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(54) Titre : SIGNATURE D'EXPRESSION GENIQUE PRONOSTIQUE POUR LES PATIENTS SOUFFRANT DU CANCER DU POUMON A CELLULES NON PETITES

(54) Title: PROGNOSTIC GENE EXPRESSION SIGNATURE FOR NON SMALL CELL LUNG CANCER PATIENTS

(57) Abrégé/Abstract:
The invention relates to a method of typing non-small cell lung cancer cells by determining RNA levels for a set of genes. Said typing can be used for determining a metastasizing potential of the cancer cells. The invention further relates to a set of probes and a set of primers that can be used for typing non-small cell lung cancer cells.

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(54) Title: PROGNOSTIC GENE EXPRESSION SIGNATURE FOR NON SMALL CELL LUNG CANCER PATIENTS

(57) Abstract: The invention relates to a method of typing non-small cell lung cancer cells by determining RNA levels for a set of genes. Said typing can be used for determining a metastasizing potential of the cancer cells. The invention further relates to a set of probes and a set of primers that can be used for typing non-small cell lung cancer cells.

Title: Prognostic gene expression signature for non small cell lung cancer patients

Field: The present invention relates to the field of cancer prognosis and diagnosis. More particular, the invention relates to a method for typing an RNA sample of an individual suffering from non-small cell lung cancer. The invention furthermore relates to a set of genes or probes for use in typing an RNA sample of said individual.

Lung cancer accounts for about 15% of all diagnosed cancers in human and causes the most cancer-related deaths in both men and women (source: Cancer facts and Figures 2007, American Cancer Society). The three main types of primary lung cancers are mesothelioma, small cell lung cancer, and non-small cell lung cancer. Mesothelioma is a rare type of cancer which affects the covering of the lung (the pleura). It is often caused by exposure to asbestos. Small cell lung cancer (SCLC), also called oat cell lung cancer, is characterized by the presence of small cells that are almost entirely composed of a nucleus. SCLC frequently occurs in (ex)smokers and is quite rare for people that never smoked. SCLC tends to spread early in development of the tumor and is often treated with chemotherapy rather than surgery. Non-small cell lung cancer (NSCLC) is the most common form of lung cancer and is diagnosed in about 85% of all lung cancer patients. NSCLC represents a diverse group of cancers with the main groups being squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. Other, minor groups comprise pleomorphic carcinoma, carcinoid tumor, salivary gland carcinoma, and unclassified carcinoma.

Adenocarcinoma is the most common subtype of NSCLC, accounting for 50% to 60% of NSCLC. It is a form which starts near the gas-exchanging surface of the lung. Most cases of the adenocarcinoma are associated with smoking.

However, among non-smokers and in particular female non-smokers, adenocarcinoma is the most common form of lung cancer. A subtype of adenocarcinoma, the bronchioalveolar carcinoma, is more common in female non-smokers and may have different responses to treatment. Squamous cell carcinoma, accounting for 20% to 25% of NSCLC, also starts in the larger breathing tubes but grows slower meaning that the size of these tumours varies on diagnosis. Large-cell carcinoma accounts for about 10% to 15% of NSCLC. It can start in any part of the lung. It tends to grow and spread quickly.

Known risk factors for developing NSCLC are smoking, actively or passively, exposure to air pollution, and exposure to radiation. When smoking is combined with other risk factors, the risk of developing lung cancer is increased.

There are multiple tests and procedures to detect, diagnose, and stage non-small cell lung cancer. Performing a chest X-ray is often the first step if a patient reports symptoms that may be suggestive of lung cancer. This may reveal an obvious mass, widening of the mediastinum (suggestive of spread to lymph nodes there), atelectasis (collapse), consolidation (infection) and pleural effusion. If there are no X-ray findings but the suspicion is high (e.g. a heavy smoker with blood-stained sputum), bronchoscopy and/or a CT scan may provide the necessary information. In any case, bronchoscopy or CT-guided biopsy is nearly always performed to identify the tumor type and to determine the stage.

If investigations have confirmed lung cancer, scan results and often positron emission tomography (PET) are used to determine whether the disease is localized and amenable to surgery or whether it has spread to the point it cannot be cured surgically.

Prognosis and treatment options depend on the stage of the cancer, the type of cancer, and the patient's general health. Early stage cancer is primarily treated by surgery, which is aimed at removing all cancer cells. Surgery can lead to the removal of all or part of a lung, depending on the location and size of the cancer.

Alternative treatment is provided by radiation therapy, or radiotherapy, comprising three-dimensional conformal radiation therapy and brachytherapy; and chemotherapy including photodynamic therapy.

In general, small-cell lung cancer (SCLC) is most commonly treated by chemotherapy in an attempt to slow or halt its spread beyond the lungs. Early stage non-small-cell lung cancer (NSCLC) is first treated by surgery and additional radiation therapy and chemotherapy to slow tumor growth and relieve symptoms, if required.

After surgery, if lymph nodes are positive in the resected lung tissues (stage II) or the mediastinum (peri-tracheal region, stage III), adjuvant chemotherapy may improve survival by up to 15%. However, the benefit of adjuvant chemotherapy for patients with stage I NSCLC is still controversial. Trials of preoperative chemotherapy in resectable NSCLC have been inconclusive (source: Clinical Evidence: concise, BMJ Publishing Group, London. 2006. ISBN 1-90554501206 ISSN 1465-9225). In the NCI Canada study JBR.10 (Pepe C. et al., J Clin Oncol. 2007; 25(12): 1553-61) patients with stage IB to IIB NSCLC were treated with vinorelbine and cisplatin chemotherapy and showed a significant survival benefit of 15% over 5 years. However subgroup analysis of patients in stage IB showed that chemotherapy did not result in any significant survival gain. Similarly, while the Italian ANITA study showed a survival benefit of 8% over 5 years with vinorelbine and cisplatin chemotherapy in stages IB to IIIA, subgroup

analysis also showed no benefit in the IB stage (Douillard, JU. et al., Lancet Oncol 2006; 7(9): 719-27).

A Cancer and Leukemia Group B (CALGB) study (protocol 9633), related to a randomized trial of carboplatin and paclitaxel in stage IB NSCLC, reported no survival advantage at the June 2006 American Society of Clinical Oncology meeting. However, subgroup analysis suggested benefit for tumors greater than 4 centimeters. For patients with resected stage II-IIIA NSCLC, standard practice is to offer adjuvant third generation platinum-based chemotherapy (e.g. cisplatin and vinorelbine).

Chemotherapeutic drugs that are used in lung cancer treatment comprise platinum alkylators, podophyllin alkaloids, vinca alkaloids, anthracyclines, topoisomerase inhibitors, taxanes, antimetabolites, tyrosine kinase inhibitors, and folate antagonists. In recent years, various molecular targeted therapies have been developed for the treatment of advanced lung cancer. Gefitinib (Iressa) targets the epidermal growth factor receptor (EGF-R) that is expressed in many cases of NSCLC. However it was not shown to increase survival, although females, Asians, non-smokers and those with the adenocarcinoma cell type appear to benefit from gefitinib.
Another drug called erlotinib (Tarceva), which also inhibits EGF-R, increases survival in lung cancer patients and has recently been approved by the FDA for second-line treatment of advanced non-small cell lung cancer.

The most common treatment for early stage SCLC is surgery if the cancer is confined to a single nodule. Surgery can be combined with either cisplatin or carboplatin together with etoposide. Chemotherapy in combination with radiation therapy improves the outcome of the therapy. Late stage SCLC is also treated by a combination of either cisplatin or carboplatin and etoposide. Other chemotherapeutic drugs, such as cyclophosphamide, doxorubicin,

vincristine, ifosfamide, topotecan, paclitaxel, methotrexate, vinorelbine, gemcitabine, irinotecan and docetaxel in various combinations, are prescribed if SCLC becomes resistant to the aforementioned drugs. Metastasis to the brain, which often occurs in SCLC is treated by radiation therapy.

Treatment of NSCLC is primarily determined by the stage of the cancer. Stage 0 cancer, in which the cancer has not spread beyond the inner lining of the lung, is often curable by surgery alone. Treatment of stage 1 cancer, which has not spread to the lymph nodes, is often also limited to surgery, either lobectomy or segmentectomy. The 5-year survival rate of patients with stage 1 is 55-70%. For stage 2 cancer, in which the cancer has spread to some lymph nodes, nowadays surgery is almost always followed by chemotherapy. Stage 3 cancer, in which the cancer has spread to nearby tissue or to distant lymph nodes, and stage 4 cancer, in which the cancer has spread to distant organs, are treated by a combination of chemotherapy and radiation therapy. Surgery is sometimes performed to remove one or more localized cancer nodules.

Chemotherapy, including adjuvant therapy, usually causes side effects, such as nausea, vomiting, loss of appetite, loss of hair, mouth sores, and severe diarrhea. For all patients, the risk of cancer recurrence has to be weighted against the severe side effects caused by aggressive treatment. This especially accounts for stage 1 NSCLC patients, where the cancer has spread beyond the inner lining of the lung, but yet has not reached the lymph nodes. Patients with an increased risk for cancer recurrence will benefit from adjuvant therapy, while patients with a reduced risk will unnecessary suffer from the severe side effects caused by adjuvant therapy. Therefore, there is a need for a method of typing NSCLC patients to determine their risk of cancer recurrence.

Therefore, the invention provides a method for typing a sample, preferably a RNA sample, of an individual suffering from non-small cell lung cancer or suspected of suffering there from, the method comprising providing a tissue sample from said individual comprising non-small cell lung cancer cells or suspected to comprise non-small cell lung cancer cells; preparing RNA from said tissue sample; determining RNA levels for a set of genes in said RNA; and typing said sample on the basis of the levels of RNA determined for said set of genes; wherein said set of genes comprises at least two of the genes listed in Table 3.

A level of RNA refers to the amount of RNA that is present in a sample, preferably relative to other RNA in said sample. Said level of RNA is a measure of the level of expression of a gene in cell of said tissue sample. It is preferred that said level of RNA refers to the amount of mRNA transcripts from a gene in a sample, preferably relative to other mRNA such as total mRNA.

The genes listed in Table 3 were identified and validated as being differentially expressed in non-small cell lung cancer samples. Non-small cell lung cancer samples were randomly divided into a training set and a validation set. In a first series of experiments, genes were identified of which the RNA level differs between a sample from an individual with a high risk for cancer recurrence versus a sample from an individual with a low risk of cancer recurrence, using the training set of cancer samples. The resulting genes were validated in a second series of experiments using the independent validation set of non small cell lung cancer samples. A gene set comprising at least two of the genes listed in Table 3 provides a prognostic signature for typing a sample of an individual suffering from non-small cell lung cancer as having a low risk or an enhanced risk of cancer recurrence. Prognostic information that can be obtained by a method of the invention comprises

three possible endpoints, which are time from surgery to distant metastases, time of disease-free survival, and time of overall survival. Kaplan-Meier plots (Kaplan and Meier. J Am Stat Assoc 53: 457-481 (1958)) can be used to display time-to-event curves for any or all of these three endpoints.

Typing refers to assessing a risk of recurrence of said non-small cell lung cancer. Said typing is intended to provide prognostic information to aid in clinical evaluation of NSCLC patients. In this respect, no recurrence within a relevant time interval is defined as "low risk", and recurrence within said relevant time interval is defined as "high risk". A relevant time interval is at least 1 year, more preferred at least two years, more preferred at least three years, more preferred at least five years, or more preferred at least ten years.

A method of the invention is particularly suited to differentiate between a high or low risk of recurrence within three years.

Cancer recurrence refers to a recurrence of the cancer in the same place as the original cancer or elsewhere in the body. A local recurrence refers to a cancer that has returned in or very close to the same place as the original cancer, while a distant recurrence means the cancer has spread, or metastasized, to organs or tissues distant from the site of the original cancer.

Said tissue sample can be derived from all or part of a cancerous growth, or of a tumor suspected to be cancerous, depending on the size of the cancerous growth. A cancerous growth can be removed by surgical treatment including lobectomy, bilobectomy or pneumonectomy, with or without part of a bronchial tube. Said tissue sample can also be derived by biopsy, comprising aspiration biopsy, needle biopsy, incisional biopsy, and excisional biopsy. It is preferred that at least 10% of the cells in a tissue sample are NSCLC cells, more preferred at least 20%, and most preferred at least 30%. Said

percentage of tumor cells can be determined by analysis of a stained section, for example hematoxylin and eosin–stained section, from the cancerous growth. Said analysis can be performed or confirmed by a pathologist.

Said individual suffering from NSCLC, or suspected of suffering from NSCLC, can be an individual suffering from stage 0 cancer, in which the cancer has macroscopically not spread beyond the inner lining of the lung, and which is often curable by surgery alone. Said individual can be suffering from stage 1 cancer, which has not spread to the lymph nodes; stage 2 cancer, in which the cancer has spread to some lymph nodes; stage 3 cancer, in which the cancer has spread to nearby tissue or to distant lymph nodes; or stage 4 cancer, in which the cancer has spread to distant organs.

It is preferred that said individual suffers from early stage NSCLC, or suspected of suffering there from. Early stage NSCLC is stage 0 cancer, stage 1 cancer, or stage 2 cancer.

In a preferred embodiment, said individual is suffering from stage 1 NSCLC, or suspected of suffering there from.

A method of the invention is preferably used to determine a risk for said patient for recurrence of the cancer. This risk may further be combined with other prognostic factors such as age, sex, tumor diameter and smoking history. A determined risk can be used by a clinician to make a decision about which patients may benefit from additional chemotherapy, and which patients are not likely to benefit from additional chemotherapy.

RNA prepared from said tissue sample preferably represents a quantitative copy of genes expressed at the time of collection of a tissue sample from the cancer. This can be achieved by processing and storing said tissue sample

under protective conditions that preserve the quality of the RNA. Examples of such preservative conditions are fixation using e.g. formaline, the use of RNase inhibitors such as RNAsin™ (Pharmingen) or RNAsecure™ (Ambion), and the use of preservative solutions such as RNAlater™ (Ambion) and RNARetain™ (Assuragen). It is further preferred that said preservative condition allows storage and transport of said tissue sample at room temperature. A preferred preservative condition is the use of RNARetain™ (Assuragen).

Said RNA sample can be isolated from said tissue sample by any technique known in the art, including but not limited to Trizol (Invitrogen; Carlsbad, California), RNAqueous® Technology (Qiagen; Venlo, the Netherlands), Total RNA Isolation method (Agilent; Santa Clara, California), and Maxwell™ 16 Total RNA Purification Kit (Promega; Madison, Wisconsin). A preferred RNA isolation procedure involves the use of RNAqueous® Technology (Qiagen; Venlo, the Netherlands).

For each of the genes listed in Table 3, a relative level of expression in a sample from an individual with a low risk of cancer recurrence was compared to the average level of expression in a reference sample comprising a mixture of non-small cell lung cancer samples. Said relative level of expression is either increased in a low risk NSCLC sample, as indicated with a positive number in the second column of Table 3, or said relative level of expression is decreased in a low risk NSCLC sample, as indicated with a negative number in the second column of Table 3.

In a preferred embodiment, one of said at least two genes is increased in a low risk NSCLC sample, compared to the average level of expression of said gene in a reference sample, while a second gene from said at least two genes

is decreased in a low risk NSCLC sample compared to the average level of expression of said gene in a reference sample.

It is furthermore preferred that said set of genes comprises at least three of the genes listed in Table 3, more preferred four of the genes listed in Table 3, more preferred five of the genes listed in Table 3, more preferred six of the genes listed in Table 3, more preferred seven of the genes listed in Table 3, more preferred eight of the genes listed in Table 3, more preferred nine of the genes listed in Table 3, more preferred ten of the genes listed in Table 3, more preferred fifteen of the genes listed in Table 3, more preferred twenty of the genes listed in Table 3, more preferred thirty of the genes listed in Table 3, more preferred forty of the genes listed in Table 3, more preferred sixty of the genes listed in Table 3, more preferred seventy of the genes listed in Table 3, more preferred seventy-two of the genes listed in Table 3, more preferred eighty of the genes listed in Table 3, more preferred ninety of the genes listed in Table 3, more preferred hundred of the genes listed in Table 3, more preferred two-hundred of the genes listed in Table 3, more preferred all of the genes listed in Table 3.

It is furthermore preferred to select genes that are increased in a low risk NSCLC sample, compared to the average level of expression of said gene in a reference sample, as well as genes that are decreased in a low risk NSCLC sample compared to the average level of expression of said gene in a reference sample.

It is particularly preferred that said set of genes comprises at least four of the genes listed in Table 3 resulting in an average accuracy of 0.598837; more preferred at least nine of the genes listed in Table 3 resulting in an average accuracy of 0.6046512; more preferred at least forty-nine of the genes listed in Table 3 resulting in an average accuracy of 0.6337209; more preferred at

least ninety of the genes listed in Table 3 resulting in an average accuracy of 0.6453488; more preferred all of the genes listed in Table 3 resulting in an average accuracy of 0.651163; as indicated in Figure 9.

The genes listed in Table 3 can be rank ordered. Ranking can be based on a correlation with overall survival time, or on a correlation with recurrence free survival time, or on a correlation with differential expression between tumor samples from low-risk and high-risk patients, or based on the selection percentages of the genes during the multiple samples approach (Michiel et al., Lancet 365: 488–92 (2005)), as is known to a skilled person. Ranking of the genes listed in Table 3 was performed according to their selection percentages during the multiple samples approach, in which the top-ranked genes represent the genes that were most often selected for development of the prognostic signature.

A preferred set of genes for use in a method of the invention comprises the first two rank-ordered genes listed in Table 3 resulting in negative predictive value of 0.7857143; more preferred the first eight rank-ordered genes listed in Table 3 resulting in negative predictive value of 0.8681319; more preferred the first thirty-six rank-ordered genes listed in Table 3 resulting in negative predictive value of 0.8829787; more preferred the first fifty-seven rank-ordered genes listed in Table 3 resulting in negative predictive value of 0.8977273; and most preferred the first seventy-two rank-ordered genes listed in Table 3 resulting in negative predictive value of 0.9166667, as indicated in Figure 8.

It is furthermore preferred that a set of genes for use in a method of the invention comprises at least two of the genes listed in Table 3, whereby one of said at least two genes is Ref Seq number XM_04626. In a more preferred embodiment, a set of genes according to the invention comprises Ref Seq

number XM_04626 and Ref Seq number NM_052966; more preferred Ref Seq number XM_04626, Ref Seq number NM_052966, and Ref Seq number NM_002664; more preferred Ref Seq number XM_04626, Ref Seq number NM_052966, Ref Seq number NM_002664, and Ref Seq number NM_004310; more preferred Ref Seq number XM_04626, Ref Seq number NM_052966, Ref Seq number NM_002664, Ref Seq number NM_004310, and Ref Seq number NM_004288; more preferred Ref Seq number XM_04626, Ref Seq number NM_052966, Ref Seq number NM_002664, Ref Seq number NM_004310, Ref Seq number NM_004288 and Ref Seq number NM_003195; more preferred Ref Seq number XM_04626, Ref Seq number NM_052966, Ref Seq number NM_002664, Ref Seq number NM_004310, Ref Seq number NM_004288, Ref Seq number NM_003195, and Ref Seq number NM_024560; more preferred Ref Seq number XM_04626, Ref Seq number NM_052966, Ref Seq number NM_002664, Ref Seq number NM_004310, Ref Seq number NM_004288, Ref Seq number NM_003195, Ref Seq number NM_024560 and Ref Seq number NM_014358; more preferred Ref Seq number XM_04626, Ref Seq number NM_052966, Ref Seq number NM_002664, Ref Seq number NM_004310, Ref Seq number NM_004288, Ref Seq number NM_003195, Ref Seq number NM_024560, Ref Seq number NM_014358, and Ref Seq number NM_201286; more preferred Ref Seq number XM_04626, Ref Seq number NM_052966, Ref Seq number NM_002664, Ref Seq number NM_004310, Ref Seq number NM_004288, Ref Seq number NM_003195, Ref Seq number NM_024560, Ref Seq number NM_014358, Ref Seq number NM_201286, and Ref Seq number NM_172006.

The genes listed in Table 3 can be identified by the gene name or by the unique identifier according to the NCBI Reference Sequences (Refseq), as provided in Table 3. Preferably, said genes can be identified by a part of the sequence of said gene which is provided in Table 3.

The RNA level of at least two of the genes listed in Table 3 can be determined by any method known in the art, including but not limited to Northern blotting, ribonuclease protection assay, multiplex technologies such as Locked Nucleic Acid-modified capture probes and multi-analyte profiling beads, quantitative polymerase chain reaction (qPCR), and microarray-mediated analyses. If required, an RNA sample can be reverse-transcribed by known methods, such as by random primed or by oligo (dT) primed reverse transcriptase reaction, into copy-DNA prior to determination of the expression level. qPCR comprises end point polymerase reaction and real-time polymerase reaction. Alternatives to PCR, such as strand-displacement amplification, branched DNA, loop-mediated isothermal amplification and nucleic-acid sequence-based amplification are specifically included in this embodiment.

In a preferred method according to the invention, RNA levels are determined by means of an array or microarray.

(Micro)array-mediated analyses to determine RNA levels of at least two of the genes listed in Table 3 in a RNA sample comprises the use of a probe on a solid surface to determine the levels of a specific RNA that is present in a RNA from a tissue sample. Said probe can be a desoxyribonucleic acid (DNA) molecule such as a genomic DNA or fragment thereof, a ribonucleic acid molecule, a cDNA molecule or fragment thereof, a PCR product, a synthetic oligonucleotide, or any combination thereof. Said probe can be a derivative or variant of a nucleic acid molecule, such as, for example, a peptide nucleic acid molecule.

Said probe is specific for a gene listed in Table 3. A probe can be specific when it comprises a continuous stretch of nucleotides that are completely complementary to a nucleotide sequence of a RNA product of said gene, or a

cDNA product thereof. A probe can also be specific when it comprises a continuous stretch of nucleotides that are partially complementary to a nucleotide sequence of a RNA product of said gene, or a cDNA product thereof. Partially means that a maximum of 5% from the nucleotides in a continuous stretch of at least 20 nucleotides differs from the corresponding nucleotide sequence of a RNA product of said gene. The term complementary is known in the art and refers to a sequence that is related by base-pairing rules to the sequence that is to be detected. It is preferred that the sequence of the probe is carefully designed to minimize nonspecific hybridization to said probe. It is preferred that the probe is or mimics a single stranded nucleic acid molecule. The length of said complementary continuous stretch of nucleotides can vary between 15 bases and several kilo bases, and is preferably between 20 bases and 1 kilobase, more preferred between 40 and 100 bases, and most preferred 60 nucleotides.

To determine the RNA level of at least two of the genes listed in Table 3, the RNA sample is preferably labeled, either directly or indirectly, and contacted with probes on the array under conditions that favor duplex formation between a probe and a complementary molecule in the labeled RNA sample. The amount of label that remains associated with a probe after washing of the microarray can be determined and is used as a measure for the level of RNA of a nucleic acid molecule that is complementary to said probe.

Systemic bias can be introduced during the handling of the sample in a microarray experiment. To reduce systemic bias, the determined RNA levels are preferably corrected for background non-specific hybridization and normalized using, for example, Feature Extraction software (Agilent Technologies). Other methods that are or will be known to a person of ordinary skill in the art, such as a dye swap experiment (Martin-Magniette et

al., Bioinformatics 21:1995-2000 (2005)) which can be performed to normalize differences introduced by dye bias, can also be applied.

In a preferred method according to the invention, the determination of the RNA levels comprises normalizing the determined levels of RNA of said set of genes in said sample.

Normalization corrects for variation due to inter-array differences in overall performance, which can be due to for example inconsistencies in array fabrication, staining and scanning, and variation between labeled RNA samples, which can be due for example to variations in purity. Conventional methods for normalization of array data include global analysis, which is based on the assumption that the majority of genetic markers on an array are not differentially expressed between samples [Yang et al., Nucl Acids Res 30: 15 (2002)]. Alternatively, the array may comprise specific probes that are used for normalization. These probes preferably detect RNA products from housekeeping genes such as glyceraldehyde-3-phosphate dehydrogenase and 18S rRNA levels, of which the RNA level is thought to be constant in a given cell and independent from the developmental stage or prognosis of said cell. Said specific probes preferably are specific for genes of which the RNA level varies over a wide range of levels.

In a preferred embodiment, a method of the invention further comprises comparing an RNA level at least two of the genes listed in Table 3 to an RNA level of said genes in a reference sample.

The reference sample can be an RNA sample isolated from a lung tissue from a healthy individual, or from so called normal adjacent tissue from an individual suffering from NSCLC, or an RNA sample from a relevant cell line or mixture of cell lines. Said reference sample can also be an RNA sample

from a cancerous growth of an individual suffering from NSCLC. Said individual suffering from NSCLC can have an increased risk of cancer recurrence, or a low risk of cancer recurrence.

It is preferred that said reference sample is an RNA sample from an individual suffering from non-small cell lung cancer and having a low risk of cancer recurrence. In a more preferred embodiment, said reference sample is a pooled RNA sample from multiple tissue samples comprising NSCLC cells from individuals suffering from non-small cell lung cancer and having a low risk of cancer recurrence. It is preferred that said multiple tissue sample comprise more than 10 tissue samples, more preferred more than 20 tissue samples, more preferred more than 30 tissue samples, more preferred more than 40 tissue samples, most preferred more than 50 tissue samples.

Comparison of a sample with a reference sample can be performed in various ways. Preferably a coefficient is determined that is a measure of the similarity of dissimilarity of a sample with said reference sample. A number of different coefficients can be used for determining a correlation between the RNA expression level in an RNA sample from an individual and a reference sample. Preferred methods are parametric methods which assume a normal distribution of the data. One of these methods is the Pearson product-moment correlation coefficient, which is obtained by dividing the covariance of the two variables by the product of their standard deviations. Preferred methods comprise cosine-angle, un-centered correlation and, more preferred, cosine correlation (Fan et al., Conf Proc IEEE Eng Med Biol Soc. 5:4810-3 (2005)).

Preferably, said correlation with a reference sample is used to produce an overall similarity score for the set of genes that are used. A similarity score is a measure of the average correlation of RNA levels of a set of genes in an RNA sample from an individual and a reference sample. Said similarity score

is a numerical value between +1, indicative of a high correlation between the RNA expression level of the set of genes in the RNA sample of the individual and the reference sample, and -1, which is indicative of an inverse correlation and therefore indicative of having an increased risk of cancer recurrence (van 't Veer et al., Nature 415: 484-5 (2002)).

In particularly preferred embodiment, an arbitrary threshold is determined for said similarity score. RNA samples that score below said threshold are indicative of an increased risk of cancer recurrence, while samples that score above said threshold are indicative of a low risk of cancer recurrence.

A similarity score and or a resultant of said score, which is a measurement of increased risk or low risk of cancer recurrence, is preferably displayed or outputted to a user interface device, a computer readable storage medium, or a local or remote computer system.

In another aspect, the invention provides a set of probes for typing a sample of an individual suffering from NSCLC, or suspected of suffering therefrom, wherein said set of probes comprises probes that are specific for at least two of the genes listed in Table 3.

The RNA level of a set of genes comprising at least two of the genes listed in Table 3 was found to be discriminative between an RNA sample from an individual suffering from NSCLC and having an increased risk for recurrence of said cancer, and an RNA sample from an individual suffering from NSCLC and having an reduced risk for recurrence of said cancer.

It is preferred that said set probes comprises probes that are specific for at least three of the genes listed in Table 3, more preferred four of the genes listed in Table 3, more preferred five of the genes listed in Table 3, more

preferred six of the genes listed in Table 3, more preferred seven of the genes listed in Table 3, more preferred eight of the genes listed in Table 3, more preferred nine of the genes listed in Table 3, more preferred ten of the genes listed in Table 3, more preferred fifteen of the genes listed in Table 3, more preferred twenty of the genes listed in Table 3, more preferred thirty of the genes listed in Table 3, more preferred forty of the genes listed in Table 3, more preferred sixty of the genes listed in Table 3, more preferred seventy of the genes listed in Table 3, more preferred seventy-two of the genes listed in Table 3, more preferred eighty of the genes listed in Table 3, more preferred ninety of the genes listed in Table 3, more preferred hundred of the genes listed in Table 3, more preferred two-hundred of the genes listed in Table 3, more preferred all of the genes listed in Table 3.

Preferably said set of probes comprises probes specific for not more than 227 different genes, more preferred not more than 150 different genes, more preferred not more than 72 different genes of the genes listed in Table 3.

In yet another aspect, the invention provides the use of a set of probes that are specific for a set of genes of the invention for determining a risk for an individual suffering of NSCLC or suspected of suffering from said cancer, for recurrence of said cancer.

According to this aspect, the invention provides the use of set of probes that are specific for a set of genes of the invention for discriminating between NSCLC cells with a low versus a high metastasizing potential by determining a nucleic acid level of expression of said set of marker genes in an RNA sample from a patient suffering from NSCLC or suspected of suffering from said cancer.

The invention furthermore provides an array comprising between 2 and 12.000 probes of which two or more probes are specific for at least two of the genes listed in Table 3. The invention furthermore provides the use of an array according to the invention for typing of NSCLC cells.

The invention also provides a set of primers for typing a sample of an individual suffering from non-small cell lung cancer or suspected of suffering there from, whereby said set of primers comprises primers specific for at least two of the genes listed in Table 3.

Said set of primer can be used for determining an RNA level for said at least two of the genes listed in Table 3 in a sample. Known methods for determining an RNA level comprise amplification methods, including but not limited to polymerase chain reaction such as multiplex PCR and multiplex ligation-dependent probe amplification, and nucleic acid sequence-based amplification.

Preferably said set of primers comprises primers specific for less than 227 different genes, more preferred not more than 150 different genes, more preferred not more than 72 different genes of the genes listed in Table 3.

According to this aspect, the invention further provides the use of a set of primers according to the invention for determining a risk for an individual suffering of NSCLC for recurrence of said cancer. The invention also provides the use of a set of primers according to the invention for discriminating between NSCLC cells with a low versus a high metastasizing potential.

In a further aspect, the invention provides a method of classifying a sample from an individual suffering from NSCLC, or suspected of suffering from NSCLC, comprising classifying a sample as derived from an individual

having a poor prognosis or a good prognosis by a method comprising providing a sample from said individual; determining a level of RNA for a set of genes comprising at least two of the genes listed in Table 3 in said sample; determining a similarity value for the level of RNA in said sample and a level of RNA for said set of genes in a patient having no recurrent disease within three years of initial diagnosis; and classifying said individual as having a poor prognosis if said similarity value is below a first similarity threshold value, and classifying said individual as having a good prognosis if said similarity value exceeds said first similarity threshold value.

Said reference sample is preferably a sample from normal lung tissue, from normal adjacent tissue, from a cell line or mixture of cell lines, or a relevant sample from an individual suffering from NSCLC. Preferably, a reference sample is from an individual suffering from non-small cell lung cancer and having a low risk of cancer recurrence. In a more preferred embodiment, said reference sample is a pooled RNA sample from multiple tissue samples comprising NSCLC cells from individuals suffering from non-small cell lung cancer and having a low risk of cancer recurrence.

A reference sample can also comprise a sample from an individual suffering from non-small cell lung cancer and having an increased risk of cancer recurrence. In that instance, the invention similarly provides a method of classifying an individual suffering from NSCLC, or suspected of suffering from NSCLC, comprising classifying a sample as derived from an individual having a poor prognosis or a good prognosis by a method comprising providing a sample from said individual; determining a level of RNA for a set of genes comprising at least two of the genes listed in Table 3 in said sample; determining a similarity value for the level of RNA in said sample and a level of RNA for said set of genes in a patient having recurrent disease within three years of initial diagnosis; and classifying said individual as having a

good prognosis if said similarity value is below a first similarity threshold value, and classifying said individual as having a poor prognosis if said similarity value exceeds said first similarity threshold value.

Legend of the Figures

Fig. 1: Kaplan-Meier plot survival estimates of overall survival of patients with a good (low-risk) profile and of patients with a poor (high-risk) profile, as identified using a leave-one-out training approach.

Fig. 2: Schematic overview of the multiple samples procedure that was used for development of a robust nearest mean classifier. A 10-fold cross validation loop was used to identify genes which expression ratios correlate with overall and recurrence free survival time.

Fig. 3: Kaplan-Meier plot survival estimates of overall survival (OS) and relapse-free survival (RFS) based on the multiple sampling outcomes of the test samples.

Fig. 4: Prognostic power (P-values) of the nearest mean classifier using different gene set sizes. The highest power (lowest p-values) for both overall survival (black line) and relapse free survival (blue line) is reached upon using a gene set size of 72 genes.

Fig. 5: Left panel; Classifier prognostic low-risk correlation outcome (leave-one-out cross validation) of 103 training samples. Correlations above -0.145 indicate samples with a low-risk profile and correlation below -0.145 indicate samples with a high-risk profile. The samples are colored according to their true survival status. Right panel, visualization of the 72-gene prognostic signature. Each row represents one sample and each column represents one gene. Samples are labeled according to their true survival status (1: relapse or death with 3 years; 0: relapse-free survival for at least 3 years). Red indicates up regulation of a gene, green indicates down regulation of a gene.

Fig. 6: Kaplan-Meier plot survival estimates of overall survival (OS) and relapse-free survival (RFS) of the 103 training samples with a low-risk 72-gene profile and of patients with a high-risk 72-gene profile.

Fig. 7: Validation of the 72-gene signature on 69 independent samples. Left panel; as right panel in figure 5 for the 69 independent validation samples. Middle and right panel; as Fig 6 for the independent validation samples.

Fig. 8: Performance of ranked subset from the 237 genes with prognostic value for overall survival for 3 years after diagnosis. Negative predictive value (NPV), positive predictive value (PPV) and total accuracy are calculated for increasing ranked subset of the 237 genes (top 2, top 3, top 4, ... top 230, all 231 genes).

Fig. 9: Prognostic performance of random subsets of different size from the total set of 237 genes. For all different subset sizes (2, 3, 4, ..., 236, 237) the mean value and 95% confidence interval were calculated for the negative predictive value (NPV), positive predictive value (PPV) and total accuracy.

Fig. 10: Kaplan-Meier plot survival estimates of overall survival (OS) and relapse-free survival (RFS) of 172 non small cell lung cancer patients based on a classification by the 72-gene signature (good profile or poor profile) and by tumor staging (stage I or stage II).

Example 1

Non small cell lung cancer samples were analyzed on Agilent 44K array

against a lung reference pool that consisted of a pool of RNA from 65 NSCLC samples. A total of 103 samples were used for training the predictive signature and 69 as an independent validation set. The samples originated from 5 different European institutes and included mainly squamous cell carcinomas and adenocarcinomas. An overview of the sample and patient characteristics is given in Table 1. All samples were taken with informed consent of the patients according to the ethical standards of the Helsinki Declaration. RNA isolation and cRNA labeling followed standard protocols (Glas et al., BMC Genomics 2006; 7: 278). Hybridization was performed on Agilent platform (Agilent 44K arrays) according to standard procedures described by the manufacturer and as described elsewhere (Glas et al., BMC Genomics 2006; 7: 278). R and Bioconductor packages, available from the Bioconductor project (www. bioconductor.org), were used for statistical analyses of the data.

A leave-one-out cross validation procedure for development of a nearest-mean classifier did not result in a signature that could be validated using this type of cross validation procedure (Fig 1). In accordance with the hierarchical clustering, this finding indicated that the gene expression data of the analyzed samples did not harbor a very consistent and striking gene expression pattern that correlated with overall survival. Apparently, due to the large heterogeneity in gene expression between tumor samples from good-outcome and from poor-outcome patients, exclusion of a single sample for training of the signature is not sufficient to identify an unbiased gene signature that also works on independent additional test samples. Instead it required a more robust multiple sampling procedure to identify an unbiased set of survival predictive signature genes.

A 10-fold cross validation procedure was used for a more robust and less biased identification of predictive genes (Fig. 2). Ten percent of the training samples were randomly removed from the training set and for all genes a cox-proportion hazard ratio was calculated together with a Log-rank survival score and a p-value for discriminatory power between those patients with and without a survival (or relapse) event (Welsh t-test). The three survival statistics were combined into a single score which was used to rank the genes according to their association with overall (or relapse-free) survival. Next, the top-ranked genes were used for prediction of the 10 left-out samples using a nearest-mean classifier. By repeating this 10-fold cross validation procedure at least 500 times, we determined the unbiased performance of the classifiers, which were all based on different training sets. The multiple classifiers as obtained from the different training sets were trained towards prediction of overall survival (OS) (P=0.001, Fig. 3A) as well as the classifiers for prediction of relapse-free survival (RFS) (P=0.011, Fig. 3B) showed a significant performance for accurate prediction of the test samples and indicated that the 10-fold cross validation procedure was not biased toward the used training samples. More importantly, this multiple sampling approach allowed us to identify those predictive genes that were most stably selected for building the signatures. These stably selected genes are most favorable for an optimal unbiased predictive signature.

To develop a classifier with optimal performance for prediction of overall survival (OS) as well as relapse-free survival (RFS), the gene selection scores generated by the multiple samples procedure for OS and RFS were ranked and genes with a high ranking in both survival analyses were selected. Starting with a minimal list of the 40 highest ranked genes, the set of predictive genes was gradually expanded to determine the optimal gene set size with the highest predictive accuracy (both for OS and RFS) on all

training samples (Fig. 4). The strongest predictive power was reached with a set of 72 predictive genes, corresponding to the highest rank-ordered seventy-two genes listed in Table 3. Investigation of the 72-gene signature performance by leave-one-out cross validation on the training samples (Fig. 5A) indicated that an optimal prediction was achieved based on the sample correlations with the good-outcome profile (Fig. 5B, threshold: 0.145). An average low-risk profile was calculated for the 72-gene signature (Table 2, second column) which served as the low-risk profile for further validation of the classifier. High and low risk training samples showed a clear difference in gene expression of the 72 signature genes (Fig. 5C). Survival analysis of the training samples confirmed that the patients of whom the lung tumor samples show a low-risk profile have a significant better survival rate for overall survival (OS) and for relapse-free survival (RFS) time than patients with high-risk tumor profile ($P<0.0001$) (Fig. 6).

The predictive signature was validated on an independent set of 69 validation samples (Table 1). The gene expression profiles of the validation samples indicated that the predictive signature is also present in independent samples (Fig. 7A). Survival analysis of the independent validation samples confirmed the discriminatory power of the 72-gene signature for identification of low- and high-risk NSCLC patients (Fig. 7B–C). The somewhat lower significance on the validation set was (partially) caused by the relative high number of censoring events within 3 years after diagnosis (lost for follow-up; other caused of death) (see also Table 1).

The sensitivity, specificity, negative predictive value (NPV), positive predictive value and overall accuracy of the classifier (Table 2) confirm the finding that the classifier is able to discriminate between patients with a low and high risk for disease progression, especially towards prediction of low-risk patients (NPV

of 93 percent on the validation set). The median overall survival time of low-risk and high-risk patients is 47 and 31 months, respectively (P<1e-4, Wilcoxon rank-sum test) and the median relapse-free survival time for both patients groups is 47 versus 24 months, respectively (P<1e-5) (Table 4).

Example 2

To determine the minimal number of signature genes that are needed for an accurate prognostic signature, the set of 237 genes was ranked according to the prognostic power of the individual genes and the set of 237 genes was sequentially reduced till a gene set comprising only the two top ranked genes. For each different gene set size (i.e. comprising from 2 genes up to 237 genes) the negative predictive value (NPV), positive predictive value (PPV) and total accuracy were determined for prognosis off overall survival for at least 3 years. Figure 8 shows that the predictive power of the signature decreases only marginally in case of a lower number of ranked signature genes; a prognostic signature that comprises of only the top 2 genes has a NPV of 80 percent and a total accuracy of 70 percent. Thus, a small number of top-ranked genes already showed a high accuracy in prediction of low-risk patients (overall survival NPV of 83%).

We further analyzed the performance of a random subset of 2 or more genes selected from the set of 237 genes. Random subsets were selected with different sizes ranging from 2 genes up to all 237 genes. In total, hundred random, computer generated subsets were selected if possible for each different size and for each different subset the NPV, PPV and total accuracy was calculated. Subsequently, the mean performance and the 95 percent confidence interval were calculated for each different subset size. The data shown in Figure 9 indicate that random subsets of two or more of the 237 signature genes show only a marginal drop of the predictive performance (Figure 9). This result confirmed that the predictive value of the signature genes does not drop substantially, also in cases when only a small number of genes are used within the prognostic signature. However, the 95% confidence interval of the predictive performances does increase upon use of smaller

signatures. This is explained by the fact that random selection of a small number of genes from the total 237 gene set will results in a much larger variation in prognostic outcome than selection of a large subset. Despite this increase in variation, the negative predictive value of the prognostic signature subsets remains between 80-90 percent. These results indicate that, although the highest performance is achieved using the complete set of 72 genes corresponding to the highest rank-ordered seventy-two genes listed in Table 3, the use of only 2 genes already results in an accurate predictive signature.

Example 3

To test whether the classifier predicted survival independently of the other two prognostic factors, tumor type and tumor grade (Fig. 1), a univariate and a multivariate analysis were performed (Table 4). In a univariate analysis, the 72-gene signature was the most significant prognostic factor with a hazard ratio of 4.83 (95% CI: 2.47-9.44, P=4.1e-6) for OS and a hazard ratio if 4.86 (95% CI: 2.40-9.50, P=3.7e-6) for RFS. In a multivariate analysis with the other two prognostics factor, the predictive power of the signature remained similar (hazard ratios of 4.70 and 4.61 for overall and relapse-free survival, respectively, Table 4). This specified that the prognostic 72-gene classifier predicted survival outcome independently of the other two factors. The multivariate analysis indicated that tumor grading has an added predictive value on top of the gene classifier (Table 4). A combination of tumor grading (grade I or II) and the signature outcome (low-risk or high-risk) resulted in highly significant overall survival classification (P=6.2e-8, Fig.10A) and relapse-free survival prediction (P=3.3e-7, Fig. 10B).

Tables

Table 1

	Training set (103)		Validation set (69)	
		(%)		(%)
Gender				
male	77	75	51	82
female	26	25	18	29
Age at diagnosis				
median	62		67	
range	41 - 77		22 - 79	
Hospital				
NKI	30	29	6	10
Heidelberg	18	17	14	23
Bailystok	12	12	1	2
Gdansk	32	31	27	44
Vumc	11	11	21	34
Smoking				
current smoker	45	44	30	48
former smoker	44	43	28	45
non-smoker	3	3	3	5
unknown	11	11	8	13
Histology				
large cell carcinoma	8	8	2	3
squamous cell carcinoma	57	55	35	56
adenocarcinoma	33	32	23	37
other	5	5	9	15
Stage				
I	72	70	45	44
II	31	30	24	23
Follow-up period (months)				
median	46		24	
range	4 - 156		0.5 - 111	
Status				
alive / censored	59	57	33	53
dead lung cancer	35	34	16	26
dead other	9	9	20	32
Relapse-free survival time (months)				
median	43		22	
range	2 - 156		0.5 - 111	
Overall survival time (months)				
median	46		24	
range	4.3 - 156		0.5 - 111	
Treatment before surgery				
yes	5	5	2	3
no	96	93	58	94
unknown	2	2	9	15

Table 2

Performance of the 72-gene classifier

	Sensitivity*	Specificity*	NPV*	PPV*	Accuracy*	P-value[a]
Training	78	66	87	51	70	2.4E-05
Validation	87	52	93	34	59	0.006
Overall	81	60	89	43	75	3.7E-07

Training			Months	P-value[b]
	median OS**	low-risk group	52	3.6E-04
		high-risk group	33	
	median RFS**	low-risk group	52	7.7E-05
		high-risk group	32	

Validation			Months	P-value[b]
	median OS**	low-risk group	33	0.02
		high-risk group	23	
	median RFS**	low-risk group	33	0.01
		high-risk group	21	

Overall			Months	P-value[b]
	median OS**	low-risk group	47	2.4E-05
		high-risk group	31	
	median RFS**	low-risk group	47	5.5E-06
		high-risk group	24	

*based on 3-year relapse-free survival
**disregarded patients that died of other other causes than lung cancer
NPV negative predictive value
PPV positive predictive value
OS overall survival time (months)
RFS relapse-free survival time (months)
[a]Log-rank test
[b]Wilcoxon rank sum test

Table 3

NSCLC associated genes. Genes are ranked according to their association with recurrence-free survival. The low-risk profile column provides the log2 ratios of each classifier gene in a low-risk profile.

Rank	Gene	Refseq	Description	low-risk profile	Sequence
1	C3orf41	XM_046264	chromosome 3 open reading frame 41	-0.477	GTCAATGCTGGGAAGACAGGAGAAAAGCTTAATTCTTGACATTTAAATACCAGTTTTCCA
2	C1orf24	NM_052966	chromosome 1 open reading frame 24	0.278	AAAGGTCCAAGGGAATTTAATCTGGAAGAGAACATATGCCAATTTTTAAACTATGACAGC
3	PLEK	NM_002664	pleckstrin	0.329	TGAGAAAGACAGCACCCATTGAAACAGATATGTGTGTGAAAGTATATTTTTCAATTCCAG
4	RHOH	NM_004310	ras homolog gene family, member H	0.418	AAAGCTTGGTGTTTTCTCTGGGTACACCCCAAGCAGCGTCTCCTTTTGGATACAGTTATT
5	PSCDBP	NM_004288	pleckstrin homology, Sec7 and coiled-coil domains, binding protein	0.570	TTCATCGTGCTGTGGAAGAGGAAGAAAGTCGCTTTTGACGGATTGTGGTGTCCTTTCAAA
6	TCEA2	NM_003195	transcription elongation factor A (SII), 2	-0.236	ATCGAGGAATGCATCTTCCGGGACGTTGGAAACACAGACATGAAGTATAAGAACCGTGTA
7	FLJ21963	NM_024560	NA	-0.202	GCAAGATCCCCCGATCAGCTTTATCTGCCATTGTCAATGGCAAGCCATACAAGATAACTT
8	CLEC4E	NM_014358	C-type lectin domain family 4, member E	0.317	GCAAAATTGGAATGATGTAACCTGTTTCCTCAATTATTTTCGGATTTGTGAAATGGTAGG
9	USP51	NM_201286	ubiquitin specific peptidase 51	-0.458	AAAGCAGCACCATTTAGCTGTAGACCTTTATCATGGGGTCATATATTGCTTCATGTGTAA
10	WFDC10B	NM_172006	WAP four-disulfide core domain 10B	0.380	GCGACCCAGCATAGATCTATGCATCCACCACTGTTCATATTTCCAAAAGTGTGAAACAAA
11	IGH@	NA	immunoglobulin heavy locus	0.219	CGTGAGGATGCTTGGCACGTACCCCGTGTACATACTTCCCAGGCACCCAGCATGGAAATA
12	SLC4A3	NM_005070	solute carrier family 4, anion exchanger, member 3	-0.269	GATGCTGAACCAAACTTCGATGAGGATGGCCAGGATGAGTACAATGAGCTGCACATGCCA
13	CD53	NM_000560	CD53 molecule	0.627	ACCATAGGGCTATGATCTGCAGTAGTTCTGTGGTGAAGAGACTTGTTTCATCTCCGGAAA
14	LOC401431	NM_001008745	NA	-0.466	AGGTCTGATGCAGTAGCTTTTACTATTGGTGGAAATCGATGTTTTTTCCTTGAAAGTCTA
15	SCFV	XM_941394	NA	0.597	GGGGCTGGAATGGGTGGCAGTTATATCACATGATGGAAGTAATAAATACTACGCAGACTC

16	THRAP2	NM_015335	thyroid hormone receptor associated protein 2	-0.171	AACTTCCTACCACTCACCCTAGCATTACTTATATGATATGTCTCCATACCCATTACAATC
17	PRDM13	NM_021620	PR domain containing 13	-0.981	TAATGACTGCTGTACAGTGGGTATAGTATTTTGGTTTTGGTTCCAGATTGTGCAATCTTT
18	OBSL1	XM_051017	obscurin-like 1	-0.455	TTTGCATTCCATTGCATATTTCCAAGTCGGCTTTGCTATAAACACAAATATTCTCCAGAA
19	C7orf40	NA	chromosome 7 open reading frame 40	-0.322	CTGTGTTAATACACCTAGTGAGGAGTGGAGCTGAATTTGAATGCAAGCCTTGGCACCTTA
20	TAGAP	NM_054114	T-cell activation GTPase activating protein	0.425	GGCCATACGCCATGCCATAGCTTGTGCTATCTGTAAATATGAGACTTGTAAAGAACTGCC
21	MGC11271	NM_024323	NA	-0.229	TTGCAAATTTTAGGGTCCTGAGCCAAGTATGGATGGTTCAGAATTTGTTTCTTTCCTGGA
22	IGLV6-57		immunoglobulin lambda variable 6-57	0.768	AACTCTGCCTCCCTCACCATCTCTGGACTGAGGACTGAGGACGAGGCTGACTACTACTGT
23	CD38	NM_001775	CD38 molecule	0.649	TGAAAAATCCTGAGGATTCATCTTGCACATCTGAGATCTGAGCCAGTCGCTGTGGTTGTT
24	FKBP9	NM_007270	FK506 binding protein 9, 63 kDa	-0.217	TACTGATGTAGCCCTGAGGTAGTTCATGAAAATGCTGTGCACTCATTCCATGGAATAAAT
25	ADAMTSL2	NM_014694	ADAMTS-like 2	-0.322	GGCCCAGGGCCCACAGCCAGCGGTGGAGGTGTCTTGCTCCGGGCCCGTAGCCCACGCCCT
26	CD48	NM_001778	CD48 molecule	0.470	CATCATGAGGGTGTTGAAAAAGACTGGGAATGAGCAAGAATGGAAGATCAAGCTGCAAGT
27	GNPTAB	NM_024312	N-acetylglucosamine-1-phosphate transferase, alpha and beta subunits	0.484	CAGCAATCATTGCAGACTAACTTTATTAGGAGAAGCCTATGCCAGCTGGGAGTGATTGCT
28	DHRS8	NM_016245	dehydrogenase/reductase (SDR family) member 8	0.293	CACCTAGTTTTCTGAAAACTGATTTACCAGGTTTAGGTTGATGTCATCTAATAGTGCCAG
29	LOC388886	NM_207644	NA	-0.302	CTACTGACTTGTGATGCTCTCAAGCACATGATAGTGGGCGATGAAGGTCAAGGAGGACTC
30	CNIH3	NM_152495	cornichon homolog 3 (Drosophila)	-0.457	CTCCCATCTGAAACCTGTGACTCAGGTTTATGAATGGTGTTTGTGTAGCAACACATTGTG
31	PSMA6	NM_002791	proteasome (prosome, macropain) subunit, alpha type, 6	0.016	TAGCAGAGAGAGACTAAACATTGTCGTTAGTTTACCAGATCCGTGATGCCACTTACCTGT
32	CCRK	NM_001039803	cell cycle related kinase	-0.165	AGGATGAGCGTGAGCCAGAAGCAGCTGTGTATTTAAGGAAACAAGCGTTCCTGGAATTAA
33	SHROOM1	NM_133456	shroom family member 1	-0.281	GTCTCTGCTTTTCCCTTGAGGGATTGGGGAGGACCCAGTCCAGGCCTTTCTAAGATACTC
34	GPSM1	NM_015597	G-protein signalling modulator 1 (AGS3-like, C. elegans)	-0.428	GTCTGTGCCATGTTGTCAATGGGTCCTTTCCAACCCAAGAGGTACATTTGTTTTTCTGTT
35	TRO	NM_001039705	trophinin	-0.397	CCCCATGTTTACAGATACCGCTAATAAATTGCAGTAGTCCTTCCCATGGAGCCAAAGTAC
36	GSTT2	NM_000854	glutathione S-transferase theta 2	-0.614	GTAACATGAAGAACACTCAAAAATTGGCAAATGTCATCAGTGTTTTAAACAGAATAAAGA
37	NQO2	NM_000904	NAD(P)H dehydrogenase, quinone 2	0.099	TCACAGTGTCTGATTTGTATGCCATGAACTTTGAGCCGAGGGCCACAGACAAAGATATCA

38	EAF2	NM_018456	ELL associated factor 2	0.706	CAGGATTCCTGATATAGATGCCAGTCATAATAGATTTCGAGACAACAGTGGCCTTCTGAT
39	MUM1L1	NM_152423	melanoma associated antigen (mutated) 1-like 1	-0.034	ATGATATAAATGCCAACTGGCAAGTCATTCCAAACTGCTTGAAGGAGTAGATGAACCAGA
40	MUC4	NM_004532	mucin 4, cell surface associated	0.396	TGGGGCGAGCACTGTGAGCACCTGAGCATGAAACTCGACGCGTTCTTCGGCATCTTCTTT
41	C13orf21	NM_001010897	chromosome 13 open reading frame 21	-0.150	CCTCTGAACGATCACTGGTTTACTTTCTATGGATACATCTCTCCTCCATTGAGAATTGAT
42	PABPC1	NM_002568	poly(A) binding protein, cytoplasmic 1	0.040	CAGAACTTCTTCATATGCTCAAGTCTCCAGAGTCACTCCATTCTAAGGTTGATGAAGCTG
43	PLA2G7	NM_005084	phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	0.494	AAAGCATTTAGGACTTCATAAAGATTTTGATCAGTGGGACTGCTTGATTGAAGGAGATGA
44	PARK2	NM_004562	Parkinson disease (autosomal recessive, juvenile) 2, parkin	0.421	GATGTTTTAATTCCAAACCGGATGAGTGGTGAATGCCAATCCCCACACTGCCCTGGGACT
45	AOAH	NM_001637	acyloxyacyl hydrolase (neutrophil)	0.157	TTTACAAACTTCAATCTTTTCTACATGGATTTTGCCTTCCATGAAATCATACAGGAGTGG
46	IGL@		immunoglobulin lambda locus	0.357	CCCAAGGCATCAAGCCCTTCTCCCGTGCACTCAATAAACCCTCAATAAATATTCTCATTT
47	LOC642480	XM_925983	NA	0.097	GCTGGTAAAATCATTGGTATGTTGTTGGAGATTGGTAATTTGGAACTCCTTCATATGCTT
48	TMSB4X	NM_021109	thymosin, beta 4, X-linked	0.760	CCGATATGGCTGAGATTGAGAAATTCGATAAGTCGAAACTGAAGAAGACAGAGATGCAAG
49	LOC390712	XM_372630	NA	0.855	CTGTGAAGGGCAGATTCACCATCTCCACAGACAACTCAAAGAACACGCTCTACCTGCAAA
50	ACOT8	NM_005469	acyl-CoA thioesterase 8	-0.132	CTATATTGGCGAGGGCGACATGAAGATGCACTGCTGCGTGGCCGCCTATATCTCCGACTA
51	GIMAP7	NM_153236	GTPase, IMAP family member 7	0.334	TTTGGGAAGTCAGCCATGAAGCACATGGTCATCTTGTTCACTCGCAAAGAAGAGTTGGAG
52	LOC375010	XM_927556	NA	-0.365	ACGTTACAACTGAGTTAGAAGAATATAAGGAAGCCTTTGCAGCAGCATTGAAAGCTAACA
53	ASAH1	NM_004315	N-acylsphingosine amidohydrolase (acid ceramidase) 1	0.790	ATGAACTCGATGCTAAGCAGGGTAGATGGTATGTGGTACAAACAAATTATGACCGTTGGA
54	TRIM45	NM_025188	tripartite motif-containing 45	-0.033	GCAGCACCACTTGAGATTTCCAGAGGACCCAGACCTTTGTTCATTCTAAAGAGACTGATA
55	C2orf30	NM_015701	chromosome 2 open reading frame 30	0.567	ACGATGGTACCCAGACAGTCAGGATGGTGTCACATTTTTATGGAAATGGAGATATTTGTG
56	EXT2	NM_000401	exostoses (multiple) 2	-0.135	TCAGGGAACCAAACCCAGAATTCGGTGCAAAAGCCAAACATCTTGGTGGGATTTGATAAA
57	IFI6	NM_002038	interferon, alpha-inducible protein 6	0.788	GCCAAGAACACGCTGTATCTGCAAATGAACAGTCTGAGAGCCGAGGACACGGCTGTGTAT
58	KCNE3	NM_005472	potassium voltage-gated channel, Isk-related family, member 3	0.084	TCATATACATTAAGTTGAGCCATATGTAATCACTGTGTTTGTAGGTTAGAAACAGCTGAG

59	CTSF	NM_003793	cathepsin F	-0.179	CCTCTCCATGTCCAGGAAACTTGTAACCACCCTTTTCTAACAGCAATAAAGAGGTGTCCT
60	SULT1C1	NM_001056	sulfotransferase family, cytosolic, 1C, member 1	0.043	GACGTCATTTGAGAAAATGAAAGAAAATCCCATGACAAATCGTTCTACAGTTTCCAAATC
61	RASL11B	NM_023940	RAS-like, family 11, member B	-0.228	TGCCTAAGGGTGGCTGAAATACTAAAACACTATCTTACAGCAAGTGAACAGGGGCTACCT
62	LOC148898	NM_001008896	NA	-0.108	AGGGTCTCCAATTTAGGCTTTCAACATTATCTCTAAAGAAGGTTATACATTATGTCGGCT
63	HMGCL	NM_000191	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase (hydroxymethylglutaricaciduria)	0.014	GGACATGGAAATGAGAATAGGTTAAATGGTGCAGGTACCTCATAGCCAGCTCTACACAGA
64	IGHA1		immunoglobulin heavy constant alpha 1	0.940	TGCTGAGTTGGGTTTTCCTTGCTGCTATTTTAAAAGGTGTCCAGTGTGAGGTGCAGCTGG
65	C1QTNF3	NM_030945	C1q and tumor necrosis factor related protein 3	-0.180	GTTGAGGGTTTTACATTGCTGTATTCAAAAAATTATTGGTTGCAATGTTGTTCACGCTAC
66	NKI		0	-0.292	CATACGGTTTTGTTTGGAGGATGGCTTCTGCTGCTAAAAATACAAAAGTTTGGAAACCGC
67	IL11RA	NM_004512	interleukin 11 receptor, alpha	-0.075	GAGCCCATTTCTGTGAGACCCTGTATTTCAAATTTGCAGCTGAAAGGTGCTTGTACCTCT
68	ADRA2C	NM_000683	adrenergic, alpha-2C-, receptor	-0.685	TAGTCGGGGGGTGGCTGCCAGGGGGCAAGGAGAAAGCACCGACAATCTTTGATTACTGAA
69	IGKC		immunoglobulin kappa constant	0.943	CCATCAGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCAGTATAATAACT
70	CEACAM5	NM_004363	carcinoembryonic antigen-related cell adhesion molecule 5	-0.252	AGTTCTCTTTATCGCCAAAATCACGCCAAATAATAACGGGACCTATGCCTGTTTTGTCTC
71	PURB	NM_033224	purine-rich element binding protein B	-0.108	TCTGTGAATGGAACTGAAGTGAACGTGAATATGCTGACTATATCCTGGAAGCATTTTTAT
72	TPD52	NM_001025252	tumor protein D52	0.181	AACATTGCCAAAGGGTGGCAAGACGTGACAGCAACATCTGCTTACAAGAAGACATCTGAA
73	SLAMF1	NM_003037	signaling lymphocytic activation molecule family member 1	0.432	AGGCGCAGAACAGAGCGTTACTTGATAACAGCGTTCCATCTTTGTGTTGTAGCAGATGAA
74	GCH1	NM_000161	GTP cyclohydrolase 1 (dopa-responsive dystonia)	0.191	TATTCCATGAAGTTTAGTATTTGGTTGACATAGTGCTCTTCAAATTGATCCCATTACCCT
75	KLRB1	NM_002258	killer cell lectin-like receptor subfamily B, member 1	0.243	TCAACCCTTGGAATAACAGTCTAGCTGATTGTTCCACCAAAGAATCCAGCCTGCTGCTTA
76	TRIB2	NM_021643	tribbles homolog 2 (Drosophila)	0.064	ACGGCTTTTCTATTGCTGTATGATACAGAACTCTTTTGGCATAAATATTTGTGTTCCCAG

77	DNAJB9	NM_012328	DnaJ (Hsp40) homolog, subfamily B, member 9	0.430	ATTTCTTTCTTAGTTGTTGGCACTCTTAGGTCTTAGTATGGATTTATGTGTTTGTGTGTG
78	KHDRBS3	NM_006558	KH domain containing, RNA binding, signal transduction associated 3	-0.436	ATGATGAACAGAGTTATGATTCCTATGATAACAGCTATAGCACCCCAGCCCAAAGTGGTG
79	TUB	NM_003320	tubby homolog (mouse)	0.045	CTCTAGGTCCATTTTCCTAACCACAAGATAAAGATGTTACATTGTCAAAGCTTGCCGTAG
80	VNN2	NM_004665	vanin 2	0.358	AAAGAGCCTGGGTGTTTGGGTCAGATAAATGAAGATCAAACTCCAGCTCCAGCCTCATTT
81	PDLIM4	NM_003687	PDZ and LIM domain 4	-0.240	TGCTCCCACGCCTGCTTCTTAAGGTCCCTGCTCGGCCGGTGTAAATATGTTTCACCCTGT
82	ARHGAP15	NM_018460	Rho GTPase activating protein 15	0.460	AATGCATTGAAGCTGTTGAGAAAAGAGGTCTAGATGTTGATGGAATATATCGAGTTAGTG
83	SLC16A12	NM_213606	solute carrier family 16, member 12 (monocarboxylic acid transporter 12)	-0.111	TTATAGTGGGATAATTTTACATCTTAAATATTTCTTTCTACTACTGTAAGCTCTACTTTG
84	IGKV1D-13		immunoglobulin kappa variable 1D-13	1.032	GAAAGCTCCTAAGCTCCTGATCTATGATGCCTCCAGTTTGGAAAGTGGGGTCCCATCAAG
85	TBRG4	NM_004749	transforming growth factor beta regulator 4	-0.247	CCATTCTATGAGTGGCTGGAACTCAAGTCTGAATGGCAGAAAGGCGCCTACCTCAAGGAC
86	MEGF6	NM_001409	multiple EGF-like-domains 6	-0.446	AGGCAGGCTTTTTGGTGCTAGGCCCTGGGACTGGAAGTCGCCCAGCCCGTATTTATGTAA
87	FCRLM1	NM_032738	Fc receptor-like and mucin-like 1	0.477	GACATACCAGTCTTTAGCTGGTGCTATGGTCTGTTCTTTAGTTCTAGTTTGTATCCCCTC
88	FCGR2B	NM_001002273	Fc fragment of IgG, low affinity IIb, receptor (CD32)	0.323	AATCCCACTAATCCTGATGAGGCTGACAAAGTTGGGGCTGAGAACACAATCACCTATTCA
89	C1orf24	NM_052966	chromosome 1 open reading frame 24	0.126	AAATCGACACTGTGGATTGACTTTCCCGGTCACTATATAAAGCAAATAAACTTAAAACAC
90	ANKRD38	NM_181712	ankyrin repeat domain 38	-0.307	ATGCCATATGTACAGTCTTGACTATTTCTGAGTCATCTAGTGGCTCCAATTTGCTCCAGG
91	POU2AF1	NM_006235	POU domain, class 2, associating factor 1	0.937	TTTTCTGGGAAATGACTTTTCTGGGAAATGACAGTTTCTTTGACATATTTTCTTTGCCCA
92	LOC441212	NM_001039754	NA	-0.325	TCTTTATCAAAGACAACCAAAAGTTACAACAGTTCAGAGTAGCACATGAGGATTTCATGT
93	CTSS	NM_004079	cathepsin S	0.445	TCTGTTGGTGTAGATGCGCGTCATCCTTCTTTCTTCCTCTACAGAAGTGGTGTCTACTAT
94	L3MBTL	NM_015478	l(3)mbt-like (Drosophila)	0.004	TTTGCTTGCCAAACTTAGCTTTGCCAGTGATAGTCAATATTAAAGTGTACTTTTTTCCCC
95	CDKN1C	NM_000076	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	-0.239	GTATTCTGCACGAGAAGGTACACTGGTCCCAAAGTGTAAAGCTTTAAGAGTCATTTATAT
96	AMPD1	NM_000036	adenosine monophosphate deaminase 1 (isoform M)	0.395	GGAATTTCTCATGAGGAGAAAGTAAAGTTTCTGGGCGACAATTACCTTGAGGAAGGCCCT

97	TMED4	NM_182547	transmembrane emp24 protein transport domain containing 4	-0.117	CAGTTGCTTGATCAGGTGGAACAGATTCAGAAGGAGCAGGATTACCAAAGGTATCGTGAA
98	LAMB2	NM_002292	laminin, beta 2 (laminin S)	-0.227	CCCACATGCATGTCTGCCTATGCACTGAAGAGCTCTTGGCCCGGCAGGGCCCCCCATAAA
99	DTX3	NM_178502	deltex 3 homolog (Drosophila)	-0.212	CTGTGAGGAACCTCCTTACCCTGTTCTGGAATCGCTGCCAGACTGTAGCTTTTAATTTAA
100	MAP2K6	NM_002758	mitogen-activated protein kinase kinase 6	0.036	ACAGCATCAATAGAAAGTCATCTTTGAGATAATTTAACCCTGCCTCTCAGAGGGTTTTCT
101	PDGFRB	NM_002609	platelet-derived growth factor receptor, beta polypeptide	0.719	TAGGTGATTATATCTTTGGTACCGTATTGAGAACCCACTCTCCCTCCTTGGACCAACTCT
102	IGLV2-14		immunoglobulin lambda variable 2-14	0.968	CATCACTGGTCTCCAGGCTGAGGACGAGGCTGATTATTACTGCAGCTCATATACAAGCAG
103	ANKH	NM_054027	ankylosis, progressive homolog (mouse)	-0.166	TTATTGGCAGCAGTTTTATAAAGTCCGTCATTTGCATTTGAATGTAAGGCTCAGTAAATG
104	XBP1	NM_005080	X-box binding protein 1	0.644	CCTTTTTGGCATCCTGGCTTGCCTCCAGTTTTAGGTCCTTTAGTTTGCTTCTGTAAGCAA
105	LOC283174	NA	NA	-0.115	CCCGGGAGTGTTGCAAGTTAAACTGATGAAAAGACGTTTAGTATTTAATTGCTCCTCATG
106	PGM5	NM_021965	phosphoglucomutase 5	0.330	CTAACAGCCAGCCACTGCCCTGGAGGACCAGGGGGAGAGTTTGGAGTGAAGTTTAATGTT
107	ISYNA1	NM_016368	NA	-0.247	TACCCTATGTTGAACAAGAAAGGACCGGTACCCGCTGCCACCAATGGCTGCACCGGTGAT
108	PGRMC1	NM_006667	progesterone receptor membrane component 1	0.161	TGCCCGGAAAAATGATTAAAGCATTCAGTGGAAGTATATCTATTTTTGTATTTTGCAAAA
109	IGL@		immunoglobulin lambda locus	0.950	AAGATAGCAGCCCCGTCAAGCGGGAGTGGAGACCACCACACCCTCCAAACAAAGCAACAA
110	EFHA2	NM_181723	EF-hand domain family, member A2	-0.457	GCCACATGCAGGGTTCAGAATAGCTTTCAACATGTTTGACACTGATGGCAATGAGATGGT
111	CCM2	NM_001029835	cerebral cavernous malformation 2	-0.009	TCGGCACCCTCAGAGGGGGATGAGTGGGACCGCATGATCTCGGACATCAGCAGCGACATT
112	CTA-246H3.1	NM_001013618	NA	0.923	AACAAGGCCACACTGGTGTGTCTCATGAATGACTTCTATCTGGGAATCTTGACGGTGACC
113	SMR3A	NM_012390	submaxillary gland androgen regulated protein 3 homolog A (mouse)	0.863	CACCCTATGGTCCAGGGAGAATTCAATCACACTCTCTTCCTCCTCCTTATGGCCCAGGTT
114	TNFRSF17	NM_001192	tumor necrosis factor receptor superfamily, member 17	0.902	GATCTCTTTAGGATGACTGTATTTTTCAGTTGCCGATACAGCTTTTTGTCCTCTAACTGT
115	PDE6B	NM_000283	phosphodiesterase 6B, cGMP-specific, rod, beta (congenital stationary night blindness 3, autosomal dominant)	-0.156	ACTGAGAACATTTGCAGCCACACATGTACATATGTGTACACAGGTAGACAGATGGACACA
116	BEX2	NM_032621	brain expressed X-linked 2	-1.281	ATTTCTTGTGGGTCTCCTATTACCAGCTTCTAAATGAATGTTGTTTTTGACCCAGTTTGT

117	PTPN21	NM_007039	protein tyrosine phosphatase, non-receptor type 21	0.006	TTACTGAAGCTATGCTGGGCAATTCTGGCAATCATTAAAGTGCATAGATTTCTATCTTAA
118	LGR6	NM_001017403	leucine-rich repeat-containing G protein-coupled receptor 6	-0.096	TAAGCTTTGGAAGAGATTACACATGATGTCTTTTTCTTAGAGATTCACAGTGCATGTTAG
119	BAI2	NM_001703	brain-specific angiogenesis inhibitor 2	-0.008	ATATATATATCTCTCTATTTTCAGACTCCACTTTGGAACTACCCAGGAGCCAGCGCCCTC
120	FLJ25006	NM_144610	NA	0.033	TGTTTGTACTGATACTAGACCATTTAGAGCCCAATTTGTGGTCTACCTTCAGCAAGTGTT
121	CPNE5	NM_020939	copine V	0.608	TGGTTCTGTGCCCGTCTCTGAGACAGTCTCTGTGTGGAATTTGCCTTAAACTGAAGTAAA
122	LOC647115	XM_930136	NA	0.507	CGAGACCCACCTTCCTCTTCCTTTAGCAGCTGGGAAATTGGGGGCGTTTATGGCGCCCCG
123	KCNN3	NM_002249	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3	0.495	AGTGACCAAGCCAACACTCTGGTGGACCTTTCCAAGATGCAGAATGTCATGTATGACTTA
124	SLAMF7	NM_021181	SLAM family member 7	0.617	GGAGACCTCCCTACCAAGTGATGAAAGTGTTGAAAAACTTAATAACAAATGCTTGTTGGG
125	TTC22	NM_017904	tetratricopeptide repeat domain 22	0.571	AGAACCAACCTCCCATCCTGAATCGCCTGGCAAAAATCTTCTACTTCCTGGGAAAGCAGG
126	LIPA	NM_000235	lipase A, lysosomal acid, cholesterol esterase (Wolman disease)	0.195	TATAATTACTTTAGCTGCACTAACAGTACAATGCTTGTTAATGGTTAATATAGGCAGGGC
127	DHRS8	NM_016245	dehydrogenase/reductase (SDR family) member 8	0.044	TGCACAGGGAAGCTAGAGGTGGATACACGTGTTGCAAGTATAAAAGCATCACTGGGATTT
128	SLC2A14	NM_153449	solute carrier family 2 (facilitated glucose transporter), member 14	-0.263	CTGACTTAGGGTTAGAATGGCCCAATGATCCTACAACTTTTTGATGCTATTTCATTTGAT
129	PRG1	NM_002727	proteoglycan 1, secretory granule	0.337	AGGACTTGGGTCAACATGGATTAGAAGAGGATTTTATGTTATAAAAGAGGATTTTCCCAC
130	dJ222E13.2	NR_002184	NA	-0.158	ACGGAAGCGCAGCCAAAAAGAGCTGCTCAACTACGCCTGGCAGCATCGAGAGAGCAAGAT
131	FYB	NM_001465	FYN binding protein (FYB-120/130)	0.248	GATCAAGAGAATATTTCAGAGTTTTGGTTTACACATCAAGAAACAGACACACATACCTAG
132	PTPRC	NM_002838	protein tyrosine phosphatase, receptor type, C	0.391	TCAATGGTCCTGCAAGTCCAGCTTTAAATCAAGGTTCATAGGAAAAGACATAAATGAGGA
133	ICAM5	NM_003259	intercellular adhesion molecule 5, telencephalin	-0.089	GACGCCCCCCAGACTCACACGGGGGCTTATTTATTGCTTTATTTATTTACTTATTCATTT
134	CCRL2	NM_003965	chemokine (C-C motif) receptor-like 2	0.172	GTGAGCTAACATTTGCTAAGCACTGAATTTGTCTCAGGCACCGTGCAAGGCTCTTTACAA
135	CCR5	NM_000579	chemokine (C-C motif)	0.177	AACAGTAGCATAGGACCCTACCCTCTGGGCCAAGTCAAAGACATTCTGACATCTTAGTAT

			receptor 5		
136	CLIC2		chloride intracellular channel 2	0.276	GAGAGTGAGCATATCAGAGAGGCAAATTCTTAAAGAATGATTTTTAAAATCAGCTCTAGG
137	WNT11	NM_004626	wingless-type MMTV integration site family, member 11	-0.449	TTTGCTTTTTCTTCCTTTGGGATGTGGAAGCTACAGAAATATTTATAAAACATAGCTTTT
138	SAMSN1	NM_022136	SAM domain, SH3 domain and nuclear localisation signals, 1	0.615	CTCTGGTTGCTATATCTCATCAGGAAATTCAGATAATGGCAAAGAGGATCTGGAGTCTGA
139	PRLR	NM_000949	prolactin receptor	-0.087	CTCTTGTTATCATCAGGTTCACATTAAAAACAGATACTTACAAACTGACTTGAAGCACAG
140	LRRC18	NM_001006939	leucine rich repeat containing 18	-0.271	ACAGGAAACCAAGGGCTCCCCTGTGGCTGCAGCAGCTCTTTCAGCCAAGCCCATAAAACT
141	LOXL4	NM_032211	lysyl oxidase-like 4	-0.227	GTCTCAACCAAGTGTCTGAAGTGAACTTTGCATTGAATAAATTTTTGCCATGGAAAGAAC
142	CD3G	NM_000073	CD3g molecule, gamma (CD3-TCR complex)	0.109	GTTCCCAGAGATGACAAATGGAGAAGAAAGGCCATCAGAGCAAATTTGGGGGTTTCTCAA
143	RAB2	NM_002865	RAB2, member RAS oncogene family	0.345	ACACTACAAAGTCATCTTGAGTATTTTAAATCGGTTTGTGTAGTTAGGTTTCCCAACATC
144	NPDC1	NM_015392	neural proliferation, differentiation and control, 1	-0.511	CACTAAAAACATGTTTTGATGCTGTGTGCTTTTGGCTGGGCCTCGGGCTCCAGGCCCTGG
145	AMACR	NM_014324	alpha-methylacyl-CoA racemase	-0.114	ACGAGCTGCTGATCAAAGGACTTGGACTAAAGTCTGATGAACTTCCCAATCAGATGAGCA
146	PRAME		preferentially expressed antigen in melanoma	0.032	GTGATGAACCCCTTGGAAACCCTCTCAATAACTAACTGCCGGCTTTCGGAAGGGGATGTG
147	CCR2	NM_000647	chemokine (C-C motif) receptor 2	0.553	ATGAAGTCATGCGTTTAATCACATTCGAGTGTTTCAGTGCTTCGCAGATGTCCTTGATGC
148	SLC25A22	NM_024698	solute carrier family 25 (mitochondrial carrier: glutamate), member 22	-0.308	TTTTTTCTTTTGAAGAGTTTTAAGAAGTTGTAACTTTTTGTGTCTTGTCATGTCAGAGAA
149	MC1R	NM_002386	melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)	-0.401	CAGTCGCCCAAGCAGACAGCCCTGGCAAATGCCTGACTCAGTGACCAGTGCCTGTGAGCA
150	RHOD	NM_014578	ras homolog gene family, member D	-0.091	TCATCGTCGTGGGCTGCAAGACTGACCTGCGCAAGGACAAATCACTGGTGAACAAGCTCC
151	FCGR3B	NM_000570	Fc fragment of IgG, low affinity IIIb, receptor (CD16b)	0.368	TGGTGATGGTACTCCTTTTTGCAGTGGACACAGGACTATATTTCTCTGTGAAGACAAACA
152	SAMM50	NM_015380	sorting and assembly machinery component 50 homolog (S. cerevisiae)	-0.130	CTTTGGAGAACTTTTCCGAACACACTTCTTTCTCAACGCAGGAAACCTCTGCAACCTCAA

CA 02689714 2009-11-30

153	PABPC3	NM_030979	poly(A) binding protein, cytoplasmic 3	-0.071	ATTGATCAGAGACCACGAAAAGAAATTTGTGCTTCACCGAAGAAAAATATCTAAACATCG
154	CXCR4	NM_001008540	chemokine (C-X-C motif) receptor 4	0.361	TGCTGGTTTTTCAGTTTTCAGGAGTGGGTTGATTTCAGCACCTACAGTGTACAGTCTTGT
155	TMEM154	NM_152680	transmembrane protein 154	0.072	GCATTTTCGTACATTTTAAGCAAACTAGGTTAACAACAACATAGCCTAGTCAAACTTCTC
156	MAFA	NM_201589	v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (avian)	-0.224	GTTCGAGGTGAAGAAGGAGCCTCCCGAGGCCGAGCGCTTCTGCCACCGCCTGCCGCCAGG
157	PAX8	NM_003466	paired box gene 8	0.590	CAAGCTTCCTTCTTTCTAACCCCCAGACTTTGGCCTCTGAGTGAAATGTCTCTCTTTGCC
158	LIN7A	NM_004664	lin-7 homolog A (C. elegans)	-0.265	TTGAGGGAAAGCTACTTGATCAAACATCCGATAGTCACAAATTTGAAACCGTGCTTCAGA
159	CRTAM	NM_019604	cytotoxic and regulatory T cell molecule	0.168	AAGCAGAATAGATGTTTGTTTTTCTAGTGGTTATACCAAGCTATACTTCCTGTTTTCACG
160	SLC22A5	NM_003060	solute carrier family 22 (organic cation transporter), member 5	0.018	TTCAGAGTAGCTCACTTTAGTCCTGTAACTTTATTGGGTGATATTTTGTGTTCAGTGTAA
161	LOC402176	NM_001011538	NA	0.308	ATGAACACAAAGGGGGAAGAGGAGAGGCACCGGTATACATTCTCTAGGCCTTTTAGAAAA
162	EBI2	NM_004951	Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)	0.510	CTGAAACGGCAAGTCAGTGTATCGATTTCTAGTGCTGTGAAGTCAGCCCCTGAAGAAAAT
163	REEP4	NM_025232	receptor accessory protein 4	-0.231	CCACATGCAGGGATGCACCCACAATGTACCAAAGCAGGCTGGGCCCAGGGTTCTATTTAT
164	KIAA1946	NM_177454	KIAA1946	-0.627	TTTGAATCCTCTGGTATCAATACGTATTATAGGGTTTTAGAGATCTGTGGGTCAAATGAT
165	PABPC1	NM_002568	poly(A) binding protein, cytoplasmic 1	-0.077	TGTTCCAACTGTTTAAAATTGATCAGGGACCATGAAAAGAAACTTGTGCTTCACCGAAGA
166	LOC652106	XM_941436	NA	0.749	CTCCAGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTAGTAGTAGTACCATATACT
167	IGLL1	NM_020070	immunoglobulin lambda-like polypeptide 1	0.916	TCCAAGCCAACAAGGCTACACTGGTGTGTCTCATGAATGACTTTTATCCGGGAATCTTGA
168	MEG3	NR_002766	maternally expressed 3	-1.459	CCGCAGGAACCCTGAGGCCTAGGGGAGCTGTTGAGCCTTCAGTGTCTGCATGTGGGAAGT
169	PEPD	NM_000285	peptidase D	-0.032	ATGCTGTTCTTTAGTAGCAACTAAAATGTGTCTTGCTGTCATTTATATTCCTTTTCCCAG
170	OAT	NM_000274	ornithine aminotransferase (gyrate atrophy)	0.162	TAATGTAATGGCATCTATATTCAGTTGAAGTGTTTTGATGTGCATGTGTACTTCCTAAGG
171	FBXL13	NM_145032	F-box and leucine-rich repeat protein 13	-0.059	ATGCCATTACCTGCACATTTTGGATATCTCTGGTTGTGTCTTGCTTACTGACCAAATCCT
172	IFI6	NM_002038	interferon, alpha-inducible protein 6	0.056	AGTAGCCAGCAGCTCCCAGAACCTCTTCTTCCTTCTTGGCCTAACTCTTCCAGTTAGGAT
173	IL2RB	NM_000878	interleukin 2 receptor, beta	0.248	TTGAGGTTGTCTGAGTCTTGGGTCTATGCCTTGAAAAAAGCTGAATTATTGGACAGTCTC

174	PRKAB2	NM_005399	protein kinase, AMP-activated, beta 2 non-catalytic subunit	0.111	GGGAATTAAATATGTGAGTCCTCTTTTTAATGGTGCTTTTTGTAACCTTTAATGCTGAGG
175	FKSG44	NM_031904	NA	0.057	ACTCATTCTTTGAATGTTCTCATTCTTTTGTATCATGTGACTTATTAAAATCAGTTTCTA
176	TPD52	NM_001025252	tumor protein D52	0.111	AACTGCTTACTCAACACTACCACCTTTTCCTTATACTGTATATGATTATGGCCTACAATG
177	RIMS2	NM_014677	regulating synaptic membrane exocytosis 2	-0.668	GATGAACTAGAGCTATCCAATATGGTGATTGGATGGTTCAAACTTTTCCCACCTTCCTCC
178	APCDD1	NM_153000	adenomatosis polyposis coli down-regulated 1	-0.621	GTTTTATATGCTGGAATCCAATGCAGAGTTGGTTTGGGACTGTGATCAAGACACCTTTTA
179	Rgr	NM_153615	NA	0.121	CCATGGGACTTTTGTGAGTCAGGCGGGAGACCATTTTATGTTTATTTTCTTTAGTGTATA
180	C2orf27	NM_013310	chromosome 2 open reading frame 27	-0.120	GGATTTATTTATAGCTTAACTAAGAATTTCAAATTTCTACCACAACACTGAAATAAAGTT
181	TRAK1	NM_001042646	trafficking protein, kinesin binding 1	0.576	TAAGAAACATCAACCAGGTTGTCAAGCAGAGATCTCTGACCCCTTCTCCCATGAACATCC
182	MMP11	NM_005940	matrix metallopeptidase 11 (stromelysin 3)	-0.602	GGCCAAAAAGTTCACAGTCAAATGGGGAGGGGTATTCTTCATGCAGGAGACCCCAGGCCC
183	COL6A3	NM_004369	collagen, type VI, alpha 3	0.054	GACCCTCGCTCTCTGTCTCCAGCAGTTCTCTCGAATACTTTGAATGTTGTGTAACAGTTA
184	UTX	NM_021140	ubiquitously transcribed tetratricopeptide repeat, X chromosome	0.182	AATGCTGTTATTTTTTCCAGATTTACCTGCCATTGAAATTTTAAGGAGTTCTGTAATTTC
185	PCSK5	NM_006200	proprotein convertase subtilisin/kexin type 5	-0.248	TGCCAACGGAAGGTTCTTCAACAACTTTGCTGCAAAACATGTACATTTCAAGGCTGAGCA
186	AYTL1	NM_017839	acyltransferase like 1	-0.057	GAAGAATTCGCCAAGTATTTAAAGTTGCCTGTTTCAGATGTCTTGAGACAACTTTTTGCA
187	RNF13	NM_007282	ring finger protein 13	0.357	CTGTCTCATCTTGATAGTCATTTTCATGATCACAAAATTTTTCCAGGATAGACATAGAGC
188	CTA-126B4.3	NM_015703	NA	-0.285	TGCTGTGATTGTATCCGAAGTAGTCCTCGTGAGAAAAGATAATGAGATGACGTGAGCAGC
189	LRAT	NM_004744	lecithin retinol acyltransferase (phosphatidylcholine--retinol O-acyltransferase)	0.130	AGGAAGAGTCAACAGACTTTAGCAAAATCCTTTTATTTGATTCATGCATAACTCCTGATG
190	C9orf127	NM_001042589	chromosome 9 open reading frame 127	-0.270	AGCCTTCCCAAGACATGGATTCCTTCCCAGGGAGACAAAGCCCTGTCAGGAGCACAGCAT
191	LCOR	NM_032440	ligand dependent nuclear receptor corepressor	-0.038	TTCATGTCTGTGAAGCTTTTAAACATTACACTTGAGATCAGTCATGACTTGATATTCAGG
192	SPN	NM_001030288	sialophorin (leukosialin, CD43)	0.682	TCCTCACCCACCTCTTCACTCTGAATCCTCATGAGGCTTCTCAGCCCTGGATTTCCTGCT
193	CAMK2N1	NM_018584	calcium/calmodulin-dependent protein kinase II inhibitor 1	-0.750	TGTTATTGAAGATGATAGGATTGATGACGTGCTGAAAAATATGACCGACAAGGCACCTCC

194	BCL2A1	NM_004049	BCL2-related protein A1	-0.069	TGTAACCATATTTGCATTTGAAGGTATTCTCATCAAGAAACTTCTACGACAGCAAATTGC
195	TP53TG3	NM_016212	NA	-0.389	TCTTGTGTATTTATTACATTTTCACGTGTCTTCACGCATCTCTTGAATTGGAAATTGTGC
196	CD247	NM_000734	CD247 molecule	0.321	CAAAGTGGCATAAAAAACATGTGGTTACACAGTGTGAATAAAGTGCTGCGGAGCAAGAGG
197	MAP1B	NM_005909	microtubule-associated protein 1B	-0.775	TTGCAGTAATGATATTTATTAAAAACCCATAACTACCAGGAATAATGATACCTCCCACCC
198	CREB3	NM_006368	cAMP responsive element binding protein 3	-0.079	GAGGGGCTTATTCTGCCTGAGACACTTCCTCTCACTAAGACAGAGGAACAAATTCTGAAA
199	FLJ20054	NM_019049	NA	0.206	GGTACTAGTTTGTATGTATGTTTAAAGTATGTATTGACCATGAGATTTCCCAGTGTTTGG
200	ACSL1	NM_001995	acyl-CoA synthetase long-chain family member 1	0.806	ACTCGGTTCTCCAGGCCTGATTCCCCGACTCCATCCTTTTTCAGGGTTATTTAAAAATCT
201	GIMAP2	NM_015660	GTPase, IMAP family member 2	0.518	ATGACCAAGTGAAGGAACTAATGGACTGTATTGAGGATCTGTTGATGGAGAAAAATGGTG
202	IGHG1		immunoglobulin heavy constant gamma 1 (G1m marker)	0.296	GTTGGACCACAAACTATGCACAGAAGTTTCAGGGGAAGGTCACCATGACCAAGGACACGT
203	MAZ	NM_001042539	MYC-associated zinc finger protein (purine-binding transcription factor)	-0.413	GCTGTGCACCTTCATGTGGTCCGAAATATAAGCCGAGCTCAGCATCTTGCCACACACGTG
204	LOC648674	XM_937741	NA	0.027	CTCATAAGTGGGGCTATACTGTGAAGGGCATTCAGAAATACAAAGCAAAGGTTATTTCCG
205	P2RY8	NM_178129	purinergic receptor P2Y, G-protein coupled, 8	0.483	AACACAGGTCTATTGACTCACACACATGTTTTAAGATGGAAAACTTTACTTCTGTTCTTG
206	TMEM158	NM_015444	transmembrane protein 158	-0.304	CCAACGCGCACGGCCGCGCTTTCTTCGCCGCCGCCTTCCACCGCGTCGGGCCGCCGCTGC
207	RAB1A	NM_004161	RAB1A, member RAS oncogene family	0.637	CAAAATAAGAACTATAGAGTTAGACGGGAAAACAATCAAGCTTCAAATATGGGACACAGC
208	LOC399959	NA	NA	0.029	CATTTCTAACAAGCATCTTCTTAACCAACTTTATGCACAGTGTATGTTTGTAAGTGCTTC
209	LMAN1	NM_005570	lectin, mannose-binding, 1	0.794	TTGACTACCATTTTCCTGTGTACTTCATCTATTTGTGTACAAAATGATGTCGTTTTGAGG
210	OPHN1	NM_002547	oligophrenin 1	0.096	TTATCATGGGAAAGTATTCTCTTTTCAAGAAGTTCTTTGATTCTGTAATAACTAGAACAA
211	SGK3	NM_001033578	serum/glucocorticoid regulated kinase family, member 3	0.239	GTATGTCTTGAGAAAGAAATCACAGAAGCATTTCTCACCAATACTCTTTGGCTTAAAATG
212	DUSP15	NM_001012644	dual specificity phosphatase 15	-0.314	AAGCGCTGCCGGCAGGGCTCCGCGACCTCGGCCTCCTCCGCCGGGCCGCACTCAGCAGCC
213	DEAF1	NM_021008	deformed epidermal autoregulatory factor 1 (Drosophila)	-0.251	CGGGCACATGGACATGGGCGCCGAGGCCCTGCCCGGCCCCGACGAGGCCGCCGCTGCCGC
214	NEUROG3	NM_020999	neurogenin 3	0.156	CATTCAAAGAATACTAGAATGGTAGCACTACCCGGCCGGAGCCGCCCACCGTCTTGGGTC

CA 02689714 2009-11-30

215	TLX2	NM_016170	T-cell leukemia homeobox 2	-0.261	ACGGAGCCTCGGGCTACGGTCCCGCCGGCTCACTTGCCCCGCTGCCCGGCAGCTCCGGAG
216	LAMC1	NM_002293	laminin, gamma 1 (formerly LAMB2)	0.040	ACCTTAATTACACTCCCGCAACACAGCCATTATTTTATTGTCTAGCTCCAGTTATCTGTA
217	NKI			-0.145	#N/A
218	BBS2	NM_031885	Bardet-Biedl syndrome 2	0.025	TGGGGACAGCTTCTTCCTAGGTGAGGAAAATACAGGTCATGAAGTTCCTGGCAAAGATTT
219	CKB	NM_001823	creatine kinase, brain	-0.193	AGAAATGAAGCCCGGCCCACACCCGACACCAGCGCCTGCTGCTTCCTAACTTATTGCCTGG
220	LOC389199	NM_203423	NA	-0.310	CCCGCCCCACGAGTGGGTCTTCGCAGGGCCCCCTCTGACGCACACGGGGACCAGCCACGC
221	SALL1	NM_002968	sal-like 1 (Drosophila)	-0.373	CCTCAGTGATGCATTAGATCTCTAATAAAGTCTGTATATACATGTACACTTTGATCCTGC
222	ANKRD20A2	NM_001012421	ankyrin repeat domain 20 family, member A2	-0.043	AGACGGTCAGCTCTCATGCTTGCTGTATACTATGACTCACCAGGTATTGTCAGTATCCTT
223	FOXC2	NM_005251	forkhead box C2 (MFH-1, mesenchyme forkhead 1)	-0.202	TCAACCACAGCGGGGACCTGAACCACCTCCCCGGCCACACGTTCGCGGCCCAGCAGCAAA
224	SMEK2	NM_020463	NA	0.259	TGTGGAAGATACTTTGAAATCACTTTCTACTTTGTTAGTAAAGTTCTGTCTTTCCAGAGC
225	CYP2R1	NM_024514	cytochrome P450, family 2, subfamily R, polypeptide 1	0.172	CTCATCTGTGCTGAAAGACGCTGAAACTGCCTGGGATGTTTTCGGGAACAAGAATGTATA
226	ZNF205	NM_001042428	zinc finger protein 205	-0.315	TACGTGTGCGACCGCTGCGCCAAGCGCTTCACCCGCCGCTCGGACTTGGTCACCCACCAG
227	ATP5L	NM_006476	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit G	0.176	ACACGTCTGTTTAGCCCGCAATTGGAAAGGATATATGTGGCAATATTAACCTGGTACATG
228	FANCC	NM_000136	Fanconi anemia, complementation group C	0.016	CTATTTGCGACACGAACTGTGCCCAATGTGTGCCCAAGGACAAGGCTATTAACAAATTCA
229	ZDHHC4	NM_018106	zinc finger, DHHC-type containing 4	-0.128	CTTCGGAGCAACCTTCAAGAGATCTTTCTACCTGCCTTTCCATGTCATGAGAGGAAGAAA
230	PRR5	NM_001017528	proline rich 5 (renal)	-0.382	AAAGCGCCTCCTCCGCCGCTCCCGCTCGGGGGACGTGCTGGCCAAGAACCCTGTGGTGCG
231	MIER2	NM_017550	mesoderm induction early response 1, family member 2	0.333	CATCCCTCACCCCACCAAGGACCACACTGTGAAGTGATAACTGCCTTGAACCCCCCTTTG
232	NT5C2	NM_012229	5'-nucleotidase, cytosolic II	0.278	CGTTGCTTTAGGGCAGGATTCTATTTTGAGGGAAAAGACAGTATCCTTATTACCTTTTGT
233	BEX1	NM_018476	brain expressed, X-linked 1	-2.105	TGAACCAGTCTGTAAGATTTTTGTTAGCAGAAGAATTTTACCTATTGCATGGAAAGATGC
234	OSTalpha	NM_152672	NA	0.038	ACGAATGTACTACCGAAGGAAAGACCACAAGGTTGGGTATGAAACTTTCTCTTCTCCAGA
235	TMSL3	NM_183049	thymosin-like 3	0.557	CTTTTAGCTGTTTAACTTTGTAAGATGCAAAGAGGTTGGATCAAGTTTAAATGACTGTGC
236	CCL3	NM_002983	chemokine (C-C motif) ligand 3	0.003	TGGGAAACATGCGTGTGACCTCCACAGCTACCTCTTCTATGGACTGGTTGTTGCCAAACA
237	CSF3	NM_000759	colony stimulating factor 3 (granulocyte)	0.195	GGGTCCCACGAATTTGCTGGGGAATCTCGTTTTTCTTCTTAAGACTTTTGGGACATGGTT

Table 4

Univariate and multivariate analysis for overall and relapse-free survival

	Cox-Ranked Univariate			Cox-Ranked Multivariate		
	Hazard ratio	*(95% CI)*	P-value	Hazard ratio	*(95% CI)*	P-value
Overall survival						
72-gene classifier *(low-risk vs. high-risk)*	4.83	*(2.47-9.44)*	4.1E-06	4.70	*(2.40-9.21)*	6.4E-06
Histology *(squamous, adeno or other)*	0.82	*(0.55-1.21)*	0.31	0.89	*(0.57-1.40)*	0.62
Tumor Stage *(Grade I vs. II)*	2.22	*(1.27-3.88)*	0.0049	2.13	*(1.21-3.73)*	0.0084
Relapse-free survival						
72-gene classifier *(low-risk vs. high-risk)*	4.86	*(2.49-9.50)*	3.7E-06	4.61	*(2.36-9.03)*	8.4E-06
Histology *(squamous, adeno or other)*	0.79	*(0.53-1.18)*	0.25	0.87	*(0.55-1.37)*	0.54
Tumor Stage *(Grade I vs. II)*	2.27	*(1.30-3.97)*	0.004	2.08	*(1.19-3.64)*	0.011

72-gene classifier & Tumor Stage	Overall survival	6.2E-08
	Relapse-free survival	3.3E-07

Claims

1. A method for typing a sample of an individual suffering from non-small cell lung cancer or suspected of suffering therefrom, the method comprising

a. providing a tissue sample from said individual comprising non-small cell lung cancer cells or suspected to comprise non-small cell lung cancer cells;

b. preparing RNA from said tissue sample;

c. determining RNA levels for a set of genes in said RNA; and

d. typing said sample on the basis of the levels of RNA determined for said set of genes,

wherein said set of genes comprises at least two of the genes listed in Table 3.

2. Method according to claim 1, whereby said sample is a sample of an individual suffering from early stage NSCLC or suspected of suffering therefrom.

3. Method according to claim 1 or claim 2, whereby said sample is a sample of an individual suffering from stage 1 NSCLC or suspected of suffering therefrom.

4. Method according to any of claims 1-3, wherein typing said samples on the basis of the RNA levels determined for said set of genes comprises comparing the RNA level at least two of the genes listed in Table 3 to the RNA level of said genes in a reference sample.

5. Method according to any of claims 1-4, whereby one of said at least two genes is induced in a low risk NSCLC sample, compared to the average level of expression of said gene in a reference sample, while a second gene from said at

least two genes is repressed in a low risk NSCLC sample compared to the average level of expression of said gene in a reference sample.

6. Method according to any of claims 1-4, whereby said set of genes comprises seventy-two of the genes listed in Table 3, which genes are ranked 1-72.

7. Method according to any of the previous claims, further comprising normalizing the determined RNA levels of said set of genes in said sample.

8. A set of probes for typing a sample of an individual suffering from non-small cell lung cancer or suspected of suffering therefrom, whereby said set of probes comprises probes specific for at least two of the genes listed in Table 3.

9. Use of the set of probes according to claim 8 for determining a risk for an individual suffering of NSCLC for recurrence of said cancer.

10. Use of the set of probes according to claim 8 for discriminating between NSCLC cells with a low versus a high metastasizing potential.

11. A set of primers for typing a sample of an individual suffering from non-small cell lung cancer or suspected of suffering therefrom, whereby said set of primers comprises primers specific for at least two of the genes listed in Table 3.

12. Use of the set of primers according to claim 11 for determining a risk for an individual suffering of NSCLC for recurrence of said cancer.

13. Use of the set of primers according to claim 11 for discriminating between NSCLC cells with a low versus a high metastasizing potential.

14. Method of classifying a sample from an individual suffering from NSCLC, or suspected of sufffering from NSCLC, comprising

classifying a sample as derived from an individual having a poor prognosis or a good prognosis by a method comprising

providing a sample from said individual;

determining a level of RNA for a set of genes comprising at least two of the genes listed in Table 3 in said sample;

determining a similarity value for the level of RNA in said sample and a level of RNA for said set of genes in a patient having no recurrent disease within three years of initial diagnosis; and

classifying said individual as having a poor prognosis if said similarity value is below a first similarity threshold value, and classifying said individual as having a good prognosis if said similarity value exceeds said first similarity threshold value.

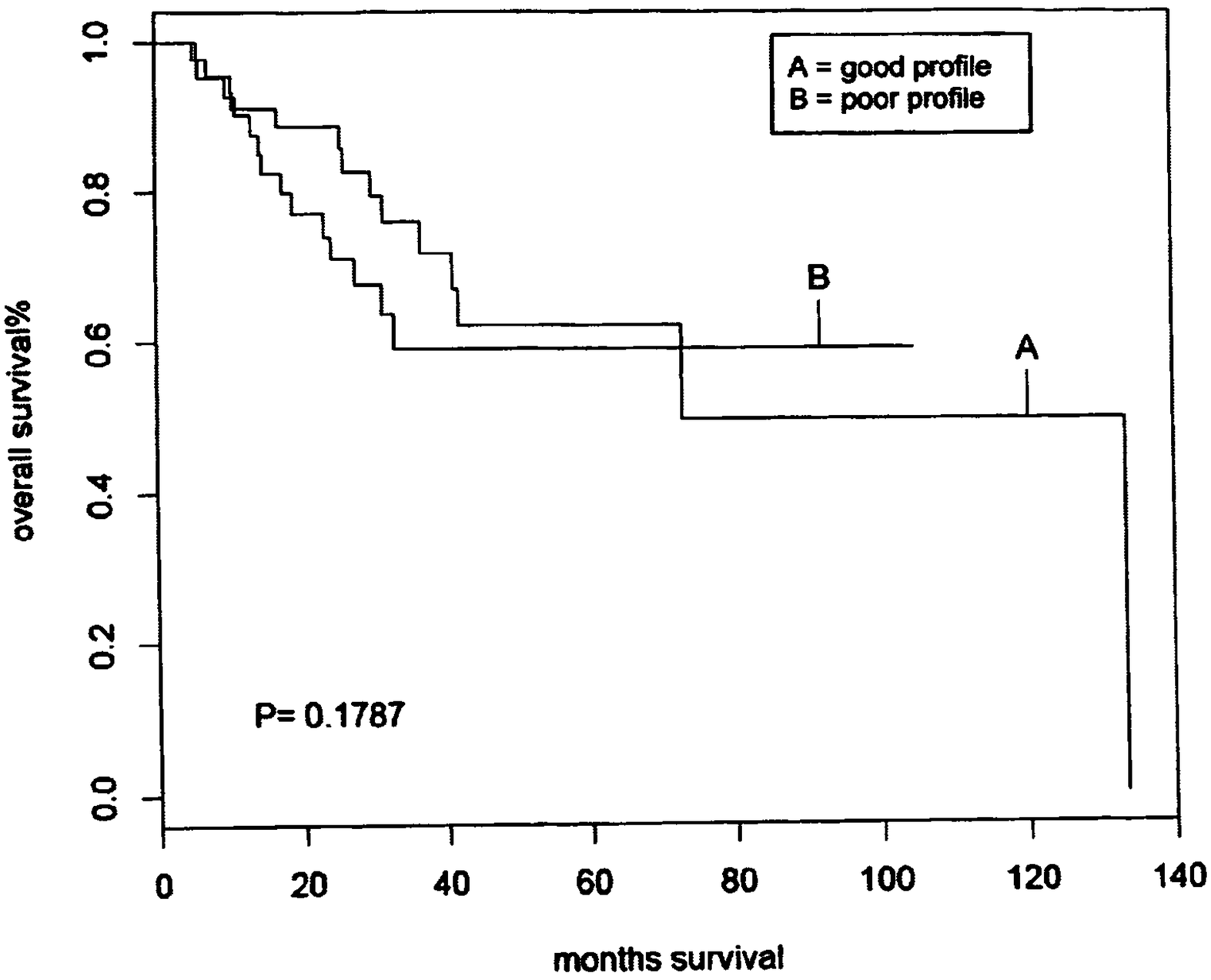
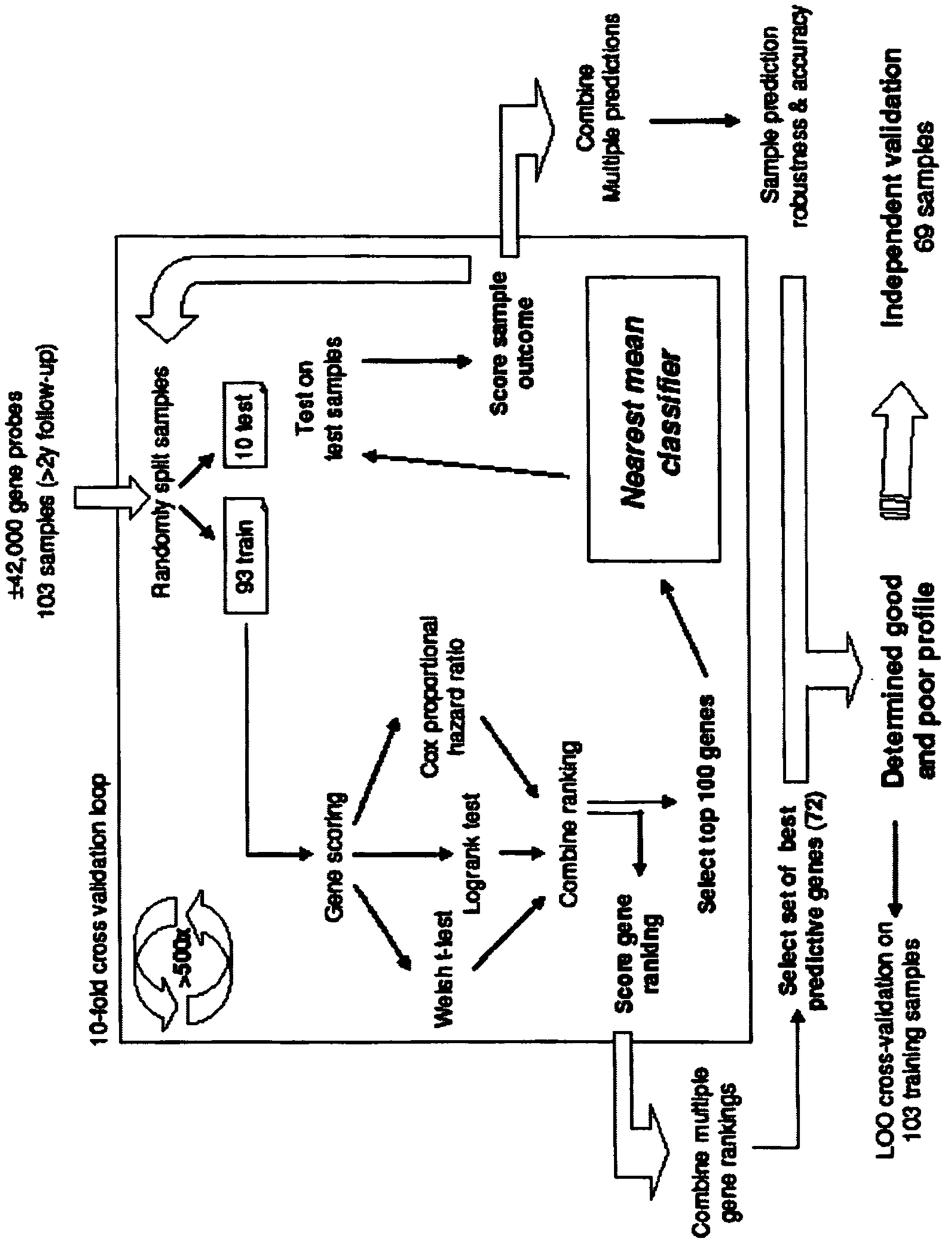

A = good profile
B = poor profile
overall survival%
1.0
0.8
0.6
0.4
0.2
0.0
B
A
P= 0.1787
0
20
40
60
80
100
120
140
months survival

Fig. 1

Fig. 2


±42,000 gene probes
103 samples (>2y follow-up)
Randomly split samples
93 train
10 test
10-fold cross validation loop
>500x
Gene scoring
Welch t-test
Cox proportional hazard ratio
Logrank test
Combine ranking
Score gene ranking
Select top 100 genes
Test on test samples
Score sample outcome
Nearest mean classifier
Combine multiple gene rankings
Select set of best predictive genes (72)
LOO cross-validation on 103 training samples
Determined good and poor profile
Combine Multiple predictions
Sample prediction robustness & accuracy
Independent validation 69 samples

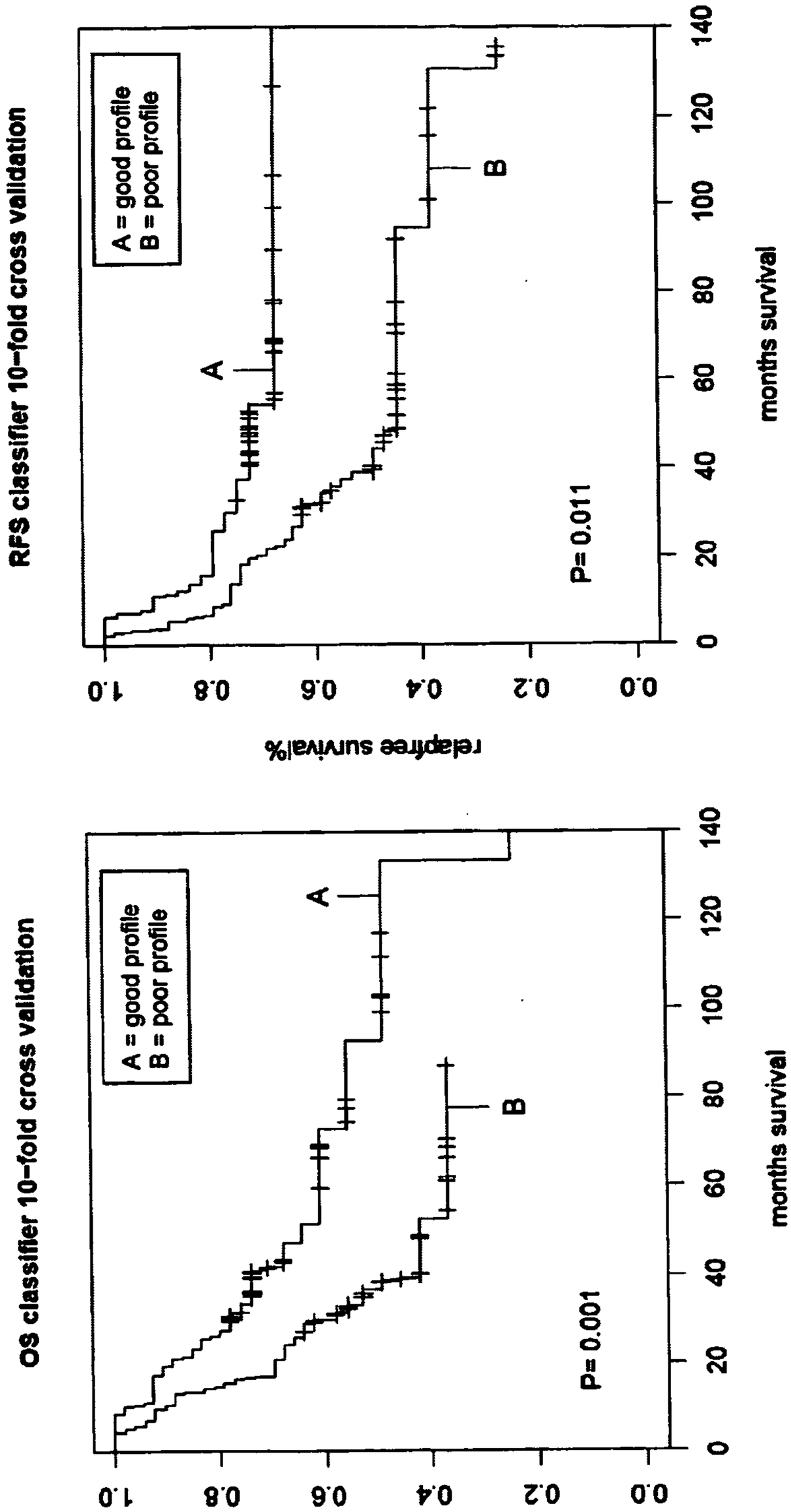

RFS classifier 10-fold cross validation
A = good profile
B = poor profile
A
B
P= 0.011
relapfree survival%
months survival
OS classifier 10-fold cross validation
A = good profile
B = poor profile
A
B
P= 0.001
overall survival%
months survival

Fig. 3

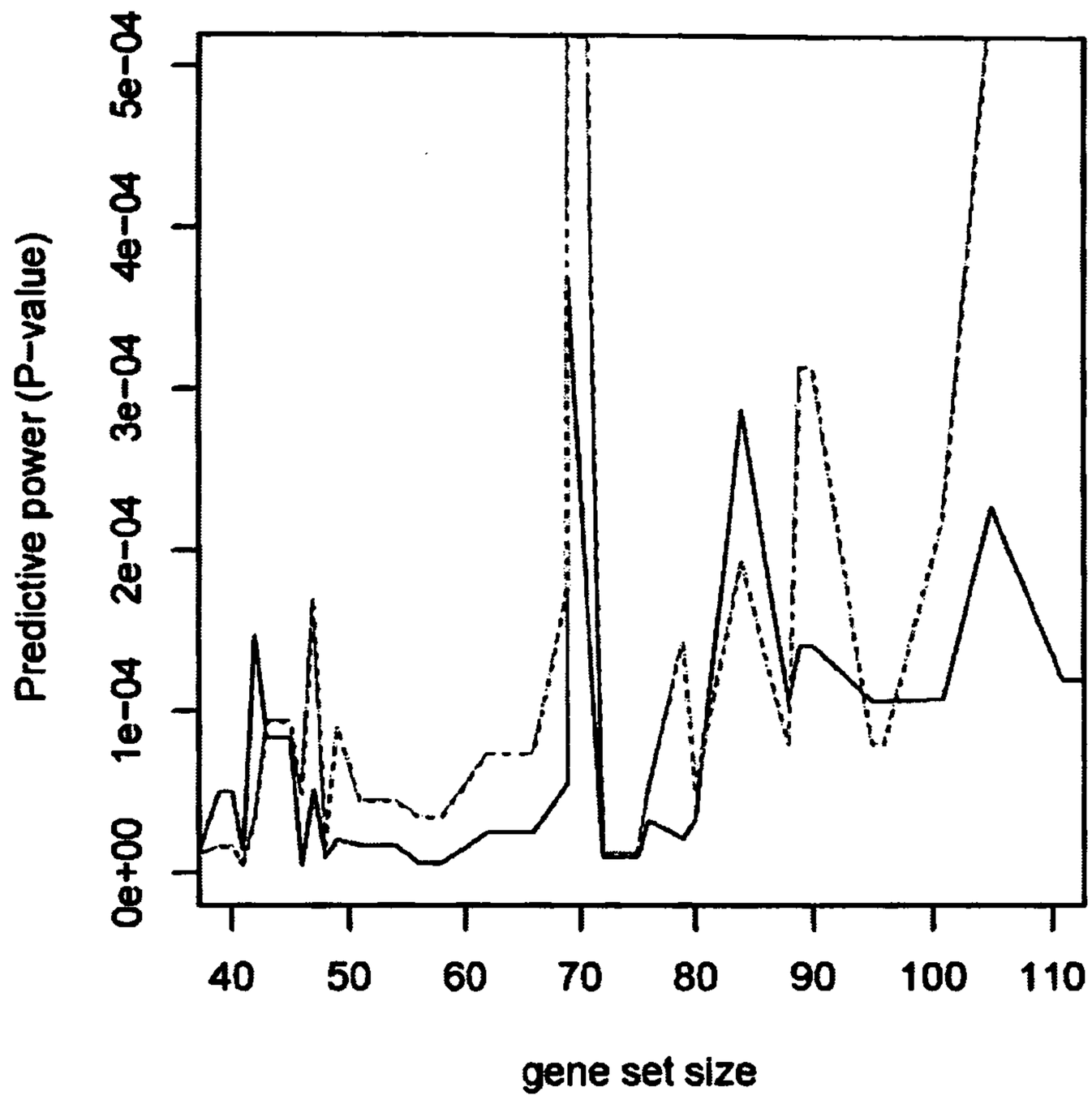

Predictive power (P-value)
0e+00
1e-04
2e-04
3e-04
4e-04
5e-04
40
50
60
70
80
90
100
110
gene set size

Fig. 4

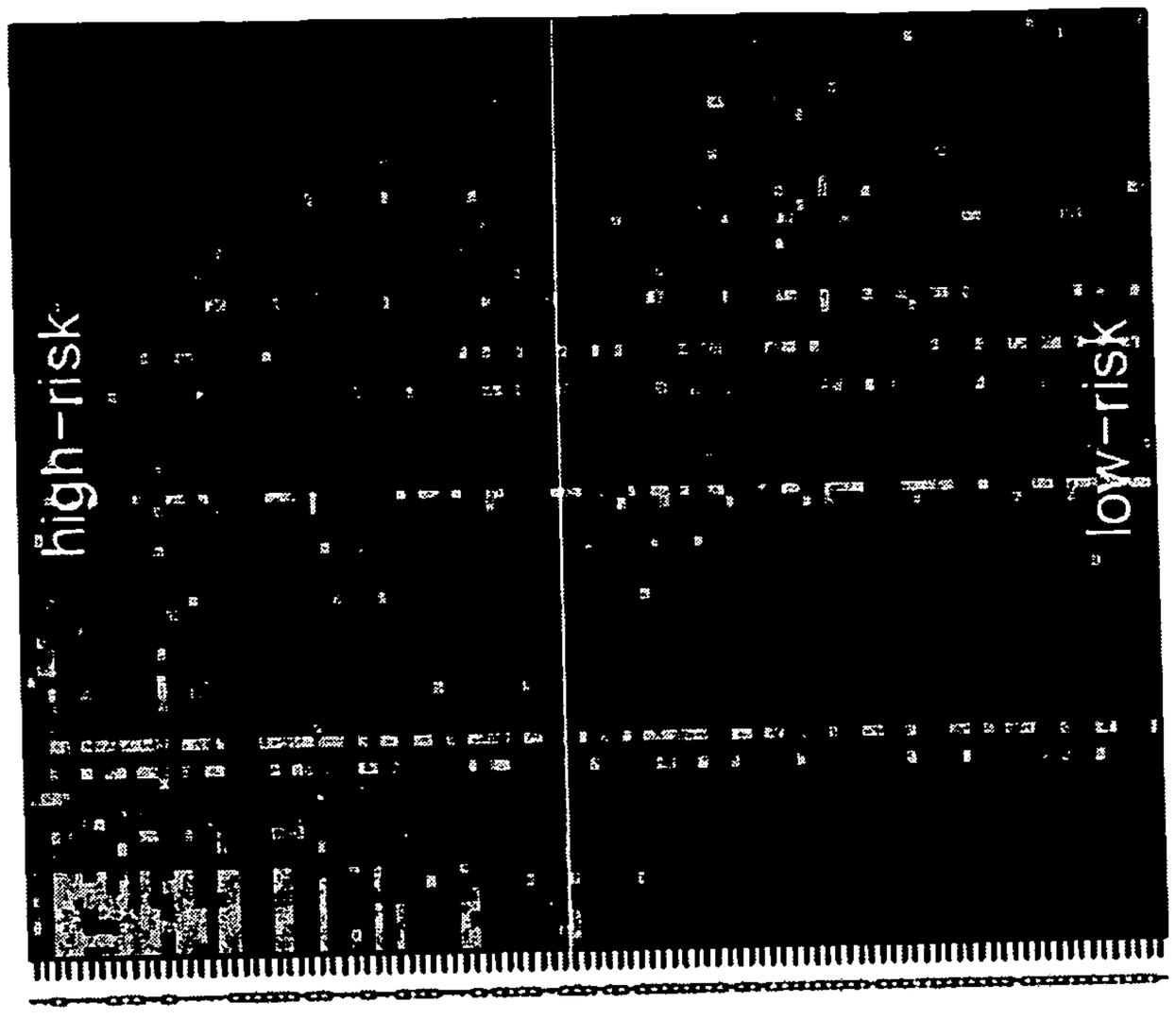
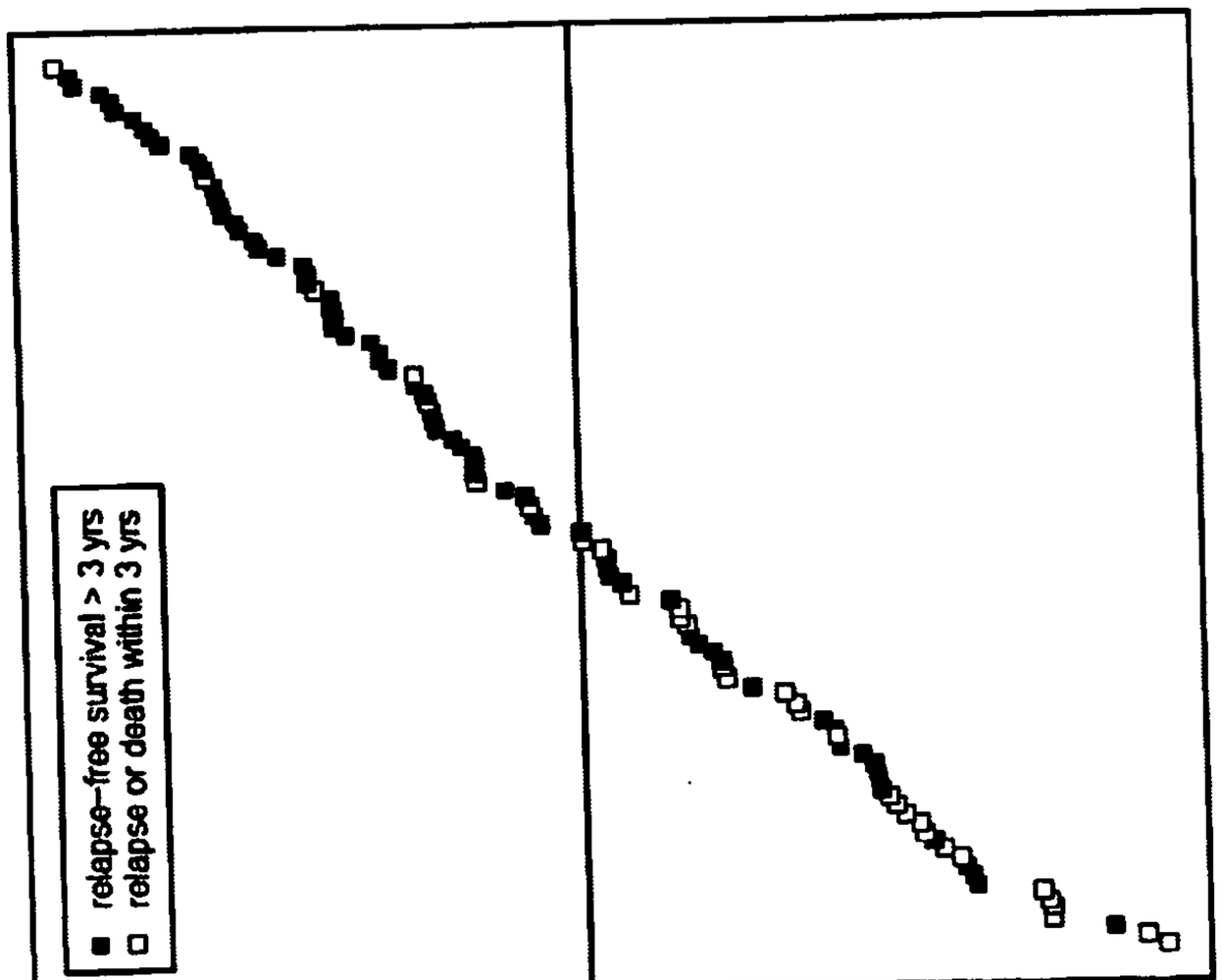

high-risk
low-risk

Fig. 5


relapse-free survival > 3 yrs
relapse or death within 3 yrs
correlation with low-risk profile
-0.5
0.0
0.5

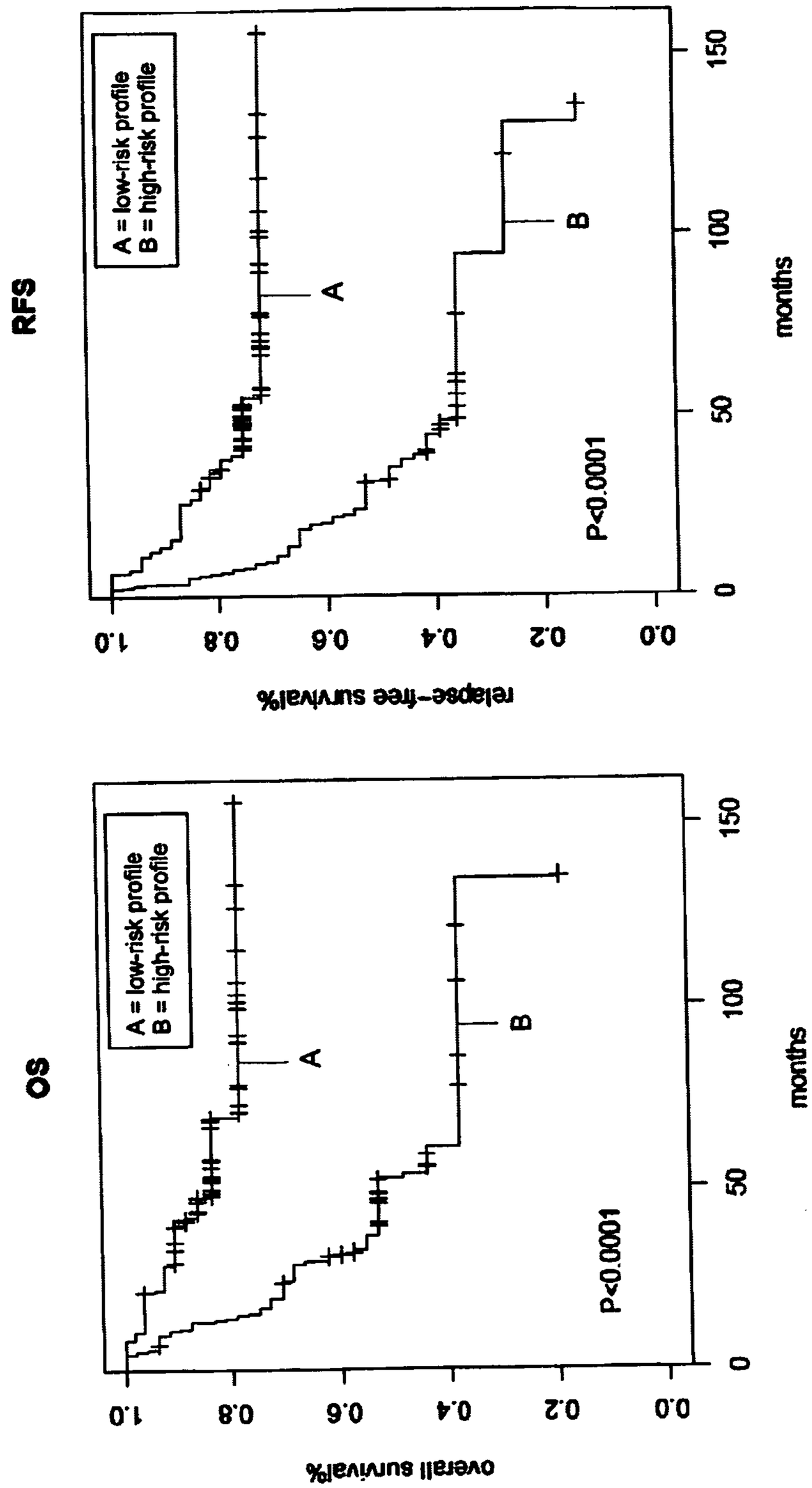

RFS
A = low-risk profile
B = high-risk profile
A
B
P<0.0001
relapse-free survival%
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
months

Fig. 6

OS
A = low-risk profile
B = high-risk profile
A
B
P<0.0001
overall survival%
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
months

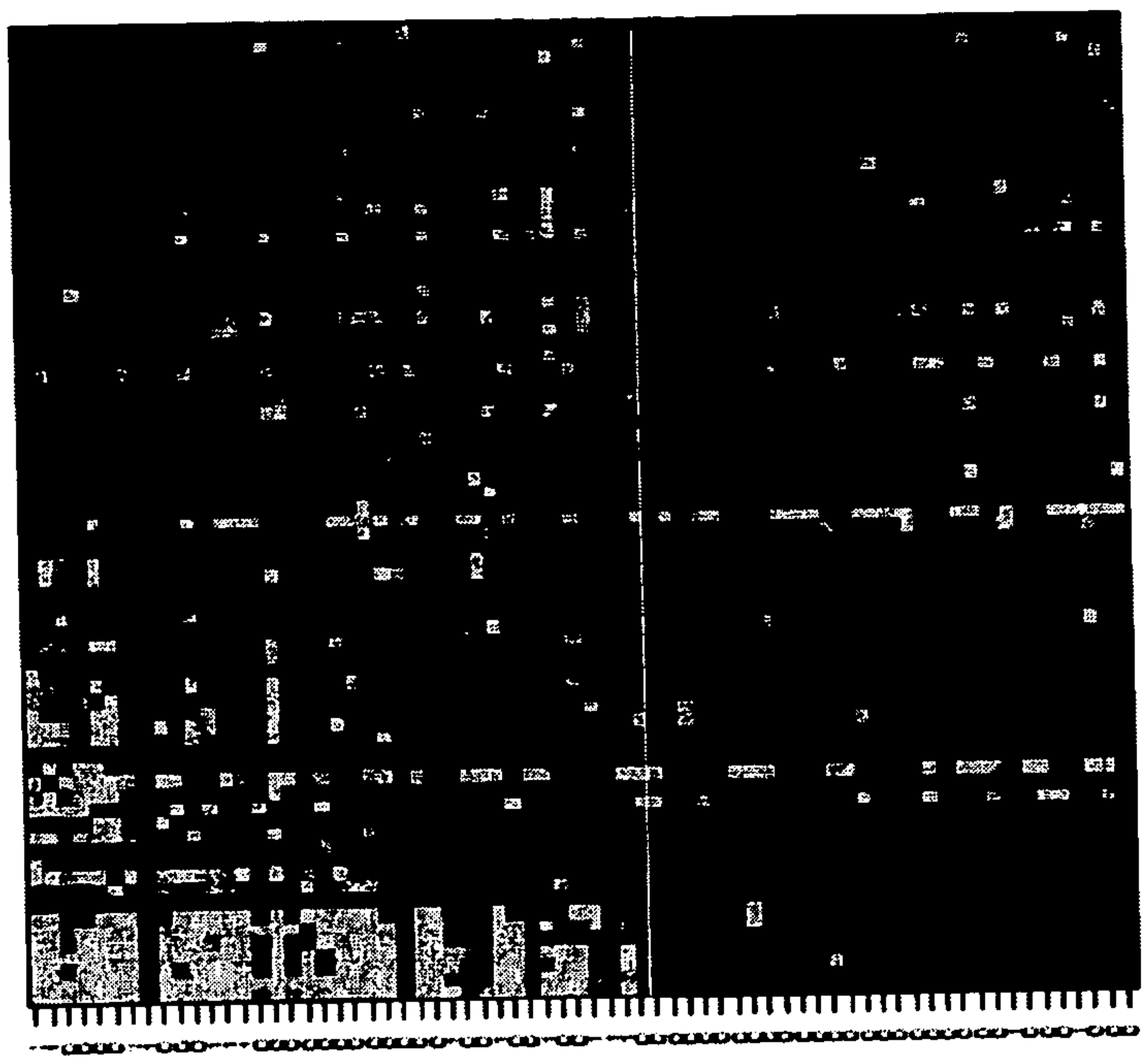


Fig. 7.1

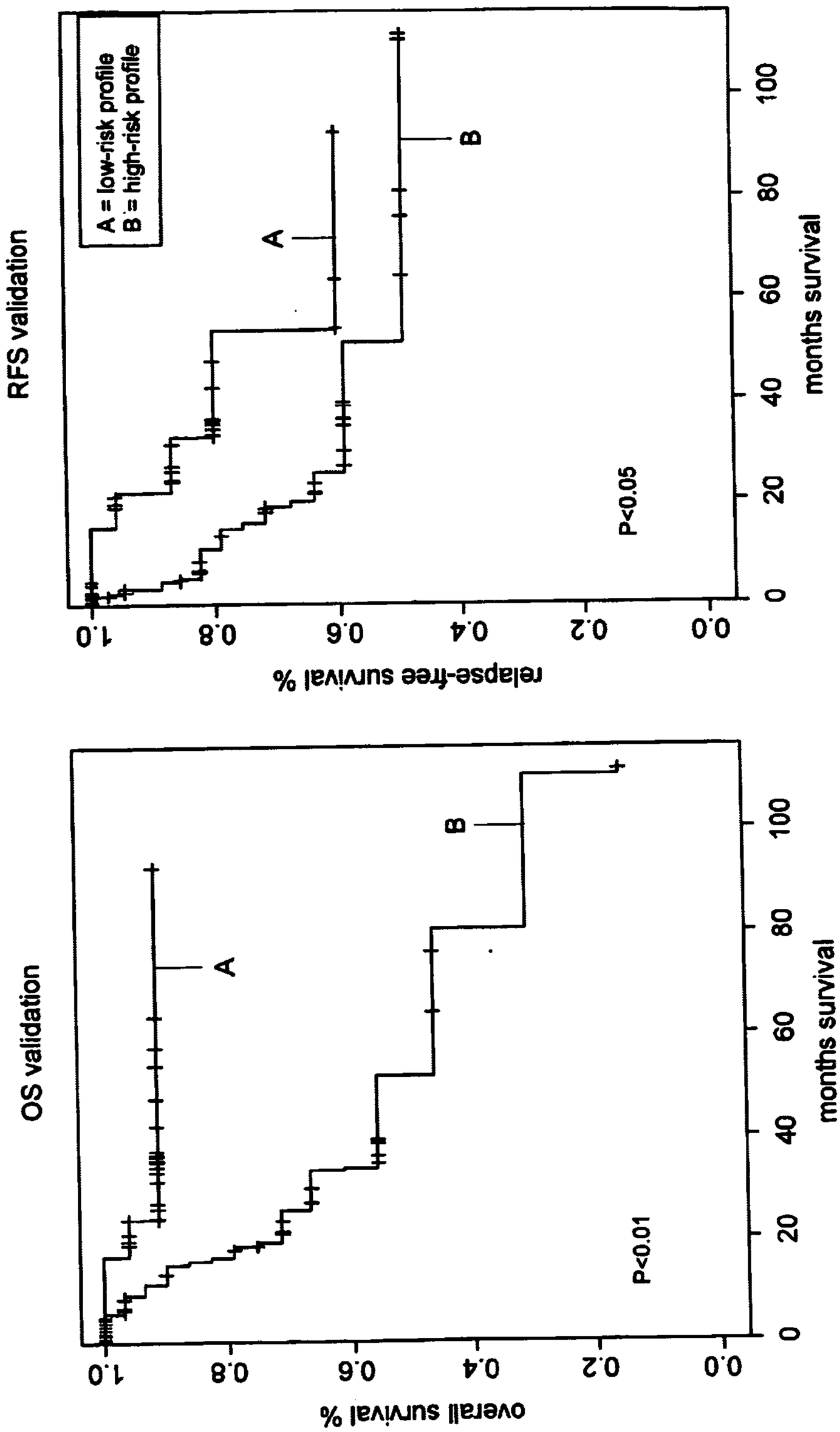

A = low-risk profile
B = high-risk profile
A
B
P<0.05
RFS validation
relapse-free survival %
months survival
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
OS validation
P<0.01
overall survival %

Fig. 7.2

Fig. 8

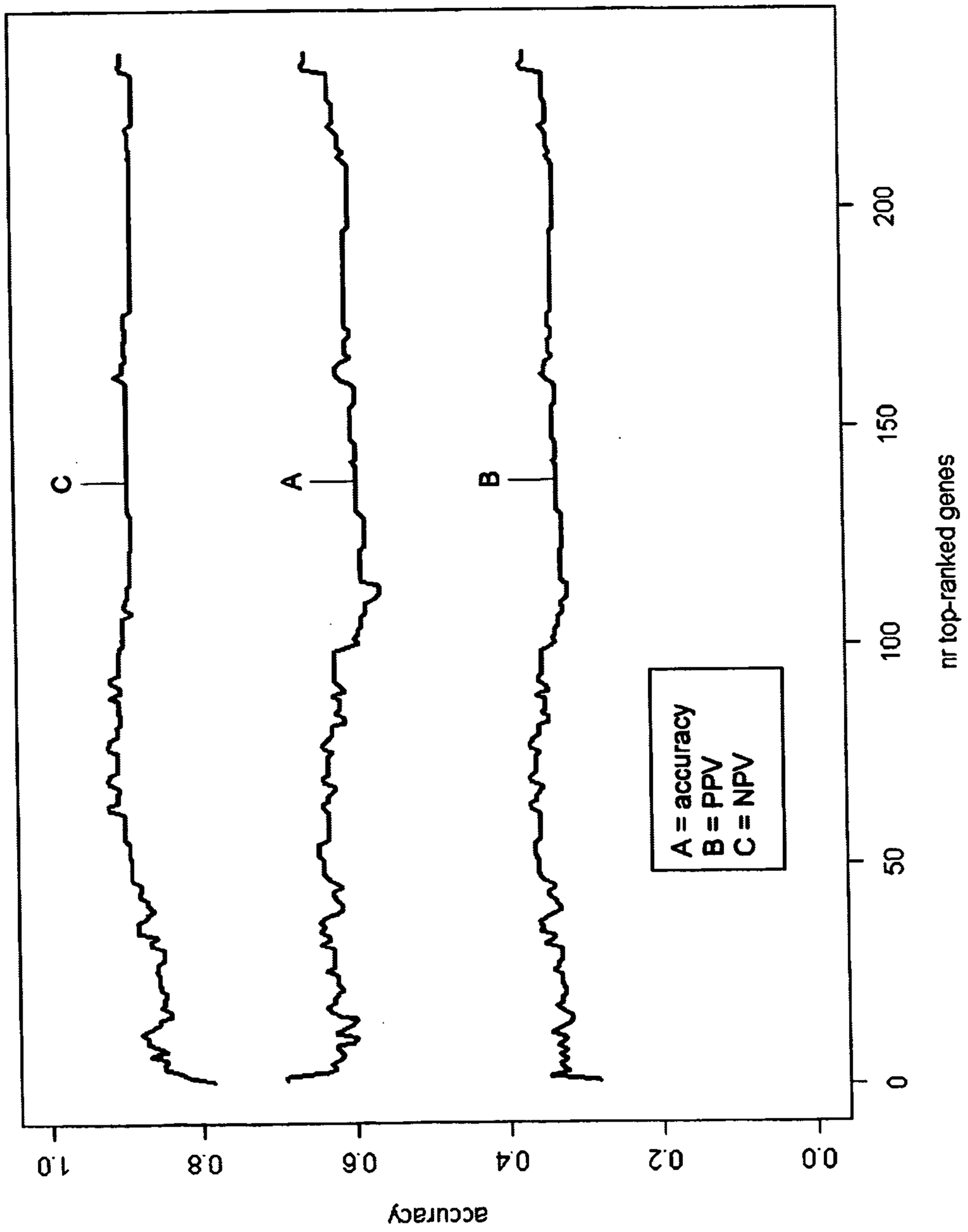
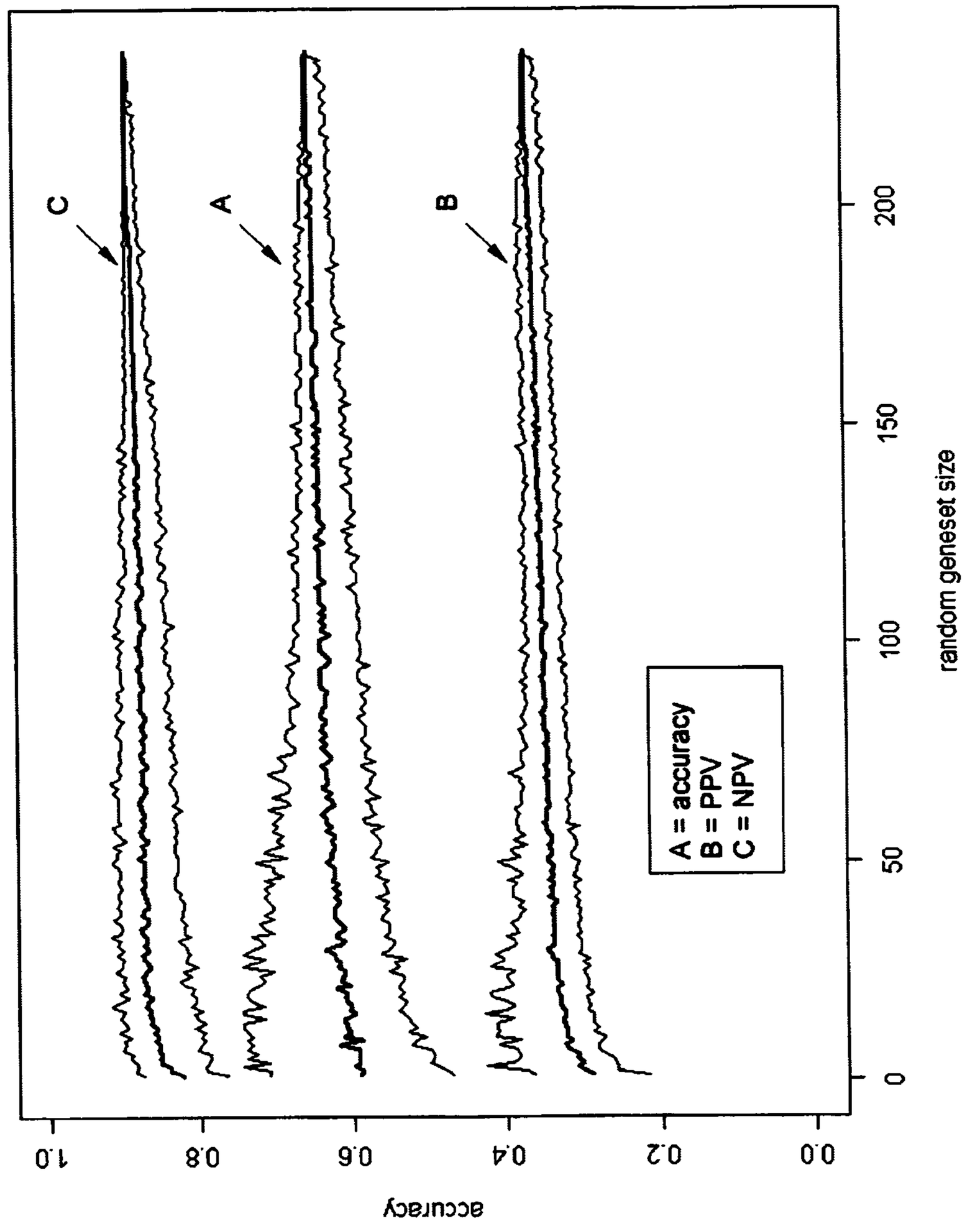

overall survival > 3 yrs
C
A
B
A = accuracy
B = PPV
C = NPV
0
50
100
150
200
nr top-ranked genes
0.0
0.2
0.4
0.6
0.8
1.0
accuracy

Fig. 9


overall survival > 3 yrs
C
A
B
A = accuracy
B = PPV
C = NPV
0
50
100
150
200
random geneset size
0.0
0.2
0.4
0.6
0.8
1.0
accuracy

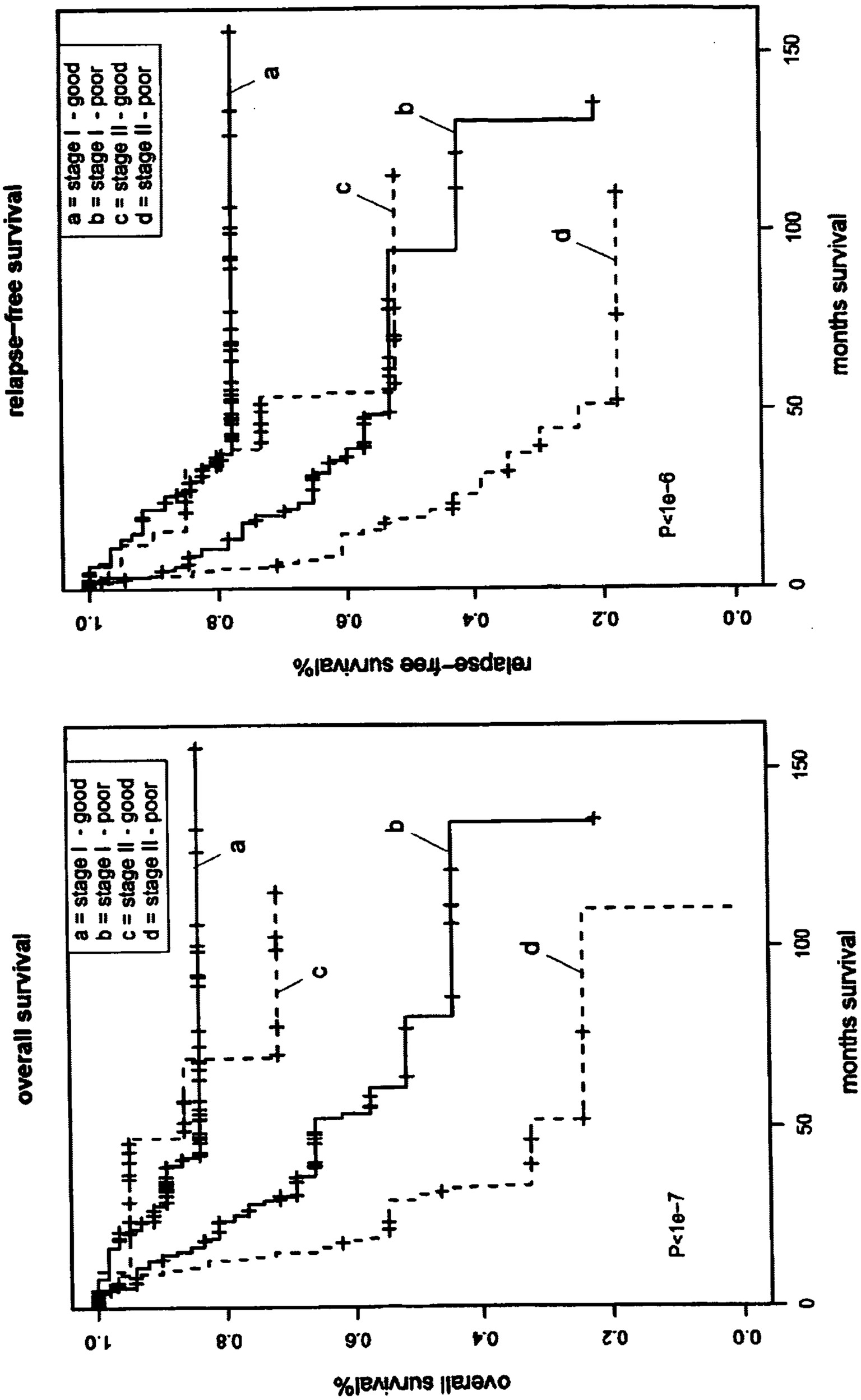

a = stage I - good
b = stage I - poor
c = stage II - good
d = stage II - poor
a
b
c
d
P<1e-6
relapse-free survival
relapse-free survival%
months survival
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150

Fig. 10

a = stage I - good
b = stage I - poor
c = stage II - good
d = stage II - poor
a
b
c
d
P<1e-7
overall survival
overall survival%
months survival
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150