.

In another embodiment, the invention provides for an oligonucleotide microarray of maximal 500 probesets, comprising at least 1, preferably at least 2, more preferably at least 25, still more preferably at least 100 oligonucleotide probes which each are capable of hybridizing under stringent conditions to different genes of the oligodendroglial tumor-associated genes selected from Table 3. Alternatively, the invention provides for an oligonucleotide microarray of maximal 500 probesets, comprising at least 1, preferably at least 2, more preferably at least 25, still more preferably at least 100 oligonucleotide probes which each are capable of hybridizing under stringent conditions to different genes of the oligodendroglial tumor-associated genes selected from Table 4.

In oligodendrogliomas there is a strong correlation between LOH on 1p/19q and response to treatment. In another embodiment, the invention provides for a method using an oligonucleotide microarray, which can be used for the determination of the presence of 1p LOH, 19q LOH or 1p/19q LOH. Particularly, the microarray for these determination should comprise the genesets of Table 5, 6 and 7, respectively. Accordingly, the invention also comprises an oligonucleotide microarray of maximal 500 probesets, comprising at least 1, preferably at least 2, more preferably at least 25, still more preferably at least 50 oligonuclaotide probes which each are capable of hybridizing under stringent conditions to different genes of the oligodendroglial tumor-associated genes selected form Table 5, 6 and 7, respectively.

For the above described methods, the invention also comprises a kit-of-parts comprising an oligonucleotide microarray as described above and means for comparing a gene expression profile determined by using said microarray with a database of oligodendroglial tumor reference expression profiles.

LEGENDS TO THE FIGURES

Figure 1.

Correlation plot of all samples. Samples are plotted against each other to determine the degree of similarity based on expressed genes. Red and blue denote high and low similarity respectively (scale bar). Below the correlation plot is a graphic representation of histological and patient data. *Tissue*: origin of sample ■ control cortex, □ control white matter, □ low-grade oligodendroglioma, ░ anaplastic oligodendroglioma. *1p, 19q, 10q LOH*: ▓ no LOH, ░ LOH. (LOH: loss of heterozygosity). *EGFR ampl:* amplification of the EFGR chromosomal locus: ■ no amplification, ░ amplification, *Response*: response to therapy ■ complete response, □ partial response, □ stable disease, ░ progressive disease. *Surv tot*: survival (years) from time of diagnosis. ■ >10, □ 7-10, □ 3-7, ░ <3.A: patient alive at time of analysis.

Figure 2.

Principle components analysis (PCA) and hierarchical clustering of 60 probesets differentially expressed between oligodendroglial tumors with combined 1p and 19q LOH and those that have retained both 1p and 19q arms. A: samples are separated on their 1p and 19q chromosomal status by the first principle component axis (PCA1) whereas PCA2 separates control brain from anaplastic oligodendroglial tumors. The 1p and 19q status are color coded with ▩ = no LOH on 1p and 19q, ⬚ = LOH on 1p and 19q, and ☐ LOH on either 1p or 19q. B: Hierarchical clustering shows relative expression levels of individual genes (columns) plotted against individual tumor samples (rows). For clarity, control brain samples were omitted from the clustering analysis. Gene expression levels are color coded with red and green indicating high (+2) and low green (-2) expression respectively (on a log2 scale). Dendrograms denote hierarchical clustering (Euclidian distance) of samples (top) and genes (left). The 1p and 19q status in indicated below the hierarchical clustering (▩ = no LOH, ☐ = LOH). As can be seen, hierarchical clustering clearly identifies two main subgroups associated with 1p/19q LOH.

Figure 3.

PCA and hierarchical clustering based on 16 probesets differentially expressed between chemosensitive (CR+PR (complete response, partial response)) and chemoresistant (SD+PD, stable disease, progressive disease)) oligodendroglial tumors. A: samples are separated on their response to chemotherapy by the first principle component axis (PCA1) whereas PCA2 separates control brain from anaplastic oligodendroglial tumors. B: Hierarchical clustering based on 16 differentially expressed probesets. Relative expression levels of individual genes (columns) are plotted against individual tumor samples (rows). Gene expression levels are color coded with red and green indicating high (+1.8) and low green (-1.8) expression respectively. Dendrograms denote hierarchical clustering of samples (top) and genes (left) using Wards method. Hierarchical clustering separates tumors that fully respond to chemotherapy (CR) from tumors that do not respond (SD+PD). Furthermore, hierarchical clustering also clearly separates tumors with poor prognosis (subgroup III in figure 1) from other oligodendroglial tumors. Responses in oligodendroglial tumors are color coded with ▨ complete response, ☐ partial response, ☐ stable disease, ☐ progressive disease, ☐ control brain. 1p chromosomal status is depicted as ▨ no loss of 1p and ☐ 1p LOH.

Figure 4.

PCA hierarchical clustering based on 103 probesets associated with survival after diagnosis. A: PCA identifies three main clusters of samples: oligodendroglial tumors with short survival, oligodendroglial tumors with long survival and control samples. Two low-grade samples (38 and 42, survival < 10 years ☐) cluster between control and tumor samples. PCA analysis separates short vs. long survivors on the first principle component axis (PCA1) whereas control and tumor samples are separated by the second PCA axis. B: Hierarchical clustering based on 103 differentially expressed probesets. Relative expression levels of individual genes (columns) are plotted against individual tumor samples (rows). Gene expression levels are color coded with red and green indicating high (+2) and low green (-2) expression respectively. Dendrograms denote hierarchical clustering (Euclidian distance) of samples (top) and genes (left). Interestingly, the subgroups identified by hierarchical clustering are virtually identical to the subgroups that were identified by unsupervised clustering (figure 1). Survival after diagnosis is depicted as ▨ >10 years survival, ☐ <10 years survival, ☐ <7 years survival, ▨ <4 years survival, ☐ patient still alive or, ☐ control brain.

Detailed Description of the Invention

The current inventors performed expression profiling on oligodendroglial tumors and correlated the results to response to treatment, survival after diagnosis and common chromosomal aberrations. One of the findings was that the chromosomal aberrations led to ~50% expression of some but not all of the genes that had been deleted, Thus, this means that it is not straightforward to use the expression data of the genes from the 1p and 19q loci for the determination of the presence of a loss of heterozygosity (LOH) in these areas. Yet, the present inventors have found that a subset of genes, which show a reduced expression when one of the chromosomal arms 1p and 19q are deleted can be used to detect these chromosomal aberrations. The genes, which can distinguish between the presence or absence of 1p have been listed in Table 5, for LOH of 19q the genes are listed in Table 6, and Table 7 gives the list of discriminating genes for combined 1p and 19q LOH.

This means that gene expression data can be used for the determination of LOH of 1p and/or 19q. This is advantageous, since currently for said determination a FISH (Fluorescence In Situ Hybridisation) or LOH (loss of heterozygosity)-PCR is used, which are specialised tests, using labelled probes. Now it has been established that a similar determination can be achieved by using standard array technology.

Further, the present study shows that the currently used predictions, based on loss of 1p, were only correctly assigned to the correct treatment response group in 20/28 (71%) of the cases, both because of positive and negative misclassifications

The term "classifying" is used in its art-recognized meaning and thus refers to arranging or ordering items, *i.c.* gene expression profiles, by classes or categories or dividing them into logically hierarchical classes, subclasses, and sub-subclasses based on the characteristics they have in common and/or that distinguish them. In particular "classifying" refers to assigning, to a class or kind, an unclassified item. A "class" then being a grouping of items, based on one or more characteristics, attributes, properties, qualities, effects, parameters, etc., which they have in common, for the purpose of classifying them according to an established system or scheme.

The term "classification scheme" is used in its art-recognized meaning and thus refers to a list of classes arranged according to a set of pre-established principles, for the purpose of organizing items in a collection or into groups based on their similarities and differences.

The term "clustering" refers to the activity of collecting, assembling and/or uniting into a cluster or clusters items with the same or similar elements, a "cluster" referring to a group or number of the same or similar items, *i.c.* gene expression profiles, gathered or occurring closely together based on similarity of characteristics. "Clustered" indicates an item has been subjected to clustering.

The term "clustered position" refers to the location of an individual item, *i.c.* a gene expression profile, in amongst a number of clusters, said location being determined by clustering said item with at least a number of items from known clusters.

The process of clustering used in a method of the present invention may be any mathematical process known to compare items for similarity in characteristics, attributes, properties, qualities, effects, parameters, etc.. Statistical analysis, such as for instance multivariance analysis, or other methods of analysis may be used. Preferably methods of analysis such as self-organising maps, hierarchical clustering, multidimensional scaling, principle component analysis, supervised learning, k-nearest neighbours, support vector machines, discriminant analysis, partial least square methods and/or Pearson's correlation coefficient analysis are used. In another preferred embodiment of a method of the present invention Pearson's correlation coefficient analysis, significance analysis of microarrays (SAM) and/or prediction analysis of microarrays (PAM) are used to cluster gene expression profiles according to similarity. A highly preferred method of clustering comprises similarity clustering of gene expression profiles wherein the expression level of differentially-expressed genes, having markedly lower or higher expression than the geometric mean expression level determined for all genes in all profiles to be clustered, is log(2) transformed, and wherein the transformed expression levels of all differentially-expressed genes in all profiles to be clustered is clustered by using K-means. A numerical query may then be used to select a subset of genes used in the process of hierarchical clustering (Eisen et al., 1998), thus, numerical queries may be run to select differentially expressed genes relative to the calculated geometric mean to select a smaller group of genes for hierarchical clustering.

Unsupervised sample clustering using genes obtained by numerical or threshold filtering is used to identify discrete clusters of samples as well as the gene-signatures associated with these clusters. The term gene signatures is used herein to refer to the set of genes that define the discrete position of the cluster apart from all other clusters, and includes cluster-specific genes. A numerical or threshold filtering is used to select genes for the analysis that are most likely of diagnostic relevance. Hierarchical clustering allows for visualization of large variation in gene expression across samples or present in most samples, and these genes could be used for unsupervised clustering so that clustering results are not affected by the noise from absent or non-changed genes.

Thus, while K-means clustering may be performed on all genes, the Pearson correlation is preferably calculated based on a subset of genes. Generally speaking the larger the threshold for accepting a deviation or change from the geometric mean, the smaller the number of genes that is selected by this filtering procedure. Different cut-off or threshold values were used to prepare lists with different numbers of genes. The higher the number of genes selected and included on such lists, the more noise is generally encountered within the dataset, because there will be a relatively large contribution of non-tumor pathway related genes in such lists. The filtering and selection procedure is preferably optimized such that the analysis is performed on as many genes as possible, while minimizing the noise.

All genes with changed expression values in at least one sample higher than or equal to 1.5 times the log(2) transformed expression values and genes with changed expression values lower than or equal to -1.5 times the log(2) transformed expression value means are selected for unsupervised clustering.

The subset of genes showing a markedly higher or lower expression than the geometric mean may for instance be a value that is more than 1.5 times the geometric mean value, preferably more than 2 times the geometric mean value, Likewise, a markedly lower expression than the geometric mean expression level may for instance be a value that is less than 0.8 times the geometric mean value, preferably less than 0.6 times the geometric mean value.

Independently (see Fig. 1) a Pearson correlation coefficient analysis was performed on the samples (1881 probesets), which showed that clustering of patients is feasible.

The present invention now provides several methods to accurately identify known as well as newly discovered diagnostically, prognostically and therapeutically relevant subgroups of oligodendroglial tumors, as well as methods that can predict if treatment is likely to be effective. The basis of these methods resides in the measurement of (oligodendroglial tumor-specific) gene expression in subjects suffering from brain tumors. The methods and compositions of the invention thus provide tools useful in choosing a therapy for brain tumor patients, including methods for assigning an brain tumor patient to a brain tumor class or cluster, methods of choosing a therapy for a brain tumor patient, and methods of determining the survival prognosis for a brain tumor patient.

The methods of the invention comprise in various aspects the steps of establishing a gene expression profile of subject samples, for instance of reference subjects suffering from a brain tumor or of a subject diagnosed or classified as having a brain tumor. The expression profiles of the present invention are generated from samples from subjects having a brain tumor. The samples from the subject used to generate the expression profiles of the present invention can be derived from a tumor biopsy, wherein the sample comprises preferably more than 75% tumor cells.

“Gene expression profiling” or “expression profiling” is used herein in its art-recognised meaning and refers to a method for measuring the transcriptional state (mRNA) or the translational state (protein) of a plurality of genes in a cell. Depending on the method used, such measurements may involve the genome-wide assessment of gene expression, but also the measurement of the expression level of selected genes, resulting in the establishment of a “gene expression profile” or “expression profile”, which terms are used in that meaning hereinbelow. As used herein, an "expression profile" comprises one or more values corresponding to a measurement of the relative abundance of a gene expression product. Such values may include measurements of RNA levels or protein abundance. Thus, the expression profile can comprise values representing the measurement of the transcriptional state or the translational state of the gene. In relation thereto, reference is made to U.S. Pat. Nos. 6,040,138, 5,800,992, 6,020135, 6,344,316, and 6,033,860.

The transcriptional state of a sample includes the idensities and relative abundance of the RNA species, especially mRNAs present in the sample. Preferably, a substantial fraction of all constituent RNA species in the sample are measured, but at least a sufficient fraction to characterize the transcriptional state of the sample is measured. The transcriptional state can be conveniently determined by measuring transcript abundance by any of several existing gene expression technologies.

Translational state includes the identities and relative abundance of the constituent protein species in the sample. As is known to those of skill in the art, the transcriptional state and translational state are often related.

Each value in the expression profiles as determined and embodied in the present invention is a measurement representing the absolute or the relative expression level of a differentially-expressed gene. The expression levels of these genes may be determined by any method known in the art for assessing the expression level of an RNA or protein molecule in a sample. For example, expression levels of RNA may be monitored using a membrane blot (such as used in hybridization analysis such as Northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). *See* U.S. Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, to which explicit reference is made. The gene expression monitoring system may also comprise nucleic acid probes in solution.

In one embodiment of the invention, microarrays are used to measure the values to be included in the expression profiles. Microarrays are particularly well suited for this purpose because of the reproducibility between different experiments. DNA microarrays provide one method for the simultaneous measurement of the expression levels of large numbers of genes. Each array consists of a reproducible pattern of capture probes attached to a solid support. Labeled RNA or DNA is hybridized to complementary probes on the array and then detected by laser scanning. Hybridization intensities for each probe on the array are determined and converted to a quantitative value representing relative gene expression levels. *See,* the Experimental section. *See* also, U.S. Pat. Nos. 6,040,138, 5,800,992 and 6,020,135, 6,033,860, and 6,344,316, to which explicit reference is made. High-density oligonucleotide arrays are particularly useful for determining the gene expression profile for a large number of RNA's in a sample.

In one approach, total RNA isolated from the sample is converted to labeled cRNA and then hybridized to an oligonucleotide array. Each sample is hybridized to a separate array. Relative transcript levels are calculated by reference to appropriate controls present on the array and in the sample. *See,* for example, the Experimental section.

In another embodiment, the values in the expression profile are obtained by measuring the abundance of the protein products of the differentially-expressed genes. The abundance of these protein products can be determined, for example, using antibodies specific for the protein products of the differentially-expressed genes. The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')2 fragments which can be generated by treating the antibody with an enzyme such as pepsin. The antibody can be a polyclonal, monoclonal, recombinant, e.g., a chimeric or humanized, fully human, non-human, e.g., murine, or single chain antibody. In a preferred embodiment it has effector function and can fix complement. The antibody can be coupled to a toxin or imaging agent. A full-length protein product from a differentially-expressed gene, or an antigenic peptide fragment of the protein product can be used as an immunogen. Preferred epitopes encompassed by the antigenic peptide are regions of the protein product of the differentially-expressed gene that are located on the surface of the protein, e.g., hydrophilic regions, as well as regions with high antigenicity. The antibody can be used to detect the protein product of the differentially-expressed gene in order to evaluate the abundance and pattern of expression of the protein. These antibodies can also be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given therapy. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labeling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, (3-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, quantum dots or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

Once the values comprised in the subject expression profile and the reference expression profile or expression profiles are established, the subject profile is compared to the reference profile to determine whether the subject expression profile is sufficiently similar to the reference profile. Alternatively, the subject expression profile is compared to a plurality of reference expression profiles to select the reference expression profile that is most similar to the subject expression profile. Any method known in the art for comparing two or more data sets to detect similarity between them may be used to compare the subject expression profile to the reference expression profiles. In some embodiments, the subject expression profile and the reference profile are compared using a supervised learning algorithm such as the support vector machine (SVM) algorithm, prediction by collective likelihood of emerging patterns (PCL) algorithm, the k-nearest neighbour algorithm, or the Artificial Neural Network algorithm. To determine whether a subject expression profile shows "statistically significant similarity" or "sufficient similarity" to a reference profile, statistical tests may be performed to determine whether the similarity between the subject expression profile and the reference expression profile is likely to have been achieved by a random event. Any statistical test that can calculate the likelihood that the similarity between the subject expression profile and the reference profile results from a random event can be used. The accuracy of assigning a subject to an oligodendroglial tumor class based on similarity between differentially-expressed genes is affected largely by the heterogeneity within the patient population, as is reflected by the deviation from the geometric mean. Therefore, when more accurate diagnoses are required, the stringency in evaluating the similarity between the subject and the reference profile should be increased by changing the numerical query.

The method used for comparing a subject expression profile to one or more reference profiles is preferably carried out by re-running the subsequent analyses in a (n+1) modus by performing clustering methods as described herein. Also, in order to identify the oligodendroglial tumor class reference profile that is most similar to the subject expression profile, as performed in the methods for establishing the oligodendroglial tumor class of a subject having a brain tumor, *i.e.* by diagnosing presence of an oligodendroglial tumor in a subject or by classifying the oligodendroglial tumor in a subject, profiles are clustered according to similarity and it is determined whether the subject profile corresponds to a known class of reference profiles. In assigning a subject oligodendroglial tumor to a specific oligodendroglial tumor class for instance, this method is used wherein the clustered position of the subject profile, obtained after performing the clustering analysis of the present invention, is compared to any known oligodendroglial tumor class. If the clustered position of the subject profile is within a cluster of reference profiles, i.e. forms a cluster therewith after performing the similarity clustering method, it is said that the oligodendroglial tumor of the subject corresponds to the oligodendroglial tumor class of reference profiles.

In some embodiments of the present invention, the expression profiles comprise values representing the expression levels of genes that are differentially-expressed in oligodendroglial tumor classes. The term "differentially-expressed" as used herein means that the measured expression level of a particular gene in the expression profile of one subject differs at least n-fold from the geometric mean calculated from all patient profiles. The expression level may be also be up-regulated or down-regulated in a sample from a subject in comparison with a sample from a normal brain sample , or in comparison with the mean of all oligodendroglial tumor patients. Examples of genes that are differentially expressed in brain tumor patients which respond to therapy and brain tumor patients which do not respond to therapy, short vs. long survivors and 1p and/or 19q LOH vs no loss are listed in Tables 3, 4, 5, 6 and 7.

It should be noted that many genes will occur, of which the measured expression level differs at least n-fold from the geometric mean expression level for that gene of all reference profiles. This may for instance be due to the different physiological state of the measured cells, to biological variation or to the presence of other diseased states. Therefore, the presence of a differentially-expressed gene is not necessarily informative for determining the presence of different oligodendroglial tumor classes, nor is every differentially-expressed gene suitable for performing diagnostic tests. Moreover, a cluster-specific differential gene expression, as defined herein, is most likely to be informative only in a test among subjects having brain tumors. Therefore, a diagnostic test performed by using cluster-specific gene detection should preferably be performed on a subject in which the presence of an oligodendroglial tumor is confirmed. This confirmation may for instance be obtained by standard macroscopic and microscopic detection methods.

The present invention provides groups of genes that are differentially-expressed in diagnostic oligodendroglial tumor biopsy and surgical resection samples of patients in different therapeutic groups (i.e. responders/non-responders, or short-survivors/long-survivors). Values representing the expression levels of the nucleic acid molecules detected by the probes were analyzed as described in the Experimental section using Omniviz and SAM analysis tools. Omniviz software was used to perform all clustering steps such as K-means, Hierarchical and Pearson correlation tests. SAM was

used specifically to identify the genes underlying the clinically relevant groups identified in the Pearson correlation analysis. PAM is used to decide the minimum number of genes necessary to diagnose all individual patients within the given groups of the Pearson correlation.
In short, expression profiling was carried out on biopsy material from 28 brain tumor patients. Unsupervised clustering was used to identify novel (sub)groups within the Pearson correlation following the hierarchical clustering. After running the SAM analysis the diagnostic gene-signatures (incl. cluster-specific genes) were obtained.

It appeared that a clustering separating the different groups of patients could be performed on the basis of differential expression of a plurality of genes.

The present invention thus provides a method of classifying oligodendroglial tumors. Using this method, a total of 28 brain tumor samples analysed on a DNA microarray consisting of 54675 probe sets, representing approximately 23000 genes, could be classified. The classification into patient groups was performed on the basis of strong correlation between their individual differential expression profiles within a group for 1881 probe sets (~1413 genes). The methods used to analyze the expression level values to identify differentially-expressed genes were employed such that optimal results in clustering, *i.e.* unsupervised ordering, were obtained. The genes that defined the position or clustering of these patient groups could be determined and the minimal sets of genes required to accurately predict the prognostically important classes could be derived. It should be understood that the method for classifying oligodendroglial tumors according to the present invention may result in a distinct pattern and therefore in a different classification scheme when other (numbers of) subjects are used as reference, or when other types of oligonucleotide microarrays for establishing gene expression profiles are used.

The present invention thus provides a comprehensive classification of oligodendroglial tumors covering previously identified therapeutically defined classes. Further analysis of classes by significance analysis of microarrays (SAM) to determine the minimum number of genes that defined or predicted these classes resulted in the establishment of cluster-specific genes or signature genes.

The methods of the present invention comprise in some aspects the step of defining cluster-specific genes by selecting those genes of which the expression level characterizes the clustered position of the corresponding oligodendroglial tumor class within a classification scheme of the present invention. Such cluster-specific genes are selected preferably on the basis of SAM analysis. This method of selection comprises the following.

The methods of the present invention comprise in some aspects the step of establishing whether the level of expression of cluster-specific genes in a subject shares sufficient similarity to the level of expression that is characteristic for an individual oligodendroglial tumor class. This step is necessary in determining the presence of that particular oligodendroglial tumor class in a subject under investigation, in which case the expression of that gene is used as a prognostic marker. Whether the level of expression of cluster-specific genes in a subject shares sufficient similarity to the level of expression of that particular gene in an individual oligodendroglial tumor class may for instance be determined by setting a threshold value.

The present invention also reveals genes with a high differential level of expression in specific oligodendroglial tumor classes compared to the geometric mean of all reference subjects. These highly differentially-expressed genes are selected from the genes shown in Tables 3-7, These genes and their expression products are useful as markers to predict the responsiveness to treatment, 1p and/or 19q loss of heterozygosity or survival chance in a patient. Antibodies or other reagents or tools may be used to detect the presence of these markers of brain tumor.

The present invention also reveals gene expression profiles comprising values representing the expression levels of genes in the various identified oligodendroglial tumor classes. In a preferred embodiment, these expression profiles comprise the values representing the differential expression levels. Thus, in one embodiment the expression profiles of the invention comprise one or more values representing the expression level of a gene having differential expression in a defined oligodendroglial tumor class. Each expression profile contains a sufficient number of values such that the profile can be used to distinguish treatment response groups, to distinguish groups with different survival, an to distinguish groups with 1p and/or 19q LOH. The expression profile comprises more than one or two values corresponding to a differentially-expressed gene, for example at least 3 values, at least 4 values, at least 5 values, at least 6 values, at least 7 values, at least 8 values, at least 9 values, at least 10 values, at least 11 values, at least 12 values, at least 13 values, at least 14 values, at least 15 values, at least 16 values, at least 17 values, at least 18 values, at least 19 values, at least 20 values, at least 22 values, at least 25 values, at least 27 values, at least 30 values, at least 35 values , at least 40 values, at least 45 values, at least 50 values, at least 75 values, at least 100 values, at least 125 values, at least 150 values, at least 175 values, at least 200 values, at least 250 values, at least 300 values, at least 400 values, at least 500 values, at least 600 values, at least 700 values, at least 800 values, at least 900 values, at least 1000 values, at least 1200 values, at least 1500 values, or at least 2000 or more values.

It is recognized that the diagnostic accuracy of assigning a subject to an oligodendroglial tumor class will vary based on the number of values contained in the expression profile. Generally, the number of values contained in the expression profile is selected such that the diagnostic accuracy is at least 85%, at least 87%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least

99%, as calculated using methods described elsewhere herein, with an obvious preference for higher percentages of diagnostic accuracy.

It is recognized that the diagnostic accuracy of assigning a subject to an oligodendroglial tumor class will vary based on the strength of the correlation between the expression levels of the differentially-expressed genes within that specific oligodendroglial tumor class. When the values in the expression profiles represent the expression levels of genes whose expression is strongly correlated with that specific oligodendroglial tumor class, it may be possible to use fewer number of values (genes) in the expression profile and still obtain an acceptable level of diagnostic or prognostic accuracy.

The strength of the correlation between the expression level of a differentially-expressed gene and a specific oligodendroglial tumor class may be determined by a statistical test of significance. For example, the chi square test used to select genes in some embodiments of the present invention assigns a chi square value to each differentially-expressed gene, indicating the strength of the correlation of the expression of that gene to a specific oligodendroglial tumor class. Similarly, the T-statistics metric and the Wilkins' metric both provide a value or score indicative of the strength of the correlation between the expression of the gene and its specific oligodendroglial tumor class. These scores may be used to select the genes of which the expression levels have the greatest correlation with a particular oligodendroglial tumor class to increase the diagnostic or prognostic accuracy of the methods of the invention, or in order to reduce the number of values contained in the expression profile while maintaining the diagnostic or prognostic accuracy of the expression profile. Preferably, a database is kept wherein the expression profiles of reference subjects are collected and to which database new profiles can be added and clustered with the already existing profiles such as to provide the clustered position of said new profile among the already present reference profiles. Furthermore, the addition of new profiles to the database will improve the diagnostic and prognostic accuracy of the methods of the invention. Preferably, in a method of the present invention SAM or PAM analysis tools are used to determine the strength of such correlations.

The methods of the invention comprise the steps of providing an expression profile from a sample from a subject affected by oligodendroglial tumor and comparing this subject expression profile to one or more reference profiles that are associated with a particular oligodendroglial tumor class with a known prognosis, or a class with a favourable response to therapy. By identifying the oligodendroglial tumor class reference profile that is most similar to the subject expression profile, e.g. when their clustered positions fall together, the subject can be assigned to an oligodendroglial tumor class. The oligodendroglial class assigned is that with which the reference profile(s) are associated. Similarly, the prognosis of a subject affected by an oligodendroglial tumor can be predicted by determining whether the expression profile from the subject is sufficiently similar to a reference profile associated with an established prognosis, such as a good prognosis or a bad prognosis. Whenever a subject's expression profile can be assigned to one of the reference profile(s), a preferred intervention strategy, or therapeutic treatment can then be proposed for said subject, and said subject can be treated according to said assigned strategy. As a result, treatment of a subject with an oligodendroglial can be optimized according to the identified cluster.

In one aspect, the present invention provides a method of determining the prognosis for a brain tumor patient, said method comprising the steps of providing a classification scheme for oligodendroglial tumors by producing such a scheme according to a method of the invention for reference subjects having known post-therapy lifetimes. The present invention provides for the assignment of the various clinical data recorded to reference subjects affected by brain tumors. This assignment preferably occurs in a database. This has the advantage that once a new subject is identified as belonging to a particular oligodendroglial tumor class, then the prognosis that is assigned to that class may be assigned to that subject.

The present invention provides compositions that are useful in determining the gene expression profile for a subject affected by an oligodendroglial tumor and selecting a reference profile that is similar to the subject expression profile. These compositions include arrays comprising a substrate having capture probes that can bind specifically to nucleic acid molecules that are differentially-expressed in oligodendroglial tumor classes. Also provided is a computer-readable medium having digitally encoded reference profiles useful in the methods of the claimed invention.

The present invention provides arrays comprising capture probes for detection of polynucleotides (transcriptional state) or for detection of proteins (translational state) in order to detect differentially-expressed genes of the invention. By "array" is intended a solid support or substrate with peptide or nucleic acid probes attached to said support or substrate. Arrays typically comprise a plurality of different nucleic acid or peptide capture probes that are coupled to a surface of a substrate in different, known locations. These arrays, also described as "microarrays" or colloquially "chips" have been generally described in the art, and reference is made U.S. Patent. Nos. 5,143,854, 5,445,934, 5,744,305, 5,677,195, 6,040,193, 5,424,186,6,329,143, and 6,309,831 and Fodor *et al.* (1991) *Science* 251:767-77. These arrays may generally be produced using mechanical synthesis methods or light directed synthesis methods which incorporate a combination of photolithographic methods and solid phase synthesis methods. Typically, "oligonucleotide microarrays" will be used for determining the transcriptional state, whereas "peptide microarrays" will be used for determining the translational state of a cell.

"Nucleic acid" or "oligonucleotide" or "polynucleotide" or grammatical equivalents used herein means at least two nucleotides covalently linked together. Oligonucleotides are typically from about 5, 6, 7, 8, 9, 10, 12, 15, 25, 30, 40, 50 or more nucleotides in length, up to about 100 nucleotides in length. Nucleic acids and polynucleotides are a polymers of any length, including longer lengths, e.g., 200, 300, 500, 1000, 2000, 3000, 5000, 7000, 10,000, etc. A nucleic acid of the present invention will generally contain phosphodiester bonds, although in some cases, nucleic acid analogs are included that may have alternate backbones, comprising, e.g., phosphoramidate, phosphorothioate, phosphorodithioate, or O-methylphophoroamidite linkages (see Eckstein, Oligonucleotides and Analogues: A Practical Approach, Oxford University Press); and peptide nucleic acid backbones and linkages. Other analog nucleic acids include those with positive backbones; non-ionic backbones, and non-ribose backbones, including those described in U.S. Pat. Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, Carbohydrate Modifications in Antisense Research, Sanghui & Cook, eds. Nucleic acids containing one or more carbocyclic sugars are also included within one definition of nucleic acids. Modifications of the ribose-phosphate backbone may be done for a variety of reasons, e.g. to increase the stability and half-life of such molecules in physiological environments or as probes on a biochip. Mixtures of naturally occurring nucleic acids and analogues can be made; alternatively, mixtures of different nucleic acid analogues, and mixtures of naturally occurring nucleic acids and analogues may be made.

Particularly preferred are peptide nucleic acids (PNA) which includes peptide nucleic acid analogues. These backbones are substantially non-ionic under neutral conditions, in contrast to the highly charged phosphodiester backbone of naturally occurring nucleic acids. This results in two advantages. First, the PNA backbone exhibits improved hybridization kinetics. PNAs have larger changes in the melting temperature (T_m) for mismatched versus perfectly matched basepairs. DNA and RNA typically exhibit a 2-4 °C drop in T_m for an internal mismatch. With the non-ionic PNA backbone, the drop is closer to 7-9°C. Similarly, due to their non-ionic nature, hybridization of the bases attached to these backbones is relatively insensitive to salt concentration. In addition, PNAs are not degraded by cellular enzymes, and thus can be more stable.

The nucleic acids may be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. As will be appreciated by those in the art, the depiction of a single strand also defines the sequence of the complementary strand; thus the sequences described herein also provide the complement of the sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine, isoguanine, etc.

"Transcript" typically refers to a naturally occurring RNA, e.g., a pre-mRNA, hnRNA, or mRNA. As used herein, the term "nucleoside" includes nucleotides and nucleoside and nucleotide analogues, and modified nucleosides such as amino modified nucleosides. In addition, "nucleoside" includes non-naturally occurring analogue structures. Thus, e.g. the individual units of a peptide nucleic acid, each containing a base, are referred to herein as a nucleoside.

As used herein a "nucleic acid probe or oligonucleotide" is defined as a nucleic acid capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. As used herein, a probe may include natural (i.e., A, G, C, or T) or modified bases (7-deazaguanosine, inosine, etc.). In addition, the bases in a probe may be joined by a linkage other than a phosphodiester bond, so long as it does not functionally interfere with hybridization. Thus, e.g., probes may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages. It will be understood by one of skill in the art that probes may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. The probes are preferably directly labeled such as with isotopes, chromophores, lumiphores, chromogens, or indirectly labeled such as with biotin to which a streptavidin complex may later bind or with enzymatic labels. By assaying for the hybridization of the probe to its target nucleic acid sequence, one can detect the presence or absence of the select sequence or subsequence. Diagnosis or prognosis may be based at the genomic level, or at the level of RNA or protein expression.

The skilled person is capable of designing oligonucleotide probes that can be used in diagnostic methods of the present invention. Preferably, such probes are immobilised on a solid surface as to form an oligonucleotide microarray of the invention. The oligonucleotide probes useful in methods of the present invention are capable of hybridizing under stringent conditions to oligodendroglial tumor-associated nucleic acids, such as to one or more of the genes selected from Table 2 or Table 3.

Techniques for the synthesis of arrays using mechanical synthesis methods are described in, e.g., U.S. Patent No. 5,384,261, to which reference is made herein. Although a planar array surface is preferred, the array may be fabricated on a surface of virtually any shape or even a multiplicity of surfaces. Arrays may be peptides or nucleic acids on beads, gels, polymeric surfaces, fibers such as fiber optics, glass or any other appropriate substrate, for the purpose of which reference is made to U.S. Pat. Nos. 5,770,358, 5,789,162, 5,708,153, 6,040,193 and 5,800,992. Arrays may be packaged in such a manner as to allow for diagnostics or other manipulation of an all-inclusive device. Reference is for example made to U.S. Pat. Nos. 5,856,174 and 5,922,591.

The arrays provided by the present invention comprise capture probes that can specifically bind a nucleic acid molecule that is differentially-expressed in oligodendroglial tumor classes. These arrays can be used to measure the expression levels of nucleic acid molecules to thereby create an expression profile for use in methods of determining the therapeutic treatment and prognosis for oligodendroglial tumor patients.

In some embodiments, each capture probe in the array detects a nucleic acid molecule selected from the nucleic acid molecules designated in Tables 2 or Table 3. The designated nucleic acid molecules include those differentially-expressed in oligodendroglial tumor classes.

The arrays of the invention comprise a substrate having a plurality of addresses, where each address has a capture probe that can specifically bind a target nucleic acid molecule. The number of addresses on the substrate varies with the purpose for which the array is intended. The arrays may be low-density arrays or high-density arrays and may contain 4 or more, 8 or more, 12 or more, 16 or more, 20 or more, 24 or more, 32 or more, 48 or more, 64 or more, 72 or more 80 or more, 96, or more addresses, or 192 or more, 288 or more, 384 or more, 768 or more, 1536 or more, 3072 or more, 6144 or more, 9216 or more, 12288 or more, 15360 or more, or 18432 or more addresses. In some embodiments, the substrate has no more than 12, 24, 48, 96, or 192, or 384 addresses, no more than 500, 600, 700, 800, or 900 addresses, or no more than 1000, 1200, 1600, 2400, or 3600 addresses.

The invention also provides a computer-readable medium comprising one or more digitally encoded expression profiles, where each profile has one or more values representing the expression of a gene that is differentially-expressed in an oligodendroglial tumor class. The preparation and use of such profiles is well within the reach of the skilled person (see e.g. WO 03/083140). In some embodiments, the digitally-encoded expression profiles are comprised in a database. See, for example, U.S. Patent No. 6,308,170.

The present invention also provides kits useful for predicting the responsiveness to therapy in subjects affected by an oligodendroglial tumor. These kits comprise an array and a computer readable medium. The array comprises a substrate having addresses, where each address has a capture probe that can specifically bind a nucleic acid molecule (by using an oligonucleotide array) or a peptide (by using a peptide array) that is differentially-expressed in an oligodendroglial tumor class. The results are converted into a computer-readable medium that has digitally-encoded expression profiles containing values representing the expression level of a nucleic acid molecule detected by the array.

By using the array described above, the amounts of various kinds of nucleic acid molecules contained in a nucleic acid sample can be simultaneously determined. In addition, there is an advantage such that the determination can be carried out even with a small amount of the nucleic acid sample. For instance, mRNA in the sample is labeled, or labeled cDNA is prepared by using mRNA as a template, and the labeled mRNA or cDNA is subjected to hybridization with the array, so that mRNAs being expressed in the sample are simultaneously detected, whereby their expression levels can be determined.

Genes each of which expression is altered due to an oligodendrioglial tumor can be found by determining expression levels of various genes in the tumor cells and classified into certain types as described above and comparing the expression levels with the expression level in a control tissue.

The method for determining the expression levels of genes is not particularly limited, and any of techniques for confirming alterations of the gene expressions mentioned above can be suitably used. Among all, the method using the array is especially preferable because the expressions of a large number of genes can be simultaneously determined. Suitable arrays are commercially available, e.g., from Affymetrix.

For instance, mRNA is prepared from tumor cells, and then reverse transcription is carried out with the resulting mRNA as a template. During this process, labeled cDNA can be obtained by using, for instance, any suitable labeled primers or labeled nucleotides.

As to the labeling substance used for labeling, there can be used substances such as radioisotopes, fluorescent substances, chemiluminescent substances and substances with fluophor, and the like. For instance, the fluorescent substance includes Cy2, FluorX, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, fluorescein isothiocyanate (FITC), Texas Red, Rhodamine and the like. In addition, it is desired that samples to be tested (cancer samples to be tested in the present selection method) and a sample to be used as a control are each labeled with different fluorescent substances, using two or more fluorescent substances, from the viewpoint of enabling simultaneous detection. Here, labeling of the samples is carried out by labeling mRNA in the samples, cDNA derived from the mRNA, or nucleic acids produced by transcription or amplification from cDNA.

Next, the hybridization is carried out between the above-mentioned labeled cDNA and the array to which a nucleic acid corresponding to a suitable gene or its fragment is immobilized. The hybridization may be performed according to any known processes under conditions that are appropriate for the array and the labeled cDNA to be used. For instance, the hybridization can be performed under the conditions described in Molecular Cloning, A laboratory manual, 2nd ed., 9.52-9.55 (1989).

The hybridization between the nucleic acids derived from the samples and the array is carried out, under the above-mentioned hybridization conditions. When much time is needed for the time period required for procedures from the collection of samples to the determination of expression levels of genes, the degradation of mRNA may take place due to actions of ribonuclease. In order to determine the difference in the gene expressions in the samples to be tested (i.e., tumor cells or biopsies from oligodendroglial tumor patients) and the gene expressions in a control sample, it is preferable that the mRNA levels in both of these samples are adjusted using a standard gene with relatively little alterations in expressions.

Thereafter, by comparing the hybridization results of the samples to be tested with those of the control sample, genes exhibiting differential expression levels in both samples can be detected. Concretely, a signal which is appropriate depending upon the method of labeling used is detected for the array which is subjected to hybridization with the nucleic acid sample labeled by the method as described above, whereby the expression levels in the samples to be tested can be compared with the expression level in the control sample for each of the genes on the array.

The genes thus obtained which have a significant difference in signal intensities are genes each of which expression is altered specifically for certain oligodendroglial tumor classes.

The present invention also provides a computer-readable medium comprising a plurality of digitally-encoded expression profiles wherein each profile of the plurality has a plurality of values, each value representing the expression of a gene that is differentially-expressed in an oligodendroglial tumor class. The invention also provides for the storage and retrieval of a collection of data relating to oligodendroglial tumor specific gene expression data of the present invention, including sequences and expression levels in a computer data storage apparatus, which can include magnetic disks, optical disks, magneto-optical disks, DRAM, SRAM, SGRAM, SDRAM, RDRAM, DDR RAM, magnetic bubble memory devices, and other data storage devices, including CPU registers and on-CPU data storage arrays. Typically, the data records are stored as a bit pattern in an array of magnetic domains on a magnetizable medium or as an array of charge states or transistor gate states, such as an array of cells in a DRAM device (e.g., each cell comprised of a transistor and a charge storage area, which may be on the transistor).

For use in diagnostic, research, and therapeutic applications suggested above, kits are also provided by the invention. In the diagnostic and research applications such kits may include any or all of the following: assay reagents, buffers, oligodendroglial tumor class-specific nucleic acids or antibodies, hybridization probes and/or primers, antisense polynucleotides, ribozymes, arrays, antibodies, Fab fragments, capture peptides etc. In addition, the kits may include instructional materials containing directions (i.e., protocols) for the practice of the methods of this invention. While the instructional materials typically comprise written or printed materials, they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials. One such internet site may provide a database of oligodendroglial tumor reference expression profiles useful for performing similarity clustering of a newly determined subject expression profiles with a large set of reference profiles of oligodendroglial subjects comprised in said database. Preferably the database includes clinically relevant data such as patient prognosis, effects of methods of treatment and other characteristics relating to the oligodendroglial tumor patient.

The invention encompasses for instance kits comprising an array of the invention and a computer-readable medium having digitally-encoded reference profiles with values representing the expression of nucleic acid molecules detected by the arrays. These kits are useful for assigning a brain tumor patient subject to an oligodendroglial tumor class.

In a preferred embodiment a kit-of-parts according to the invention comprises an oligonucleotide microarray according to the invention and means for comparing a gene expression profile determined by using said microarray with a database of oligodendroglial reference expression profiles. The present invention also comprises kits of parts suitable for performing a method of the invention as well as the use of the various products of the invention, including databases, microarrays, oligonucleotide probes and classification schemes in diagnostic or prognostic methods of the invention.

The present invention discloses a number of genes that are differentially-expressed in oligodendroglial tumor classes. These differentially-expressed genes are shown in Tables 3-7. Many of the treatment sensitivity-associated transcripts (Table 3) are involved in transcriptional regulation, interaction with the extracellular matrix or affect cytoskeletal dynamics. For example genes involved in regulation of transcription include: i) PAX8, a member of the paired box gene family of transcription factors; ii) Sp110, a protein that can function as an activator of transcription; iii) RENT1, a protein involved in mRNA nuclear export and nonsense-mediated mRNA decay; and iv) TNFSF13, a member of the tumor necrosis factor ligand family that activate transcription via e.g. NF-κB. TNFSF13 transgenic mice develop lymphoid tumors (Planelles, L. et al., (2004) Cancer Cell 6:399-408). Transcripts involved in the cellular interaction with the extracellular matrix include: i) MAN1C1, an α-mannosidase involved in the maturation of N-linked glycans; ii) CHSY1, which synthesizes chondroitin sulfate, a widely expressed glycosaminoglycan and iii) LGALS9, a member of the tandem-repeat type galectins that bind beta-galactoside. LGALS9 is expressed at high levels in distant metastasis of breast cancer (for review see (Hirashima, M. et al., (2004) Glycoconj. J. 19:593-600). Also two treatment sensitivity associated transcripts that are involved in regulation of cytoskeletal dynamics were identified: i) ARPC1B, involved in the branching of actin filaments and downregulated in gastric cancers; and ii) IQGAP1, a scaffolding protein that interacts with components of the cytoskeleton. Overexpression of IQGAP1 enhances cell migration (Mataraza, J.M. et al., (2003) J. Biol. Chem. 278: 41237-41245). Other genes expressed at high levels in chemoresistant oligodendroglial tumors include i) AQP1, a water channel often highly expressed in malignant gliomas that plays a role in migration and neovascularization of tumors; ii) TRIM56, a member of the tripartite motif family and iii) ARH, an adaptor protein that interacts with the LDL receptor. In summary, the genes identified in this invention that are associated with treatment sensitivity (Table 3) are involved in several discrete cellular processes and further study on these transcripts may help identify the molecular mechanisms that underlie treatment sensitivity.

Comparison of expression profiles to patient survival after diagnosis identified 103 differentially expressed probesets (Table 4). The observation that many genes are differentially expressed suggests that different molecular pathways are affected in the tumors of short and long survivors. The genetic background of the tumor therefore appears to be an important factor in determining the prognosis of the patient, although other factors also can contribute significantly to patient survival (e.g. tumor location). Therefore, genes that are differentially expressed between long and short survivors can help identify patient subgroups that are associated with favorable prognosis. Functional analysis reveals that many transcripts upregulated in short survivors are involved in the regulation of transcription. Examples include, i) BTEB1, a member of the SP1-like/KLF family of transcription regulators, ii) BCL10, an activator NF-κB, iii) DR1, a transcriptional repressor, iv) JUN, part of the AP1 transcription factor complex, v) PTPN12 and vi) PTP4A2, members of the protein tyrosine phosphatase family that regulate processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation, vii) SFRS4, a member of the SR family of splicing factors, and viii) LMO4, a LIM domain containing protein that may play a role as a transcriptional regulator. In contrast, transcripts encoding proteins involved in RNA translation are downregulated in short survivors. They include five ribosomal proteins (RPL24, RPL3, PRL7, RPLP2 and RPS3) and proteins involved in post-transcriptional modification like CUGBP1 and RBM4.

This invention shows that expression profiling can identify transcripts associated with chromosomal aberrations, therapeutic response and survival after diagnosis in patients suffering from oligodendroglial tumors.
As described above this knowledge can be used to identify patient classes with a high likelihood to respond to treatment and patient classes with favorable prognosis.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE

Methods:

Tumor samples:

Patients were chosen with (anaplastic) oligodendroglioma or mixed oligoastrocytoma with enhancing disease at the time of chemotherapy. Patients were treated in a single institution (Erasmus MC) in clinical trials evaluating the efficacy of Temozolomide or PCV. Only patients with an evaluable for response to chemotherapy were included in this study. Treatment response was evaluated by MRI and scored according to McDonald's criteria (Macdonald D.R. et al., (1990) J. Clin. Oncol. 8:1277-1280). Tumor size was defined as the product of the two largest perpendicular tumor diameters. Complete response (CR) was defined as disappearance of all contrast-enhancing tumor on two subsequent scans at least one month apart, the patient being off steroids and neurologically stable or improved. Partial response (PR) was defined as ≥50 % reduction in tumor area on two subsequent scans at least one-month apart, steroids stable or decreased and neurologically stable or improved. Progressive disease (PD) was defined as ≥25 % increase in tumor area, new tumor on MRI or neurological deterioration and steroids stable or increased. All other situations were considered stable disease (SD). Samples were collected immediately after surgical resection, snap frozen, and stored at -800C in the Erasmus MC brain tumor tissue bank. Samples were visually inspected on 10 µm H&E stained frozen sections by the neuropathologist (J.M.K). Samples with less than 80% tumor were omitted from this study. Tissue adjacent to the inspected sections was subsequently used for nucleic acid isolation. Using these criteria, 28 oligodendroglial tumors were selected (Table 1). Four additional tumor samples with insufficient RNA quantity for array analysis were selected for confirmation of differentially expressed genes using QPCR.

Nucleic acid isolation:

Tissues were homogenized using a polytron following which RNA and genomic DNA were extracted using Trizol (Life-Technologies) according to the manufacturers instructions. Total RNA, present in the aqueous phase after extraction, was precipitated in isopropanol, redissolved in diethyl-pyrocarbonate treated water and further purified on RNeasy mini columns (Qiagen). Genomic DNA present in the organic phase was precipitated using ethanol, washed in 0.1M Na-citrate, 10% ethanol and dissolved in 8 mM NaOH whereafter the pH was adjusted to 8.4 using 1M Hepes (free acid).

cDNA synthesis and array hybridization

RNA quality was assessed on agarose gel and Bioanalyser (Agilent). cDNA synthesis and cRNA labeling was performed using the alternative protocol for one-cycle cDNA synthesis. Biotin-labeled cRNA was generated using the ENZO Highyield RNA transcript labeling kit (ENZO life sciences inc, NY). Affymetrix (Santa Clara, CA) HG U133-plus2 microarrays were hybridized overnight with 15 µg biotin labeled cRNA. 54.675 probesets (a probeset is a set of oligonucleotide probes that examines the expression of a single transcript) are spotted on these arrays allowing expression profiling of virtually all human transcripts. Multiple probesets may be directed against the same transcript. Microarrays were then washed using fluidics stations according to standard Affymetrix protocols.

Microsatellite analysis

Microsatellites were amplified by PCR on 10 ng genomic DNA using forward and reversed primers and a fluorescently labeled M13 (-21) primer. Primers and cycling conditions are stated in supplementary table 1. PCR products were precipitated, dissolved in formamide and run on an ABI 3100 genetic analyzer (Applied Biosystems). Samples were analyzed using Genescan 3.7 software (Applied Biosystems) and scored by two independent researchers. Since non-neoplastic tissues were not available for most of the tumor samples, allelic losses were statistically determined as described (Harkes I.C., et al. (2003) Br. J. Cancer 89:2289-2292). Allelic loss was assumed when the tumor sample had a homozygous allele pattern for all microsatellites within the locus ($P<0.05$ for each locus).

Fluorescence In Situ Hybridization

1p/19q status of samples with non-informative microsatellite analysis was determined using Fluorescence In Situ Hybridization (FISH) as previously described (Stege E.M. et al., (2005) Cancer 103:802-809). Locus-specific probes for 1p36 (D1S32), centromere 1 (pUC1.77), 19q13.4 (Bac clone 426G3), and 19p13 (Bac clones 957I1, 153P24, and 959O6) were labeled with either biotin-16-dUTP, digoxigenin-16-dUTP (Roche Diagnostics, Mannheim, Germany) or Spectrum Orange (Vysis Illinois, USA) as previously described (23). Probes were detected using FITC-labeled sheep-anti-digoxigenin (Roche Diagnostics) and/or CY3-labeled avidin (Brunschwig Chemie, Amsterdam, The Netherlands). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Sixty non-overlapping nuclei were enumerated per hybridization. Ratios were calculated as the number of signals of the marker divided by the number of signals of the reference. Ratio <0.80 were considered allelic loss.

Semi-Quantitative RT-PCR

Semi-quantitative RT-PCR was performed using SYBR Green PCR master mix (Applied Biosystems) according to the manufacturers instructions. Expression levels were evaluated relative to HPRT and PDGB controls. Intron spanning primers were designed against 16 genes (supplementary table 2). All primers had an amplification efficiency >80% (determined by serial dilution) and generated a single amplification product at a temperature above 77 °C (determined by melting point analysis). Cycling was performed on an ABI7700 sequence detection system (Applied Biosystems); cycling conditions are stated in supplementary table 2. Amplification of the EFGR receptor was determined by semi-quantitative PCR using identical conditions as described above. 20 ng genomic DNA was used for each reaction. The amount of product amplified using genomic EGFR primers was compared to the amount of product amplified using primers on different chromosomes lying within the F3 and the FGFR3 loci. Statistical analysis was performed using the Mann Whitney U test (eatworms.swmed.edu/~leon/stats/utest.cgi), values are ± SEM.

Data analysis:

Arrays were omitted from the analysis when the number of present calls < 35% and when the 5'/3' ratio of GAPDH controls >3. Probesets that were absent (according to Affymetrix MAS5.0 software) in at least 33 of the 34 microarrays were omitted from further analysis. Raw intensities of the remaining probesets (36875) of each chip were log2 transformed and normalized using quantile normalization. For each probeset, the geometric mean of the hybridization intensities of all samples was calculated. The level of expression of each probeset was determined relative to this geometric mean and log2 transformed. The geometric mean of the hybridization signal of all samples was used to ascribe equal weight to gene-expression levels. Unsupervised clustering was performed using Omniviz version 3.6.0 (Omniviz, Maynard, MA) software. Probesets whose expression levels differed more than 2 fold from the geometric mean in at least one sample were selected for the unsupervised clustering analysis. Similarities between samples is plotted using Omniviz software as Pearson's correlations.

Differentially expressed genes were identified using statistical analysis of microarrays (SAM analysis) (Tusher V.G. et al., Proc. Natl. Acad. Sci. U. S. A. 98:5116-5121). Such supervised analysis correlates gene expression with an external variable. SAM calculates a score for each probeset on the basis of the change in expression relative to the SD of all measurements. Unless otherwise indicated, analyses were performed using stringent statistical parameters with a false discovery rate (FDR) of less than 1 probeset. Differentially expressed probesets were imported into Spotfire DecisionSite (Spotfire, Somerville, MA) to perform principle components analysis (PCA) and hierarchical clustering. Data were log2 transformed followed by calculation of the z-score for each probeset. PCA structures a dataset using as few variables as possible and is a mathematical way to reduce data dimensionality. PCA summarizes the most important variance in a dataset as principle components. For more information on the use of PCA in microarray analysis microarrays see (Raychaudhuri S. et al. (2000), In: Hunter L, Altman B, Dunker AK, Klein TE, Lauderdale K, editors. Pacific Symposium on Biocomputing 1999. Honolulu, Hawaii: World Scientific Press; 2000) and references therein. Hierarchical clustering groups data based on their similarities in gene expression profiles. Weighted average was used to perform most clustering analysis, in which the distance between two clusters is defined as the average of distances between all pairs of objects. Unlike clustering based on unweighted averages, the weighted average ascribes equal weight to the two branches of the dendrogram that are about to be fused. Ward's hierarchical clustering method forms groups in a manner that minimizes the loss associated with each grouping. At each step in this analysis, the two clusters whose fusion results in minimum increase in information loss are combined.

Results

Samples:

Patient data, histological diagnosis, chromosomal aberrations, and response to chemotherapy are summarized in table 1. In total we performed expression analysis on 28 oligodendroglial tumors (2 lowgrade and 26 anaplastic oligodendrogliomas), and 6 control brain samples (4 samples from whole cortex, 2 from white matter only). We identified 14/28 samples (50%) with loss of most/all of the short arm of chromosome 1 (sample 18 had a predicted loss distal to 1p33) and 16/28 (57%) samples with loss of 19q (see Table 1). Most tumors showed combined loss or retention of 1p and 19q: only three tumors showed loss of 19q without loss of 1p, one showed LOH on 1p35.2 without loss of 19q. EGFR amplification and LOH on 10q was identified in 4/28 (14%) oligodendroglial tumors, three of which showed combined EGFR amplification and 10q LOH. When comparing the response rate (CR+PR vs. PD+SD) to loss of the telomeric end of chromosome 1, a response to chemotherapy was observed in 12/14 (86%) samples with 1p35.2 LOH and 6/14 (43%) without loss of 1p35.2. Similar results were obtained when comparing the response rate to LOH on 19q or to combined LOH on 1p and 19q (table 1). All four tumors in which the EGFR genomic region was amplified had retained both copies of 1p and 19q and showed no response to chemotherapy (progressive disease for all). 3/4 tumors with 10q LOH showed no response to treatment.

Unsupervised clustering:

Unsupervised clustering identifies a number of subgroups, summarized in Figure 1. A first subgroup consists mainly of control samples but also includes low-grade tumor samples. Because the amount of tumor present in all samples was high (determined by visual inspection of sections prior to the sample used for expression profiling), this close homology to control brain tissue is likely to reflect an intrinsic property of low-grade oligodendroglial tumors. The low-grade oligodendroglioma samples have a higher homology to samples from whole cortex than to samples from white matter. Group II consists of tumor samples that have LOH on 1p and 19q and has a relatively good prognosis: All but one sample respond favorably to chemotherapy and most (4/6) patients with CR are found in this group. Patients in this group also have a relatively long survival both after diagnosis (15.3±3.6 years) and after surgical resection of the tumor (4.8±1.5 years). Group III has the worst prognosis: None of the tumors respond to chemotherapy, the average time of survival after diagnosis was short (1.9±0.2 years) as was the average time after surgical resection (1.5±0.3 years). All tumors of this subgroup have retained both copies of 1p and 19q and are characterized by an amplification of the EGFR locus. The samples between groups II and III have a more mixed appearance, there is some degree of correlation with both groups I and group III. Many samples with PR and all samples with SD are found in this group. Survival after diagnosis and surgical resection is intermediate between groups II and III: 8.3±1.5 and 2.3±0.3 years respectively.

Supervised clustering: tumor vs. controls

We first performed supervised clustering to identify genes that are differentially expressed between control and tumors tissue. SAM analysis identified 1881 differentially expressed probesets (~1413 genes). Strongest downregulated transcripts in oligodendroglial tumors include those that encode proteins expressed in mature oligodendrocytes: myelin associated oligodendrocyte basic protein (MOBP), myelin oligodendrocyte glycoprotein (MOG), myelin associated glycoprotein (MAG), claudin 11 (CLDN11) and myelin basic protein (MBP). These transcripts are expressed (±SD) at 0.052±0.021 (4 probesets), 0.10±0.013 (4 probesets), 0.086 (1 probeset), 0.30±0.25 (2 probesets), and 0.21±0.17 (7 probesets) levels of control brain mRNA respectively. This downregulation was observed in each sample. The strong downregulation in low-grade samples confirms the hypothesis that their homology to control brain tissue (see figure 1) is a result of the genes expressed by the tumor. The downregulation of MOG was confirmed using RT-PCR (table 2).

It has been reported that *PDGFRa* is often highly expressed in oligodendroglial tumors (Riemenschneider M.J. et al., (2004) Acta Neuropathol. (Berlin) 107:277-282.). However, this gene was not present in the set of tumor-associated genes identified by our screen. Closer inspection reveals that, although *PDGFRa* is on average upregulated 4.1 fold, the high variation of upregulation (4.1±4.7) indicates that this transcript is not a reliable marker for the amount of tumor present in the sample. In fact, we failed to observe any upregulation in 10/28 samples. The select upregulation of *PDGFRa* in a subset of samples was confirmed using RTPCR.

Supervised clustering on chromosomal aberrations

Supervised clustering was performed to identify genes associated with specific chromosomal losses. For this we compared expression profiles of samples with i) 1p LOH (n=9) vs. no loss (n=9), ii) 19q LOH (n=11) vs. no loss (n=7), and iii) combined 1p and 19q LOH (n=6) with no loss on either arm (n=6). SAM analysis identified 376, 64 and 60 probesets as being differentially expressed following loss of 1p, 19q or 1p and 19q respectively. Probesets are listed in supplementary table 3. Interestingly, many of the identified probesets are located on the lost chromosomal arm(s): 136/376 (36.1%) probesets are located on 1p, 25/64 (39.1%) on 19q and 49/60 (82%) on 1p or 19q. Of the differentially expressed genes located on the lost chromosomal arm(s), the ratio (±SD) loss vs. no loss is 0.53±0.22 (1p), 0.54±0.07 (19q) and 0.53±0.09 (1p and 19q) indicating that loss of one allele reduces expression levels by ~50%. In fact, all but two of the differentially expressed probesets that are located on the lost chromosomal(s) are downregulated. This correlation between chromosomal loss and expression level therefore suggest that these genes have an allele-number dependent expression level. Furthermore, the differentially expressed genes can be identified across the entire chromosomal arms and suggests the entire arms have been lost.

Principle components analysis (PCA) and hierarchical clustering of genes associated with LOH on 1p and 19q is depicted in figure 2. All anaplastic oligodendrogliomas with combined loss/retention of 1p and 19q were correctly distributed by the first principal component axis, PCA1. This correct distribution includes 7 samples (2 samples that have retained both 1p and 19q copies and 5 samples with LOH on 1p and 19q) that were omitted from the clustering analysis. Further confirmation of a subset of differentially expressed genes by RT-PCR is shown in table 2 (including 4 additional oligodendroglial tumors).

Genes associated with chemosensitivity

We next performed supervised clustering to identify genes that are associated with response to chemotherapy. For this analysis we compared mRNA expression levels between tumors that show a response to chemotherapy (CR+PR), and those that do not (SD+PD). Such comparison using SAM (FDR<1 gene) identified 16 differentially expressed probesets that are listed in the supplementary table 3. 160 differentially expressed probesets (137 genes) were identified using less stringent statistical analysis (FDR=4.9%), of which 31 (27 genes) are located on chromosomes 1p or 19q (19%). Confirmation of differentially expressed genes was performed using RT-PCR on IQGAP, MAN1C1, TRIM56 and AQP1 transcripts (table 2).

PCA based on the 16 genes associated with chemotherapeutic response identifies three main subgroups (figure 3): Samples with no response to chemotherapy (SD and PD, red), samples with response to treatment (CR and PR, green), and control samples (gray). Similarly, hierarchical clustering also separates the majority of oligodendroglial tumors with response to chemotherapy from those that show no or little response to treatment (figure 3). Similar results were obtained when clustering was performed on 160 differentially expressed probesets identified using FDR=4.9%. Most oligodendroglial tumors were correctly distributed on their response to treatment by the first principal component axis, PCA1: PCA1>0 in 14/18 samples that respond to treatment whereas PCA1<0 in 10/10 samples with no response to treatment. Only 4/28 samples were therefore incorrectly classified based on expression of genes associated with chemosensitivity. In comparison, 8/28 samples are incorrectly classified when predicting response to treatment based on the 1p chromosomal status: 6/14 tumors without LOH on 1p show response to treatment and 2/14 with LOH on 1p do not respond to treatment.

Genes associated with survival

We next performed supervised clustering to identify genes associated with overall survival after diagnosis. For this analysis we compared expression profiles of tumors from patients with the shortest survival time (2.0 ± 0.3 years, n=7) with those with the longest survival time (17.6 ± 4.4 years, n=8) after diagnosis. SAM analysis identified 103 probesets (92 genes, see supplementary data) associated with patient survival. 30 (29%) of these probesets are located on either 1p or 19q chromosomal arms. PCA of survival-associated genes identifies three main clusters of samples: oligodendroglial tumors with short survival, oligodendroglial tumors with long survival and control samples. Low-grade samples cluster between control and tumor samples. Similar subgroups were identified by hierarchical clustering using these probesets (figure 4). It is interesting to note that the subgroups identified by hierarchical clustering are virtually identical to the subgroups that were identified by unsupervised clustering (figure 1). Most oligodendroglial tumors were correctly distributed on survival after diagnosis by the first principal component axis, PCA1: PCA1>0 in 12/14 samples with favorable prognosis (i.e. survival time > 7 years after diagnosis) whereas PCA1<0 in 8/11 samples with relatively short survival after diagnosis (i.e. < 7 years).

Table 1. Summary of patient data, histological diagnosis and response to chemotherapy of samples used in this study. M: male; F: female; ctr: normal brain; ctr/w: control brain white matter; OD oligodendroglioma (grade II); AOD: anaplastic oligodendroglioma, AOA anaplastic oligoastrocytoma; LOH: loss of heterozygosity; ampl: amplification of the EGFR locus; ther.: therapy: PCV: combination therapy of procarbazine, CCNU, and vincristine; temo: temozolomide. Treatment response was scored according to McDonald's criteria (20) CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease. Surv tot: patient survival after diagnosis (years); Surv op: patient survival after surgical resection of the sample used in this study.

Sample	sex	age	sample type	1p status	19q status	10q status	EGFR ampl	Response	ther.	surv tot	surv op	alive
1	F	39	control									no
3	F		control									no
4	M	63	control									no
7	M	63	control									no
8	F	45	AOD	LOH	LOH	no LOH	no	CR	PCV	15	8.5	yes
9	M	35	AOD	LOH	LOH	no LOH	no	PR	temo	13	2.7	no
10	M	59	AOD	LOH	LOH	no LOH	no	PR	temo	9.8	1.5	no
11	M	44	AOD	LOH	LOH	no LOH	no	PR	PCV	12	3.2	no
12	F	57	AOD	no LOH	LOH	no LOH	no	PR	PCV	19	1.9	no
13	M	40	AOD	LOH	LOH	no LOH	no	PR	PCV	24	1.9	no
14	M	59	AOD	no LOH	no LOH	LOH	yes	PD	PCV	2	1.6	no

15	F	19	AOD	no LOH	no LOH	no LOH	no	CR	temo	3.7	3.7	no
16	M	49	AOA	no LOH	LOH	no LOH	no	SD	PCV	10.9	1.2	no
17	M	47	AOD	no LOH	no LOH	no LOH	no	PD	PCV	4	3.9	no
18	M	34	AOD	LOH	no LOH	no LOH	no	PR	PCV	1.8	0.4	no
20	M	50	AOD	LOH	LOH	no LOH	no	SD	temo	11	1.3	no
21	M	32	AOD	LOH	LOH	no LOH	no	CR	PCV	3.9	3.6	yes
22	M	55	AOD	no LOH	no LOH	LOH	yes	PD	PCV	1.5	1.4	no
23	F	45	AOD	LOH	LOH	no LOH	no	PR	PCV	19	6.1	no
24	M	43	AOD	no LOH	no LOH	no LOH	no	PR	PCV	11	1.0	no
25	M	51	AOD	LOH	LOH	no LOH	no	PD	temo	10	3.0	no
28	M	35	AOD	LOH	LOH	no LOH	no	CR	PCV	2.2	2.2	yes
29	M	52	AOD	no LOH	no LOH	LOH	yes	PD	temo	2.3	2.1	no
30	M	88	control									no
31	F	68	control									no
34	M	45	AOD	no LOH	no LOH	no LOH	yes	PD	PCV	1.8	1.0	no
36	F	21	AOA	no LOH	LOH	no LOH	no	PR	temo	2.5	2.4	no
37	F	33	AOD	LOH	LOH	no LOH	no	CR	PCV	23	11.1	no

38	F	39	OD	LOH	LOH	no LOH	no	CR	PCV	9.7	6.6	no
40	M	45	AOD	LOH	LOH	no LOH	no	PR	PCV	16	3.5	no
41	F	39	AOA	no LOH	no LOH	LOH	no	PR	PCV	4.8	4.1	no
42	F	37	OD	no LOH	no LOH	no LOH	no	PR	PCV	8	8.0	yes
44	M	39	AOD	no LOH	no LOH	no LOH	no	SD	temo	2.7	2.7	no
46	M	30	AOD	no LOH	no LOH	no LOH	no	SD	PCV	6.3	2.5	no
Additional samples used for RT-PCR confirmation												
26	M	52	OD	LOH	no loss	no loss	no	MR	PCV			yes
27	M	44	AOD	no LOH	loss	no loss	no	stopped	PCV			yes
32	M	72	control									no
33	M	49	AOD	LOH	loss	no loss	no	PR	PCV			yes
45			AOD	no LOH	no loss	no loss	no	unknown				

Table 2. Confirmation of a subset of differentially expressed genes identified by expression profiling. Differential expression of most transcripts was reconfirmed by RT-PCR. The relative expression levels between control (either no loss of 1p, 19q, no tumor or CR/PR) and test set (either LOH on 1p, 19q, tumor or SD/PD) also remained similar on the array (rel expr array) and by RT-PCR (rel expr QPCR). QPCR ctr: expression of the examined transcript in control samples (either no loss of 1p, 19q, no tumor or CR/PR) relative to PDGB expression levels; QPCR marker: expression of the examined transcript in test samples (either LOH on 1p, 19q, no tumor or CR/PR) relative to PDGB expression levels. Statistical analysis was performed on QPCR ctr vs. marker using the Mann Whitney U test (two tailed), values are ±SE.

gene	marker for	rel expr array	rel expr QPCR	QPCR ctr	QPCR marker	P
F3	1p LOH	5.4	6.9	0.52±0.21	3.63±2.88	p<0.001
IQGAP	1p LOH	2.8	4.6	0.48±0.15	2.19±1.09	p<0.001
PPAP2B	1p LOH	3.3	4.4	2.38±0.75	10.4±8.1	p<0.005
GNG12	1p LOH	2.8	5.4	0.61±0.23	3.28±1.19	p<0.001
MOG	tumor	11.6	21.9	2.44±0.84	53.4±5.0	p<0.00001
LANCL2	EGFR ampl	9.6	15.7	4.84±0.56	76.1±25.6	p<0.005
EGFR	EGFR ampl	6.3	14.4	33.3±4.6	480±112	p<0.005
CASP3	19q LOH	2.0	1.7	2.57±0.83	4.47±1.41	ns
ZNF222	19q LOH	2.4	1.6	0.34±0.10	0.54±0.17	ns
DCDT	19q LOH	4.0	4.8	0.65±0.29	3.14±0.79	p<0.005
MAN1C1	response	3.5	3.0	2.09±0.50	6.24±1.51	p<0.05

IQGAP1	response	2.4	2.3	0.99±0.41	2.32±1.28	$p<0.05$
TRIM56	response	2.3	2.7	0.17±0.05	0.47±0.21	$p<0.05$
AQP1	response	9.7	7.5	2.42±1.21	18.2±13.2	$p<0.02$

Table 3.: Differentially expressed probesets, which are able to discriminate on basis of response tot treatment

Probe Set ID	Title	Gene Symbol	Location
1552506_at	hypothetical protein FLJ38464	FLJ38464	Chr:9q34.11
1554830_a_at	dudulin 2	TSAP6	Chr:2q14.2
1555600_s_at	apolipoprotein L, 4	APOL4	Chr:22q11.2-q13.2
1555852_at	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	TAP1	Chr:6p21.3
1555997_s_at	insulin-like growth factor binding protein 5	IGFBP5	Chr:2q33-q36
1556643_at	hypothetical protein BC011840	LOC93343	Chr:19p13.12
1567628_at	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	CD74	Chr:5q32
1568619_s_at	hypothetical protein LOC162073	LOC162073	Chr:16p13.11
200660_at	S100 calcium binding protein A11 (calgizzarin)	S100A11	Chr:1q21
200673_at	lysosomal-associated protein transmembrane 4 alpha	LAPTM4A	Chr:2p24.3
200791_s_at	IQ motif containing GTPase activating protein 1	IQGAP1	Chr:15q26.1
200867_at	zinc finger protein 313	ZNF313	Chr:20q13.13
200887_s_at	signal transducer and activator of transcription 1, 91kDa	STAT1	Chr:2q32.2
201053_s_at	proteasome (prosome, macropain) inhibitor subunit 1 (PI31)	PSMF1	Chr:20p13
201125_s_at	integrin, beta 5	ITGB5	Chr:3q21.2
201136_at	proteolipid protein 2 (colonic epithelium-enriched)	PLP2	Chr:Xp11.23
201259_s_at	synaptophysin-like protein	SYPL	Chr:7q22.2

201319_at	myosin regulatory light chain MRCL3	MRCL3	Chr:18p11.31
201324_at	epithelial membrane protein 1	EMP1	Chr:12p12.3
201325_s_at	epithelial membrane protein 1	EMP1	Chr:12p12.3
201336_at	vesicle-associated membrane protein 3 (cellubrevin)	VAMP3	Chr:1p36.23
201339_s_at	sterol carrier protein 2	SCP2	Chr:1p32
201464_x_at	v-jun sarcoma virus 17 oncogene homolog (avian)	JUN	Chr:1p32-p31
201465_s_at	v-jun sarcoma virus 17 oncogene homolog (avian)	JUN	Chr:1p32-p31
201531_at	zinc finger protein 36, C3H type, homolog (mouse)	ZFP36	Chr:19q13.1
201560_at	chloride intracellular channel 4	CLIC4	Chr:1p36.11
201590_x_at	annexin A2	ANXA2	Chr:15q21-q22
201817_at	ubiquitin-protein isopeptide ligase (E3)	KIAA0010	Chr:7q36.3
201887_at	interleukin 13 receptor, alpha 1	IL13RA1	Chr:Xq24
201954_at	actin related protein 2/3 complex, subunit 1B, 41kDa	ARPC1B	Chr:7q22.1
201963_at	fatty-acid-Coenzyme A ligase, long-chain 2	FACL2	Chr:4q34-q35
202096_s_at	benzodiazapine receptor (peripheral)	BZRP	Chr:22q13.31
202132_at	transcriptional co-activator with PDZ-binding motif (TAZ)	TAZ	Chr:3q23-q24
202133_at	transcriptional co-activator with PDZ-binding motif (TAZ)	TAZ	Chr:3q23-q24
202193_at	LIM domain kinase 2	LIMK2	Chr:22q12.2
202377_at	leptin receptor	LEPR	Chr:1p31
202803_s_at	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)	ITGB2	Chr:21q22.3
202863_at	nuclear antigen Sp100	SP100	Chr:2q37.1
203044_at	carbohydrate (chondroitin) synthase 1	CHSY1	Chr:15q26.3
203132_at	retinoblastoma 1 (including osteosarcoma)	RB1	Chr:13q14.2
203153_at	interferon-induced protein with tetratricopeptide repeats 1	IFIT1	Chr:10q25-q26

203236_s_at	lectin, galactoside-binding, soluble, 9 (galectin 9)	LGALS9	Chr:17q11.2
203275_at	interferon regulatory factor 2	IRF2	Chr:4q34.1-q35.1
203379_at	ribosomal protein S6 kinase, 90kDa, polypeptide 1	RPS6KA1	Chr:3
203426_s_at	insulin-like growth factor binding protein 5	IGFBP5	Chr:2q33-q36
203567_s_at	tripartite motif-containing 38	TRIM38	Chr:6p21.3
203735_x_at	Homo sapiens transcribed sequence with weak similarity to protein ref:NP_060312.1 (H.sapiens) hypothetical protein FLJ20489 [Homo sapiens]	---	---
203879_at	phosphoinositide-3-kinase, catalytic, delta polypeptide	PIK3CD	Chr:1p36.2
203973_s_at	KIAA0146 protein	KIAA0146	Chr:8q11.21
204017_at	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3	KDELR3	Chr:22q13.1
206515_at	cytochrome P450, family 4, subfamily F, polypeptide 3	CYP4F3	Chr:19p13.2
207542_s_at	aquaporin 1 (channel-forming integral protein, 28kDa)	AQP1	Chr:7p14
207753_at	zinc finger protein 304	ZNF304	Chr:19q13.4
208540_x_at	---	---	---
208789_at	polymerase I and transcript release factor	PTRF	Chr:17q21.31
208966_x_at	interferon, gamma-inducible protein 16	IFI16	Chr:1q22
209047_at	aquaporin 1 (channel-forming integral protein, 28kDa)	AQP1	Chr:7p14
209091_s_at	SH3-domain GRB2-like endophilin B1	SH3GLB1	Chr:1p22
209619_at	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	CD74	Chr:5q32
209762_x_at	SP110 nuclear body protein	SP110	Chr:2q37.1
209823_x_at	major histocompatibility complex, class II, DQ beta 1	HLA-DQB1	Chr:6p21.3
209949_at	neutrophil cytosolic factor 2 (65kDa, chronic	NCF2	Chr:1q25

	granulomatous disease, autosomal 2)		
209969_s_at	signal transducer and activator of transcription 1, 91kDa	STAT1	Chr:2q32.2
210426_x_at	RAR-related orphan receptor A	RORA	Chr:15q21-q22
210427_x_at	annexin A2	ANXA2	Chr:15q21-q22
210582_s_at	LIM domain kinase 2	LIMK2	Chr:22q12.2
210829_s_at	single-stranded DNA binding protein 2	SSBP2	Chr:5q14.1
210840_s_at	IQ motif containing GTPase activating protein 1	IQGAP1	Chr:15q26.1
211168_s_at	regulator of nonsense transcripts 1	RENT1	Chr:19p13.2-p13.11
211366_x_at	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	CASP1	Chr:11q23
211429_s_at	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	SERPINA1	Chr:14q32.1
211495_x_at	tumor necrosis factor (ligand) superfamily, member 13	TNFSF13	Chr:17p13.1
211561_x_at	mitogen-activated protein kinase 14	MAPK14	Chr:6p21.3-p21.2
211612_s_at	interleukin 13 receptor, alpha 1	IL13RA1	Chr:Xq24
211656_x_at	major histocompatibility complex, class II, DQ beta 1	HLA-DQB1	Chr:6p21.3
211733_x_at	sterol carrier protein 2	SCP2	Chr:1p32
211749_s_at	vesicle-associated membrane protein 3 (cellubrevin)	VAMP3	Chr:1p36.23
211924_s_at	plasminogen activator, urokinase receptor	PLAUR	Chr:19q13
211959_at	insulin-like growth factor binding protein 5	IGFBP5	Chr:2q33-q36
212203_x_at	interferon induced transmembrane protein 3 (1-8U)	IFITM3	Chr:11p15.5
212268_at	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	SERPINB1	Chr:6p25
212687_at	LIM and senescent cell antigen-like domains 1	LIMS1	Chr:2q12.3
212859_x_at	metallothionein 1E (functional)	MT1E	Chr:16q13
213293_s_at	tripartite motif-containing 22	TRIM22	Chr:11p15

213446_s_at	IQ motif containing GTPase activating protein 1	IQGAP1	Chr:15q26.1
213503_x_at	annexin A2	ANXA2	Chr:15q21-q22
213504_at	COP9 subunit 6 (MOV34 homolog, 34 kD)	COPS6	Chr:7q22.1
213698_at	zinc finger protein 258	ZNF258	Chr:1p34.2
214087_s_at	myosin binding protein C, slow type	MYBPC1	Chr:12q23.3
214180_at	Homo sapiens transcribed sequence with weak similarity to protein ref:NP_060265.1 (H.sapiens) hypothetical protein FLJ20378 [Homo sapiens]	---	---
214257_s_at	hypothetical protein FLJ21272	FLJ21272	Chr:1q21.2
214684_at	MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)	MEF2A	Chr:15q26
214791_at	hypothetical protein BC004921	LOC93349	Chr:2q37.1
216526_x_at	major histocompatibility complex, class I, C	HLA-C	Chr:6p21.3
216598_s_at	chemokine (C-C motif) ligand 2	CCL2	Chr:17q11.2-q21.1
217388_s_at	kynureninase (L-kynurenine hydrolase)	KYNU	Chr:2q22.3
217730_at	PP1201 protein	PP1201	Chr:2p24.3-p24.1
217746_s_at	programmed cell death 6 interacting protein	PDCD6IP	Chr:3p22.3
217788_s_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GalNAc-T2)	GALNT2	Chr:1q41-q42
218154_at	hypothetical protein FLJ12150	FLJ12150	Chr:8q24.3
218162_at	HNOEL-iso protein	HNOEL-iso	Chr:1p13.1
218247_s_at	hypothetical protein LOC51320	LOC51320	Chr:18q21.1
218418_s_at	KIAA1518 protein	KIAA1518	Chr:19p13.2
218673_s_at	ubiquitin activating enzyme E1-like protein	GSA7	Chr:3p25.2
218802_at	hypothetical protein FLJ20647	FLJ20647	Chr:4q25
218918_at	mannosidase, alpha, class 1C, member 1	MAN1C1	Chr:1p35
218943_s_at	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide	RIG-I	Chr:9p12
219505_at	cat eye syndrome chromosome region, candidate 1	CECR1	Chr:22q11.2
219706_at	chromosome 20 open	C20orf29	Chr:20p13

	reading frame 29		
219751_at	hypothetical protein FLJ21148	FLJ21148	Chr:16q13
220088_at	complement component 5 receptor 1 (C5a ligand)	C5R1	Chr:19q13.3-q13.4
220407_s_at	transforming growth factor, beta 2	TGFB2	Chr:1q41
220477_s_at	chromosome 20 open reading frame 30	C20orf30	Chr:20p13
221773_at	ELK3, ETS-domain protein (SRF accessory protein 2)	ELK3	Chr:12q23
221790_s_at	LDL receptor adaptor protein	ARH	Chr:1p36-p35
222448_s_at	UMP-CMP kinase	UMP-CMPK	---
223047_at	chemokine-like factor super family 6	CKLFSF6	Chr:3p22.3
223165_s_at	inositol hexaphosphate kinase 2	IHPK2	Chr:3p21.31
223376_s_at	brain protein I3	BRI3	Chr:7q22.1
223642_at	Zic family member 2 (odd-paired homolog, Drosophila)	ZIC2	Chr:13q32
223681_s_at	InaD-like protein	INADL	Chr:1p32.1
224584_at	chromosome 20 open reading frame 30	C20orf30	Chr:20p13
224840_at	FK506 binding protein 5	FKBP5	Chr:6p21.3-21.2
224856_at	FK506 binding protein 5	FKBP5	Chr:6p21.3-21.2
225267_at	karyopherin alpha 4 (importin alpha 3)	KPNA4	Chr:3q25.33
225415_at	rhysin 2	LOC151636	Chr:3q21.1
225869_s_at	unc-93 homolog B1 (C. elegans)	UNC93B1	Chr:11q13
226040_at	Homo sapiens cDNA FLJ11958 fis, clone HEMBB1000996.	---	---
226074_at	hypothetical protein FLJ32332	FLJ32332	Chr:3p21.31
226621_at	fibrinogen, gamma polypeptide	FGG	Chr:4q28
226628_at	THO complex 2	THOC2	Chr:Xq25-q26.3
226694_at	A kinase (PRKA) anchor protein 2	AKAP2	Chr:9q31-q33
227013_at	LATS, large tumor suppressor, homolog 2 (Drosophila)	LATS2	Chr:13q11-q12
227066_at	similar to MOB-LAK	LOC148932	Chr:1p34.1
227474_at	paired box gene 8	PAX8	Chr:2q12-q14
227792_at	Homo sapiens cDNA: FLJ22994 fis, clone KAT11918.	---	---
227801_at	tumor suppressor TSBF1	TSBF1	Chr:3q26.1
227837_at	hypothetical protein FLJ20309	FLJ20309	Chr:2q33.3
227882_at	fukutin-related protein	FKRP	Chr:19q13.33

228042_at	ADP-ribosylarginine hydrolase	ADPRH	Chr:3q13.31-q13.33
228229_at	KIAA1951 protein	KIAA1951	Chr:19q13.31
228369_at	trinucleotide repeat containing 5	TNRC5	Chr:6pter-p12.1
228410_at	GRB2-associated binding protein 3	GAB3	Chr:Xq28
228425_at	Homo sapiens, clone IMAGE:4820851, mRNA	---	---
228651_at	hypothetical gene supported by AK075366		Chr:1
228949_at	putative NFkB activating protein 373	FLJ23091	Chr:1p31.2
228980_at	hypothetical gene supported by AK091492; AL831912		Chr:17q21.1
229101_at	hypothetical protein LOC150166	LOC150166	Chr:22q11.21
229143_at	CCR4-NOT transcription complex, subunit 3	CNOT3	Chr:19q13.4
229812_at	ubiquitin specific protease 31	USP31	Chr:1p36.12
230636_s_at	basic transcription element binding protein 1	BTEB1	Chr:9q13
231876_at	tripartite motif-containing 56	TRIM56	Chr:7q22.1
233103_at	Homo sapiens cDNA FLJ14109 fis, clone MAMMA1001322, moderately similar to B-CELL GROWTH FACTOR PRECURSOR.	---	---
240277_at	solute carrier family 30 (zinc transporter), member 7	SLC30A7	Chr:1p21.2
240656_at	Homo sapiens transcribed sequences	---	---
242521_at	Homo sapiens, similar to Alu subfamily SQ sequence contamination warning entry, clone IMAGE:4342162, mRNA	---	---
40524_at	protein tyrosine phosphatase, non-receptor type 21	PTPN21	Chr:14q31.3
57163_at	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 1	ELOVL1	Chr:1p34.1
AFFX-HUMISGF3A/M97935_3_at	---	---	---
AFFX-HUMISGF3A/M97935_MB_at	---	---	---

Table 4.: Differentially expressed probesets, which are able to discriminate on basis of patient survival

Probe Set ID	Title	Gene Symbol	Location	ratio short/long
200902_at	15 kDa selenoprotein	SEP15	Chr:1p31	1.92
231057_at	Myotubularin related protein 2	MTMR2	Chr:11q21	0.38
232929_at	Homo sapiens cDNA FLJ13240 fis, clone OVARC1000496.	---	Chr:3q13.31	0.40
213156_at	Homo sapiens, clone IMAGE:4214654, mRNA	IMAGE:4214654	Chr:3q13.31	0.44
227082_at	Homo sapiens mRNA; cDNA DKFZp586K1922 (from clone DKFZp586K1922)	---	Chr:3q13.31	0.39
227121_at	Homo sapiens mRNA; cDNA DKFZp586K1922 (from clone DKFZp586K1922)	---	Chr:3q13.31	0.43
239545_at	O-acetyltransferase	CAS1	Chr:7q21.3	0.47
229624_at	similar to OPA3 protein; Optic atrophy 3 (Iraqi-Jewish optic atrophy plus)	LOC401922	Chr:19q13.32	1.96
235384_at	similar to RP2 protein, testosterone-regulated - ricefield mouse (Mus caroli)	LOC390916	Chr:19q13.11	2.37
229075_at	Homo sapiens transcribed sequences	---	Chr:4q28.1	1.61
237803_x_at	Homo sapiens transcribed sequences	---		0.34
241435_at	V-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	ETS1	Chr:11q23.3	0.36
240216_at	CDNA FLJ25794 fis, clone TST07014	---	Chr:3q13.31	0.42
239577_at	Homo sapiens, clone IMAGE:4182817, mRNA	---	---	0.42
226189_at	Homo sapiens, clone IMAGE:4794726, mRNA	IMAGE:4794726	Chr:7p21.1	2.38
218694_at	ALEX1 protein	ALEX1	Chr:Xq21.33-q22.2	0.66
226291_at	amyotrophic lateral sclerosis 2 (juvenile)	ALS2	Chr:2q33.2	0.78
223251_s_at	ankyrin repeat domain 10	ANKRD10	Chr:13q34	0.42
224810_s_at	ankyrin repeat domain 13	ANKRD13	Chr:12q24.12	0.65
200782_at	annexin A5	ANXA5	Chr:4q28-q32	2.92
205711_x_at	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	ATP5C1	Chr:10q22-q23	0.69
208870_x_at	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	ATP5C1	Chr:10q22-q23	0.68
205263_at	B-cell CLL/lymphoma 10	BCL10	Chr:1p22	1.75
203543_s_at	basic transcription element binding protein 1	BTEB1	Chr:9q13	2.66
217928_s_at	chromosome 11 open reading frame 23	C11orf23	Chr:11q13	0.57

218796_at	chromosome 20 open reading frame 42	C20orf42	Chr:20p12.3	0.09
217752_s_at	cytosolic nonspecific dipeptidase (EC 3.4.13.18)	CN2	Chr:18q22.3	1.59
222409_at	coronin, actin binding protein, 1C	CORO1C	Chr:12q24.1	0.52
204264_at	carnitine palmitoyltransferase II	CPT2	Chr:1p32	1.59
209489_at	CUG triplet repeat, RNA binding protein 1	CUGBP1	Chr:11p11	0.67
225434_at	death effector domain-containing DNA binding protein 2	DEDD2	Chr:19q13.31	2.33
212131_at	DKFZP434D1335 protein	DKFZP434D1335	Chr:19q13.12	1.99
224436_s_at	DKFZp564D177 protein	DKFZp564D177	Chr:9q31.2	3.49
201681_s_at	discs, large (Drosophila) homolog 5	DLG5	Chr:10q23	0.46
209187_at	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	DR1	Chr:1p22.1	1.93
204363_at	coagulation factor III (thromboplastin, tissue factor)	F3	Chr:1p22-p21	5.40
209004_s_at	F-box and leucine-rich repeat protein 5	FBXL5	Chr:4p15.33	1.59
208933_s_at	hypothetical protein FLJ10359	FLJ10359	Chr:1q42.3	2.98
240239_at	hypothetical protein FLJ14779	FLJ14779	Chr:19q13.13	1.64
221518_s_at	hypothetical protein FLJ20727	FLJ20727	Chr:11p15.3	0.59
228950_s_at	putative NFkB activating protein 373	FLJ23091	Chr:1p31.2	3.13
212558_at	ganglioside-induced differentiation-associated protein 1-like 1	GDAP1L1	Chr:20q12	2.80
201864_at	GDP dissociation inhibitor 1	GDI1	Chr:Xq28	0.60
238119_at	GL004 protein	GL004	Chr:2q36.3	0.50
212294_at	guanine nucleotide binding protein (G protein), gamma 12	GNG12	Chr:1p31.2	4.08
207157_s_at	guanine nucleotide binding protein (G protein), gamma 5	GNG5	Chr:1p22	2.77
212211_at	gene trap ankyrin repeat	GTAR	Chr:4q21.1-q21.21	1.43
225784_s_at	hepatocellular carcinoma-associated antigen 127	HCA127	Chr:Xq11.2	0.31
223042_s_at	hepatitis C virus core-binding protein 6	HCBP6	Chr:Xq28	0.66
219288_at	HT021	HT021	Chr:3p21.1	3.24
209185_s_at	insulin receptor substrate 2	IRS2	Chr:13q34	2.40
201464_x_at	v-jun sarcoma virus 17 oncogene homolog (avian)	JUN	Chr:1p32-p31	2.55
201466_s_at	v-jun sarcoma virus 17 oncogene homolog (avian)	JUN	Chr:1p32-p31	2.89
213340_s_at	KIAA0495	KIAA0495	Chr:1p36.32	2.95
213271_s_at	KIAA1117 protein	KIAA1117	Chr:6q15	0.60
208935_s_at	lectin, galactoside-binding, soluble, 8 (galectin 8)	LGALS8	Chr:1q42-q43	2.56
209205_s_at	LIM domain only 4	LMO4	Chr:1p22.3	2.62
225479_at	hypothetical protein LOC116064	LOC116064	Chr:3q13.33	0.67

227466_at	hypothetical protein LOC285550	LOC285550	Chr:4p15.33	1.54
1558700_s_at	hypothetical protein LOC339324	LOC339324	Chr:19q13.13	2.05
235940_at	hypothetical protein MGC10999	MGC10999	Chr:9q21.33	5.16
228326_at	hypothetical protein MGC43690	MGC43690	Chr:6q27	0.54
213259_s_at	similar to RIKEN cDNA 1110002C08 gene	MGC9564	Chr:17q11.2	0.55
224874_at	hypothetical protein MGC9850	MGC9850	Chr:13q12.2	3.26
212080_at	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	MLL	Chr:11q23	0.51
208709_s_at	nardilysin (N-arginine dibasic convertase)	NRD1	Chr:1p32.2-p32.1	1.69
209791_at	peptidyl arginine deiminase, type II	PADI2	Chr:1p35.2-p35.1	4.02
207769_s_at	polyglutamine binding protein 1	PQBP1	Chr:Xp11.23	0.65
214527_s_at	polyglutamine binding protein 1	PQBP1	Chr:Xp11.23	0.65
224909_s_at	KIAA1415 protein	PRex1	Chr:20q13.13	2.89
208615_s_at	protein tyrosine phosphatase type IVA, member 2	PTP4A2	Chr:1p35	1.88
208616_s_at	protein tyrosine phosphatase type IVA, member 2	PTP4A2	Chr:1p35	1.87
216988_s_at	protein tyrosine phosphatase type IVA, member 2	PTP4A2	Chr:1p35	1.97
202006_at	protein tyrosine phosphatase, non-receptor type 12	PTPN12	Chr:7q11.23	1.76
201165_s_at	pumilio homolog 1 (Drosophila)	PUM1	Chr:1p35.2	1.42
225251_at	RAB24, member RAS oncogene family	RAB24	Chr:5q35.3	0.53
213718_at	RNA binding motif protein 4	RBM4	Chr:11q13	0.47
212197_x_at	Rho interacting protein 3	RHOIP3	Chr:17p11.2	0.66
214143_x_at	ribosomal protein L24	RPL24	Chr:3q12	0.67
211073_x_at	ribosomal protein L3	RPL3	Chr:22q13	0.64
200717_x_at	ribosomal protein L7	RPL7	Chr:8q13.3	0.71
200909_s_at	ribosomal protein, large P2	RPLP2	Chr:11p15.5-p15.4	0.72
208692_at	ribosomal protein S3	RPS3	Chr:11q13.3-q13.5	0.55
202361_at	SEC24 related gene family, member C (S. cerevisiae)	SEC24C	Chr:10q22.3	0.53
201696_at	splicing factor, arginine/serine-rich 4	SFRS4	Chr:1p35.2	1.48
220298_s_at	spermatogenesis associated 6	SPATA6	Chr:1p33	5.60
238459_x_at	spermatogenesis associated 6	SPATA6	Chr:1p33	6.03
220299_at	spermatogenesis associated 6	SPATA6	Chr:1p33	4.09
46256_at	SPRY domain-containing SOCS box protein SSB-3	SSB3	Chr:16p13.3	0.66
209022_at	stromal antigen 2	STAG2	Chr:Xq25	0.73
201519_at	translocase of outer mitochondrial membrane 70 homolog A (yeast)	TOMM70A	Chr:3q12.3	0.65
208661_s_at	tetratricopeptide repeat domain 3	TTC3	Chr:21q22.2	0.46
208662_s_at	tetratricopeptide repeat domain 3	TTC3	Chr:21q22.2	0.51
210645_s_at	tetratricopeptide repeat domain 3	TTC3	Chr:21q22.2	0.50

219043_s_at	IAP-associated factor VIAF1	VIAF1	Chr:2q12.1	0.78
201294_s_at	SOCS box-containing WD protein SWiP-1	WSB1	Chr:17q11.2	0.39
201296_s_at	SOCS box-containing WD protein SWiP-1	WSB1	Chr:17q11.2	0.55
207090_x_at	likely ortholog of mouse zinc finger protein 30	ZFP30	Chr:19q13.13	1.63
228157_at	zinc finger protein 207	ZNF207	Chr:17q12	0.56
222357_at	zinc finger protein 288	ZNF288	Chr:3q13.2	0.30
226252_at	hypothetical gene supported by AK022228		Chr:3q13.31	0.44
227388_at	hypothetical gene supported by BC017510; BC036931; BC028316		Chr:9p21.1	3.48
244740_at	LOC342935		Chr:19q13.43	1.70

Table 5.: Differentially expressed probesets, which are able to discriminate on basis of loss of heterozygosity (LOH) on the 1p locus

Probe Set ID	Title	Gene Symbol	Location	ratio loss/no loss
1553954_at	hypothetical protein MGC19780	MGC19780	chr1p21.3	0.55
1554433_a_at	zinc finger protein 146	ZNF146	chr19q13.1	0.57
1554479_a_at	caspase recruitment domain family, member 8	CARD8	chr19q13.32	0.59
1555832_s_at	---	---	---	0.50
1558256_at	hypothetical protein LOC148189	LOC148189	chr19q12	0.45
1558604_a_at	H.sapiens mRNA; clone CD 43T7	---	---	0.47
1558700_s_at	hypothetical protein LOC339324	LOC339324	chr19q13.12	0.49
200006_at	Parkinson disease (autosomal recessive, early onset) 7	PARK7	chr1p36.33-p36.12	0.70
200020_at	TAR DNA binding protein	TARDBP	chr1p36.22	0.70
200050_at	zinc finger protein 146	ZNF146	chr19q13.1	0.52
200087_s_at	coated vesicle membrane protein	RNP24	chr12q24.31	0.84
200620_at	chromosome 1 open reading frame 8	C1orf8	chr1p36-p31	0.58
200625_s_at	CAP, adenylate cyclase-associated protein 1 (yeast)	CAP1	chr1p34.2	0.61
200636_s_at	protein tyrosine phosphatase, receptor type, F	PTPRF	chr1p34	0.39
200650_s_at	lactate dehydrogenase A	LDHA	chr11p15.4	0.26
200686_s_at	splicing factor, arginine/serine-rich 11	SFRS11	chr1p31	0.44
200777_s_at	basic leucine zipper and W2 domains 1	BZW1	chr2q33	0.75
200791_s_at	IQ motif containing GTPase activating protein 1	IQGAP1	chr15q26.1	0.27
200902_at	15 kDa selenoprotei	SEP15	chr1p31	0.53
201064_s_at	poly(A) binding protein, cytoplasmic 4 (inducible form)	PABPC4	chr1p32-p36	0.64
201080_at	phosphatidylinositol-4-phosphate 5-kinase, type II, beta	PIP5K2B	chr17q12	1.63
201155_s_at	mitofusin 2	MFN2	chr1p36.22	0.64
201164_s_at	pumilio homolog 1 (Drosophila)	PUM1	chr1p35.2	0.52
201165_s_at	pumilio homolog 1 (Drosophila)	PUM1	chr1p35.2	0.74
201177_s_at	SUMO-1 activating enzyme subunit 2	UBA2	chr19q12	0.43
201179_s_at	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3	GNAI3	chr1p13	0.60

201181_at	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3	GNAI3	chr1p13	0.57
201209_at	histone deacetylase 1	HDAC1	chr1p34	0.44
201225_s_at	serine/arginine repetitive matrix 1	SRRM1	chr1p36.11	0.71
201274_at	proteasome (prosome, macropain) subunit, alpha type, 5	PSMA5	chr1p13	0.60
201323_at	EBNA1 binding protein 2	EBNA1BP2	chr1p35-p33	0.56
201339_s_at	sterol carrier protein 2	SCP2	chr1p32	0.56
201398_s_at	translocation associated membrane protein 1	TRAM1	chr8q13.3	0.66
201426_s_at	vimentin	VIM	chr10p13	0.39
201445_at	calponin 3, acidic	CNN3	chr1p22-p21	0.40
201519_at	translocase of outer mitochondrial membrane 70 homolog A (yeast)	TOMM70A	chr3q12.2	1.52
201667_at	gap junction protein, alpha 1, 43kDa (connexin 43)	GJA1	chr6q21-q23.2	0.19
201674_s_at	A kinase (PRKA) anchor protein 1	AKAP1	chr17q21-q23	1.85
201696_at	splicing factor, arginine/serine-rich 4	SFRS4	chr1p35.3	0.61
201864_at	GDP dissociation inhibitor 1	GDI1	chrXq28	1.56
201948_at	guanine nucleotide binding protein-like 2 (nucleolar)	GNL2	chr1p34.3	0.49
202049_s_at	zinc finger protein 262	ZNF262	chr1p32-p34	0.51
202096_s_at	benzodiazapine receptor (peripheral)	BZRP	chr22q13.31	0.36
202149_at	neural precursor cell expressed, developmentally down-regulated 9	NEDD9	chr6p25-p24	0.49
202250_s_at	WD repeat domain 42A	WDR42A	chr1q22-q23	1.78
202260_s_at	syntaxin binding protein 1	STXBP1	chr9q34.1	1.84
202299_s_at	hepatitis B virus x interacting protein	HBXIP	chr1p13.3	0.57
202300_at	hepatitis B virus x interacting protein	HBXIP	chr1p13.3	0.59
202361_at	SEC24 related gene family, member C (S. cerevisiae)	SEC24C	chr10q22.2	1.73
202362_at	RAP1A, member of RAS oncogene family	RAP1A	chr1p13.3	0.51
202412_s_at	ubiquitin specific protease 1	USP1	chr1p32.1-p31.3	0.43
202413_s_at	ubiquitin specific protease 1	USP1	chr1p32.1-p31.3	0.41
202471_s_at	isocitrate dehydrogenase 3 (NAD+) gamma	IDH3G	chrXq28	1.54
202502_at	acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain	ACADM	chr1p31	0.57
202625_at	v-yes-1 Yamaguchi sarcoma viral related oncogene	LYN	chr8q13	0.46

	homolog			
202626_s_at	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	LYN	chr8q13	0.43
202668_at	ephrin-B2	EFNB2	chr13q33	0.42
202669_s_at	ephrin-B2	EFNB2	chr13q33	0.50
202868_s_at	POP4 (processing of precursor , S. cerevisiae) homolog	POP4	chr19q12	0.63
202939_at	zinc metalloproteinase (STE24 homolog, yeast)	ZMPSTE24	chr1p34	0.53
202950_at	crystallin, zeta (quinone reductase)	CRYZ	chr1p31-p22	0.43
203069_at	synaptic vesicle glycoprotein 2A	SV2A	chr1q21.2	1.96
203221_at	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	TLE1	chr9q21.32	0.31
203222_s_at	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	TLE1	chr9q21.32	0.33
203283_s_at	heparan sulfate 2-O-sulfotransferase 1	HS2ST1	chr1p31.1-p22.1	0.29
203284_s_at	heparan sulfate 2-O-sulfotransferase 1	HS2ST1	chr1p31.1-p22.1	0.51
203288_at	KIAA0355	KIAA0355	chr19q13.11	0.60
203289_s_at	chromosome 16 open reading frame 35	C16orf35	chr16p13.3	2.09
203303_at	t-complex-associated-testis-expressed 1-like	TCTE1L	chrXp21	0.33
203310_at	syntaxin binding protein 3	STXBP3	chr1p13.3	0.48
203347_s_at	likely ortholog of mouse metal response element binding transcription factor 2	M96	chr1p22.1	0.44
203364_s_at	KIAA0652 gene product	KIAA0652	chr11p11.2	1.59
203389_at	kinesin family member 3C	KIF3C	chr2p23	2.12
203401_at	phosphoribosyl pyrophosphate synthetase 2	PRPS2	chrXp22.3-p22.2	0.35
203511_s_at	trafficking protein particle complex 3	TRAPPC3	chr1p34.3	0.55
203560_at	gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase)	GGH	chr8q12.3	0.37
203611_at	telomeric repeat binding factor 2	TERF2	chr16q22.1	1.59
203765_at	grancalcin, EF-hand calcium binding protein	GCA	chr2q24.2	0.32
203787_at	single-stranded DNA binding protein 2	SSBP2	chr5q14.1	0.38
203819_s_at	IGF-II mRNA-binding protein 3	IMP-3	chr7p11	0.05
203928_x_at	microtubule-associated protein tau	MAPT	chr17q21.1	2.57
203930_s_at	microtubule-associated	MAPT	chr17q21.1	2.44

	protein tau			
204011_at	sprouty homolog 2 (Drosophila)	SPRY2	chr13q31.1	0.33
204022_at	Nedd-4-like ubiquitin-protein ligase	WWP2	chr16q22.1	1.93
204036_at	endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2	EDG2	chr9q31.3	0.15
204228_at	peptidyl prolyl isomerase H (cyclophilin H)	PPIH	chr1p34.1	0.49
204299_at	FUS interacting protein (serine-arginine rich) 1	FUSIP1	chr1p36.11	0.52
204363_at	coagulation factor III (thromboplastin, tissue factor)	F3	chr1p22-p21	0.16
204379_s_at	fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	FGFR3	chr4p16.3	0.20
204400_at	embryonal Fyn-associated substrate	EFS	chr14q11.2-q12	2.55
204451_at	frizzled homolog 1 (Drosophila)	FZD1	chr7q21	0.38
204722_at	sodium channel, voltage-gated, type III, beta	SCN3B	chr11q24.1	4.57
204984_at	glypican 4	GPC4	chrXq26.1	0.40
205095_s_at	ATPase, H+ transporting, lysosomal V0 subunit a isoform 1	ATP6V0A1	chr17q21	1.80
205130_at	renal tumor antigen	RAGE	chr14q32	0.52
205140_at	fucose-1-phosphate guanylyltransferase	FPGT	chr1p31.1	0.36
205173_x_at	CD58 antigen, (lymphocyte function-associated antigen 3)	CD58	chr1p13	0.22
205176_s_at	integrin beta 3 binding protein (beta3-endonexin)	ITGB3BP	chr1p31.3	0.48
205260_s_at	acylphosphatase 1, erythrocyte (common) type	ACYP1	chr14q24.3	0.44
205263_at	B-cell CLL/lymphoma 10	BCL10	chr1p22	0.54
205292_s_at	heterogeneous nuclear ribonucleoprotein A2/B1	HNRPA2B1	chr7p15	0.72
205497_at	zinc finger protein 175	ZNF175	chr19q13.4	0.63
205852_at	cyclin-dependent kinase 5, regulatory subunit 2 (p39)	CDK5R2	chr2q35	2.45
205996_s_at	adenylate kinase 2	AK2	chr1p34	0.59
206095_s_at	FUS interacting protein (serine-arginine rich) 1	FUSIP1	chr1p36.11	0.43
206401_s_at	microtubule-associated protein tau	MAPT	chr17q21.1	2.72
206993_at	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit s (factor B)	ATP5S	chr14q21.3	1.42
207090_x_at	zinc finger protein KIAA0961	KIAA0961	chr19q13.13	0.61
207236_at	zinc finger protein 345	ZNF345	chr19q13.12	0.45
207358_x_at	microtubule-actin crosslinking factor 1	MACF1	chr1p32-p31	0.54

208095_s_at	signal recognition particle 72kDa	SRP72	chr4q11	0.66
208374_s_at	capping protein (actin filament) muscle Z-line, alpha 1	CAPZA1	chr1p13.2	0.55
208615_s_at	protein tyrosine phosphatase type IVA, member 2	PTP4A2	chr1p35	0.51
208680_at	peroxiredoxin 1	PRDX1	chr1p34.1	0.33
208709_s_at	nardilysin (N-arginine dibasic convertase)	NRD1	chr1p32.2-p32.1	0.60
208723_at	ubiquitin specific protease 11	USP11	chrXp11.23	1.92
208728_s_at	cell division cycle 42 (GTP binding protein, 25kDa)	CDC42	chr1p36.1	0.55
208766_s_at	heterogeneous nuclear ribonucleoprotein R	HNRPR	chr1p36.12	0.67
208924_at	ring finger protein 11	RNF11	chr1pter-p22.1	0.65
208971_at	uroporphyrinogen decarboxylase	UROD	chr1p34	0.63
209001_s_at	anaphase promoting complex subunit 13	ANAPC13	chr3q22.1	1.32
209045_at	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble	XPNPEP1	chr10q25.3	1.44
209099_x_at	jagged 1 (Alagille syndrome)	JAG1	chr20p12.1-p11.23	0.38
209117_at	WW domain binding protein 2	WBP2	chr17q25	2.05
209120_at	nuclear receptor subfamily 2, group F, member 2	NR2F2	chr15q26	0.33
209187_at	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	DR1	chr1p22.1	0.46
209355_s_at	phosphatidic acid phosphatase type 2B	PPAP2B	chr1pter-p22.1	0.25
209537_at	exostoses (multiple)-like 2	EXTL2	chr1p21	0.61
209669_s_at	PAI-1 mRNA-binding protein	PAI-RBP1	chr1p31-p22	0.48
209707_at	phosphatidylinositol glycan, class K	PIGK	chr1p31.1	0.62
209711_at	solute carrier family 35 (UDP-glucuronic acid/UDP-N-acetylgalactosamine dual transporter), member D1	SLC35D1	chr1p32-p31	0.49
209875_s_at	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	chr4q21-q25	0.22
210092_at	mago-nashi homolog, proliferation-associated (Drosophila)	MAGOH	chr1p34-p33	0.40
210093_s_at	mago-nashi homolog, proliferation-associated (Drosophila)	MAGOH	chr1p34-p33	0.51
210137_s_at	dCMP deaminase	DCTD	chr4q35.1	0.17
210178_x_at	FUS interacting protein (serine-arginine rich) 1	FUSIP1	chr1p36.11	0.54
210191_s_at	putative homeodomain transcription factor 1	PHTF1	chr1p13	0.65

210371_s_at	retinoblastoma binding protein 4	RBBP4	chr1p35.1	0.48
210502_s_at	peptidylprolyl isomerase E (cyclophilin E)	PPIE	chr1p32	0.54
210517_s_at	A kinase (PRKA) anchor protein (gravin) 12	AKAP12	chr6q24-q25	0.36
210645_s_at	tetratricopeptide repeat domain 3	TTC3	chr21q22.2	1.94
210754_s_at	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	LYN	chr8q13	0.57
210770_s_at	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	CACNA1A	chr19p13.2-p13.1	3.12
210829_s_at	single-stranded DNA binding protein 2	SSBP2	chr5q14.1	0.33
210840_s_at	IQ motif containing GTPase activating protein 1	IQGAP1	chr15q26.1	0.32
211383_s_at	WD repeat domain 37	WDR37	chr10p15.3	1.34
211474_s_at	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	SERPINB6	chr6p25	0.54
211488_s_at	integrin, beta 8	ITGB8	chr7p21.1	0.55
211662_s_at	voltage-dependent anion channel 2	VDAC2	chr10q22	1.62
211703_s_at	beta-amyloid binding protein precursor	BBP	chr1p31.3	0.44
211733_x_at	sterol carrier protein 2	SCP2	chr1p32	0.64
211755_s_at	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1	ATP5F1	chr1p13.2	0.67
212131_at	family with sequence similarity 61, member A	FAM61A	chr19q13.11	0.48
212132_at	family with sequence similarity 61, member A	FAM61A	chr19q13.11	0.35
212192_at	potassium channel tetramerisation domain containing 12	KCTD12	chr13q22.3	0.36
212226_s_at	phosphatidic acid phosphatase type 2B	PPAP2B	chr1pter-p22.1	0.33
212230_at	phosphatidic acid phosphatase type 2B	PPAP2B	chr1pter-p22.1	0.32
212245_at	multiple coagulation factor deficiency 2	MCFD2	chr2p21	0.66
212294_at	guanine nucleotide binding protein (G protein), gamma 12	GNG12	chr1p31.2	0.24
212355_at	KIAA0323 protein	KIAA0323	chr14q11.2	0.49
212370_x_at	family with sequence similarity 21, member B	FAM21B	chr10q11.22 /// chr10q11.23	1.61
212383_at	ATPase, H+ transporting, lysosomal V0 subunit a isoform 1	ATP6V0A1	chr17q21	1.74
212393_at	SET binding factor 1	SBF1	chr22q13.33	1.77
212491_s_at	DnaJ (Hsp40) homolog, subfamily C, member 8	DNAJC8	chr1p35.3	0.56

212503_s_at	KIAA0934 protein	KIAA0934	chr10p15.3	1.29
212513_s_at	ubiquitin specific protease 33	USP33	chr1p31.1	0.53
212515_s_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked	DDX3X	chrXp11.3-p11.23	0.74
212628_at	Protein kinase N2	PKN2	chr1p22.2	0.47
212698_s_at	septin 10	SEPT10	chr2q13	0.43
212699_at	secretory carrier membrane protein 5	SCAMP5	chr15q23	2.34
212893_at	zinc finger, ZZ domain containing 3	ZZZ3	chr1p31.1	0.49
212920_at	Homo sapiens transcribed sequence with weak similarity to protein ref:NP_060312.1 (H.sapiens) hypothetical protein FLJ20489 [Homo sapiens]	---	---	0.40
212928_at	TSPY-like 4	TSPYL4	chr6q22.1	1.41
213001_at	angiopoietin-like 2	ANGPTL2	chr9q34	3.95
213004_at	angiopoietin-like 2	ANGPTL2	chr9q34	4.16
213156_at	Homo sapiens mRNA; cDNA DKFZp586B211 (from clone DKFZp586B211)	---	---	1.89
213158_at	Homo sapiens mRNA; cDNA DKFZp586B211 (from clone DKFZp586B211)	---	---	1.66
213170_at	glutathione peroxidase 7	GPX7	chr1p32	0.52
213186_at	zinc finger DAZ interacting protein 3	DZIP3	chr3q13.13	1.48
213259_s_at	sterile alpha and TIR motif containing 1	SARM1	chr17q11	1.87
213340_s_at	KIAA0495	KIAA0495	chr1p36.32	0.35
213351_s_at	transmembrane and coiled-coil domains 1	TMCC1	chr3q21.3	1.62
213424_at	KIAA0895 protein	KIAA0895	chr7p14.1	0.66
213436_at	cannabinoid receptor 1 (brain)	CNR1	chr6q14-q15	0.32
213439_x_at	RaP2 interacting protein 8	RPIP8	chr17q21.31	2.77
213464_at	SHC (Src homology 2 domain containing) transforming protein 2	SHC2	chr19p13.3	2.03
213467_at	FALSE	---	---	2.59
213557_at	CDC2-related protein kinase 7	CRK7	chr17q12	1.67
213798_s_at	CAP, adenylate cyclase-associated protein 1 (yeast)	CAP1	chr1p34.2	0.57
213883_s_at	beta-amyloid binding protein precursor	BBP	chr1p31.3	0.52
214241_at	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, 19kDa	NDUFB8	chr10q23.2-q23.33	1.66
214383_x_at	kelch domain containing 3	KLHDC3	chr6p21.1	1.44
214894_x_at	microtubule-actin crosslinking factor 1	MACF1	chr1p32-p31	0.58
214933_at	calcium channel, voltage-dependent, P/Q type, alpha	CACNA1A	chr19p13.2-p13.1	2.72

	1A subunit			
215017_s_at	formin binding protein 1-like	FNBP1L	chr1p22.1	0.20
215222_x_at	microtubule-actin crosslinking factor 1	MACF1	chr1p32-p31	0.50
215691_x_at	chromosome 1 open reading frame 41	C1orf41	chr1p32.1-p33	0.44
216268_s_at	jagged 1 (Alagille syndrome)	JAG1	chr20p12.1-p11.23	0.39
216903_s_at	calcium binding atopy-related autoantigen 1	CBARA1	chr10q22.1	1.67
217724_at	PAI-1 mRNA-binding protein	PAI-RBP1	chr1p31-p22	0.67
217877_s_at	hypothetical protein SP192	SP192	chr1p34.1	0.44
217893_s_at	hypothetical protein FLJ12666	FLJ12666	chr1p34.3	0.50
217921_at		---	---	0.56
217968_at	tumor suppressing subtransferable candidate 1	TSSC1	chr2p25.2	1.52
218011_at	ubiquitin-like 5	UBL5	chr19p13.3	0.57
218097_s_at	CUE domain containing 2	CUEDC2	chr10q24.32	1.46
218302_at	presenilin enhancer 2 homolog (C. elegans)	PSENEN	chr19q13.12	0.50
218370_s_at	hypothetical protein FLJ12903	FLJ12903	chr1p35.1	0.61
218462_at	RNA processing factor 1	RPF1	chr1p22.3	0.44
218490_s_at	zinc finger protein 302	ZNF302	chr19q13.11	0.49
218577_at	hypothetical protein FLJ20331	FLJ20331	chr1p31.1	0.62
218640_s_at	pleckstrin homology domain containing, family F (with FYVE domain) member 2	PLEKHF2	chr8q22.1	0.32
218712_at	hypothetical protein FLJ20508	FLJ20508	chr1p34.3	0.48
218924_s_at	chitobiase, di-N-acetyl-	CTBS	chr1p22	0.37
218938_at	F-box and leucine-rich repeat protein 15	FBXL15	chr10q24.32	2.59
219094_at	armadillo repeat containing 8	ARMC8	chr3q22.3	1.53
219314_s_at	zinc finger protein 219	ZNF219	chr14q11	1.88
219372_at	carnitine deficiency-associated, expressed in ventricle 1	CDV1	chr12q24.13	0.60
219375_at	choline/ethanolaminephospho transferase	CEPT1	chr1p13.3	0.58
219494_at	RAD54B homolog	RAD54B	chr8q21.3-q22	0.34
219818_s_at	G patch domain containing 1	GPATC1	chr19q13.11	0.52
219848_s_at	zinc finger protein 432	ZNF432	chr19q13.41	0.53
219939_s_at	upstream of NRAS	UNR	chr1p22	0.65
220358_at	Jun dimerization protein p21SNFT	SNFT	chr1q32.3	0.47
220443_s_at	ventral anterior homeobox 2	VAX2	chr2p13	2.58
221024_s_at	solute carrier family 2 (facilitated glucose transporter), member 10	SLC2A10	chr20q13.1	0.09
221432_s_at	solute carrier family 25, member 28	SLC25A28	chr10q23-q24	1.72

221486_at	endosulfine alpha	ENSA	chr1q21.2	1.66
221522_at	ankyrin repeat domain 27 (VPS9 domain)	ANKRD27	chr19q13.11	0.62
221679_s_at	abhydrolase domain containing 6	ABHD6	chr3p14.3	1.90
221958_s_at	putative NFkB activating protein 373	FLJ23091	chr1p31.2	0.35
222409_at	coronin, actin binding protein, 1C	CORO1C	chr12q24.1	1.60
222452_s_at	hypothetical protein SP192	SP192	chr1p34.1	0.50
222459_at	hypothetical protein FLJ12666	FLJ12666	chr1p34.3	0.59
222495_at	protein x 013	AD-020	chr1p13.3	0.54
222580_at	zinc finger protein 644	ZNF644	chr1p22.2	0.57
222654_at	myo-inositol monophosphatase A3	IMPA3	chr8q12.1	0.60
222699_s_at	pleckstrin homology domain containing, family F (with FYVE domain) member 2	PLEKHF2	chr8q22.1	0.34
222833_at	hypothetical protein FLJ20481	FLJ20481	chr16q12.2	0.25
222834_s_at	guanine nucleotide binding protein (G protein), gamma 12	GNG12	chr1p31.2	0.40
222893_s_at	hypothetical protein FLJ13150	FLJ13150	chr1p22.1	0.55
222975_s_at	upstream of NRAS	UNR	chr1p22	0.62
223017_at	endoplasmic reticulum thioredoxin superfamily member, 18 kDa	TLP19	chr1p32.3	0.48
223042_s_at	FUN14 domain containing 2	FUNDC2	chrXq28	1.47
223066_at	SNARE associated protein snapin	SNAPAP	chr1q21.3	0.65
223103_at	START domain containing 10	STARD10	chr11q13	2.40
223120_at	fucosidase, alpha-L- 2, plasma	FUCA2	chr6q24	0.34
223125_s_at	chromosome 1 open reading frame 21	C1orf21	chr1q25	0.51
223132_s_at	tripartite motif-containing 8	TRIM8	chr10q24.3	1.84
223159_s_at	NIMA (never in mitosis gene a)-related kinase 6	NEK6	chr9q33.3-q34.11	0.36
223230_at	hypothetical protein FLJ14936	FLJ14936	chr1p33-p32.1	0.58
223296_at	mitochondrial carrier protein	MGC4399	chr1p36.22	0.65
223331_s_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 20	DDX20	chr1p21.1-p13.2	0.53
223398_at	chromosome 9 open reading frame 89	C9orf89	chr9q22.31	0.22
223418_x_at	hypothetical protein DKFZp566D1346	DKFZP566D1346	chr1p32.3-p31.3	0.58
223435_s_at	protocadherin alpha 9///protocadherin alpha subfamily C, 2///protocadherin alpha subfamily C, 1///protocadherin alpha	PCDHA9 /// PCDHAC2 /// PCDHAC1 /// PCDHA13 /// PCDHA12 ///	chr5q31	2.25

	13///protocadherin alpha 12///protocadherin alpha 11///protocadherin alpha 10///protocadherin alpha 8///protocadherin alpha 7///protocadherin alpha 6///protocadherin alpha 5///protocadherin alpha 4///protocadherin alpha 3///protocadherin alpha 2///protocadherin alpha 1	PCDHA11 /// PCDHA10 /// PCDHA8 /// PCDHA7 /// PCDHA6 /// PCDHA5 /// PCDHA4 /// PCDHA3 /// PCDHA2 /// PCDHA1		
223500_at	complexin 1	CPLX1	chr4p16.3	3.79
223603_at	zinc finger protein 179	ZNF179	chr17p11.2	2.71
223824_at	chromosome 10 open reading frame 59	C10orf59	chr10q23.31	0.60
224212_s_at	protocadherin alpha 9///protocadherin alpha subfamily C, 2///protocadherin alpha subfamily C, 1///protocadherin alpha 13///protocadherin alpha 12///protocadherin alpha 11///protocadherin alpha 10///protocadherin alpha 8///protocadherin alpha 7///protocadherin alpha 6///protocadherin alpha 5///protocadherin alpha 4///protocadherin alpha 3///protocadherin alpha 2///protocadherin alpha 1	PCDHA9 /// PCDHAC2 /// PCDHAC1 /// PCDHA13 /// PCDHA12 /// PCDHA11 /// PCDHA10 /// PCDHA8 /// PCDHA7 /// PCDHA6 /// PCDHA5 /// PCDHA4 /// PCDHA3 /// PCDHA2 /// PCDHA1	chr5q31	2.12
224280_s_at	hypothetical protein RP1-317E23	LOC56181	chr1p36.11	0.49
224315_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 20	DDX20	chr1p21.1-p13.2	0.58
224565_at	trophoblast-derived noncoding RNA	TncRNA	chr11q13.1	0.32
224591_at	HP1-BP74	HP1-BP74	chr1p36.12	0.60
224686_x_at	Homo sapiens transcribed sequence with strong similarity to protein ref:NP_060471.1 (H.sapiens) hypothetical protein FLJ10120 [Homo sapiens]	---	chr17q21.32	1.47
224867_at	similar to protein of fungal metazoan origin like (11.1 kD) (2C514)	LOC440574	chr1p36.13	0.51
224909_s_at	KIAA1415 protein	PREX1	chr20q13.13	0.37
224925_at	KIAA1415 protein	PREX1	chr20q13.13	0.34
224937_at	prostaglandin F2 receptor negative regulator	PTGFRN	chr1p13.1	0.44
224985_at	neuroblastoma RAS viral (v-ras) oncogene homolog	NRAS	chr1p13.2	0.63
225222_at	hippocampus abundant gene transcript 1	HIAT1	chr1p21.3	0.58
225327_at	hypothetical protein	FLJ10980	chr15q21.2-	1.81

	FLJ10980		q21.3	
225379_at	microtubule-associated protein tau	MAPT	chr17q21.1	1.89
225382_at	zinc finger protein 275	ZNF275	chrXq28	2.37
225384_at	dedicator of cytokinesis 7	DOCK7	chr1p31.3	0.40
225475_at	mesoderm induction early response 1	MI-ER1	chr1p31.2	0.51
225479_at	CDNA FLJ32247 fis, clone PROST1000120	---	---	1.46
225612_s_at	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5	B3GNT5	chr3q28	0.30
225633_at	hypothetical protein LOC147991	LOC147991	chr19q13.11	0.64
225878_at	kinesin family member 1B	KIF1B	chr1p36.2	0.59
225925_s_at	ubiquitin specific protease 48	USP48	chr1p36.12	0.58
226000_at	hypothetical protein DKFZp547A023	DKFZp547A023	chr1p13.2	0.43
226116_at	Homo sapiens cDNA FLJ12540 fis, clone NT2RM4000425.	---	---	0.72
226189_at	Homo sapiens, clone IMAGE:4794726, mRNA	---	---	0.46
226294_x_at	hypothetical protein FLJ23790	FLJ23790	chr8q24.13	0.70
226411_at	ecotropic viral integration site 5-like	EVI5L	chr19p13.2	2.15
226458_at	Homo sapiens, clone IMAGE:4449283, mRNA	---	---	0.53
226487_at	hypothetical protein FLJ14721	FLJ14721	chr12q24.11	3.21
226517_at	branched chain aminotransferase 1, cytosolic	BCAT1	chr12pter-q12	0.17
226532_at	Homo sapiens transcribed sequence with weak similarity to protein ref:NP_055301.1 (H.sapiens) neuronal thread protein [Homo sapiens]	---	---	0.49
226601_at	solute carrier family 30 (zinc transporter), member 7	SLC30A7	chr1p21.2	0.65
226630_at	chromosome 14 open reading frame 106	C14orf106	chr14q21.3	0.49
226760_at	hypothetical protein LOC203411	LOC203411	chrXp22.13	1.38
226909_at	KIAA1729 protein	KIAA1729	chr4p16.1	0.20
226976_at	Karyopherin alpha 6 (importin alpha 7)	KPNA6	chr1p35.1-p34.3	0.55
227081_at	dynein, axonemal, light intermediate polypeptide 1	DNALI1	chr1p35.1	0.34
227091_at	KIAA1505 protein	KIAA1505	chr7p12.3	0.59
227112_at	---	---	---	1.96
227154_at	hypothetical protein MGC15730	MGC15730	chr1p36.13	2.74

227199_at	Chromosome 21 open reading frame 106	C21orf106	chr21q22.3	1.53
227222_at	F-box only protein 10	FBXO10	chr9p13.2	1.73
227270_at	hypothetical protein LOC285550	LOC285550	chr4p15.33	0.47
227278_at	Homo sapiens transcribed sequence with weak similarity to protein ref:NP_055301.1 (H.sapiens) neuronal thread protein [Homo sapiens]	---	---	0.48
227334_at	ubiquitin specific protease 54	USP54	chr10q22.2	2.18
227361_at	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1	HS3ST3B1	chr17p12-p11.2	0.08
227388_at	tumor suppressor candidate 1	TUSC1	chr9p21.1	0.39
227449_at	EPH receptor A4	EPHA4	chr2q36.1	0.32
227456_s_at	chromosome 6 open reading frame 136	C6orf136	chr6p21.33	1.59
227573_s_at	KIAA0657 protein	KIAA0657	chr2q35	1.71
227639_at	phosphatidylinositol glycan, class K	PIGK	chr1p31.1	0.51
227674_at	zinc finger protein 585A	ZNF585A	chr19q13.12	0.32
227680_at	zinc finger protein 326	ZNF326	chr1p22.2	0.56
227812_at	tumor necrosis factor receptor superfamily, member 19	TNFRSF19	chr13q12.11-q12.3	0.25
227845_s_at	src homology 2 domain-containing transforming protein D	SHD	chr19p13.3	5.98
227889_at	hypothetical protein FLJ20481	FLJ20481	chr16q12.2	0.40
227898_s_at	hypothetical protein FLJ38705	FLJ38705	chr8q24.3	1.73
228020_at	hypothetical protein FLJ20758	FLJ20758	chr2p11.2	1.64
228135_at	chromosome 1 open reading frame 52	C1orf52	chr1p22.3	0.52
228165_at	hypothetical protein DKFZp547D2210	DKFZp547D2210	chr12p13.31	2.36
228190_at	---	---	---	0.43
228284_at	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	TLE1	chr9q21.32	0.45
228415_at	adaptor-related protein complex 1, sigma 2 subunit	AP1S2	chrXp22.2	0.35
228422_at	Homo sapiens, clone IMAGE:5300488, mRNA	---	---	2.08
228538_at	zinc finger protein 662	ZNF662	chr3p22.1	0.33
228600_x_at	hypothetical protein MGC72075	MGC72075	chr7p15.3	0.12
228652_at	hypothetical protein FLJ38288	FLJ38288	chr19q13.43	0.55
228730_s_at	secernin 2	SCRN2	chr17q21.32	1.63
228805_at	FLJ44216 protein	FLJ44216	chr5q35.2	0.41
228813_at	histone deacetylase 4	HDAC4	chr2q37.2	2.68

228949_at	putative NFkB activating protein 373	FLJ23091	chr1p31.2	0.30
228950_s_at	putative NFkB activating protein 373	FLJ23091	chr1p31.2	0.40
228970_at	archease	ARCH	chr1p35.1	0.54
229228_at	cAMP responsive element binding protein 5	CREB5	chr7p15.1	0.34
229299_at	hypothetical protein FLJ30596	FLJ30596	chr5p13.2	0.35
229318_at	Homo sapiens transcribed sequences	---	---	1.71
229435_at	GLIS family zinc finger	GLIS3	chr9p24.2	0.20
229498_at	Homo sapiens transcribed sequences	---	---	0.29
230258_at	GLIS family zinc finger	GLIS3	chr9p24.2	0.34
230350_at	Homo sapiens transcribed sequence with moderate similarity to protein ref:NP_060312.1 (H.sapiens) hypothetical protein FLJ20489 [Homo sapiens]	---	---	1.87
230352_at	Phosphoribosyl pyrophosphate synthetase 2	PRPS2	chrXp22.3-p22.2	0.25
230637_at	sideroflexin 4	SFXN4	chr10q26.11	2.62
231118_at	ankyrin repeat domain 35	ANKRD35	chr1q21.1	0.33
231183_s_at	Jagged 1 (Alagille syndrome)	JAG1	chr20p12.1-p11.23	0.44
231774_at	calsenilin, presenilin binding protein, EF hand transcription factor	CSEN	chr2q21.1	2.40
231924_at	Homo sapiens cDNA FLJ10570 fis, clone NT2RP2003117.	---	chr11p15.2	0.45
231940_at	zinc finger protein 529	ZNF529	chr19q13.13	0.64
232195_at	G protein-coupled receptor 158	GPR158	chr10p12.1	3.45
232322_x_at	START domain containing 10	STARD10	chr11q13	1.93
234140_s_at	stromal interaction molecule 2	STIM2	chr4p15.2	0.48
234672_s_at	hypothetical protein FLJ10407	FLJ10407	chr1p32.3	0.49
235015_at	zinc finger, DHHC domain containing 9	ZDHHC9	chrXq26.1	1.79
235058_at	Hypothetical protein FLJ10349	FLJ10349	chr1p36.11	0.64
235414_at	zinc finger protein 383	ZNF383	chr19q13.12	0.48
235431_s_at	pellino 3 alpha	MGC35521	chr11q13.2	2.20
235500_at	heterogeneous nuclear ribonucleoprotein C (C1/C2)	HNRPC	chr14q11.2	1.82
235509_at	hypothetical protein MGC40214	MGC40214	chr8q22.1	0.37
235648_at	zinc finger protein 567	ZNF567	chr19q13.12	0.47
235721_at	deltex 3 homolog (Drosophila)	DTX3	chr12q13.3	1.67
235759_at	EF hand calcium binding protein 1	EFCBP1	chr8q21.3	0.19

235916_at	yippee-like 4 (Drosophila)	YPEL4	chr11q12.1	2.86
235940_at	chromosome 9 open reading frame 64	C9orf64	chr9q21.32	0.25
235969_at	hypothetical protein FLJ33996	FLJ33996	chr12q13.13	1.67
238547_at	hypothetical protein MGC39389	HEXIM2	chr17q21.31	1.58
239108_at	Male sterility domain containing 1	MLSTD1	chr12p11.22	0.41
239442_at	KIAA0582 protein	KIAA0582	chr2p14	1.93
240841_at	insulinoma-associated 2	INSM2	chr14q13.2	2.38
241858_at	fucose-1-phosphate guanylyltransferase	FPGT	chr1p31.1	0.40
242263_at	CGI-100 protein	CGI-100	chr1pter-q31.3	0.57
242269_at	FLJ42875 protein	FLJ42875	chr1p36.32	0.40
242429_at	zinc finger protein 567	ZNF567	chr19q13.12	0.51
243042_at	FLJ35093 protein	FLJ35093	chr1p31.1	0.55
244462_at	zinc finger protein 224	ZNF224	chr19q13.2	0.53
244740_at	hypothetical protein MGC9913	MGC9913	chr19q13.43	0.64
33760_at	peroxisomal biogenesis factor 14	PEX14	chr1p36.22	0.60
38398_at	MAP-kinase activating death domain	MADD	chr11p11.2	1.50
38710_at	OTU domain, ubiquitin aldehyde binding 1	OTUB1	chr11q13.1	1.44

Table 6.: Differentially expressed probesets, which are able to discriminate on basis of loss of heterozygosity (LOH) on the 19q locus

Probe Set ID	Title	Gene Symbol	location	ratio loss/no loss
200650_s_at	lactate dehydrogenase A	LDHA	Chr:11p15.4	0.31
221058_s_at	chemokine-like factor	CKLF	Chr:16q22.1	0.67
218624_s_at	hypothetical protein MGC2752	MGC2752	Chr:19p13.2	0.56
200826_at	small nuclear ribonucleoprotein D2 polypeptide 16.5kDa	SNRPD2	Chr:19q13.2	0.49
219603_s_at	zinc finger protein 226	ZNF226	Chr:19q13.2	0.35
222028_at	zinc finger protein 45 (a Kruppel-associated box (KRAB) domain polypeptide)	ZNF45	Chr:19q13.2	0.55
229123_at	zinc finger protein 224	ZNF224	Chr:19q13.2	0.54
244462_at	zinc finger protein 224	ZNF224	Chr:19q13.2	0.52
219495_s_at	zinc finger protein 180 (HHZ168)	ZNF180	Chr:19q13.2	0.57
206175_x_at	zinc finger protein 222	ZNF222	Chr:19q13.2	0.43
228131_at	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)	ERCC1	Chr:19q13.2-q13.3	0.51
201194_at	selenoprotein W, 1	SEPW1	Chr:19q13.3	0.48
225434_at	death effector domain-containing DNA binding protein 2	DEDD2	Chr:19q13.31	0.49
227689_at	zinc finger protein 227	ZNF227	Chr:19q13.32	0.57
202153_s_at	nucleoporin 62kDa	NUP62	Chr:19q13.33	0.47
209751_s_at	spondyloepiphyseal dysplasia, late	SEDL/SEDLP	Chr:19q13.4	0.54
207753_at	zinc finger protein 304	ZNF304	Chr:19q13.4	0.53
205497_at	zinc finger protein 175	ZNF175	Chr:19q13.4	0.62
1556678_a_at	Homo sapiens full length insert cDNA clone ZD41C11	---	Chr:19q13.41	0.59
219848_s_at	zinc finger protein 432	ZNF432	Chr:19q13.41	0.51
202408_s_at	PRP31 pre-mRNA processing factor 31 homolog (yeast)	PRPF31	Chr:19q13.42	0.49
229614_at	hypothetical protein LOC162967	LOC162967	Chr:19q13.42	0.62
225256_at	Homo sapiens transcribed sequence with weak similarity to protein ref:NP_071431.1 (H.sapiens) cytokine		Chr:19q13.43	0.55

	receptor-like factor 2; cytokine receptor CRL2 precusor [Homo sapiens]			
238436_s_at	Homo sapiens transcribed sequences	---	Chr:19q13.43	0.64
238437_at	Homo sapiens transcribed sequences	---	Chr:19q13.43	0.60
228652_at	hypothetical protein FLJ38288	FLJ38288	Chr:19q13.43	0.51
244741_s_at	LOC342935		Chr:19q13.43	0.61
244740_at	LOC342935		Chr:19q13.43	0.65
201274_at	proteasome (prosome, macropain) subunit, alpha type, 5	PSMA5	Chr:1p13	0.60
211755_s_at	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1	ATP5F1	Chr:1p13.2	0.68
224729_s_at	ATP synthase mitochondrial F1 complex assembly factor 1	ATPAF1	Chr:1p33	0.48
218080_x_at	Fas (TNFRSF6) associated factor 1	FAF1	Chr:1p33	0.51
213622_at	collagen, type IX, alpha 2	COL9A2	Chr:1p33-p32	0.38
203359_s_at	c-myc binding protein	MYCBP	Chr:1p33-p32.2	0.51
228970_at	archease	ARCH	Chr:1p34.3	0.53
202139_at	aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)	AKR7A2	Chr:1p35.1-p36.23	0.58
212491_s_at	DnaJ (Hsp40) homolog, subfamily C, member 8	DNAJC8	Chr:1p35.3	0.61
201225_s_at	serine/arginine repetitive matrix 1	SRRM1	Chr:1p36.11	0.71
224867_at	similar to Putative protein of fungal and metazoan origin (11.1 kD)		Chr:1p36.13	0.54
212401_s_at	cell division cycle 2-like 2	CDC2L2	Chr:1p36.3	0.70
222000_at	hypothetical protein LOC339448	LOC339448	Chr:1p36.32	0.66
213340_s_at	KIAA0495	KIAA0495	Chr:1p36.32	0.38
220526_s_at	mitochondrial ribosomal protein L20	MRPL20	Chr:1p36.3-p36.2	0.56
202297_s_at	RER1 homolog (S. cerevisiae)	RER1	Chr:1p36.32	0.50
236369_at	Homo sapiens transcribed sequence with weak similarity to protein prf:2109260A (H.sapiens) 2109260A	---	Chr:20q11.21	1.38

	B cell growth factor [Homo sapiens]			
202096_s_at	benzodiazapine receptor (peripheral)	BZRP	Chr:22q13.31	0.39
228538_at	similar to Zinc finger protein 7 (Zinc finger protein KOX4) (Zinc finger protein HF.16)		Chr:3p21.33	0.43
202763_at	caspase 3, apoptosis-related cysteine protease	CASP3	Chr:4q34	0.51
201572_x_at	dCMP deaminase	DCTD	Chr:4q35.1	0.28
210137_s_at	dCMP deaminase	DCTD	Chr:4q35.1	0.18
201571_s_at	dCMP deaminase	DCTD	Chr:4q35.1	0.28
233103_at	Homo sapiens cDNA FLJ14109 fis, clone MAMMA1001322, moderately similar to B-CELL GROWTH FACTOR PRECURSOR.	---	Chr:5q14.1	0.40
203787_at	single-stranded DNA binding protein 2	SSBP2	Chr:5q14.1	0.45
210829_s_at	single-stranded DNA binding protein 2	SSBP2	Chr:5q14.1	0.38
210059_s_at	mitogen-activated protein kinase 13	MAPK13	Chr:6p21.31	0.46
231067_s_at	A kinase (PRKA) anchor protein (gravin) 12	AKAP12	Chr:6q24-q25	0.55
203819_s_at	IGF-II mRNA-binding protein 3	IMP-3	Chr:7p11	0.13
218640_s_at	pleckstrin homology domain containing, family F (with FYVE domain) member 2	PLEKHF2	Chr:8q22.1	0.35
222699_s_at	pleckstrin homology domain containing, family F (with FYVE domain) member 2	PLEKHF2	Chr:8q22.1	0.37
228284_at	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	TLE1	Chr:9q21.32	0.50
203222_s_at	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	TLE1	Chr:9q21.32	0.39
223398_at	hypothetical protein MGC11115	MGC11115	Chr:9q22.32	0.25
226809_at	Homo sapiens cDNA FLJ30428 fis, clone BRACE2008941.	---	Cross Hyb Matching Probes	0.17

Table 7.: Differentially expressed probesets, which are able to discriminate on basis of loss of heterozygosity (LOH) on both the 1p and 19 q loci

Probe Set ID	Title	Gene Symbol	Location	ratio loss/no loss
201177_s_at	SUMO-1 activating enzyme subunit 2	UBA2	Chr:19q12	0.45
215019_x_at	KIAA1827 protein	KIAA1827	Chr:19q13	0.56
201258_at	ribosomal protein S16	RPS16	Chr:19q13.1	0.55
226131_s_at	ribosomal protein S16	RPS16	Chr:19q13.1	0.71
212131_at	DKFZP434D1335 protein	DKFZP434D1335	Chr:19q13.12	0.49
218490_s_at	zinc finger protein 302	ZNF302	Chr:19q13.12	0.50
219818_s_at	evolutionarily conserved G-patch domain containing	ECGP	Chr:19q13.12	0.57
231940_at	KIAA1615 protein	KIAA1615	Chr:19q13.13	0.60
235648_at	hypothetical protein MGC45586	MGC45586	Chr:19q13.13	0.47
219495_s_at	zinc finger protein 180 (HHZ168)	ZNF180	Chr:19q13.2	0.55
206175_x_at	zinc finger protein 222	ZNF222	Chr:19q13.2	0.38
235702_at	Homo sapiens transcribed sequences	---	Chr:19q13.31	0.59
205497_at	zinc finger protein 175	ZNF175	Chr:19q13.4	0.61
1556678_a_at	Homo sapiens full length insert cDNA clone ZD41C11	LOC284371	Chr:19q13.41	0.58
219848_s_at	zinc finger protein 432	ZNF432	Chr:19q13.41	0.51
228652_at	hypothetical protein FLJ38288	FLJ38288	Chr:19q13.43	0.51
242140_at	similar to envelope protein	LOC113386	Chr:19q13.43	0.45
244740_at	LOC342935		Chr:19q13.43	0.61
208374_s_at	capping protein (actin filament) muscle Z-line, alpha 1	CAPZA1	Chr:1p13.1	0.57
211755_s_at	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1	ATP5F1	Chr:1p13.2	0.61
226000_at	hypothetical protein DKFZp547A023	DKFZp547A023	Chr:1p13.2	0.48
230300_at	Homo sapiens transcribed sequences	---	Chr:1p13.3	0.49
222495_at	protein x 013	AD-020	Chr:1p13.3	0.52
223331_s_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 20	DDX20	Chr:1p21.1-p13.2	0.54
228661_s_at	Homo sapiens, clone IMAGE:4821863, mRNA	---	Chr:1p21.2	0.54
219939_s_at	NRAS-related gene	D1S155E	Chr:1p22	0.65
205263_at	B-cell CLL/lymphoma 10	BCL10	Chr:1p22	0.56
209187_at	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	DR1	Chr:1p22.1	0.50
215017_s_at	hypothetical protein FLJ20275	FLJ20275	Chr:1p22.1	0.24

218462_at	RNA processing factor 1	RPF1	Chr:1p22.3	0.43
228135_at	gm117	gm117	Chr:1p22.3	0.57
200902_at	15 kDa selenoprotein	15-sep	Chr:1p31	0.56
202502_at	acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain	ACADM	Chr:1p31	0.51
212893_at	DKFZP564I052 protein	DKFZP564I052	Chr:1p31.1	0.49
208709_s_at	nardilysin (N-arginine dibasic convertase)	NRD1	Chr:1p32.2-p32.1	0.63
223017_at	endoplasmic reticulum thioredoxin superfamily member, 18 kDa	TLP19	Chr:1p32.3	0.51
218080_x_at	Fas (TNFRSF6) associated factor 1	FAF1	Chr:1p33	0.48
242086_at	spermatogenesis associated 6	SPATA6	Chr:1p33	0.29
223230_at	hypothetical protein FLJ14936	FLJ14936	Chr:1p33-p32.1	0.63
213798_s_at	CAP, adenylate cyclase-associated protein 1 (yeast)	CAP1	Chr:1p34.2	0.57
228970_at	archease	ARCH	Chr:1p34.3	0.50
212491_s_at	DnaJ (Hsp40) homolog, subfamily C, member 8	DNAJC8	Chr:1p35.3	0.52
235058_at	Homo sapiens transcribed sequence with weak similarity to protein ref:NP_060265.1 (H.sapiens) hypothetical protein FLJ20378 [Homo sapiens]	---	Chr:1p36.11	0.62
204299_at	FUS interacting protein (serine-arginine rich) 1	FUSIP1	Chr:1p36.11	0.53
206095_s_at	FUS interacting protein (serine-arginine rich) 1	FUSIP1	Chr:1p36.11	0.51
224867_at	similar to Putative protein of fungal and metazoan origin (11.1 kD)		Chr:1p36.13	0.49
202675_at	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	SDHB	Chr:1p36.1-p35	0.68
226532_at	Full-length cDNA clone CS0DD009YD14 of Neuroblastoma Cot 50-normalized of Homo sapiens (human)	---	Chr:1p36.22	0.50
222000_at	hypothetical protein LOC339448	LOC339448	Chr:1p36.32	0.63
214611_at	glutamate receptor, ionotropic, kainate 1	GRIK1	Chr:21q22.11	0.39
203787_at	single-stranded DNA binding protein 2	SSBP2	Chr:5q14.1	0.42
231067_s_at	A kinase (PRKA) anchor protein (gravin) 12	AKAP12	Chr:6q24-q25	0.51
218640_s_at	pleckstrin homology domain containing, family F (with FYVE domain) member 2	PLEKHF2	Chr:8q22.1	0.26

222699_s_at	pleckstrin homology domain containing, family F (with FYVE domain) member 2	PLEKHF2	Chr:8q22.1	0.30
202241_at	phosphoprotein regulated by mitogenic pathways	C8FW	Chr:8q24.13	0.32
223796_at	cell recognition molecule CASPR3	CASPR3	Chr:9p12	0.41
203222_s_at	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	TLE1	Chr:9q21.32	0.38
223398_at	hypothetical protein MGC11115	MGC11115	Chr:9q22.32	0.19
229498_at	Homo sapiens transcribed sequences	MRNA; cDNA DKFZp779M2422 (from clone DKFZp779M2422)	Chr:Xq26.2	0.26
226411_at	similar to ecotropic viral integration site 5; Neuroblastoma stage 4S gene	LOC115704	Chr:19p13.3	2.24

Claims

1. A method for producing a classification scheme for oligodendroglial tumors comprising the steps of:

a) providing a plurality of reference samples, said reference samples comprising cell samples from a plurality of reference subjects suffering from oligodendroglial tumors, with known responsiveness to therapy and survival or with known loss of heterozygosity of 1p and/or 19q;

b) providing reference profiles by establishing a gene expression profile, matched with parameters for treatment sensitivity, survival and loss of heterozygosity for each of said reference samples individually;

c) clustering said individual reference profiles according to a statistical procedure, comprising:

(i) K-means clustering;

(ii) hierarchical clustering; and

(iii) Pearson correlation coefficient analysis; and

d) assigning an oligodendroglial tumor class according to treatment sensitivity and/or survival and/or loss of heterozygosity to each cluster.

2. Method according to claim 1, wherein the clustering of said gene expression profiles is performed based on the information of differentially-expressed genes and the treatment sensitivity and/or survival and/or loss of heterozygosity of the subject.

3. Method according to claim 1 or 2, wherein the clustering of said gene expression profiles with respect to treatment response is performed based on the information of the genes of Table 3.

4. Method according to claim 1 or 2, wherein the clustering of said gene expression profiles with respect to survival is performed based on the information of the genes of Table 4.

5. Method according to claim 1 or 2, wherein the clustering of said gene expression profiles with respect to loss of heterozygosity of 1p is performed based on the information of the genes of Table 5.

6. Method according to claim 1 or 2, wherein the clustering of said gene expression profiles with respect to loss of heterozygosity of 19q is based on the information of the genes of Table 6.

7. Method according to claim 1 or 2, wherein the clustering of said gene expression profiles with respect to loss of heterozygosity of 1p and 19q is performed based on the information of the genes of Table 7.

8. A method for classifying an oligodendroglial tumor of a subject suffering from oligodendroglioaml tumor, comprising the steps of:

a) providing a classification scheme for oligodendroglial tumors according to the method of any one of claims 1-7;

b) providing a subject profile by establishing a gene expression profile for said subject;

c) clustering the subject profile together with reference profiles;

d) determining in said scheme the clustered position of said subject profile among the reference profiles, and

e) assigning to said oligodendroglial tumor the oligodendroglial tumor class that corresponds to said clustered position.

9. Method according to claim 8, wherein gene expression profile with respect to treatment response comprises the expression parameters of a set of genes according to table 3, still more preferably 1 to 50 genes of the genes of table 3.

10. Method according to claim 8, wherein gene expression profile with respect to survival comprises the expression parameters of a set of genes according to table 4, still more preferably 1 to 50 genes of the genes of table 4.

11. Method according to claim 8, wherein gene expression profile with respect to 1p loss of heterozygosity comprises the expression parameters of a set of genes according to table 5, still more preferably 1 to 50 genes of the genes of table 5.

12. Method according to claim 8, wherein gene expression profile with respect to 19q heterozygosity comprises the expression parameters of a set of genes according to table 6, still more preferably 1 to 50 genes of the genes of table 6.

13. Method according to claim 8, wherein gene expression profile with respect to 1p and 19q loss of heterozygosity comprises the expression parameters of a set of genes according to table 7, still more preferably 1 to 50 genes of the genes of table 7.

14. A method of determining the prognosis for a subject suffering from an oligodendroglial tumor, said method comprising the steps of:

a) providing a classification scheme for oligodendroglial tumors by producing such a scheme according to the method of any one of claims 1-7;

b) determining the prognosis for each olidendroglial tumor class in said scheme based on clinical records for the subjects comprised in said class;

c) establishing the oligodendroglial class of a subject suffering from an oligodendroglial tumor by classifying the oligodendroglial tumor in said subject according to a method of any of claims 8-13, and

d) assigning to said subject the prognosis corresponding to the established oligodendroglial tumor class of said subject.

15. A method of determining the prognosis for a subject suffering from an oligodendroglial tumor, said method comprising the steps of:

a) isolation of RNA from tumor cells of said subject;

b) preparation of antisense, biotinylated RNA to the RNA of step a);

c) hybridisation of said antisense, biotinylated DNA on Affymetrix U133A or U133 Plus2.0 GeneChips®;

d) normalising the measured values for the gene set of Table 3;

e) clustering the obtained data together with the reference data, obtained from a reference set of patient with known prognosis; and

f) determining the prognosis on basis of the subgroup/cluster to which the data of the subject are clustering.

16. Oligonucleotide microarray of maximal 500 probesets, comprising at least 1, preferably at least 2, more preferably at least 25, still more preferably at least 100 oligonucleotide probes which each are capable of hybridizing under stringent conditions to different genes of the oligodendroglial tumor-associated genes selected from Table 3.

17. Oligonucleotide microarray of maximal 500 probesets, comprising at least 1, preferably at least 2, more preferably at least 25, still more preferably at least 100 oligonucleotide probes which each are capable of hybridizing under

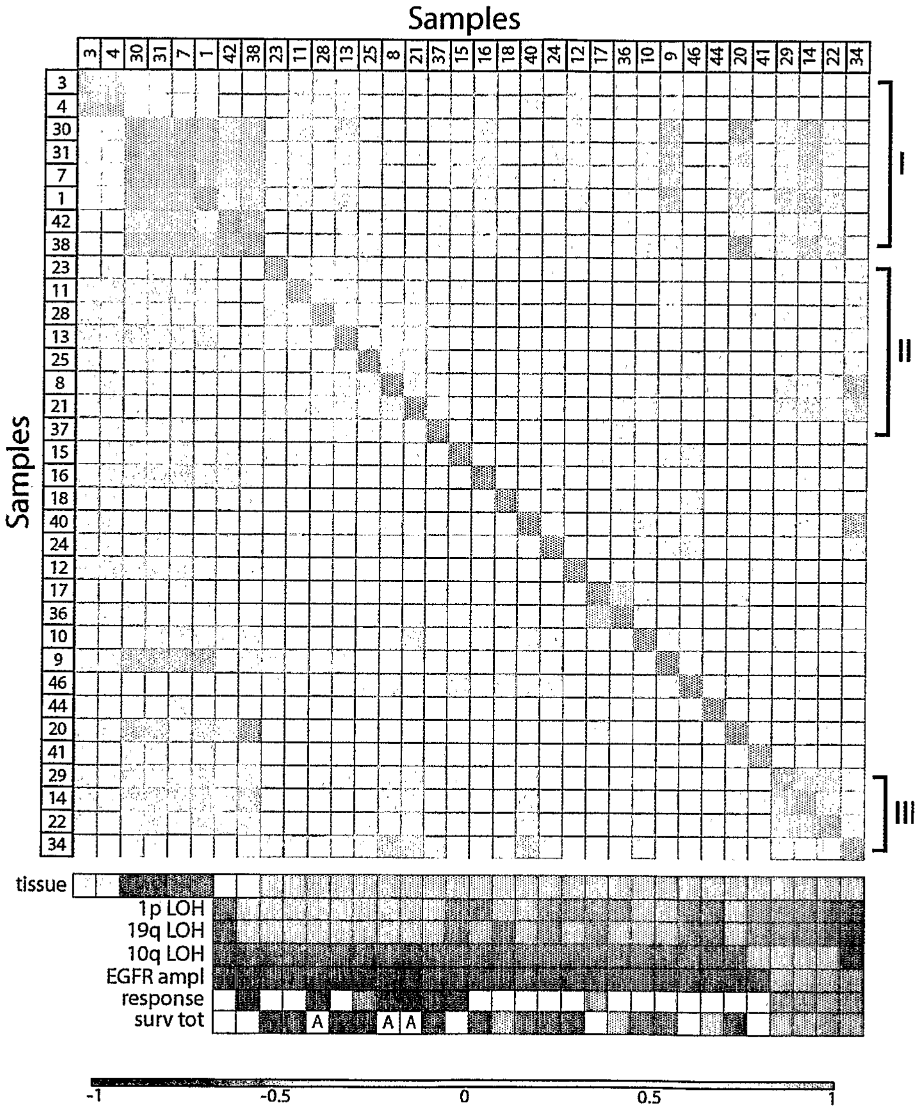
stringent conditions to different genes of the oligodendroglial tumor-associated genes selected from Table 4.

18. Oligonucleotide microarray of maximal 500 probesets, comprising at least 1, preferably at least 2, more preferably at least 25, still more preferably at least 100 oligonucleotide probes which each are capable of hybridizing under stringent conditions to different genes of the oligodendroglial tumor-associated genes selected from Table 5.

19. Oligonucleotide microarray of maximal 500 probesets, comprising at least 1, preferably at least 2, more preferably at least 25, still more preferably at least 100 oligonucleotide probes which each are capable of hybridizing under stringent conditions to different genes of the oligodendroglial tumor-associated genes selected from Table 6.

20. Oligonucleotide microarray of maximal 500 probesets, comprising at least 1, preferably at least 2, more preferably at least 25, still more preferably at least 100 oligonucleotide probes which each are capable of hybridizing under stringent conditions to different genes of the oligodendroglial tumor-associated genes selected from Table 7.

21. Kit-of-parts comprising an oligonucleotide microarray according to any of claims 16 to 20 and means for comparing a gene expression profile determined by using said microarray with a database of oligodendroglial tumor reference expression profiles.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III
tissue
1p LOH
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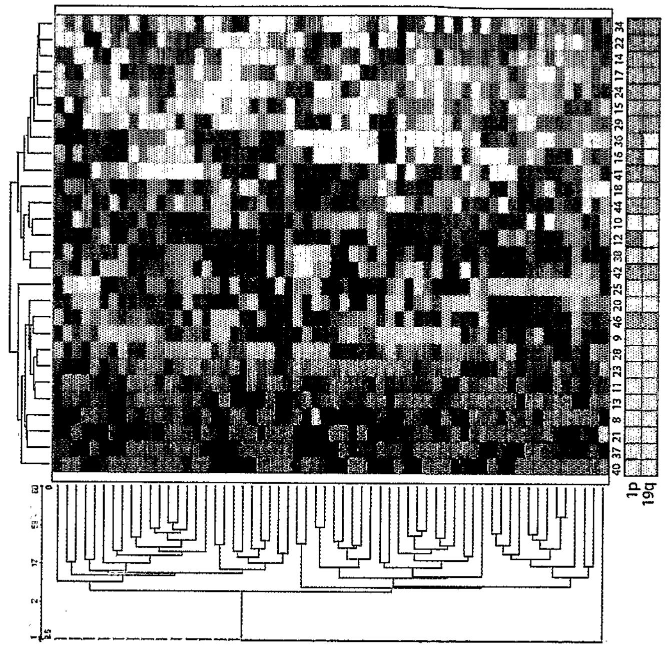
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1p
19q

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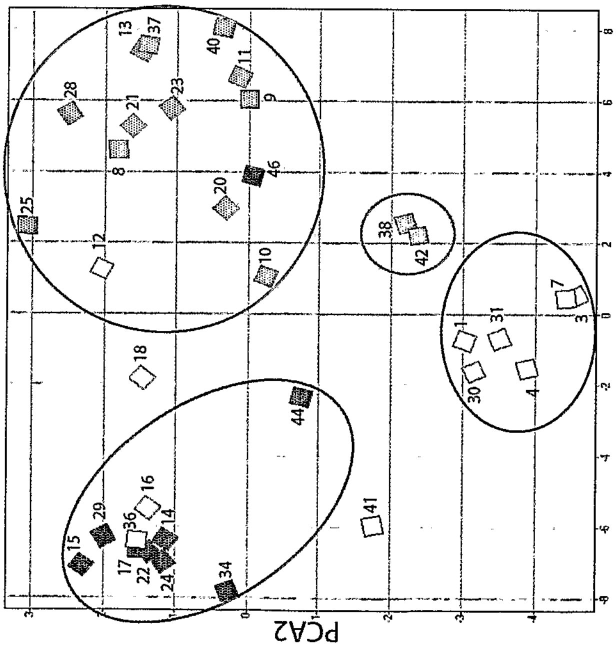
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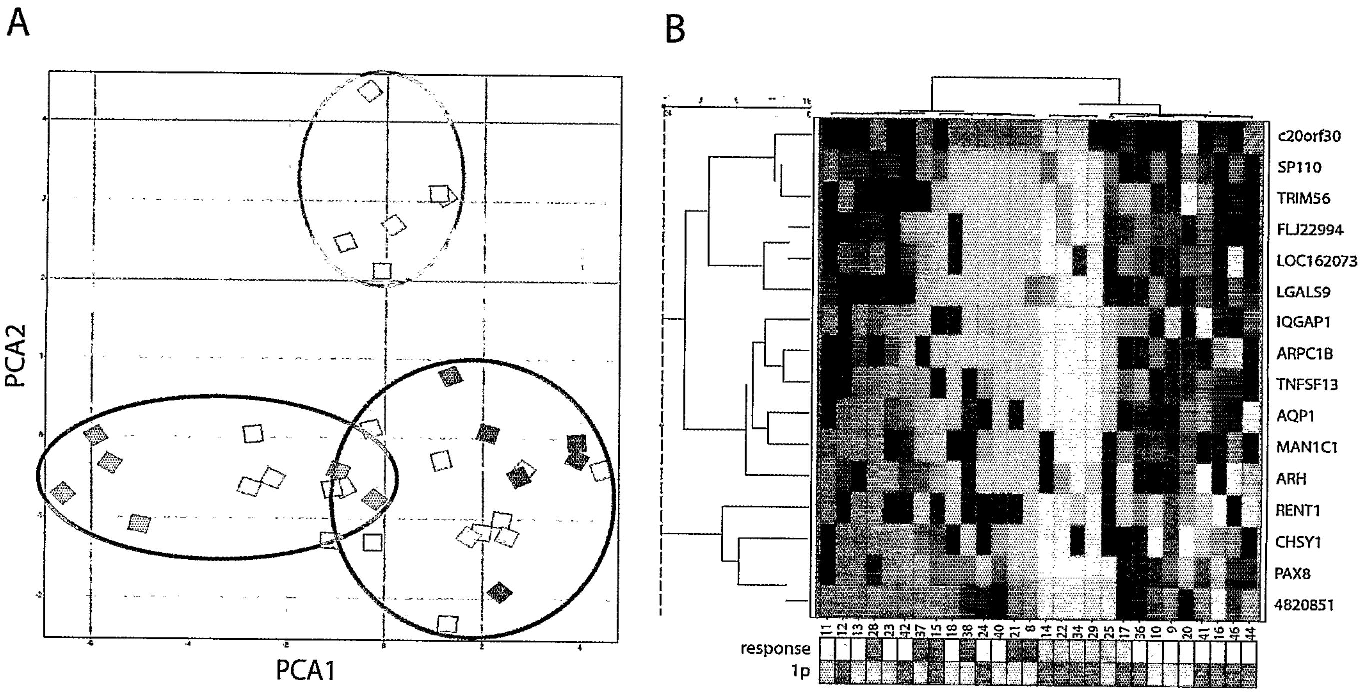
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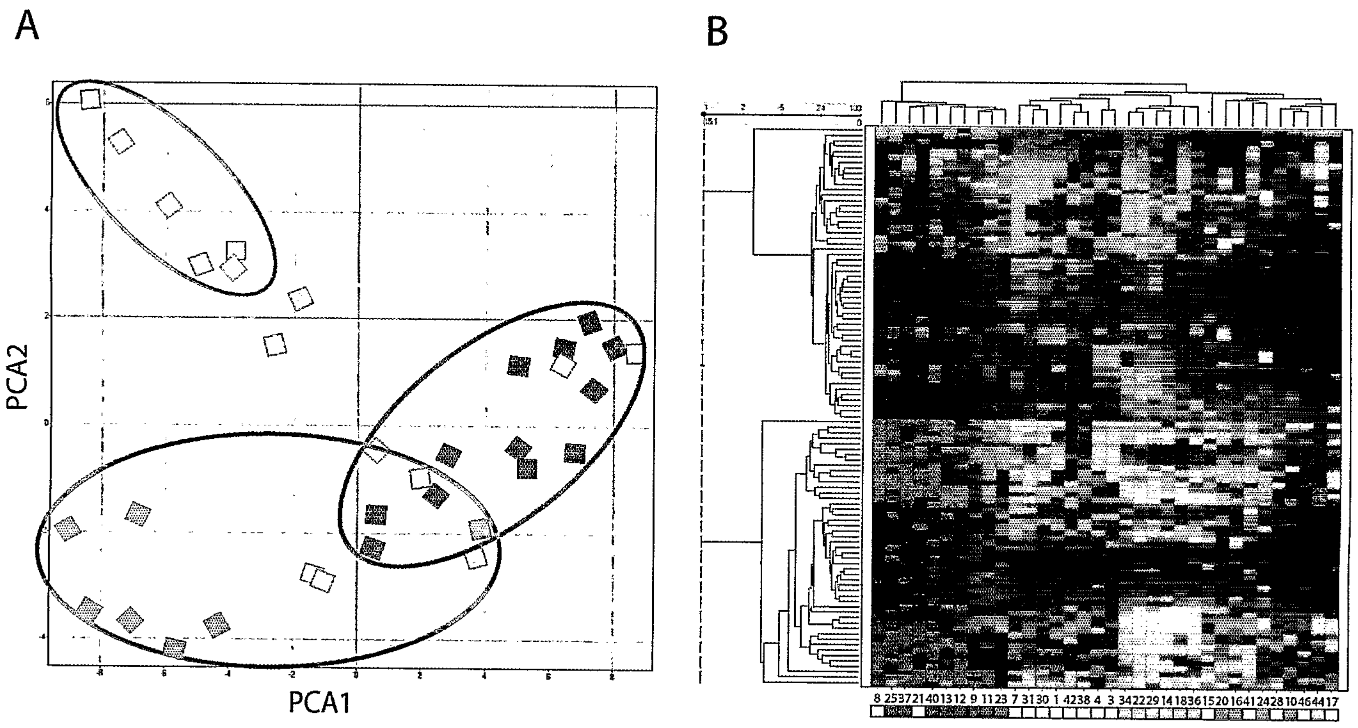
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INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2005/000855

A. CLASSIFICATION OF SUBJECT MATTER
G06F19/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G06F

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

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

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MUKASA AKITAKE ET AL: "Distinction in gene expression profiles of oligodendrogliomas with and without allelic loss of 1p" ONCOGENE, vol. 21, no. 25, 6 June 2002 (2002-06-06), pages 3961-3968, XP002374750 ISSN: 0950-9232 abstract; figure 1; tables 2,3 page 3961, column 2, paragraph 1 - page 3964, column 2, paragraph 2 page 3697, column 1, paragraph 2 - column 2, paragraph 1 ----- -/--	1-21

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2006	19/04/2006

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/005601 A (THE REGENTS OF THE UNIVERSITY OF MICHIGAN; CLARKE, MICHAEL, F; LIU, RU) 20 January 2005 (2005-01-20) abstract; tables 4-8 page 3, paragraph 2 - page 4, paragraph 1 -----	15-21
X	KOHLMANN ALEXANDER ET AL: "Molecular characterization of acute leukemias by use of microarray technology." GENES CHROMOSOMES AND CANCER, vol. 37, no. 4, August 2003 (2003-08), pages 396-405, XP002375048 ISSN: 1045-2257 abstract; figures 1B,2; tables 1-3 page 396, column 1, paragraph 1 - page 397, column 1, paragraph 2 page 402, column 1, paragraph 2 - column 2, paragraph 4 -----	1-21
A	KUROKAWA YUKINORI ET AL: "Molecular prediction of response to 5-fluorouracil and interferon-alpha combination chemotherapy in advanced hepatocellular carcinoma." CLINICAL CANCER RESEARCH, vol. 10, no. 18, 15 September 2004 (2004-09-15), pages 6029-6038, XP002374752 ISSN: 1078-0432 abstract page 6030, column 2, paragraph 2 - page 6033, column 1, paragraph 2 -----	1-21
A	US 2005/209786 A1 (CHEN TZONG-HAO ET AL) 22 September 2005 (2005-09-22) abstract; figures 1-5 paragraph [0008] paragraph [0019] - paragraph [0037] -----	1-21

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INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2005/000855

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		CA 2528669 A1	20-01-2005
		US 2006019256 A1	26-01-2006
US 2005209786 A1	22-09-2005	NONE	