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Fortsættes ...

DESCRIPTION

Technical field

[0001] The present invention refers to a method for in vitro predicting survival and/or metastatic outcome of gastrointestinal stromal tumors (GISTs). **It describes** a kit for (i) the in vitro prediction of the survival outcome of a patient suffering from GIST, (ii) and/or the development of metastases in a patient treated for or suffering from GIST, (ii) and/or the prediction of the efficacy of a treatment for GIST. The present invention also **describes** a method for screening for compounds for the use in the treatment of GISTs, and to a compound for its use in the treatment of GISTs.

[0002] Therefore, the present invention has utility in the medical and pharmaceutical fields, especially in the field of diagnosis.

[0003] In the description below, the references into brackets ([]) refer to the listing of references situated at the end of the text.

Background of the Invention

[0004] Gastrointestinal stromal tumors (GISTs) are the most frequent mesenchymal tumors of the gastrointestinal tract and account for approximately 25% of soft tissue sarcomas. GISTs are thought to arise from the intestinal cells of Gajal (1), or from a common progenitor cell (2).

[0005] The KIT tyrosine kinase or the platelet-derived factor receptor a (PDGFRA) activating mutations are early oncogenic events in GISTs. Most GISTs (80%) are characterized by activating mutations of the *KIT* tyrosine kinase receptor, while a subset (8%) harbours platelet-derived factor receptor α (*PDGFRA* mutations (3,4). In addition to these mutations, other genetic changes do occur, the most frequent alterations reported being 14q, 22q and 1p deletions (5). Overall, GIST cytogenetics is quite simple and imbalances manly involve full chromosomes or chromosome arms. Notably, GIST molecular and cytogenetic profiles correlate with disease progression. Nevertheless, it has been observed that changes are more frequent and more complex in advanced tumors (6). Furthermore, the genetic basis of the metastatic outcome of GISTs is still poorly understood.

[0006] Clinical management of GISTs consists mainly of surgical resection with adjuvant or neo-adjuvant targeted therapy with Imatinib Mesylate (Gleevec, formerly STI571, Novartis Pharma AG) which has been demonstrated to target the KIT- or PDGFRA-aberrant signaling induced by activating mutations (7). The majority of cases can be cured by surgical resection alone, but 20-40% of patients relapse with distant liver metastasis being the most common manifestation of the recurrent disease.

[0007] Many pathological criteria based on tumor site, size, cell type, degree of necrosis and mitotic rate have been proposed for predicting the outcome of patients with GISTs. A consensus was found by the National Institute of Health (NIH) in 2001 to estimate the relative risk of GISTs based on tumor size and mitotic count (8) and in 2006, the Armed Forces Institute of Pathology (AFIP) proposed an updated system taking into account also a tumor location (9). Even if these two systems are particularly efficient in determining the metastatic risk of GISTs, they are based on an indirect histopathological reflection of tumor aggressiveness. Moreover, the cutoff values for these criteria have been determined empirically leading to subjectivity that is inevitable in skilled pathologists' assessments. Hence, there is a need to more deeply understand the biology underlining the aggressiveness of GISTs in order to identify objective biomarkers that enhance the specificity and the reproducibility of outcome prediction.

[0008] The development of a valid and reliable, investigator-independent method of GIST prognostication is essential for the proper clinical management of GIST patients, especially in the context of adjuvant treatment, where many patients are exposed to imatinib while only a small proportion will likely benefit from such treatment (19).

[0009] To achieve this purpose, genomic and expression profiling has already been used but only partial and heterogeneous results have been reported. At the genomic level, it has been shown that the genome complexity level increases with tumor stage (6, 10), but no threshold has ever been defined and no specific alteration has been proposed except for $p16^{INK4A}$ alterations whose role in metastasis development is still controversial (11-16). At the expression level, Yamaguchi and colleagues have proposed a gene-expression signature : they identified CD26 as a prognostic marker but only in GISTs of gastric origin. Nevertheless, the authors concluded that CD26 might not be the cause of malignant progression of gastric GISTs. Moreover, this signature is limited as it has been established on only a few cases (32 GISTs), it predicts outcome only in gastric GISTs (but not in GIST of the small intestine) and it has not been compared to histopathological grading methods considered as "gold standard" (17).

[0010] In the few genome or expression profiling analyses using smaller numbers of GISTs that have been conducted before this study, only one (35) presents an integrative analysis gathering genome and transcription profiling as we present here. This study was based on 25 patients and aimed to identify target genes located in altered regions described within the last 15 years. Essentially, many studies have described GISTs genome and demonstrated that GISTs cytogenetics is quite simple, reflected by only few aberrations, deletions being more frequent than gains (6, 10, 11, 12, 36, 37). All these studies concluded that chromosome 14, 22 and 1p deletions are the most frequent aberrations. It has also been shown that changes are more frequent in high-risk and overly malignant GISTs than in low/intermediate-risk GISTs (6, 10), but a strong association between one alteration and prognosis has not yet been identified, except *CDKN2A* alterations as discussed above. At the expression level, most of the studies have been set up to enhance delineation of diagnosis (38, 39) or to identify expression differences according to *KIT* or *PDGFRA* mutation status (40-42).

[0011] The AURKA, encoded for a gene that maps to chromosome 20q13, is a mitotic centrosomal protein kinase (20). It is a well known oncogene, which main role in tumor development is the control of chromosome segregation during mitosis (21). Gene amplification and AURKA overexpression have been widely described in many cancer types (22). In particular, as it has been clearly demonstrated that AURKA overexpression induces centrosome duplication-distribution abnormalities and aneuploidy leading to transformation in breast cancer cells (23). Actually, centrosomes maintain genomic stability through the establishment of the bipolar spindle body during cell division, ensuring equal segregation of replicated chromosomes to two daughter cells. The AURKA expression has also been associated with poor prognosis mainly in breast carcinoma (24), colon carcinoma (25, 26), neuroblastoma (27) and head and neck squamous cell carcinoma (28). Taken together, these data indicate that up-regulation of AURKA expression could be a major driving event in establishing genome complexity leading to wild gene expression reprogramming, creating optimal conditions for development of metastasis. AURKA inhibitors are currently under clinical studies (29-33).

[0012] Yamashita et al. ("Chromosomal numerical abnormality profiles of gastrointestinal stromal tumors", Japanese Journal of clinical oncology, vol. 36, no. 2, 2006-02, pages 85-92) describe the examination by means of FISH for chromosomal abnormality in GISTs, in order to test chromosomal numerical abnormality (CNA)

as a possible biological predictor of biological behaviour of GISTs. Yamashita et al. also describe that CNA in the primary sites was more extensive in the GISTs with recurrence and metastasis than in those without, especially as to the loss of chromosome 20 and genomic imbalance of AURKA -containing BAC probe on 20q in the cases with metastases.

[0013] WO2010/068951 document describes the use of an inhibitor of AURKA and an inhibitor of Src in *in vitro* synergy assays to test whether C1368 (a selective AURKA inhibitor) and dasatinib (Src kinase inhibitor) synergize to induce cell death in HCT116 human cancer cell line, which is a colorectal cancer cell line (see p. 3, 1. 27-28). GIST is not a cancer in which the association between AURKA and Src inhibitor is tested.

[0014] WO2006/129064 document teaches that AURKA inhibitors that may be useful for the treatment of tumours. However, this document describes no result of such inhibitors on cancer cell lines or patient; D3 is only putative.

[0015] In view of all these elements, it clearly still exists a need of new tools allowing to predict outcome of GISTs, notably palliating the failures, drawbacks and obstacles of the state of the art.

Description of the invention

[0016] After important researches, the Applicant found surprisingly a one gene-expression signature prognostic for clinical outcome of primary GISTs.

[0017] Surprisingly, it has been demonstrated by the Applicant that the CINSARC signature (CINSARC for Complexity INdex in SARComa, a 67 genes-expression prognostic signature related to genome complexity in sarcomas, PCT/FR2010/000323, [55]) and/or a new one-gene-expression signature predict metastatic outcome in GIST and that the combination of each of these signatures with genome imbalances outperforms current histopathological grading method in determining patient prognosis. More specifically, these molecular signatures identify "at-risk patients" within cases stratified as intermediate-risk according to the Armed Forces Institut of Pathology classification.

[0018] The Applicant manages to show that a positive correlation exists between GI (Genomic Index) and *AURKA* expression.

[0019] The Applicant surprisingly manages to construct a decisional algorithm based on GI and *AURKA* expression.

[0020] Advantageously, application of the signature permits more selective imatinib therapy leading to decreased iatrogenic morbidity and improved outcomes for individual patients.

[0021] Accordingly, in a first aspect, the invention **describes** a method for *in vitro* predicting survival and/or metastatic outcome of gastrointestinal stromal tumors (GISTs), the method comprising the measure of the level, in a patient-derived biological sample of GIST, of a pool of polypeptides or polynucleotides consisting in Aurora kinase A (AURKA).

[0022] Method for in vitro predicting survival and/or metastatic outcome of gastrointestinal stromal tumors (GISTs), characterized in that it comprises the measure of the level, in a patient-derived biological sample of GIST, of a pool of polypeptides or polynucleotides consisting in Aurora kinase A (AURKA), wherein GIST is classified in a group with high risk to develop metastases within 5 years, i.e. with a risk to develop metastases within 5 years of more than 80%, when AURKA is up-regulated compared to a group with no risk to develop metastases within 5 years, wherein AURKA is down-regulated and less than 9.13, wherein 9.13 is calculated from the AURKA mean expression of stratified tumor samples.

[0023] "Predicting survival and/or metastatic outcome" refers herein to predicting whether a patient has a chance to survive, or a risk to develop metastases following the outcome of a GIST. The survival or development of metastases may be calculated from invention, GIST may be classified in a group with high risk to develop metastases within 5 years of an outcome of GIST, or in a group with no risk to develop metastases within 5 years, or in an intermediate group. More particularly, the group of patient with high risk to develop metastases within 5 years is characterized by a risk to develop metastases within 5 years of more than 80%, when AURKA is up-regulated, compared to a group with no risk to develop metastases within 5 years when AURKA is down-regulated.

[0024] "Patient-derived biological sample of GIST" refers herein to any biological sample containing GIST cells and obtained from a patient treated for or suffering from GIST. For example, GIST may be primary untreated tumors.

[0025] "Polypeptide" refers herein to the AURKA protein (Genbank accession number NM_198433; SEQ ID NO: 1), a AURKA protein fragment, a AURKA protein region or a derivative of AURKA protein. For example, the polypeptide may be a polypeptide having at least 70% of sequence identity with the peptidic sequence of AURKA protein, or a polypeptide having at least 80% of sequence identity with the peptidic sequence of AURKA protein, or a polypeptide having at least 90% of sequence identity with the peptidic sequence of AURKA protein.

[0026] "Polynucleotide" refers herein to any polynucleotide coding for the polypeptide as defined above, or to any polynucleotide hybridizing under stringent conditions to a polypeptide coding for the polypeptide as defined above. The polynucleotide of the invention may be any of DNA and RNA, for example the sequence SEQ ID NO: 2. The DNA may be in any form of genomic DNA, a genomic DNA library, cDNA or a synthetic DNA. Moreover, the polynucleotide of the present invention may be any of those amplified directly by an RT-PCR method using total RNA or an mRNA fraction prepared from a GIST. The polynucleotide of the present invention includes a polynucleotide that hybridizes under stringent conditions to a polynucleotide.

[0027] The measure of the level of polypeptides may be realized by any appropriate technique known by the man skilled in the art. It may be, for example, an immunohistochemistry technique, in which the expression of the protein is measured after hybridization of an antibody recognizing specifically the AURKA protein.

[0028] The measure of the level of polynucleotides may be realized be any appropriate technique known by the man skilled in the art. It may be, for example, a method of genomic qPCR (quantitative polymerization chain reaction), CGH-array (Comparative Genomic Hibridization) or RT-qPCR (real time qPCR) in order to check copy number of genomic DNA or quantify expression of genomic DNA.

[0029] Advantageously, the AURKA expression allows to predicting survival and/or metastatic outcome of GISTs, and no other gene or protein expression has to be measured. The method of the invention may comprise the calculation of the Genomic Index (GI), i.e. the number and type of alterations of the GIST genome, according to the formula as follows:

$GI = A^2 \times C$,

wherein A is the number of alterations in GIST genome and C is the number of involved chromosomes in GIST.

[0030] "Number of alterations in GIST genome" refers herein to different numerical and segmental gains and losses. The alterations may for example involve whole chromosome arms or chromosome without rearrangement, or intra-chromosome gains or losses. It may be measured by techniques known in the art, such as CGH-array.

[0031] "Number of involved chromosomes in GIST" refers herein to the number of chromosomes of GIST cells having an alteration. The number of chromosome may be measured by CGH-array.

[0032] Advantageously, GIST is classified in a group of metastasis- and disease-free survival group when AURKA is down-regulated and the GI is equal or less than 10. In this case, AURKA expression may be less than 9.13, or less than 9, or less than 8, or less than 7, or less than 6, or less than 5. In this case, there is a survival of 5 years, i.e. there are no metastasis or disease during 5 years after GIST outcome or after the end of a treatment. In a particular embodiment, GIST may be classified in a group with low risk to develop metastases within 5 years, i.e. with a risk to develop metastases within 5 years comprises between 0% and 10%, when AURKA expression is equal or less than the mean of AURKA expression, and GI is equal or less than 10, said mean being the mean of AURKA expression/level in several GISTs, for example a series of 50 to 70 GISTs, for example 60 GISTs. In this case, there is no metastasis or disease during 5 years.

[0033] Alternatively, GIST may be classified in a group with high risk to develop metastases within 5 years, i.e. with a risk to develop metastases within 5 years more than 75%, when AURKA expression is more than the mean of AURKA expression and GI is more than 10, said mean being the mean of AURKA expression in several GISTs, for example in a series of 50 to 70 GISTs, for example 60 GISTs. In this case, there are 75% of cases of metastasis or disease during 5 years after GIST outcome or after the end of a treatment.

[0034] A kit for the *in vitro* prediction of the survival outcome of a patient suffering from GIST, and/or the development of metastases in a patient treated for or suffering from GIST, and/or the prediction of the efficacy of a treatment for GIST, characterized in that it comprises means for detecting and/or quantify, in a sample, AURKA expression/level, and means for the calculation of the GI is described.

[0035] "Means for detecting and/or AURKA expression/level" may be any means for detecting levels of proteins or of polynucleotides known by the man skilled in the art. The means may be for example the means to realize an immunohistochemistry analysis, a western blot or a q-PCR.

[0036] "Means for the calculation of the GI" refers herein to any means allowing the calculation of the number of alterations in GIST genome and of the number of involved chromosomes in GIST.

[0037] A method for screening for compounds for the use in the treatment of GISTs **is described**, comprising the steps of :

1. a. Contacting a test compound with a patient-derived biological sample containing GISTs cells,
2. b. Measuring the expression or the level of AURKA,
3. c. Comparing said expression or level of AURKA with the expression of AURKA before the contact between said test compound and said sample,
4. d. Selecting said test compound that allows a down-regulation of the expression/level of AURKA.

[0038] "Down-regulation" refers herein to any diminution of the expression or level of AURKA protein or polynucleotide.

[0039] The method may also comprise the steps of:

e. Calculating GI,

f. Comparing said GI with the GI before the contact between said test compound and said sample,

g. Selecting said test compound allowing a down-regulation of the GI to 10 or less.

[0040] An AURKA inhibitor for its use in the treatment of GISTs **is described**.

[0041] "Inhibitor" refers herein to any compound allowing a decrease of the expression/level of AURKA protein, or in a decrease of a biological effect of AURKA, when the inhibitor is contacted with GIST. The AURKA inhibitor may be PHA-739358 (29, 54), MLN8237 (30), MK-5108 (33).

[0042] Another object of the invention is a method of treatment of GIST, in a subject in need thereof, comprising the step of administering to the patient a pharmaceutically effective dose of a inhibitor as defined above.

Brief description of the figures

[0043]

- Figure 1: Kaplan-Meyer analysis of metastasis-free (MFS) and disease-free (DFS) survival according to CINSARC stratification. Centroids have been retrained in a previously published (Yamaguchi *et al,* 2008) series of 32 GISTs (a) and then applied to the present series of 60 GISTs (b).
- Figure 2: Kaplan-Meyer analysis of metastasis-free (MFS) and disease-free (DFS) survival according to *AURKA* expression. *AURKA* has been identified in the present 60-GISTs series, this series is considered as (a) the "training set" and the Yamaguchi's series as (b) the "validation set". A1 corresponds to tumors with below-average *AURKA* expression.
- Figure 3: CGH profiles of four cases representing GISTs with very few rearrangements (GIST #8), GISTs moderately rearranged (GISTs #49 and #11) and GISTs highly rearranged (GIST #38). Genomic alterations are presented and organized from chromosome 1 to 22 on the X axis and log ratio values are reported on the Y axis. Significant gains or losses are indicated by blue lines and blue areas above or below each profile, respectively.
- Figure 4: Kaplan-Meyer analysis of metastasis-free (MFS) and disease-free (DFS) survival according to (a) GI, (b) AFIP classification and (c) GI in the subgroup of AFIP intermediate cases.

- Figure 5: Kaplan-Meyer analysis of metastasis-free (MFS) and disease-free (DFS) survival according to both GI and *AURKA* expression. **AG1** corresponds to tumors with GI below 10 and *AURKA* expression below average. **AG2** corresponds to tumors with GI over 10 and *AURKA* expression above average.
- Figure 6: Volcano-plot representation of t-test comparing expression profiles of GISTs with or without metastasis. Venn diagram at the bottom indicates the number of genes overlapping with CINSARC signature.
- Figure 7: Scatter-plot presenting the association between Genomic Index (GI, Y axis) and *AURKA* expression (log2, X axis). Horizontal and vertical **black** lines correspond to GI threshold of 10 and to *AURKA* mean expression, respectively. r = Pearson correlation coefficient. **Black** circles indicate metastatic cases.
- Figure 8: Histogram presenting the 4000 more frequently deleted probe sets in metastatic (M) cases (blue). Corresponding frequencies for non-metastatic (NM) cases are in red. Y axes represent the deletion frequency. Bottom panels are detailed views of the probe sets with the highest differences between M and NM cases.
- Figure 9: Chromosome 9 genomic profile of the 18 metastatic GISTs (upper panel). Deletions and gains are indicated in green and red, respectively; and color intensity is proportional to copy number changes. A detailed view is given (bottom panel) for the 6 cases presenting a homozygous 9p21 deletion targeting *CDKN2A* locus (dark green).
- Figure 10: prognostic values of protein expression of AURKA gene. Kaplan-Meyer analysis of metastasis-free (MFS) survival according to *AURKA* expression. The expression of the AURKA protein has been measured after specific hybridization with an antibody recognizing specifically AURKA protein. The hybridization of the antibody was then revealed by a chromogenic process.

Examples

Example 1: Material and methods

Tumor samples

[0044] Sixty seven fresh frozen GIST tumors were selected from the European GIST database CONTICAGIST (www.conticagist.org) which contains data from GIST tissues, including information regarding patients, primary tumor, treatment, follow-up and availability of tumor samples. All GISTs selected were primary untreated tumors. Their characteristics are presented in supplementary table 1. Most GISTs (59/67) were studied by both CGH-array and gene expression profiling (a combination of 66 by CGH-array and 60 by gene expression profiling).

DNA isolation and array-CGH

[0045] (Agilent arrays (G4450A). Briefly, for each sample, 350 ng of DNA were fragmented by a double enzymatic digestion (AluI + RsaI) and checked with LabOnChip (2100 Bioanalyzer System, Agilent Technologies) before labeling and hybridization. Tumor and control DNAs were labeled by random priming with CY5-dUTPs and CY3-dUTP, respectively, hybridized at 65°C for 24h and rotating at 20 rpm. Arrays were scanned using an Agilent G2585CA DNA Microarray Scanner and image analysis was done using the Feature-Extraction V10.1.1.1 software (Agilent Technologies). Normalization was done using the ranking-mode method available in the Feature-Extraction V10.1.1 software, with default value for any parameter. Raw copy number ratio data were transferred to CGH Analytics v4.0.76 software. The ADM-2 algorithm of CGH Analytics v4.0.76 software (Agilent) was used to identify DNA copy number anomalies at the probe level. A low-level copy number gain was defined as a log 2 ratio >0.25 and a copy number loss was defined as a log 2 ratio <-0.25. A high-level gain or amplification was defined as a log 2 ratio >1.5 and a homozygous deletion is suspected when ration is below -1. To establish decision criteria for prognosis, alterations involving more than 100 probes have been automatically computed using an aberration filter.

Real-time genomic quantitative PCR and sequencing

[0046] To determine the copy number status of *p16, p15* and *p14,* real-time PCR was performed on genomic DNA using TaqMan® Universal Master Mix (Applied Biosystems). For normalizing the results, we used three reference genes: *GAPDH, ALB* and *RPLP0,* in order to have, for each tumor, at least two of these reference genes in normal copy number (array-CGH). Primers and probe used for *RPLP0* are as follow: (F) 5'-TGGATCTGCTGGTTGTCCAA-3' (SEQ ID NO: 3); (R) 5'-CCAGTCTTGATCAGCTGCACAT-3' (SEQ ID NO: 4); (probe) 5'-AGGTGTTTACTGCCCCACTATTATCTGGTTCAGA-3' (SEQ ID NO: 5). Other primers and probes used were previously described (52). Tumor data were normalized against data obtained for normal DNA. The results were then calculated as previously described (52). A normal status corresponds to 0.8 ≤ ratio ≤ 1.2, 0.1 < ratio < 0.8 is considered as a hemizygous deletion. When ratio is inferior to 0.1, the deletion is considered as homozygous. *CDKN2A* locus has been submitted to sequencing as previously described **([52])** and *RB1* gene was sequenced using genomic DNA according to Houdayer *et al* (53) or cDNA with following primers : (F1) 5'-TCATGTCAGAGAGAGAGCTTGG-3' (SEQ ID NO: 6), (R1) 5'-CGTGCACTCCTGTTCTGACC-3' (SEQ ID NO: 7); (F2) 5'-AATGGTTCACCTCGAACACC -3' (SEQ ID NO: 8), (R2) 5'-CTCGGTAATACAAGCGAACTCC-3' (SEQ ID NO: 9); (F3) 5'-CCTCCACACACTCCAGTTAGG-3' (SEQ ID NO: 10), (R3) 5'-TGATCAGTTGGTCCTTCTCG-3' (SEQ ID NO: 11); (F4) 5'-GCATGGCTCTCAGATTCACC-3' (SEQ ID NO: 12), (R4) 5'-TCGAGGAATGTGAGGTATTGG-3' (SEQ ID NO: 13); (F5) 5'-TCTTCCTCATGCTGTTCAGG-3' (SEQ ID NO: 14), (R5) 5'-TGTACACAGTGTCCACCAAGG-3' (SEQ ID NO: 15).

RNA Isolation and gene expression profiling by one color assay

[0047] Total RNAs were extracted from frozen tumor samples with TRIzol reagent (Life Technologies, Inc.) and purified using the RNeasy® Min Elute TM Cleanup Kit (Qiagen) according to the manufacturer's procedures. RNA quality was checked on an Agilent 2100 bioanalyzer (Agilent Technologies). RNAs with a RNA Integrity Number (RIN) > 6.5 were used for microarray.

[0048] Gene expression analysis was carried out using Agilent Whole human 44K Genome Oligo Array (Agilent Technologies). This specific array represents over 41 000 human genes and transcripts, all with public domain annotations. Total RNA (500 ng) was reverse transcribed into cRNA by incorporating a T7 oligo-dT promoter primer prior to the generation of fluorescent cRNA using an Agilent Quick Amp Labelling Kit (Agilent Technologies). The labeled cRNA was purified using a Qiagen RNeasy Mini Kit (Qiagen) and quantified using a NanoDrop ND-1000 instrument. In these experiments Cy3-labeled (sample) cRNAs were hybridized to the

array using a Gene Expression Hybridization Kit (Agilent Technologies). The hybridization was incubated in Agilent SureHyb chambers for 17 hours in a Hyb Oven set to 65°C and rotating at 10 rpm. The microarray slides were washed according to the manufacturer's instructions and then scanned on an Agilent G2565BA DNA Microarray Scanner and image analysis was done using the Feature-Extraction V10.1.1.1 software (Agilent Technologies).

[0049] All microarray data were simultaneously normalized using the Quantile algorithm. The t-test was performed using Genespring (Agilent Technologies) and P-values were adjusted using the Benjamini-Hochberg procedure. The P-value and fold change cut-off for gene selection were 0.001 and 3, respectively. Gene ontology analysis was performed to establish statistical enrichment in GO terms using Genespring (Agilent Technologies).

Real-Time PCR

[0050] Reverse transcription and real-time PCR were performed as previously described (52). We used TaqMan® Gene Expression assays (Applied Biosystems): Hs01582072_m1 for *AURKA*; Hs01078066_m1 for *RB1;* Hs99999905_m1 for *GAPDH*; Hs99999903_m1 for *ACTB* and Hs99999902_m1 for *RPLP0. p14* and *p16* expression level was assessed as previously described (52). In order to normalize the results, we used *GAPDH, ACTB* and *RPLP0* genes as reference genes. Triplicates were performed for each sample for each gene. A reference CT (threshold cycle) for each sample was defined as the average measured CT of the three reference genes. Relative mRNA level of AURKA in a sample was defined as: ΔCT = CT (gene of interest) -CT (mean of the three reference genes).

Statistical analysis.

[0051] To assign prognosis, we applied the nearest centroid method. Centroids represent a centered mean of expression for the signature genes for each patient outcome (metastatic and non-metastatic). Thus, centroids were calculated from the cohort 1 samples (17) and then each sample of our series (thus considered as a validation set) was allocated to the prognostic class (centroid) with the highest Spearman correlation.

[0052] Metastasis- and disease- free survival was calculated by the Kaplan-Meier method from the date of initial diagnosis to the date of first metastasis, relapse, last follow-up or death for patients within diagnosis of metastasis. Survival curves were compared with the log rank test. Hazard ratios were performed with the Cox proportional hazard model. All statistical analyses were performed using R software version 2.11.11 and the package "survival".

Example 2: Results

CINSARC is a significant prognosis factor in GISTs

[0053] To assess the issue whether our previously published signature could have prognostic value in GISTs, we performed expression profiling in a series of 67 GISTs (Table 1).

Table 1: Description of patients.

Percentages are indicated in brackets. nd = not determined

Sex	
Male	27 (40)
Female	40 (60)
Location	
Stomach	43 (64)
Small intestine	12 (18)
Other	12 (18)
Histological subtype	
Spindle	52 (77.5)
Epithelioid	5 (7.5)
Mixed	10 (15)
Tumor size	
≤2	5 (7.5)
2-5	25 (37)
5-10	21 (31,5)
>10	15 (22,5)
nd	1 (1.5)
Mitotic index	
≤5	42 (63)
>5	25 (37)
AFIP Risk	
Very low	15 (22)
Low	16 (24)
Intermediate	16 (24)
High	19 (28.5)
nd	1 (1.5)
Surgery margin	
R0	46 (69)
R1	4 (6)
nd	17 (25)
Mutations	
KIT	**52 (77.5)**
Ex 9	2 (3)
Ex 11	48 (71.5)
Ex 13	1 (1.5)
EX 17	1 (1.5)
PDGFRA	**12 (18)**
Ex 12	2 (3)
Ex 14	1 (1.5)
Ex 18	9 (13.5)
WT	**3 (4.5)**
Relapse events	
Local	7 (10)
Distance	18 (27)

[0054] Among them, we obtained sufficient mRNA quality for 60 cases (89.5%). We applied the CINSARC nearest-centroid signature (18) to GISTs, using a published series (17) as a training set to retrain centroids and the present series as the validation set. Kaplan-Meier analysis (figure 1) revealed that in both series the CINSARC signature split tumors into two groups with strongly distinct metastasis-free (MFS) and disease-free (DFS) survival (validation set: MFS: HR=18.3, 95%CI = [2.4 - 140], *P* = 0.005 and DFS: HR=19.6, 95%CI = [2.6 - 149.5], P = 0.004).

Gene expression changes associated with metastatic outcome

[0055] The results presented above indicate that expression of genes involved in mitosis control and chromosome integrity (CINSARC) is associated with survival outcomes in GISTs. We thus asked whether the reciprocal phenomenon was true, that is whether the differential expression between metastatic and non-metastatic cases can identify such genes. To assess this issue, we performed supervised t-test comparing tumor expression profiles stratified according to outcomes (Figure 6). Among the 297 differentially expressed genes (338 probe sets) (Table 2), 70 (86 probe sets) were down-regulated in metastatic cases and 227 (252 probe sets) were up-regulated in metastatic cases (FC > 3 and *P* < 0.001).

Table 2: 297 genes differentially expressed between GISTs with or without metastasis (t-test).

UP-regulated genes in metastatic GISts					
Probe Name	Corrected p-value	Fold Change	Genbank Accession	Gene Symbol	Description
A_23_P71558	9.44E-08	4.4	NM_004260	RECQL4	Homo sapiens RecQ protein-like 4 (RECQL4), mRNA [NM_004260]
A_23_P104651	3.83E-07	4.6	NM_080668	CDCA5	Homo sapiens cell division cycle associated 5 (CDCA5), mRNA [NM_080668]
A_23_P131866	5.70E-07	5.2	NM_198433	AURKA	Homo sapiens aurora kinase A (AURKA), transcript variant 1, mRNA [NM_198433]
A_23_P168747	5.91E-07	3.3	NM_017760	NCAPG2	Homo sapiens non-SMC condensin II complex, subunit G2 (NCAPG2), mRNA [NM_017760]
A_23_P333998	5.91E-07	5.5	AF090919	POLQ	Homo sapiens clone HQ0327 PRO0327 mRNA, complete cds. [AF090919]
A_23_P32707	5.95E-07	5.1	M_012291	ESPL1	Homo sapiens extra spindle pole bodies homolog 1 (S. cerevisiae) (ESPL1), mRNA [NM_012291]
A_32_P103633	5.95E-07	3.2	NM_004526	MCM2	Homo sapiens MCM2 minichromosome maintenance deficient 2, mitotin (S. cerevisiae) (MCM2), mRNA [NM_004526]
A_23_P29330	7.06E-07	10.5	NM_148674	SMC1 B	Homo sapiens structural maintenance of chromosomes 1 B (SMC1 B), mRNA [NM_148674]
A_24_P277576	7.78E-07	6.5	NM_004237	TRIP13	Homo sapiens thyroid hormone receptor interactor 13 (TRIP13), mRNA [NM_004237]
A_23_P385861	7.78E-07	5.7	NM_152562	CDCA2	Homo sapiens cell division cycle associated
A_23_P145657	7.78E-07	6.8	M_012447	STAG3	2 (CDCA2), mRNA [NM_152562] Homo sapiens stromal antigen 3 (STAG3), mRNA [NM_012447]
A_23_P7636	7.78E-07	5.3	NM_004219	PTTG1	Homo sapiens pituitary tumor-transforming 1 (PTTG1), mRNA [NM_004219]
A_24_P195454	7.78E-07	3.8	A_24_P195454	A_24_P195454	
A_23_P212844	7.78E-07	3.0	NM_006342	TACC3	Homo sapiens transforming, acidic coiled-coil containing protein 3 (TACC3), mRNA [NM_006342]
A_23_P18579	9.70E-07	5.2	NM_006607	PTTG2	Homo sapiens pituitary tumor-transforming 2 (PTTG2), mRNA [NM_006607]
A_23_P68610	9.70E-07	6.3	NM_012112	TPX2	Homo sapiens TPX2, microtubule-associated, homolog (Xenopus laevis) (TPX2), mRNA [NM_012112]
A_24_P507383	9.70E-07	10.6	THC2705254	THC2705254	ALU2_HUMAN (P39189) Alu subfamily SB sequence contamination warning entry, partial (13%) [THC2705254]
A_23_P74349	1.09E-06	4.7	NM_145697	NUF2	Homo sapiens NUF2, NDC80 kinetochore complex component, homolog (S. cerevisiae) (NUF2), transcript variant 1, mRNA [NM_145697]
A_23_P218827	1.15E-06	4.5	NM_199420	POLQ	Homo sapiens polymerase (DNA directed), theta (POLQ), mRNA [NM_199420]
A_23_P302654	1.16E-06	3.2	NM_018140	CEP72	Homo sapiens centrosomal protein 72kDa (CEP72), mRNA [NM_018140]
A_24_P942335	1.40E-06	6.0	BC002881	C15orf42	Homo sapiens chromosome 15 open reading frame 42, mRNA (cDNA clone IMAGE:3940845), partial cds. [BC002881]
A_23_P253661	1.48E-06	13.7	NM_024902	FLJ13236	Homo sapiens hypothetical protein FLJ13236 (FLJ13236), mRNA [NM_024902]
A_23_P122197	1.52E-06	3.4	NM_031966	CCNB1	Homo sapiens cyclin B1 (CCNB1), mRNA [NM_031966]
A_32_P188921	1.58E-06	4.2	BC007606	BC007606	Homo sapiens cDNA clone IMAGE:3351130, complete cds. [BC007606]
A_23_P429491	1.58E-06	3.9	NM_145018	FLJ25416	Homo sapiens hypothetical protein FLJ25416 (FLJ25416), mRNA [NM_145018]

UP-regulated genes in metastatic GISts					
Probe Name	Corrected p-value	Fold Change	Genbank Accession	Gene Symbol	Description
A_23_P340909	1.58E-06	5.1	BC013418	C13orf3	Homo sapiens chromosome 13 open reading frame 3, mRNA (cDNA clone MGC:4832 IMAGE:3604003), complete cds. [BC013418]
A_23_P375	1.58E-06	4.7	NM_018101	CDCA8	Homo sapiens cell division cycle associated 8 (CDCA8), mRNA [NM_018101]
A_24_P105102	1.58E-06	3.5	NM_182687	PKMYT1	Homo sapiens protein kinase, membrane associated tyrosine/threonine 1 (PKMYT1), transcript variant 2, mRNA [NM_182687]
A_23_P361419	1.58E-06	5.6	NM_018369	DEPDC1B	Homo sapiens DEP domain containing 1 B (DEPDC1 B), mRNA [NM_018369]
A_23_P133956	1.58E-06	4.8	NM_002263	KIFC1	Homo sapiens kinesin family member C1 (KIFC1), mRNA [NM_002263]
A_23_P124417	1.93E-06	5.4	NM_004336	BUB1	Homo sapiens BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast) (BUB1), mRNA [NM_004336]
A_24_P306704	2.01E-06	10.1	XR_016161	KRT18P23	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC642448), mRNA [XR_016161]
A_24_P113144	2.01E-06	3.1	NM_024857	ATAD5	Homo sapiens ATPase family, AAA domain containing 5 (ATAD5), mRNA [NM_024857]
A_24_P297539	2.06E-06	6.4	NM_181803	UBE2C	Homo sapiens ubiquitin-conjugating
A_24_P413884	2.08E-06	5.8	NM_001809	CENPA	enzyme E2C (UBE2C), transcript variant 6, mRNA [NM_181803] Homo sapiens centromere protein A (CENPA), transcript variant 1, mRNA [NM_001809]
A_23_P401	2.15E-06	4.3	NM_016343	CENPF	Homo sapiens centromere protein F, 350/400ka (mitosin) (CENPF), mRNA [NM_016343]
A_24_P76521	2.17E-06	4.9	AK056691	GSG2	Homo sapiens cDNA FLJ32129 fis, clone PEBLM2000213, weakly similar to Mus musculus genes for integrin aM290, hapsin. [AK056691]
A_32_P151800	2.36E-06	3.5	NM_207418	FAM72A	Homo sapiens family with sequence similarity 72, member A (FAM72A), mRNA [NM 207418]
A_23_P150935	2.44E-06	5.4	NM_005480	TROAP	Homo sapiens trophinin associated protein (tastin) (TROAP), mRNA [NM_005480]
A_24_P354300	2.96E-06	3.3	NM_015.426	WDR51A	Homo sapiens WD repeat domain 51 A (WDR51A), mRNA [NM_0.15426]
A_32_P217911	2.96E-06	3.8	BG951379	BG951379	MR1-CT0735-120101-003-h03 CT0735 Homo sapiens cDNA, mRNA sequence [BG951379]
A_24_P319613	3.11E-06	8.2	NM_002497	NEK2	Homo sapiens NIMA (never in mitosis gene a)-related kinase 2 (NEK2), mRNA [NM_002497]
A_24_P313504	3.16E-06	3.2	NM_005030	PLK1	Homo sapiens polo-like kinase 1 (Drosophila) (PLK1), mRNA [NM_005036]
A_23_P1.43190	3.30E-06	4.0	NM_002466	MYBL2	Homo sapiens v-myb myeloblastosis viral oncogene homolog (avian)-like 2 (MYBL2), mRNA [NM_002466]
A_23_P6001.6	3.43E-06	5.7	NR_002734	PTTG3	Homo sapiens pituitary tumor-transforming
A_32_P96719	3.61E-06	4.3	NM_024745	SHCBP1	3 (PTTG3) on chromosome 8 [NR_002734] Homo sapiens SHCSH2-domain binding protein 1 (SHCBP1), mRNA [NM_024745]
A_23_P133123	3.63E-06	3.1	NM_032117	MND1	Homo sapiens meiotic nuclear divisions 1 homolog (S. cerevisiae) (MND1), mRNA [NM_032117]
A_23_P118815	3.63E-06	6.1	NM_001012271	BIRC5	Homo sapiens baculoviral IAP repeat-containing 5 (survivin) (BIRC5), transcript variant 3, mRNA [NM_001012271]
A_24_P323598	3.63E-06	4.8	NM_001017420	ESCO2	Homo sapiens establishment of cohesion 1 homolog 2 (S. cerevisiae) (ESCO2), mRNA [NM_001017420]
A_24_P227091	3.63E-06	3.7	NM_004523	KIF11	Homo sapiens kinesin family member 11 (KIF11), mRNA [NM_004523]
A_24_P322354	3.63E-06	6.3	NM_145060	C18orf24	Homo sapiens chromosome 18 open reading frame 24 (C18orf24), transcript variant 2, mRNA [NM_145060]
A_23_P96325	3.63E-06	6.5	NM_001009954	FLJ20105	Homo sapiens FLJ20105 protein (FLJ20105), transcript variant 2, mRNA [NM_001009954]
A_23_P253752	3.86E-06	3.5	NM_138419	FAM54A	Homo sapiens family with sequence similarity 54, member A (FAM54A), mRNA [NM_138419]
A_23_P80902	4.16E-06	4.5	NM_020242	KIF15	Homo sapiens kinesin family member 15 (KIF15), mRNA [NM_020242]

UP-regulated genes in metastatic GISts					
Probe Name	Corrected p-value	Fold Change	Genbank Accession	Gene Symbol	Description
A_23_P118174	4.45E-06	3.5	NM_005030	PLK1	Homo sapiens polo-like kinase 1 (Drosophila) (PLK1), mRNA [NM_005030]
A_23_P415443	4.45E-06	3.5	NM_015341	NCAPH	Homo sapiens non-SMC condensin I complex, subunit H (NCAPH), mRNA [NM_015341]
A_24_P323434	4.47E-06	7.1	NM_152562	CDCA2	Homo sapiens cell division cycle associated
A_24_P466231	4.91E-06	3.6	THC2515749	THC2515749	2 (CDCA2), mRNA [NM_152562] Q6NWY8_HUMAN (Q6NWY8) LOC146909 protein (Fragment), partial (29%) [THC2515749]
A_23_P51085	5.23E-06	6.0	NM_020675	SPC25	Homo sapiens SPC25, NDC80 kinetochore complex component, homolog (S. cerevisiae) (SPC25), mRNA [NM_020675]
A_32_P407245	5.31E-06	4.0	NM_024902	FLJ13236	Homo sapiens hypothetical protein FLJ13236 (FLJ13236), mRNA [NM_024902]
A_23_P138507	5.32E-06	5.5	NM_001786	CDC2	Homo sapiens cell division cycle 2, G1 to S and G2 to M (CDC2), transcript variant 1, mRNA [NM_001786]
A_23_P49878	5.32E-06	7.2	NM_019013	FAM64A	Homo sapiens family with sequence similarity 64, member A(FAM64A), mRNA [NM_019013]
A_23_P345707	5.58E-06	5.6	NM_152259	C15orf42	Homo sapiens chromosome 15 open reading frame 42 (C15orf42), mRNA [NM_152259]
A_23_P52017	5.62E-06	7.4	NM_018136	ASPM	Homo sapiens asp (abnormal spindle) homolog, microcephaly associated (Drosophila) (ASPM), mRNA [NM_018136]
A_23_P88630	5.62E-06	3.3	NM_000057	BLM	Homo sapiens Bloom syndrome (BLM), mRNA [NM_000057]
A_24_P680947	5.62E-06	9.1	ENST00000335534	LOC146909	Homo sapiens hypothetical protein LOC146909, mRNA (cDNA clone IMAGE:4418755), partial cds. [BC048263]
A_32_P109296	5.67E-06	5.8	NM_152259	C15orf42	Homo sapiens chromosome 15 open reading frame 42 (C15orf42), mRNA [NM_152259]
A_24_P914479	5.84E-06	3.4	BC002724	SNX5	Homo sapiens sorting nexin 5, mRNA
					(cDNA clone IMAGE:3629947), complete cds. [BC002724]
A_24_P84970	5.88E-06	3.0	XR_016386	KRT18P42	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC391819), mRNA [XR_016386]
A_24_P161827	6.30E-06	5.1	XR_018749	LOC442405	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC442405), mRNA [XR_018749]
A_23_P388812	6.70E-06	5.7	NM_152515	CKAP2L	Homo sapiens cytoskeleton associated protein 2-like (CKAP2L), mRNA [NM_152515]
A_23_P48669	6.84E-06	4.4	NM_005192	CDKN3	Homo sapiens cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase) (CDKN3), mRNA [NM_005192]
A_23_P151150	7.46E-06	4.9	NM_202002	FOXM1	Homo sapiens forkhead box M1 (FOXM1), transcript variant 1, mRNA [NM_202002]
A_23_P52278	7.65E-06	4.0	NM_004523	KIF11	Homo sapiens kinesin family member 11 (KIF11), mRNA [NM_004523]
A_23_P34788	7.84E-06	4.7	NM_006845	KIF2C	Homo sapiens kinesin family member 2C (KIF2C), mRNA [NM_006845]
A_23_P70007	7.84E-06	5.3	NM_012484	HMMR	Homo sapiens hyaluronan-mediated mobility receptor (RHAMM) (HMMR), transcript variant 1, mRNA [NM_012484]
A_23_P161474	7.95E-06	4.5	NM_182751	MCM10	Homo sapiens MCM10 minichromosome maintenance deficient 10 (S. cerevisiae) (MCM10), transcript variant 1, mRNA [NM_182751]
A_24_P48248	7.95E-06	3.1	NM_024032	C17orf53	Homo sapiens chromosome 17 open reading frame 53 (C17orf53), mRNA [NM_024032]
A_23_P252292	8.09E-06	4.5	NM_006733	CENPI	Homo sapiens centromere protein I (CENPI), mRNA [NM_006733]
A_24_P14156	8.62E-06	5.3	NM_006101	NDC80	Homo sapiens NDC80 homolog, kinetochore complex component (S. cerevisiae) (NDC80), mRNA [NM_006101]
A_23_P356684	8.95E-06	5.9	NM_018685	ANLN	Homo sapiens anillin, actin binding protein (ANLN), mRNA [NM_018685]
A_23_P57588	9.10E-06	4.7	NM_016426	GTSE1	Homo sapiens G-2 and S-phase expressed 1 (GTSE1), mRNA [NM_016426]
A_24_P375360	9.10E-06	4.3	XR_019146	LOC651439	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC651439), mRNA [XR_019146]
A_24_P257099	9.31E-06	7.1	NM_018410	DKFZp762E1312	Homo sapiens hypothetical protein DKFZp762E1312 (DKFZp762E1312), mRNA [NM_018410]

UP-regulated genes in metastatic GISts					
Probe Name	Corrected p-value	Fold Change	Genbank Accession	Gene Symbol	Description
A_24_P247233	9.39E-06	4.9	XR_018420	KRT18P16	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC391827), mRNA [XR_018420]
A_23_P37704	9.39E-06	4.4	NM_030928	CDT1	Homo sapiens chromatin licensing and DNA replication factor 1 (CDT1), mRNA [NM_030928]
A_23_P164814	9.69E-06	3.3	NM_024323	C19orf57	Homo sapiens chromosome 19 open reading frame 57 (C19orf57), mRNA [NM_024323]
A_23_P88331	9.69E-06	6.2	NM_014750	DLG7	Homo sapiens discs, large homolog 7 (Drosophila) (DLG7), mRNA [NM_014750]
A_23_P57379	9.69E-06	4.1	NM_003504	CDC45L	Homo sapiens CDC45 cell division cycle 45-like (S. cerevisiae) (CDC45L), mRNA [NM_003504]
A_23_P259586	9.69E-06	6.7	NM_003318	TTK	Homo sapiens TTK protein kinase (TTK), mRNA [NM_003318]
A_23_P210853	9.69E-06	5.6	NM_021067	GINS1	Homo sapiens GINS complex subunit 1 (Psf1 homolog) (GINS1), mRNA [NM_021067]
A_24_P916195	9.69E-06	4.5	NM_016426	GTSE1	Homo sapiens G-2 and S-phase expressed 1 (GTSE1), mRNA [NM_016426]
A_24_P332595	9.69E-06	4.2	XR_018618	KRT18P47	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC390634), mRNA [XR_018618]
A_23_P94422	9.90E-06	5.2	NM_014791	MELK	Homo sapiens maternal embryonic leucine zipper kinase (MELK), mRNA [NM_014791]
A_23_P206441	9.94E-06	4.2	NM_000135	FANCA	Homo sapiens Fanconi anemia, complementation group A (FANCA), transcript variant 1, mRNA [NM_000135]
A_32_P62997	1.00E-05	7.4	NM_018492	PBK	Homo sapiens PDZ binding kinase (PBK), mRNA [NM_018492]
A_24_P728920	1.05E-05	6.6	BC131554	BC 131554	Homo sapiens cDNA clone IMAGE:40108029. [BC131554]
A_24_P218979	1.18E-05	3.3	NM_031299	CDCA3	Homo sapiens cell division cycle associated 3 (CDCA3), mRNA [M_031299]
A_24_P378331	1.22E-05	3.1	NM_144508	CASC5	Homo sapiens cancer susceptibility candidate 5 (CASC5), transcript variant 2, mRNA [NM_144508]
A_24_P96780	1.22E-05	4.6	NM_016343	CENPF	Homo sapiens centromere protein F, 350/400ka (mitosin) (CENPF), mRNA [NM_016343]
A_23_P256956	1.23E-05	6.9	NM_005733	KIF20A	Homo sapiens kinesin family member 20A
A_24_P195164	1.23E-05	4.3	THC2524582	THC2524582	(KIF20A), mRNA [NM_005733] Q5U0N8_HUMAN (Q5U0N8) Keratin 18 (Cell proliferation-inducing protein 46), partial (46%) [THC2524582]
A_23_P65757	1.23E-05	5.1	NM_004701	CCNB2	Homo sapiens cyclin B2 (CCNB2), mRNA [NM_004701]
A_23_P122650	1.23E-05	4.5	XR_018843	LOC649233	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC649233), mRNA [XR_018843]
					similar to Ubiquitin-conjugating enzyme E2 C (Ubiquitin-protein ligase C) (Ubiquitin carrier protein C) (UbcH10) (LOC648937), mRNA [Source:RefSeq_dna;Acc:XR_018466]
A_24_P306896	1.27E-05	5.9	ENST00000323198	ENST00000323198	[ENST00000323198]
A_23_P35219	1.27E-05	8.7	NM_002497	NEK2	Homo sapiens NIMA (never in mitosis gene a)-related kinase 2 (NEK2), mRNA [NM_002497]
A_32_P151544	1.27E-05	5.1	NM_000224	KRT18	Homo sapiens keratin 18 (KRT18), transcript variant 1, mRNA [NM_000224]
A_24_P176374	1.30E-05	3.9	NM_030928	CDT1	Homo sapiens chromatin licensing and DNA replication factor 1 (CDT1), mRNA [NM_030928]
A_24_P153003	1.41E-05	3.2	XR_019238	LOC652192	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC652192), mRNA [XR_019238]
A_23_P119254	1.44E-05	3.4	NM_018154	ASF1 B	Homo sapiens ASF1 anti-silencing function 1 homolog B (S. cerevisiae) (ASF1 B), mRNA [NM_018154]
A_24_P230486	1.44E-05	4.1	A_24_P230486	A_24_P230486	
A_23_P118834	1.46E-05	5.2	NM_001067	TOP2A	Homo sapiens topoisomerase (DNA) II alpha 170kDa (TOP2A), mRNA [NM_001067]
A_23_P155815	1.53E-05	5.2	NM_022346	NCAPG	Homo sapiens non-SMC condensin I complex, subunit G (NCAPG), mRNA [NM_022346]
A_24_P911179	1.53E-05	8.0	NM_018136	ASPM	Homo sapiens asp (abnormal spindle) homolog, microcephaly associated (Drosophila) (ASPM), mRNA [NM_018136]
A_23_P160537	1.57E-05	3.8	NM_024037	C1orf135	Homo sapiens chromosome 1 open reading frame 135 (C1orf135), mRNA [NM_024037]

UP-regulated genes in metastatic GISts					
Probe Name	Corrected p-value	Fold Change	Genbank Accession	Gene Symbol	Description
A_23_P66732	1.58E-05	3.5	NM_031965	GSG2	Homo sapiens germ cell associated 2 (haspin) (GSG2), mRNA [NM_031965]
A_24_P418687	1.62E-05	5.6	XR_015605	LOC731794	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC731794), mRNA [XR_015605]
A_23_P70249	1.68E-05	7.4	NM_001790	CDC25C	Homo sapiens cell division cycle 25 homolog C (S. pombe) (CDC25C), transcript variant 1, mRNA [NM_001790]
A_23_P258493	1.68E-05	3.5	NM_005573	LMNB1	Homosapiens lamin B1 (LMNB1), mRNA [NM_005573]
A_23_P115872	1.69E-05	5.9	NM_018131	CEP55	Homo sapiens centrosomal protein 55kDa (CEP55), mRNA [NM_018131]
A_24_P50328	1.72E-05	4.8	A_24_P50328	A_24_P50328	
A_24_P471242	1.84E-05	4.9	A_24_P471242	A_24_P471242	
A_24_P383660	1.84E-05	6.1	XR_018670	KRT18P12	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC643471), mRNA [XR_018670]
A_23_P99292	1.97E-05	3.2	NM_006479	RAD51AP1	Homo sapiens RAD51 associated protein 1 (RAD51AP1), mRNA [NM_006479]
A_23_P25069	2.13E-05	3.7	BC039117	OVOS2	Homo sapiens ovostatin 2, mRNA (cDNA clone IMAGE:4827636). [BC039117]
A_24_P161809	2.22E-05	5.2	ENST00000333983	ENST00000333983	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC391179), mRNA [XR_018953]
A_23_P259641	2.22E-05	3.1	NM_004456	EZH2	Homo sapiens enhancer of zeste homolog 2 (Drosophila) (EZH2), transcript variant 1, mRNA [NM_004456]
A_24_P255954	2.23E-05	5.5	XR_019330	LOC652370	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC652370), mRNA [XR_019330]
A_23_P206059	2.25E-05	3.5	NM_003981	PRC1	Homo sapiens protein regulator of cytokinesis 1 (PRC1), transcript variant 1, mRNA [NM_003981]
A_23_P35871	2.26E-05	5.3	NM_024680	E2F8	Homo sapiens E2F transcription factor 8 (E2F8), mRNA [NM_024680]
A_24_P416079	2.33E-05	4.4	NM_016359	NUSAP1	Homo sapiens nucleolar and spindle associated protein 1 (NUSAP1), transcript variant 1, mRNA [NM_016359]
A_24_P161733	2.43E-05	5.9	A_24_P161733	A_24_P161733	
A_24_P350060	2.47E-05	5.6	XR_016386	KRT18P42	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC391819), mRNA [XR_016386]
A_23_P63789	2.52E-05	3.0	NM_001005414	ZWINT	Homo sapiens ZW10 interactor (ZWINT), transcript variant 4, mRNA [NM_001005414]
A_24_P412088	2.52E-05	5.0	NM_182751	MCM10	Homo sapiens MCM10 minichromosome maintenance deficient 10 (S. cerevisiae) (MCM10), transcript variant 1, mRNA [NM_182751]
A_32_P108748	2.62E-05	3.5	THC2534530	THC2534530	AF235023 chromosome condensation protein G {Homo sapiens} (exp=0; wgp=1; cg=0), partial (3%) [THC2534530]
A_24_P16230	2.66E-05	6.6	XR_019037	LOC391271	PREDICTED: Homo sapiens hypothetical LOC391271 (LOC391271), mRNA [XR_019037]
A_23_P355075	2.80E-05	3.0	AK023669	CENPN	Homo sapiens cDNA FLJ13607 fis, clone PLACE1010624. [AK023669]
A_24_P25872	3.08E-05	5.4	NM_017779	DEPDC1	Homo sapiens DEP domain containing 1 (DEPDC1), mRNA [NM_017779]
A_24_P792988	3.09E-05	6.0	A_24_P792988	A_24_P792988	
A_24_P419132	3.09E-05	3.5	NM_006733	CENPI	Homo sapiens centromere protein I (CENPI), mRNA [NM_006733]
A_23_P254733	3.21E-05	3.3	NM_024629	MLF1IP	Homo sapiens MLF1 interacting protein (MLF1IP), mRNA [NM_024629]
A_24_P281374	321E-05	5.2	XR_018462	KRT18P45	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC391803), mRNA [XR_018462]
A_23_P134910	3.27E-05	3.9	NM_003878	GGH	Homo sapiens gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase) (GGH), mRNA [NM_003878]

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Probe Name	Corrected p-value	Fold Change	Genbank Accession	Gene Symbol	Description
A_23_P148475	3.48E-05	4.5	NM_012310	KIF4A	Homo sapiens kinesin family member 4A (KIF4A), mRNA [M_012310]
A_24_P358406	3.54E-05	6.2	A_24_P358406	A_24_P358406	
A_24_P169843	3.63E-05	4.8	XR_019568	KRT18P28	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC343326), mRNA [XR_019568]
A_23_P150667	3.63E-05	4.4	NM_031217	KIF18A	Homo sapiens kinesin family member 18A (KIF18A), mRNA [NM_031217]
A_24_P780319	3.69E-05	4.1	A_24_P780319	A_24_P780319	
A_23_P116123	3.69E-05	3.5	NM_001274	CHEK1	Homo sapiens CHK1 checkpoint homolog (S. pombe) (CHEK1), mRNA [NM_001274]
A_24_P584463	3.72E-05	5.4	XR_018311	LOC139060	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC139060), mRNA [XR_018311]
A_23_P323751	3.80E-05	6.8	NM 030919	FAM83D	Homo sapiens family with sequence similarity 83, member D (FAM83D), mRNA [NM_030919]
A_24_P186746	3.92E-05	6.6	XR_019198	KRT18P34	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC391589), mRNA [XR_019198]
A_24_P84711	4.01E-05	4.8	A_24_P84711	A_24_P84711	
A_24_P230466	4.03E-05	6.2	XR_018953	KRT18P32	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC391179), mRNA [XR_018953]
A_32_P9924	4.09E-05	3.5	THC2525505	THC2525505	
A_23_P149668	4.16E-05	5.8	NM_014875	KIF14	Homo sapiens kinesin family member 14 (KIF14), mRNA [NM_014875]
A_23_P25150	4.39E-05	21.2	NM_006897	HOXC9	Homo sapiens homeobox C9 (HOXC9), mRNA [NM_006897]
A_23_P58321	4.53E-05	3.4	NM_001237	CCNA2	Homo sapiens cyclin A2 (CCNA2), mRNA [NM_001237]
A_23_P99320	4.57E-05	6.6	NM_000224	KRT18	Homo sapiens keratin 18 (KRT18),
A_23_P373708	4.60E-05	5.8	NM_173624	FLJ40504	transcript variant 1, mRNA [NM_000224] Homo sapiens hypothetical protein FLJ40504 (FLJ40504), mRNA [NM_173624]
A_24_P358131	4.85E-05	6.0	XR_019148	LOC651696	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC651696), mRNA [XR_019148]
A_24_P256063	5.02E-05	6.7	XR_019231	LOC442249	PREDICTED: Homo sapiens hypothetical LOC442249 (LOC442249), mRNA [XR_019231]
A_24_P24645	5.30E-05	5.7	XR_018938	KRT18P21	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC132391), mRNA [XR_018938]
A_32_P154726	5.37E-05	6.8	THC2603239	THC2603239	Q9NJB6_TRYBR (Q9NJB6) Fibrillarin, partial (10%) [THC2603239]
A_23_P216517	5.67E-05	4.0	NM_032818	C9orf100	Homo sapiens chromosome 9 open reading frame 100 (C9orf100), mRNA [NM_032818]
A_24_P281443	5.82E-05	7.2	XR_018559	LOC649375	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC649375), mRNA [XR_018559]
A_23_P134584	5.83E-05	3.0	NM_005431	XRCC2	Homo sapiens X-ray repair complementing defective repair in Chinese hamster cells 2 (XRCC2), mRNA [NM_005431]
A_24_P6850	5.92E-05	4.7	A_24_P6850	A_24 P6850	
A_24_P264644	5.92E-05	5.6	XR_016695	KRT18P41	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC345430), mRNA [XR_016695]
A_23_P368909	6.02E-05	4.4	ENST00000328711	ENST00000328711	Uncharacterized protein C13orf29. [Source:Uniprot/SWISSPROT;Acc:Q8IVM7] [ENST00000328711]
A_24_P42136	6.22E-05	7.7	NM_000224	KRT18	Homo sapiens keratin 18 (KRT18), transcript variant 1, mRNA [NM_000224]
A_24_P230057	6.74E-05	7.2	XR_018216	LOC647913	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC647913), mRNA [XR_018216]
A_24_P314571	7.33E-05	3.5	NM_182513	SPC24	Homo sapiens SPC24, NDC80 kinetochore complex component, homolog (S. cerevisiae) (SPC24), mRNA [NM_182513]

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Probe Name	Corrected p-value	Fold Change	Genbank Accession	Gene Symbol	Description
A_23_P29723	7.60E-05	4.4	NM_001012410	SGOL1	Homo sapiens shugoshin-like 1 (S. pombe) (SGOL1), transcript variant A2, mRNA [M_001012410]
A_23_P120863	8.27E-05	5.8	NM_004861	GAL3ST1	Homo sapiens galactose-3-O-sulfotransferase 1 (GAL3ST1), mRNA [NM_004861]
A_24_P225970	8.78E-05	5.9	NM_001012409	SGOL1	Homo sapiens shugoshin-like 1 (S. pombe) (SGOL1), transcript variant A1, mRNA [NM_001012409]
A_23_P130182	1.01E-04	5.2	NM_004217	AURKB	Homo sapiens aurora kinase B (AURKB), mRNA [NM_004217]
A_23_P10385	1.05E-04	4.1	NM_016448	DTL	Homo sapiens denticleless homolog (Drosophila) (DTL), mRNA [NM_016448]
A_23_P92093	1.16E-04	3.5	NM_001407	CELSR3	Homo sapiens cadherin, EGF LAG seven-pass G-type receptor 3 (flamingo homolog, Drosophila) (CELSR3), mRNA [NM_001407]
A_24_P940678	1.22E-04	4.4	NM_170589	CASC5	Homo sapiens cancer susceptibility candidate 5 (CASC5), transcript variant 1,
					mRNA [NM_170589]
A_24_P401601	1.27E-04	5.9	XR_017288	KRT18P40	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC390904), mRNA [XR_017288]
A_23_P500464	1.31E-04	7.8	NM_001844	COL2A1	Homo sapiens collagen, type II, alpha 1 (primary osteoarthritis, spondyloepiphyseal dysplasia, congenital) (COL2A1), transcript variant 1, mRNA [NM_001844]
A_23_P373119	1.35E-04	3.2	NR_002165	HMG4L	Homo sapiens high-mobility group (nonhistone chromosomal) protein 4-like (HMG4L) on chromosome 20 [NR_002165]
A_24_P384369	1.36E-04	3.7	XR_018339	LOC648448	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC648448), mRNA [XR_018339]
A_23_P200310	1.46E-04	3.1	NM_017779	DEPDC1	Homo sapiens DEP domain containing 1 (DEPDC1), mRNA [NM_017779]
A_24_P247454	1.46E-04	7.6	XR_019026	KRT18P19	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC339781), mRNA [XR_019026]
A_23_P85441	1.51E-04	3.3	NM_020789	IGSF9	Homo sapiens immunoglobulin superfamily, member 9 (IGSF9), mRNA [NM_020789]
A_24_P144625	1.59E-04	7.3	A_24_P144625	A_24_P144625	
A_23_P431776	1.60E-04	8.6	NM_001986	ETV4	Homo sapiens ets variant gene 4 (E1A enhancer binding protein, E1AF) (ETV4), transcript variant 1, mRNA [NM_001986]
A_24_P416346	1.61E-04	8.1	NM_001986	ETV4	Homo sapiens ets variant gene 4 (E1A enhancer binding protein, E1AF) (ETV4), transcript variant 1, mRNA [NM_001986]
A_23_P408955	1.68E-04	4.2	NM_004091	E2F2	Homo sapiens E2F transcription factor 2 (E2F2), mRNA [NM_004091]
A_23_P48835	1.71E-04	3.7	NM_138555	KIF23	Homo sapiens kinesin family member 23 (KIF23), transcript variant 1, mRNA [NM_138555]
A_23_P379614	1.73E-04	3.0	NM_007280	OIP5	Homo sapiens Opa interacting protein 5 (OIP5), mRNA [NM_007280]
A_32_P119154	1.78E-04	4.5	BE138567	BE138567	xr77d10.x2 NCI_CGAP_Ov26 Homo sapiens cDNA clone IMAGE:2766163 3', mRNA sequence [BE138567]
A_23_P310	1.89E-04	4.0	NM_023009	MARCKSL1	Homo sapiens MARCKS-like 1 (MARCKSL1), mRNA [NM_023009]
A_32_P76720	1.89E-04	3.6	NM_016575	NT5DC3	Homo sapiens 5'-nucleotidase domain containing 3 (NT5DC3), transcript variant 2, mRNA [NM_016575]
A_23_P50250	1.90E-04	3.0	NM_001824	CKM	Homo sapiens creatine kinase, muscle (CKM), mRNA [NM_001824]
A_23_P217236	1.99E-04	3.1	NM_005342	HMGB3	Homo sapiens high-mobility group box 3 (HMGB3), mRNA [NM_005342]
A_24_P346855	2.00E-04	4.9	NM_002417	MKI67	Homo sapiens antigen identified by monoclonal antibody Ki-67 (MKI67), mRNA [NM_002417]
A_24_P68088	2.04E-04	10.8	NR_002947	TCAM1	Homo sapiens testicular cell adhesion molecule 1 homolog (mouse) (TCAM1) on chromosome 17 [NR_002947]
A_24_P399888	2.10E-04	4.2	NM_001002876	CENPM	Homo sapiens centromere protein M (CENPM), transcript variant 2, mRNA [NM_001002876]
A_23_P88731	2.13E-04	3.4	NM_002875	RAD51	Homo sapiens RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae) (RAD51), transcript variant 1, mRNA [NM_002875]
A_23_P350754	2.14E-04	3.5	AF238487	OR7E13P	Homo sapiens olfactory-like receptor PJCG2 (PJCG2) mRNA, partial cds. [AF238487]

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Probe Name	Corrected p-value	Fold Change	Genbank Accession	Gene Symbol	Description
A_23_P117852	2.21E-04	3.9	NM_014736	KIAA0101	Homo sapiens KIAA0101 (KIAA0101), transcript variant 1, mRNA [NM_014736]
A_23_P100127	2.25E-04	4.8	NM_170589	CASC5	Homo sapiens cancer susceptibility candidate 5 (CASC5), transcript variant 1, mRNA [NM_170589]
A_23_P155989	2.30E-04	3.0	NM_022145	CENPK	Homo sapiens centromere protein K (CENPK), mRNA [NM_022145]
A_24_P109661	2.33E-04	7.5	XR_019191	KRT18P20	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC121054), mRNA [XR_019191]
A_24_P686014	2.48E-04	6.3	XR_019186	LOC651929	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC651929), mRNA [XR_019186]
A_23_P50108	2.48E-04	3.7	NM_006101	NDC80	Homo sapiens NDC80 homolog, kinetochore complex component (S. cerevisiae) (NDC80), mRNA [NM_006101]
A_23_P108294	2.53E-04	3.6	NM_177543	PPAP2C	Homo sapiens phosphatidic acid phosphatase type 2C (PPAP2C), transcript variant 3, mRNA [NM_177543]
A_23_P110851	2.95E-04	6.4	NM_198253	TERT	Homo sapiens telomerase reverse transcriptase (TERT), transcript variant 1, mRNA [NM_198253]
A_24_P264293	3.03E-04	9.4	XR_019060	LOC644030	PREDICTED: Homo sapiens similar to Keratin, type I cytoskeletal 18 (Cytokeratin-18) (CK-18) (Keratin-18) (K18) (LOC644030), mRNA [XR_019060]
A_24_P247303	3.33E-04	8.7	A_24_P247303	A_24_P247303	
A_32_P311737	3.42E-04	3.2	AB011171	PLEKHG3	Homo sapiens mRNA for KIAA0599 protein, partial cds. [AB011171]
A_23_P166306	3.44E-04	6.9	NM_000071	CBS	Homo sapiens cystathionine-beta-synthase (CBS), mRNA [NM_000071]
A_23_P23303	3.48E-04	3.3	NM_003686	EXO1	Homo sapiens exonuclease 1 (EXO1), transcript variant 3, mRNA [NM_003686]
A_24_P255836	3.52E-04	3.5	A_24_P255836	A_24_P255836	
A_32_P168561	3.61E-04	3.1	THC2634862	THC2634862	NM_063888 TAF (TBP-associated transcription factor) family member (taf-13) {Caenorhabditis elegans} (exp=-1; wgp=0; cg=0), partial (15%) [THC2634862]
A_24_P254705	3.62E-04	3.7	NM_020394	ZNF695	Homo sapiens zinc finger protein 695 (ZNF695), mRNA [NM_020394]
A_24_P234196	3.63E-04	3.8	NM_001034	RRM2	Homo sapiens ribonucleotide reductase M2 polypeptide (RRM2), mRNA [NM_001034]
A_32_P188127	3.68E-04	6.8	A_32_P188127	A_32_P188127	
A_23_P155711	3.77E-04	5.3	NM_018248	NEIL3	Homo sapiens nei endonuclease VIII-like 3 (E. coli) (NEIL3), mRNA [NM_018248]
A_24_P85498	3.84E-04	11.6	AL117481	DKFZP434B061	Homo sapiens mRNA; cDNA DKFZp434B061 (from clone DKFZp434B061); partial cds. [AL117481]
A_23_P115444	3.97E-04	4.2	NM_005092	TNFSF18	Homo sapiens tumor necrosis factor (ligand) superfamily, member 18 (TNFSF18), mRNA [NM_005092]
A_32_P108938	4.05E-04	5.7	THC2536711	THC2536711	
A_23_P143512	4.08E-04	3.1	NM_007031	HSF2BP	Homo sapiens heat shock transcription factor 2 binding protein (HSF2BP), mRNA [NM_007031]
A_23_P252928	4.12E-04	11.3	NM_005367	MAGEA12	Homo sapiens melanoma antigen family A, 12 (MAGEA12), mRNA [NM_005367]
A_32_P147090	4.70E-04	3.3	NM_199357	ARHGAP11A	Homo sapiens Rho GTPase activating protein 11A (ARHGAP11A), transcript variant 2, mRNA [NM_199357]
A_23_P102058	4.89E-04	3.1	NM_002381	MATN3	Homo sapiens matrilin 3 (MATN3), mRNA [NM_002381]
A_32_P169500	4.92E-04	3.2	THC2537856	THC2537856	ALU1_HUMAN (P39188) Alu subfamily J sequence contamination warning entry, partial (14%) [THC2537856]
A_23_P163099	5.02E-04	3.2	NM_002692	POLE2	Homo sapiens polymerase (DNA directed), epsilon 2 (p59 subunit) (POLE2), mRNA [NM_002692]
A_23_P405267	5.04E-04	3.2	AK057922	CDH24	Homo sapiens cDNA FLJ25193 fis, clone JTH00761. [AK057922]
A_23_P74115	5.23E-04	3.1	NM_003579	RAD54L	Homo sapiens RAD54-like (S. cerevisiae) (RAD54L), mRNA [NM_003579]
A_24_P409420	5.78E-04	5.6	A_24_P409420	A_24_P409420	
A_24_P384018	5.78E-04	3.7	NR_002171	OR7E156P	Homo sapiens olfactory receptor, family 7, subfamily E, member 156 pseudogene (OR7E156P) on chromosome 13 [NR_002171]

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Probe Name	Corrected p-value	Fold Change	Genbank Accession	Gene Symbol	Description
A_24_P192727	5.85E-04	6.5	ENST00000224809	KAZALD1	Kazal-type serine protease inhibitor domain-containing protein 1 precursor. [Source: Uniprot/SWISSPROT;Acc:Q96I82] [ENST00000224809]
A_32_P210202	5.99E-04	3.2	NM_203394	E2F7	Homo sapiens E2F transcription factor 7 (E2F7), mRNA [NM_203394]
A_23_P58557	6.09E-04	3:4	NM_173800	FLJ90650	Homo sapiens laeverin (FLJ90650), mRNA [NM_173800]
A_32_P43084	6.45E-04	3.3	BM980974	BM980974	BM980974 UI-CF-EN1-ade-p-19-0-UI.s1 UI-CF-EN1 Homo sapiens cDNA clone UI-CF-EN1-ade-p-19-0-UI 3', mRNA sequence
					[BM980974]
A_23_P135061	6.68E-04	3.0	NM_003389	CORO2A	Homo sapiens coronin, actin binding protein, 2A (CORO2A), transcript variant 1, mRNA [NM_003389]
A_32_P32391	6.76E-04	3.7	NR_002171	OR7E156P	Homo sapiens olfactory receptor, family 7, subfamily E, member 156 pseudogene (OR7E156P) on chromosome 13 [NR_002171]
A_23_P7412	7.04E-04	4.2	NM_024850	BTNL8	Homo sapiens butyrophilin-like 8 (BTNL8), transcript variant 1, mRNA [NM_024850]
A_32_P46544	7.04E-04	3.0	A_32_P46544	A_32_P46544	
A_23_P113034	7.23E-04	3.5	NM_032024	C10orf11	Homo sapiens chromosome 10 open reading frame 11 (C10orf11), mRNA [NM_032024]
A_23_P50517	7.29E-04	3.9	ENST00000314121	ENST00000314121	Zinc finger protein 541. [Source:Uniprot/SWISSPROT;Acc:Q9H0D2] [ENST00000314121]
A_23_P96291	7.47E-04	5.3	NM_004988	MAGEA1	Homo sapiens melanoma antigen family A, 1 (directs expression of antigen MZ2-E) (MAGEA1), mRNA [NM_004988]
A_23_P16110	7.63E-04	3.5	NM_001079935	OR7E24	Homo sapiens olfactory receptor, family 7, subfamily E, member 24 (OR7E24), mRNA [NM_001079935]
A_32_P150891	8.60E-04	4.4	NM_001042517	DIAPH3	Homo sapiens diaphanous homolog 3 (Drosophila) (DIAPH3), transcript variant 1, mRNA [NM_001042517]
A_23_P207154	8.75E-04	5.2	NM_022644	CSH2	Homo sapiens chorionic somatomammotropin hormone 2 (CSH2), transcript variant 2, mRNA [NM_022644]
A_23_P250164	9.04E-04	4.3	NM_000187	HGD	Homo sapiens homogentisate 1,2-dioxygenase (homogentisate oxidase)
					(HGD), mRNA [NM_000187]
					Homo sapiens cDNA clone
A_24_P820087	9.47E-04	3.3	BC053669	BC053669	IMAGE:6146402, partial cds. [BC053669]
Down-regulated genes in metastatic GISTs					
Probe Name	Corrected p-value	Fold Change	Genbank Acession	Gene Symbol	Description
A_23_P167159	1.32E-06	28.6	NM_007281	SCRG1	Homo sapiens scrapie responsive protein 1 (SCRG1), mRNA [NM_007281]
A_24_P198044	5.02E-06	3.5	NM_133464	ZNF483	Homo sapiens zinc finger protein 483 (ZNF483), transcript variant 1, mRNA [NM_133464]
A_23_P45536	6.65E-06	13.6	NM_005369	MCF2	Homo sapiens MCF.2 cell line derived transforming sequence (MCF2), mRNA [NM_005369]
A_23_P79978	7.08E-06	12.7	NM_020689	SLC24A3	Homo sapiens solute carrier family 24 (sodium/potassium/calcium exchanger), member 3 (SLC24A3), mRNA [NM_020689]
A_23_P62881	7.46E-06	7.3	NM_032291	SGIP1	Homo sapiens SH3-domain GRB2-like (endophilin) interacting protein 1 (SGIP1), mRNA [NM_032291]
A_23_P73117	1.07E-05	8.9	NM_013266	CTNNA3	Homo sapiens catenin (cadherin-associated protein), alpha 3 (CTNNA3), mRNA [NM_013266]
					BX106262
A_32_P65700	1.14E-05	6.0	BX106262	BX106262	Soares_multiple_sclerosis_2NbHMSP Homo sapiens cDNA clone IMAGp998O20625, mRNA sequence [BX106262]
A_23_P394567	1.41E-05	3.1	NM_020853	KIAA1467	Homo sapiens KIAA1467 (KIAA1467),
					mRNA [NM_020853]
A_23_P259442	1.53E-05	4.4	NM_001873	CPE	Homo sapiens carboxypeptidase E (CPE), mRNA[NM_001873]
A_24P56363	1.58E-05	3.6	NM_030925	CAB39L	Homo sapiens calcium binding protein 39-like (CAB39L), transcript-variant 1, mRNA [NM_030925]
A_24_P333857	2.15E-05	7.1	NM_032291	SGIP1	sapiens SH3-domain GRB2-like (endophilin) interacting protein 1 (SGIP1), mRNA_[NM_032291]

Down-regulated genes in metastatic GISTs					
Probe Name	Corrected p-value	Fold Change	Genbank Acession	Gene Symbol	Description
A_24_P153831	2.22E-05	5.6	BC022004	CTNNA3	Homo sapiens catenin (cadherin-associated protein), alpha 3, mRNA (cDNA clone IMAGE:4823848), complete-cds. [BC022004]
A_23_P344194	2.40E-05	5.2	NM_001013635	LOC387856	Homo sapiens similar to expressed sequence AI836003 (LOC387856), mRNA [NM_001013635]
A_23_P92903	2.45E-05	7.4	NM_031908	C1QTNF2	Homo sapiens C1q and tumor necrosis factor related protein 2 (C1QTNF2), mRNA [NM_031908]
A_32_P213861	2.52E-05	3.3	AK124663	C4orf12	Homo sapiens cDNA FLJ42672 fis, clone BRAMY2026533. [AK124663]
A_23_P213810	2.66E-05	3.2	NM_015621	CCDC69	Homo sapiens coiled-coil domain containing 69 (CCDC69), mRNA [NM_015621]
A_23_P92899	2.70E-05	7.0	NM_031908	C1QTNF2	Homo sapiens C1q and tumor necrosis factor related protein 2 (C1 QTNF2), mRNA [NM_031908]
A_23_P95634	2.73E-05	7.7	NM_016599	MYOZ2	Homo sapiens myozenin 2 (MYOZ2), mRNA [NM_016599]
A_24_P45481	2.76E-05	5.2	NM_005465	AKT3	Homo sapiens v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma) (AKT3), transcript variant 1, mRNA
					[NM_005465]
A_23_P139891	3.33E-05	4.8	NM_012306	FAIM2	Homo sapiens Fas apoptotic inhibitory molecule 2 (FAIM2), mRNA [NM_012306]
A_23_P325690	3.73E-05	7.3	NM_144698	ANKRD35	Homo sapiens ankyrin repeat domain 35 (ANKRD35), mRNA [NM_144698]
A_23_P43810	4.79E-05	8.1	NM_206943	LTBP1	Homo sapiens latent transforming growth factor beta binding protein 1 (LTBP1), transcript variant 1, mRNA [NM_206943]
A_24_P37300	5.14E-05	9.3	AF052115	AF052115	Homo sapiens clone 23688 mRNA sequence. [AF052115]
A_24_P381499	7.11E-05	4.3	NM_152436	GLIPR1L2	Homo sapiens GLI pathogenesis-related 1 like 2 (GLIPR1L2), mRNA [NM_1152436]
A_23_P96285	7.67E-05	7.0	NM_022912	REEP1	Homo sapiens receptor accessory protein 1 (REEP1), mRNA [NM_022912]
A_23_P64510	8.17E-05	3.8	NM_024557	RIC3	Homo sapiens resistance to inhibitors of cholinesterase 3 homolog (C. elegans) (RIC3), mRNA [NM_024557]
A_23_P364024	8.78E-05	3.2	NM_006851	GLIPR1	Homo sapiens GLI pathogenesis-related 1 (glioma) (GLIPR1), mRNA [NM_006851]
A_32_P73991	9.58E-05	7.1	THC2667995	THC2667995	
A_24_P390096	9.75E-05	3.5	NM_006851	GLIPR1	Homo sapiens GLI pathogenesis-related 1 (glioma) (GLIPR1), mRNA [NM_006851]
A_32_P440667	1.00E-04	5.6	AK000774	AK000774	Homo sapiens cDNA FLJ20767 fis, clone COL06986. [AK000774]
A_24_P278747	1.05E-04	7.8	NM_001759	CCND2	Homo sapiens cyclin D2 (CCND2), mRNA [NM_001759]
A_23_P213288	1.11E-04	3.1	NM_001037582	SCD5	Homo sapiens stearoyl-CoA desaturase 5 (SCD5), transcript variant 1, mRNA [NM_001037582]
A_23_P150394	1.21E-04	3.5	NM_022003	FXYD6	Homo sapiens FXYD domain containing ion transport regulator 6 (FXYD6), mRNA
A_23_P47728	1.22E-04	5.2	NM_033063	MAP6	[NM_022003] Homo sapiens microtubule-associated protein 6 (MAP6), transcript variant 1, mRNA [NM_033063]
A_23_P254165	1.28E-04	9.3	NM_021785	RAI2	Homo sapiens retinoic acid induced 2 (RAI2), mRNA [NM_021785]
A_24_P67350	1.30E-04	7.0	NM_020689	SLC24A3	Homo sapiens solute carrier family 24 (sodium/potassium/calcium exchanger), member 3 (SLC24A3), mRNA [NM_020689]
A_24_P943781	1.30E-04	3.9	NM_024913	FLJ21986	Homo sapiens hypothetical protein FLJ21986 (FLJ21986), mRNA [NM_024913]
A_24_P381505	1.31E-04	3.5	NM_152436	GLIPR1L2	Homo sapiens GLI pathogenesis-related 1 like 2 (GLIPR1L2), mRNA [NM_152436]
A_32_P795513	1.86E-04	11.5	NM_198271	LMOD3	Homo sapiens leiomodin 3 (fetal) (LMOD3), mRNA [NM_198271]
A_24_P76821	1.93E-04	8.4	NM_198271	LMOD3	Homo sapiens leiomodin 3 (fetal) (LMOD3), mRNA [NM_198271]
A_23_P75915	1.99E-04	3.9	AY326436	RIC3	Homo sapiens RIC3 isoform d (RIC3) mRNA, complete cds. [AY326436]
A_32_P91005	2.13E-04	4.4	BM697215	BM697215	UI-E-DX0-ago-c-07-0-UI.r1 UI-E-DX0 Homo sapiens cDNA clone UI-E-DX0-ago-c-07-0-UI 5', mRNA sequence [BM697215]
A_32_P222695	2.13E-04	3.2	NM_001001669	FLJ41603	Homo sapiens FLJ41603 protein (FLJ41603), mRNA [NM_001001669]
A_24_P35537	2.13E-04	4.7	NM_024557	RIC3	Homo sapiens resistance to inhibitors of cholinesterase 3 homolog (C. elegans) (RIC3), mRNA [NM_024557]

Down-regulated genes in metastatic GISTs					
Probe Name	Corrected p-value	Fold Change	Genbank Acession	Gene Symbol	Description
A_24_P141520	2.18E-04	3.4	AK022297	AK022297	Homo sapiens cDNA FLJ12235 fis, clone MAMMA 1001243. [AK022297]
A_32_P174040	2.20E-04	14.5	THC2675966	THC2675966	Q9F8M7_CARHY (Q9F8M7) DTDP-glucose
					4,6-dehydratase (Fragment), partial (11 %) [THC2697639]
A_23_P5342	2.23E-04	16.2	NM_018557	LRP1B	Homo sapiens low density lipoprotein-related protein 1 B (deleted in tumors) (LRP1 B), mRNA [NM_018557]
A_32_P172803	2.28E-04	3.0	NM_001039580	MAP9	Homo sapiens microtubule-associated protein 9 (MAP9), mRNA [NM_001039580]
A_23_P94840	2.41E-04	5.2	NM_130897	DYNLRB2	Homo sapiens dynein, light chain, roadblock-type 2 (DYNLRB2), mRNA [NM_130897]
A_23_P19182	2.61E-04	4.4	NM_016606	REEP2	Homo sapiens receptor accessory protein 2 (REEP2), mRNA [NM_016606]
A_23_P77304	2.77E-04	4.2	NM_004644	AP3B2	Homo sapiens adaptor-related protein complex 3, beta 2 subunit (AP3B2), mRNA [NM_004644]
A_32_P179998	2.80E-04	9.5	NM_033053	DMRTC1	Homo sapiens DMRT-like family C1 (DMRTC1), mRNA [NM_033053]
A_24_P32085	2.82E-04	3.4	NM_024761	MOBKL2B	Homo sapiens MOB1, Mps One Binder kinase activator-like 2B (yeast) (MOBKL2B), mRNA [NM_024761]
A_24_P110983	2.95E-04	4.8	ENST00000366539	AKT3	RAC-gamma serine/threonine-protein kinase (EC 2.7.11.1) (RAC-PK-gamma) (Protein kinase Akt-3) (Protein kinase B, gamma) (PKB gamma) (STK-2). [Source:Uniprot/SWISSPROT;Acc:Q9Y243] [ENST00000366539]
A_23_P500892	3.06E-04	3.7	NM_003320	TUB	Homo sapiens tubby homolog (mouse) (TUB), transcript variant 1, mRNA [NM_003320]
A_24_P191781	3.10E-04	4.5	NM_015393	DKFZP564O0823	Homo sapiens DKFZP564O0823 protein (DKFZP564O0823), mRNA [M_015393]
A_24_P97825	3.61E-04	3.0	NM_015621	CCDC69	Homo sapiens coiled-coil domain containing 69 (CCDC69), mRNA [NM_015621]
A_32_P50943	3.76E-04	5.3	THC2734830	THC2734830	
A_24_P769588	3.76E-04	4.2	BQ428696	BQ428696	AGENCOURT_7904751 NIH_MGC_82 Homo sapiens cDNA clone IMAGE:6105895 5', mRNA sequence [BQ428696]
A_24_P810104	3.79E-04	3.0	AF052141	AF052141	Homo sapiens clone 24626 mRNA sequence. [AF052141]
A_24_P942385	4.28E-04	5.1	AK023797	KIAA0672	Homo sapiens cDNA FLJ13735 fis, clone PLACE3000155, weakly similar to Homo sapiens mRNA for KIAA0672 protein. [AK023797]
A_23_P123848	4.32E-04	3.2	NM_032552	DAB2IP	Homo sapiens DAB2 interacting protein (DAB2IP), transcript variant 1, mRNA [NM_032552]
A_24_P270235	4.37E-04	5.1	NM_001759	CCND2	Homo sapiens cyclin D2 (CCND2), mRNA [NM_001759]
A_24_P149704	4.62E-04	3.1	NM_138709	DAB2IP	Homo sapiens DAB2 interacting protein (DAB2IP), transcript variant 2, mRNA [NM_138709]
A_23_P121665	4.70E-04	6.6	NM_020777	SORCS2	Homo sapiens sortilin-related VPS10 domain containing receptor 2 (SORCS2), mRNA [NM_020777]
A_23_P133068	4.72E-04	3.6	NM_001148	ANK2	Homo sapiens ankyrin 2, neuronal (ANK2), transcript variant 1, mRNA [NM_001148]
A_32_P372337	4.98E-04	8.3	ENST00000333010	ENST00000333010	Janus kinase and microtubule-interacting protein 2. [Source:Uniprot/SWISSPROT;Acc:Q96AA8] [ENST00000333010]
A_23_P351667	5.16E-04	10.2	NM_003812	ADAM23	Homo sapiens ADAM metallopeptidase
A_24_P334300	5.22E-04	8.5	NM_004113	FGF12	domain 23 (ADAM23), mRNA [NM_003812] Homo sapiens fibroblast growth factor 12 (FGF12), transcript variant 2, mRNA [NM_004113]
A_32_P229618	5.40E-04	4.9	NM_001364	DLG2	Homo sapiens discs, large homolog 2, chapsyn-110 (Drosophila) (DLG2), mRNA [NM_001364]
A_32_P228206	5.45E-04	3.1	THC2463424	THC2463424	AA348270 EST54713 Hippocampus I Homo sapiens cDNA 3' end similar to EST containing Alu repeat, mRNA sequence [AA348270]
A_32_P21354	5.47E-04	6.9	THC2688038	THC2688038	
A_23_P368154	5.50E-04	9.4	NM_153703	PODN	Homo sapiens podocan (PODN), mRNA [NM_153703]
A_24_P84668	5.85E-04	3.8	NM_015687	FILIP1	Homo sapiens filamin A interacting protein 1 (FILIP1), mRNA [NM_015687]
A_23_P97990	7.14E-04	5.3	NM_002775	HTRA1	Homo sapiens HtrA serine peptidase 1 (HTRA1), mRNA [NM_002775]

Down-regulated genes in metastatic GISTs					
Probe Name	Corrected p-value	Fold Change	Genbank Acession	Gene Symbol	Description
A_24_P192627	7.15E-04	3.4	NM_004529	MLLT3	Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 3 (MLLT3), mRNA [NM_004529]
A_23_P110151	7.49E-04	4.0	NM_031305	ARHGAP24	Homo sapiens Rho GTPase activating protein 24 (ARHGAP24); transcript variant 2, mRNA [NM_031305]
A_24_P187799	7.72E-04	3.4	NM_024913	FLJ21986	Homo sapiens hypothetical protein FLJ21986 (FLJ21986), mRNA [NM_024913]
A_32_P60065	8.02E-04	14.2	NM_004101	F2RL2	Homo sapiens coagulation factor II (thrombin) receptor-like 2 (F2RL2), mRNA [M_004101]
A_23_P127915	8.60E-04	4.3	NM_030906	STK33	Homo sapiens serine/threonine kinase 33 (STK33), mRNA [NM_030906]
A_23_P54469	8.63E-04	4.3	NM_45805	ISL2	Homo sapiens ISL2 transcription factor, LIM/homeodomain, (islet-2) (ISL2), mRNA [NM_145805]
A_24_P100996	8.75E-04	3.6	ENST00000324559	TMEM16E	Transmembrane protein 16E (Gnathodiaphyseal dysplasia 1 protein). [Source:Uniprot/SWtSSPROT;Acc:Q75V66] [ENST00000324559]
A_23_P57155	8.86E-04	6.6	NM_001819	CHGB	Homo sapiens chromogranin B (secretogranin 1) (CHGB), mRNA [NM_001819]
A_24_P380061	9.14E-04	3.9	NM_031305	ARHGAP24	Homo sapiens Rho GTPase activating protein 24 (ARHGAP24), transcript variant 2, mRNA [NM_031305]
A_23_P382584	9.27E-04	7.3	NM_001819	CHGB	Homo sapiens chromogranin B (secretogranin 1) (CHGB), mRNA [M_001819]
A_32_P310335	9.32E-04	4.6	AK056079	AK056079	Homo sapiens cDNA FLJ31517 fis, clone NT2RI2000007. [AK056079]

[0056] Concerning the 70 down-regulated genes, no significantly enriched pathways were identified. In contrast, we observed that 45 of the 227 up-regulated genes belonged to the CINSARC signature (Figure 6). Furthermore, Gene Ontology analysis revealed that pathways enriched in this gene selection (227 metastasis up-regulated genes) were almost all the same as those enriched in the CINSARC signature. Actually, 63 of the 77 (82%) enriched pathways were common with CINSARC genes (Table 3).

Table 3: Comparison of enriched pathways (Gene Ontology analysis) in CINSARC genes and in t-test comparing tumors according to outcome and to p16/Rb1 pathway inactivation.

		CINSARC			Metastatic GISTs			GISTs with p16/R81 pathway altercation			Array	
Go Accession	**GO Term**	**corrected p-value**	**Count in Selection**	**% in Selection**	**corrected p-value**	**Count in Selection**	**% in Selection**	**corrected p-value**	**Count in Selection**	**% in Selection**	**Count in Array**	**% in Array**
GO:0000279	M phase	0	39	59,09	0	46	42,20	4,41 E-38	42	36,84	219	1,42
GO:0022402	cell cycle process	0	42	63,64	0	46	42,20	4,65E-32	42	36,84	343	2,22
GO:0022403	cell cycle phase	0	41	62,12	0	46	42,20	5,14E-37	42	36,84	267	1,73
GO:0000278	mitotic cell cycle	0	38	57,58	9,03E-41	39	35,78	1,08E-32	36	31,58	221	1,43
GO:0007049	cell cycle	0	47	71,21	1,44E-40	57	52,29	4,90E-31	54	47,37	584	3,78
GO:0000087	M phase of mitotic cell cycle	0	34	51,52	5,12E-39	38	34,86	3,26E-31	35	30,70	163	1,06
GO:000767	mitosis	0	34	51,52	9,10E-38	36	33,03	3,32E-30	33	28,95	160	1,04
GO:0051301	cell division	0	36	54,55	2,45E-27	32	29,36	1,16E-24	33	28,95	209	1,35
GO:0044427	chromosomal part	9,22E-16	14	21,21	7,80E-20	16	14,68	2,36E-11	15	13,16	270	1,75
GO:0000775	chromosome, centromeric region	1,53E-16	14	21,21	1,57E-19	16	14,68	3,65E-14	15	13,16	66	0,43
GO:0005694	chromosome	9,22E-16	17	25,76	5,47E-19	20	18,35	1,49E-11	20	17,54	318	2,06
GO:0007059	chromosome segregation	1,90E-08	6	9,09	2,43E-17	13	11,93	2,91E-12	7	6,14	58	0,38

		CINSARC			Metastatic GISTs			GISTs with p16/R81 pathway altercation			Array	
Go Accession	GO Term	corrected p-value	Count in Selection	% in Selection	corrected p-value	Count in Selection	% in Selection	corrected p-value	Count in Selection	% in Selection	Count in Array	% in Array
GO:0043228	non-membrane-bounded organelle	1,83E-23	37	56,06	1,76E-15	35	32,11	1,47E-13	41	35,96	1509	9,77
GO:0043232	intracellular non-membrane-bounded organelle	1,83E-23	37	56,06	1,76E-15	35	32,11	1,47E-13	41	35,96	1509	9,77
GO:0007346	regulation of mitotic cell cycle	2,43E-17	9	13,64	4,17E-15	8	7,34	1,12E-11	4	3,51	77	0,50
GO:0051726	regulation of cell cycle	3,31E-14	19	28,79	4,10E-15	22	20,18	8,55E-11	21	18,42	437	2,83
GO:0005634	nucleus	2,84E-08	44	66,67	1,39E-12	80	73,39	2,44E-05	82	71,93	3992	25,84
GO:0015630	microtubule cytoskeleton	2,33E-24	23	34,85	1,33E-11	18	16,51	8,53E-11	18	15,79	314	2,03
GO:0006996	organelle organization and biogenesis	6,03E-14	23	34,85	2,30E-11	27	24,77	4,50E-08	25	21,93	979	6,34
GO:0007017	microtubule-based process	2,92E-19	18	27,27	3,38E-11	16	14,68	4,07E-10	17	14,91	178	1,15
GO:0044446	intracellular organelle part	9,22E-16	33	50,00	1,15E-10	31	28,44	1,05E-04	30	26,32	2239	14,49
GO:0044422	organelle part	9,22E-16	33	50,00	1,22E-10	31	28,44	1,12E-04	30	26,32	2244	14,53
GO:0000070	mitotic sister chromatid segregation	5,21E-04	2	3,03	3,72E-10	9	8,26	7,51E-06	3	2,63	28	0,18
GO:0000819	sister chromatid segregation	6,05E-04	2	3,03	5,39E-10	9	8,26	9,60E-06	3	2,63	29	0,19
GO:0051276	chromosome organization and biogenesis	9,76E-04	6		8,55E-10	14	12,84	4,19E-05	9	7,89	347	2,25
GO:0007051	spindle organization and biogenesis	2,11E-17	10	15,15	9,02E-10	6	5,50	6,38E-07	5	4,39	21	0,14
GO:0006259	DNA metabolic process	6,01 E-05	10	15,15	1,91E-09	22	20,18	1,74E-05	12	10,53	400	2,59
GO:0005819	spindle	2,37E-22	13	19,70	7,92E-09	10	9,17	1,85E-07	8	7,02	51	0,33
GO:0044430	cytoskeletal part	4,65E-18	22	33,33	3,50E-08	17	15,60	1,49E-07	17	14,91	548	3,55
GO:0010564	regulation of cell cycle process	0,00474	2	3,03	6,07E-08	4	3,67	9,40E-07	4	3,51	45	0,29
GO:0007088	regulation of mitosis	0,00152	2	3,03	1,63E-07	4	3,67	1,88E-06	4	3,51	35	0,23
GO:0016043	cellular component organization and biogenesis	1,46E-09	23	34,85	1,89E-07	28	25,69	7,19E-05	26	22,81	1450	9,39
GO:0000226	microtubule cytoskeleton organization and biogenesis	3,57E-16	12	18,18	2,64E-07	6	5,50	6,19E-05	5	4,39	70	0,45
GO:0007126	meiosis	0,01027	2	3,03	2,99E-07	7	6,42	6,88E-05	6	5,26	53	0,34
GO:0051327	M phase of meiotic cell cycle	0,01027	2	3,03	2,99E-07	7	6,42	6,88E-05	6	5,26	53	0,34
GO:0051321	meiotic cell cycle	0,01113	2	3,03	3,54E-07	7	6,42	7,78E-05	6	5,26	54	0,35
GO:0000075	cell cycle checkpoint	7,52E-10	3	4,55	6,47E-07	4	3,67	3,89E-07	1	0,88	41	0,27
GO:0007010	cytoskeleton organization and biogenesis	2,67E-13	18	27,27	1,72E-06	16	14,68	1,88E-06	18	15,79	423	2,74

		CINSARC			Metastatic GISTs			GISTs with p16/R81 pathway altercation			Array	
Go Accession	GO Term	corrected p-value	Count in Selection	% in Selection	corrected p-value	Count in Selection	% in Selection	corrected p-value	Count in Selection	% in Selection	Count in Array	% in Array
GO:0006260	DNA replication	0,00227	8	12,12	2,94E-06	11	10,09	2,30E-02	8	7,02	169	1,09
GO:0005524	ATP binding	4,57E-10	28	42,42	3,20E-016	36	33,03	8,83E-03	35	30,70	1268	8,21
GO:0032559	adenyl ribonucleotide	5,80E-10	28	42,42	4,22E-06	36	33,03	1,09E-02	35	30,70	1282	8,30
GO:0003777	binding microtubule motor activity	2,08E-07	9	13,64	1,02E-05	10	9,17	8,79E-07	12	10,53	76	0,49
GO:0030554	adenyl nucleotide binding	1,80E-09	28	42,42	1,60E-05	36	33,03	3,12E-02	35	30,70	1349	8,73
GO:0043226	organelle	9,92E-07	54	81,82	1,61E-05	88	80,73	5,91E-02	97	85,09	6717	43,48
GO:0043229	intracellular organelle	9,92E-07	54	81,82	1,61E-05	88	80,73	5,91 E-02	97	85,09	6715	43,47
GO:0006974	response to DNA damage stimulus				1,86E-05	13	11,93	1,81 E-02	1	0,88	270	1,75
GO:0045840	positive regulation of mitosis				3,96E-05	1	0,92	1,53E-04	1	0,88	9	0,06
GO:0007018	microtubule-based movement	4,49E-08	9	13,64	6,25E-05	10	9,17	8,26E-06	12	10,53	93	0,60
GO:0043227	membrane-bounded organelle	0,002856	44	66,67	6,25E-05	80	73,39				5904	38,22
GO:0043231	intracellular membrane-bounded organelle	0,002850	44	66,67	6,25E-05	80	73,39				5901	38,20
GO:0030261	chromosome condensation				8,82E-05	5	4,59				20	0,13
GO:0005874	microtubule	7,54E-10	14	21,21	1,53E-04	12	11,01	5,35E-04	13	11,40	198	1,28
GO:0007093	mitotic cell cycle checkpoint	1,95E-06	3	4,55	1,61E-04	4	3,67				22	0,14
GO:0005856	cytoskeleton	7,31E-14	23	34,85	1,65E-04	18	16,51	2,41E-07	23	20,18	899	5,82
GO:0005875	microtubule associated complex	4,99E-06	9	13,64	2,84E-04	10	9,17	5,02E-05	8	7,02	110	0,71
GO:0030705	cytoskeleton-dependent intracellular transport	2,58E-07	9	13,64	3,32E-04	10	9,17	6,01 E-05	12	10,53	112	0,73
GO-0044424	intracellular part	6,64E-05	55	83,33	3,79E-04	88	80,73				7677	49,70
GO:0050000	chromosome localization				4,16E-04 .	1	0,92	1,42E-03	1	0,88	6	0,04
GO:0051303	establishment of chromosome localization				4,16E-04	1	0,92	1,42E-03	1	0,88	6	0,04
GO:0051656	establishment of organelle localization				5,46E-04	1	0,92	3,04E-03	1	0,88	27	0,17
GO:0006281	DNA repair				5,57E-04	12	11,01				224	1,45
GO:0051640	organelle localization				6,68E-04	1	0,92	3,76E-03	1	0,88	28	0,18
GO:0032553	ribonucleotide binding	1,01E-07	28	42,42	8,49E-04	36	33,03				1600	10,36

		CINSARC			Metastatic GISTs			GISTs with p16/R81 pathway altercation			Array	
Go Accession	GO Term	corrected p-value	Count in Selection	% in Selection	corrected p-value	Count in Selection	% in Selection	corrected p-value	Count in Selection	% in Selection	Count in Array	% in Array
GO:0032555	purine ribonucleotide binding	1,01E-07	28	42,42	8,49E-04	36	33,03				1600	10,36
GO:0007076	mitotic chromosome condensation				9,01E-04	5	4,59				16	0,10
GO:0045787	positive regulation of cell cycle				9,01E-04	1	0,92	4,14E-03	1	0,88	16	0,10
GO:0005622	intracellular	3,09E-04	56	84,85	0,00174	91	83,49				8242	53,35
GO:0003774	motor activity	3,37E-05	9	13,64	0,00183	10	9,17	6,12E-05	13	11,40	137	0,89
GO:0005876	spindle microtubule	7,68E-07	6	9,09	0,00219	5	4,59	1,03E-02	5	4,39	19	0,12
GO:0017076	purine nucleotide binding	2,58E-07	28	42,42	0,00219	36	33,03				1669	10,80
GO:0007052	mitotic spindle organization and biogenesis	6,52E-04	4	6,06	0,01084	2	1,83	3,58E-02	2	1,75	12	0,08
GO:0009110	cytokinesis	0,016369	4	6,06	0,01393	4	3,67	5,72E-02	4	3,51	27	0,17
GO:0006310	DNA recombination				0,01520	4	3,67				73	0,47
GO:0006323	DNA packaging				0,02611	5	4,59				111	0,72
GO:0000166	nucleotide binding	5,35E-06	28	42,42	0,04644	36	33,03				1913	12,38
GO:0007094	mitotic cell cycle spindle assembly checkpoint				0,04644	3	2,75				6	0,04
GO:0031577	spindle checkpoint				0,04644	3	2,75				6	0,04
GO:0000776	kinetochore	0,00036	4	6,06							26	0,17
GO:0004672	protein kinase activity	0,01408	11	16,67							566	3,66
GO:0004674	protein serine/threonine kinase activity	0,00054	11	16,67							403	2,61
GO:0005515	protein binding	0,00105	40	60,61							6165	39,91
GO:0005813	centrosome	0,00175	4	6,06							68	0,44
GO:0005815	microtubule organizing center	0,00022	4	6,06							79	0,51
GO:0006270	DNA replication initiation	0,00741	4	6,06							22	0,14
GO:0006468	protein amino acid	0,01889	12	18,18							584	3,78
GO:0007089	phosphorylation traversing start control point of mitotic cell cycle	0,00529	3	4,55							6	0,04
GO:0007096	regulation of exit from mitosis	0,03771	1	1,52							11	0,07
GO:0009987	cellular process	0,00000	60	90,91							9867	63,87
GO:0019932	second-messenger-mediated signaling	0,03778	7	10,61							182	1,18

		CINSARC			Metastatic GISTs			GISTs with p16/R81 pathway altercation			Array	
Go Accession	GO Term	corrected p-value	Count in Selection	% in Selection	corrected p-value	Count in Selection	% in Selection	corrected p-value	Count in Selection	% in Selection	Count in Array	% in Array
GO:0032991	macromolecular complex	0,04542	15	22,73							1992	12,89
GO:0043234	protein complex	0,03040	15	22,73							1493	9,66
GO:0048015	phosphoinositide-mediated signaling	0,00030	7	10,61							83	0,54
GO:0051325	interphase	0,00202	6	9,09							70	0,45
GO:0051329	interphase of mitotic cell cycle	0,00163	6	9,09							67	0,43

[0057] Moreover, gene enrichment analysis of the 182 genes not included in CINSARC showed that this gene set was also enriched by genes involved in the same pathways as CINSARC genes, i.e. mitosis control and chromosome integrity (Table 4).

Table 4: Gene Ontology analysis of the 182 genes differentially expressed between GISTs with or without metastasis and not included in CINSARC signature

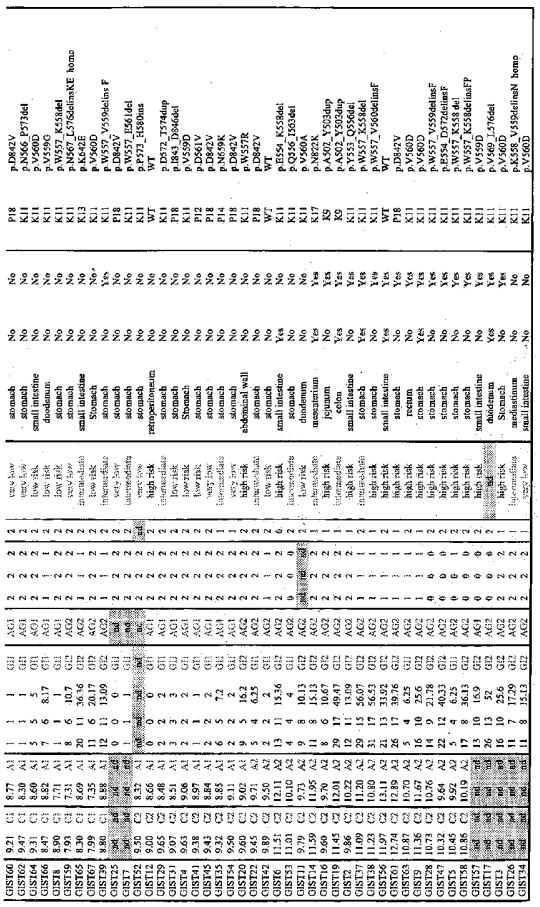
GO ACCESSION	GO Term	p-value	corrected p-value	Count in Selection	% in Selection	Count in Array	% in Array
GO:0022403	cell cycle phase	5.72E-21	1.48E-15	22	36.07	267	1.73
GO:0000279	M phase	2.61E-20	3.37E-15	22	36.07	219	1.42
GO:0007049	cell cycle	3.45E-19	2.97E-14	29	47.54	584	3.78
GO:0022402	cell cycle process	2.05E-18	1.32E-13	22	36.07	343	2.22
GO:0044427	chromosomal part	9.72E-15	5.01E-10	9	14.75	270	1.75
GO:0000278	mitotic cell cycle	6.57E-14	2.82E-09	15	24.59	221	1.43
GO:0007059	chromosome segregation	9.42E-14	3.47E-09	7	11.48	58	0.38
GO:0000087	M phase of mitotic cell cycle	1.59E-13	5.11E-09	15	24.59	163	1.06
GO:0005694	chromosome	1.88E-13	5.39E-09	12	19.67	318	2.06
GO:0007067	mitosis	2.17E-12	5.59E-08	13	21.31	160	1.04
GO:0000775	chromosome, centromeric region	1.41E-11	3.31 E-07	9	14.75	66	0.43
GO:0006259	DNA metabolic process	9.77E-11	2.10E-06	16	26.23	400	2.59
GO:0051726 GO:0000074	regulation of cell cycle	4.09E-10	8.06E-06	12	19.67	437	2.83
GO:0000070| GO:0016359	mitotic sister chromatid segregation	4.38E-10	8.06E-06	6	9.84	28	0.18
GO:0000819	sister chromatid segregation	5.74E-10	9.86E-06	6	9.84	29	0.19
GO:0051276| GO:0007001| GO:0051277	chromosome organization and biogenesis	8.43E-10	1.28E-05	9	14.75	347	2.25
GO:0005634	nucleus	8.28E-10	1.28E-05	52	85.25	3992	25.84
GO:0051301	cell division	1.00E-09	1.44E-05	12	19.67	209	1.35
GO:0051327	M phase of meiotic cell cycle	1.75E-09	2.26E-05	7	11.48	53	0.34
GO:0007126	meiosis	1.75E-09	2.26E-05	7	11.48	53	0.34
GO:0051321	meiotic cell cycle	2.05E-09	2.51E-05	7	11.48	54	0.35
GO:0007346	regulation of mitotic cell cycle	3.66E-08	4.29E-04	3	4.92	77	0.50
GO:0006260	DNA replication	1.53E-07	1.72E-03	7	11.48	169	1.09
GO:0006974	response to DNA damage stimulus	1.91E-07	2.05E-03	10	16.39	270	1.75
GO:0043232	intracellular non-membrane-bounded organelle	2.40E-07	2.38E-03	12	19.67	1509	9.77
GO:0043228	non-membrane-bounded organelle	2.40E-07	2.38E-03	12	19.67	1509	9.77
GO:0010564	regulation of cell cycle process	4.46E-07	4.25E-03	3	4.92	45	0.29
GO:0006281	DNA repair	2.04E-06	1.88E-02	9	14.75	224	1.45
GO:0007076	mitotic chromosome condensation	2.95E-06	2.50E-02	4	6.56	16	0.10
GO:0007088	regulation of mitosis	3.00E-06	2.50E-02	3	4.92	35	0.23
GO:0044446	intracellular organelle part	2.98E-06	2.50 E-02	9	14.75	2239	14.49
GO:0044422	organelle part	3.13E-06	2.52E-02	9	14.75	2244	14.53
GO:0006996	organelle organization and biogenesis	4.43E-06	3.46E-02	9	14.75	979	6.34
GO:0030261|GO:0000068	chromosome condensation	7.69E-06	5.51 E-02	4	6.56	20	0.13
GO:0006310	DNA recombination	8.03E-06	5.60E-02	5	8.20	73	0.47

AURKA is a significant marker of metastasis outcome

[0058] We took advantage of the supervised analysis results to test the possibility of reducing the CINSARC signature. Among the top-ranked significant genes sorted in the supervised t-test, *AURKA* (Aurora kinase A, previously designated STK6 or STK15) was the best ranked gene that also belonged to the CINSARC signature (Table 1). We thus tested whether *AURKA* alone could predict outcomes as well as CINSARC and we stratified samples according to their *AURKA* expression (with the mean expression of 9.13 as a cut-off, table 5).

Table 5: Expression of p16 and RB1 measured by expression array and by RT-qPCR. Expression array data are log2 transformed and RT-qPCR data are difference between tested gene and reference genes CTs, that means that the highest the value, the lowest the expression. High expressions are indicated in red and low expressions in green. Main clinical data and results are reported from table 1. *p16* and *RB1* copy number: 2 = without detectable deletion; 1 = hemizygous deletion, *indicate truncating mutation; 0 = no copy. nd = not done.

	[illegible]				CGH					CDKN2A/p16 and RB1 Copy number				Histology	Annotations			KIT and PDGFRA mutations	
GIST	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	RB1	NIH	Site of primary tumor	Local recurrence	Metastasis	Mutated gene	Mutation
GIST1	8.68	C1	8.12	A1	3	3	3	GI1	AG1	2	2	2	1	high risk	Stomach	No	No	P18	p.D842V
GIST10	7.95	C1	8.56	A1	5	4	6.25	GI1	AG1	2	2	2	2	low risk	small intestine	No	No	K11	p.V560D
GIST13	8.84	C1	8.05	A1	2	2	2	GI1	AG1	2	2	2	2	intermediate	stomach	No	No	K11	p.W557R
GIST15	9.37	C1	7.89	A1	4	3	5.33	GI1	AG1	2	2	2	2	low risk	stomach	No	No	K11	p.V559D
GIST18	8.93	C1	9.05	A1	6	4	9	GI1	AG1	2	2	2	2	intermediate	duodenum	No	No	K11	p.L576P
GIST21	9.41	C1	8.66	A1	2	2	2	GI1	AG1	2	2	2	2	intermediate	stomach	No	No	K11	p.L576P
GIST23	8.42	C1	8.39	A1	0	0	0	GI1	AG1	nd	nd	nd	2	low risk	small intestine	No	No	P12	p.Y555C
GIST24	9.04	C1	8.23	A1	4	4	4	GI1	AG1	2	2	2	2	low risk	peritoneum	No	No	K11	p.T574_R586insK
GIST27	8.67	C1	7.75	A1	1	1	1	GI1	AG1	2	2	2	2	high risk	stomach	No	No	K11	p.K581_S590dup
GIST30	8.31	C1	7.62	A1	2	2	2	GI1	AG1	2	2	2	2	intermediate	stomach	No	No	K11	p.L576_R588dup
GIST32	9.15	C1	8.09	A1	1	1	1	GI1	AG1	2	2	2	2	intermediate	stomach	No	No	K11	p.W557R
GIST33	9.02	C1	8.55	A1	3	3	3	GI1	AG1	2	2	2	2	very low	stomach	No	No	P18	p.D842V
GIST36	8.34	C1	7.61	A1	1	1	1	GI1	AG1	2	2	2	2	very low	stomach	No	No	K11	p.V559D
GIST40	8.52	C1	7.80	A1	1	1	1	GI1	AG1	2	2	2	2	low risk	stomach	No	No	K11	p.P573_T574dup; T574dup; Q575_R586dup
GIST43	9.12	C1	8.01	A1	1	1	1	GI1	AG1	2	2	2	2	very low	stomach	No	No	K11	p.T574_L589dup
GIST44	9.37	C1	8.41	A1	5	3	8.33	GI1	AG1	2	2	2	2	low risk	stomach	No	No	K11	p.Q556_V559del
GIST46	9.20	C1	8.60	A1	5	3	8.33	GI1	AG1	2	2	2	2	very low	small intestine	No	No	K11	p.Q556_V559del
GIST48	8.17	C1	8.14	A1	8	7	9.14	GI1	AG1	2	2	2	2	low risk	small intestine	No	No	K11	p.M552_E561del
GIST49	9.35	C1	8.93	A1	7	5	9.8	GI1	AG1	2	2	2	2	very low	stomach	No	No	K11	p.E554_K558del
GIST50	7.67	C1	8.36	A1	7	6	8.17	GI1	AG1	1	1	1	2	high risk	small intestine	No	No	K11	p.M552_E554delinsK
GIST51	9.31	C1	8.33	A1	0	0	0	GI1	AG1	2	2	2	2	very low	stomach	No	No	K11	p.W557R
GIST55	8.70	C1	7.72	A1	5	4	6.25	GI1	AG1	2	2	2	2	very low	stomach	No	No	K11	p.D572_D579dupinsL



[0059] For this purpose, we considered the present 67-GISTs series as the training set and the Yamaguchi's one as the validation set. Expression data were then validated by qRT-PCR and we found a high correlation between both techniques (Pearson correlation coefficient = 0.94; $P<1x10^{-15}$). Survival analyses revealed that the two groups obtained had very different outcomes, both in the training set (Present series, MFS: $P = 5.31x10^{-11}$ and DFS: $P = 3.61x10^{-12}$, fig. 2a) and in the validation set (Yamaguchi's series, MFS: $P =9.5x10^{-4}$, fig. 2b).

Chromosomal complexity is a significant prognosis factor of GISTs

[0060] We have previously shown that the CINSARC signature is associated with the genome complexity (18), therefore the question arises whether the alteration level of the GISTs genome is correlated with the CINSARC signature and with the metastatic outcome. Genome profiling with arrays containing 60 000 oligonucleotides (see material and methods) has been performed on 66 GISTs with sufficient DNA quality. Different profiles were obtained, ranging from simple, i.e. without any detectable changes, to complex, with numerical and segmental gains and losses (Figure 3). No high level amplification was detected across the 66 profiles with the exception of one case with 5p amplification (GIST17). Most of the alterations involved whole chromosome arms or chromosomes without rearrangements. In fact, when only a few changes were detected (less than eight), these always affected whole arms or chromosomes, whereas when the profile was composed of more than 10 changes, intra-chromosomal gains or losses could be observed. To define a numerical method taking into account these criteria, i.e. the number and type of alterations, a Genomic Index (GI) was calculated for each profile as follows: $GI= A^2 \times C$ (A= number of alterations and C= number of involved chromosomes). Then, tumors were assigned to two groups, GI1 or GI2, depending on whether their GI was not-greater or greater than 10, respectively (Table 5). Kaplan-Meier metastasis- and disease-free survival analyses demonstrated that this stratification split tumors into two groups with strongly distinct outcome (fig 4a). Moreover, this genomic stratification can identify GISTs with different metastatic outcomes in the intermediate-risk group of the AFIP classification (9) (fig 4b & c).

Integrative analysis allows identification of a no-risk group of patients

[0061] Considering these results as a whole, we construct a decisional algorithm based on GI and *AURKA* expression. More specifically, a positive correlation exists between GI and *AURKA* expression (Pearson correlation r=0.65, Figure 7). We observed that tumors with below-average *AURKA* expression and with GI less than 10 never develop metastasis or recurrence (Figure 7, Table 5). In line with this, Kaplan-Meier MFS and DFS analyses demonstrated that tumors with good prognostic factors (AG1: *AURKA* expression <mean and GI<10), have a 5-years MFS of 100%, whereas tumors with poor prognostic factors (AG2: *AURKA* expression > mean or GI>10) have a 5-years MFS of 23%, $P = 2.61 \times 10^{-8}$, Figure 5). Hence, this algorithm leads to the individualization of a no-risk patient group (AG 1: *A URKA* expression < 9.13 and GI<10).

P16/RB1 pathway is associated to metastatic outcome.

[0062] As a result of these findings, we reconsidered CGH array data to examine whether any specific alterations were associated with patients' outcome. We compared alteration frequency of each probe set between GISTs with or without metastatic outcome (Figure 8). No significant difference in gain frequencies was observed between those two groups (data not shown). However, among the top-ranked deletion frequencies in metastatic cases, the highest difference was observed for eight probe sets deleted in 78.9% and 9.6% of the metastatic and non-metastatic cases, respectively (Figure 8). All probe sets localize in 9p21 and target either *CDKN2A* (3 probe sets), *CDKN2B* (3 probe sets) or *MTAP* (2 probe sets) loci. 9p21 deletions were observed in 18 cases (18/66 = 27%) and among these tumors, 13 developed metastasis (13/18 = 72%). These deletions either involved the whole 9p arms or were restricted only to the *CDKN2A/B* loci and were assumed to be homozygous for 7 cases (6/7 with metastatic outcome) as indicated by the very low CGH ratios (Figure 9). The most frequently deleted region appeared to involve 3 loci (*CDKN2A*, *CDKN2B* and *MTAP*), but homozygous deletions allowed us to identify more precisely genes of interest since two tumors with homozygous deletion excluded *MTAP* (GISTs #5 and #17). Accordingly, we checked *CDKN2A* and *CDKN2B* copy number status by genomic qPCR and fully confirmed all CGH results. Notably, suspected homozygous deletions were confirmed and refined, we observed that homozygous deletion in GIST #5 involved only *CDKN2A* but not *CDKN2B* (of which one copy was retained) (Table 5). Subsequently, all GISTs without homozygous deletion and from which DNA was available (58 cases) were submitted to *CDKN2A* sequencing. We did not detect any mutations (data not shown) and only three SNPs (RS3731249, RS11515 and one unreferenced silent SNP: c.*56G>A) were indentified in 4, 14 and 20 cases respectively. To precisely quantify the *p14* and *p16* expressions (both mRNA hybridized to the same (Agilent probe sets), we quantified expression using specific probes for *p14* and *p16* RT-qPCR (Table 5). In all the tumors without any copy of *p16* (7 cases) and in three tumors with only one copy, *p16* mRNA was nearly absent; and no protein was detected by IHC in the two cases with homozygous and the three cases with hemizygous deletion for which histological blocs were available (data not shown). The lack of *p16* mRNA and/or protein is therefore evidenced in 10 cases with nine belonging to the metastatic group (18 cases).

[0063] We thus hypothesized that another genomic alteration could lead to p16 pathway inactivation in cases without *p16* homozygous deletion. We observed one homozygous deletion and 13 hemizygous deletion of the *RB1* locus (Table 6).

Table 6: Results of the CINSARC analysis, *AURKA* expression (A = 9.13 as cut-off), CGH analysis (GI = 10 as cut-off) and *CDKN2A/2B* and *RB1* copy number determined by genomic qPCR and array-CGH, respectively (2 = without detectable deletion; 1 = hemizygous deletion; 0 = no copy). P = *PDGFRA*, K = *KIT*, WT= Wild type, nd = not done.

	Exp array		CGH						*p16* Expression		*RB1* Expression	
	CINSARC Grading	AURKA Stratification	Genomic Index	AGI	*p16* copy number	*RB1* copy number	Local recurrence	Metastasis	cDNA array	qPCR	cDNA array	qPCR
GIST10	C1	A1	GI1	AG1	2	2	No	No	10,67	10,13	6,82	6,13
GIST13	C1	A1	GI1	AG1	2	2	No	No	8,66	10,21	7,94	4,98
GIST15	C1	A1	GI1	AG1	2	2	No	No	8,36	11,80	8,45	4,68
GIST18	C1	A1	GI1	AG1	2	2	No	No	11,86	6,89	6,96	5,58
GIST21	C1	A1	GI1	AG1	2	2	No	No	12,43	6,81	8,19	4,39
GIST23	C1	A1	GI1	AG1	2	2	No	No	8,75	nd	7,00	nd
GIST24	C1	A1	GI1	AG1	2	2	No	No	9,26	11,06	8,17	4,91
GIST27	C1	A1	GI1	AG1	2	2	No	No	9,57	12,03	7,62	5,32
GIST30	C1	A1	GI1	AG1	2	2	No	No	8,68	14,01	7,81	5,20
GIST32	C1	A1	GI1	AG1	2	2	No	No	10,90	8,96	8,02	4,46
GIST33	C1	A1	GI1	AG1	2	2	No	No	9,31	12,47	7,11	5,53
GIST38	C1	A1	GI1	AG1	2	2	No	No	10,39	10,01	7,53	5,08
GIST40	C1	A1	GI1	AG1	2	2	No	No	10,02	9,15	8,67	4,35
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GIST52	C2	A1	nd	nd	2	nd	No	No	9,72	13,49	7,20	5,30
GIST45	C2	A1	GI1	AG1	2	2	No	No	7,44	15,35	6,93	5,77
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GIST59	C1	A1	GI2	AG2	2	2	No	No	13,72	1,71	7,78	4,30
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GIST22	C2	A2	GI1	AG2	2	2	No	No	11,59	7,42	8,05	4,91
GIST11	C2	A2	GI2	AG2	nd	2	No	No	10,75	9,47	6,87	5,78
GIST65	C1	A1	GI2	AG2	1	2	No	No	9,22	9,23	8,06	5,20
GIST25	nd	nd	GI1	nd	2	2	No	No	nd	nd	nd	nd
GIST7	nd	nd	GI1	nd	2	2	No	No	nd	nd	nd	nd
GIST1	C1	A1	GI1	AG1	2	1	No	No	9,96	10,33	6,06	6,93
GIST35	C2	A1	GI1	AG1	2	1	No	No	10,42	10,39	6,28	6,75
GIST54	C2	A1	GI1	AG1	2	1	No	No	9,39	11,79	6,61	6,02
GIST28	nd	nd	GI2	AG2	2	1	No	No	nd	nd	nd	nd
GIST34	nd	nd	GI2	AG2	2	1	No	No	nd	nd	nd	nd
GIST42	C2	A2	GI1	AG2	1	2	No	No	12,27	12,51	9,02	5,32
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GIST3	nd	nd	GI2	AG2	2	1	No	Yes	nd	nd	nd	nd
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GIST14	C2	A2	GI2	AG2	2	1	Yes	Yes	14,13	5,91	8,91	7,19
GIST16	C2	A2	GI2	AG2	2	1	No	Yes	11,73	9,67	7,22	5,47
GIST19	C2	A2	GI2	AG2	2	1	Yes	Yes	12,64	8,02	7,22	5,96
GIST2	C2	A2	GI2	AG2	2	1	No	Yes	13,77	4,95	7,17	6,76
GIST61	C2	A2	GI2	AG2	1	1*	No	Yes	14,59	5,16	7,63	7,58
GIST50	C2	A2	GI2	AG2	1	2	Yes	Yes	11,95	14,13	8,89	5,05
GIST9	C2	A2	GI2	AG2	1	1	Yes	Yes	8,51	12,92	6,97	4,96
GIST57	nd	nd	GI2	AG1	0	2	No	Yes	nd	nd	nd	nd
GIST26	C2	A2	GI2	AG2	0	2	No	Yes	5,69	15,29	8,01	4,62
GIST47	C2	A2	GI2	AG2	0	2	No	Yes	6,08	13,37	7,48	4,80
GIST5	C2	A2	GI1	AG2	0	2	No	Yes	3,80	18,20	8,56	5,09
GIST56	C2	A2	GI2	AG2	0	2	No	Yes	6,17	14,57	7,89	5,22
GIST17	nd	nd	GI2	AG2	0	2	Yes	Yes	nd	nd	nd	nd

[0064] Eleven tumors harboring *RB1* deletions are classified AG2 and eight developed metastases. Interestingly tumors with a *p16* homozygous deletion are without any *RB1* deletion although they are highly rearranged. Sequencing *PB1* in all patients with available RNA and DNA (66 cases) we indentified one mutation (c.1959_1960del/ p.Lys653AsnfsX14) in the retained copy in GIST #61. qPCR analysis confirmed that deleted tumors had a significantly down regulated expression of *RB1* (Table 6).

Conclusion:

[0065] We demonstrated that CINSARC is a very powerful signature to predict metastatic outcome. CINSARC is composed of 67 genes which are all involved in chromosome integrity and mitosis control pathways, indicating that such mechanisms appear to be driving the development of metastasis in this tumor type, as we recently demonstrated in sarcomas (18). This is in line with results from the reciprocal approach, which is the identification of genes differentially expressed between GISTs with or without metastatic outcome. Actually, among the 227 up-regulated genes in the 18 metastasizing tumors, 45 were common with the CINSARC signature and the activated pathways were almost all the same. These results indicate that genome integrity and mitosis control are the effective restrain mechanisms underlying development of metastasis, and moreover, that these mechanisms appear to be sufficient, or at least the strongest. In line with this, we show that expression of the top ranked gene in both approaches, *AURKA*, is as efficient as CINSARC to predict metastatic outcome in both series of GISTs.

[0066] Following our results demonstrating the central role of the genome integrity control, we hypothesized that a defect of such mechanisms should lead to chromosome imbalances and that the resulting genome complexity should also predict outcome in GISTs. This is exactly what we show here since tumor stratification according to a CGH Genome Index (GI) which integrates the number of alterations and of altered chromosomes forms two groups of clearly distinct outcomes. This is clearly in agreement with the *AURKA* expression results, since whole chromosome losses are the most frequent alterations observed in GISTs and these are assumed to originate from the chromosome segregation deficiency induced by mitosis check-point defects, such as the AURKA overexpression (34).

[0067] In contrast to results of Yamagushi and colleagues, our study demonstrates that CINSARC, *AURKA* expression or CGH prognostic values are irrespective to tumor location. Furthermore, as mentioned above, the biological meaning of CINSARC and its association to genomic changes strongly indicate that CINSARC genes are involved in malignant progression and are not just a consequence of the process. This hypothesis is supported by the association we observe here between *CDKN2A* deletions (homozygous deletions in 6 cases and hemizygous deletions in 9 cases among 18 cases with metastatic outcome *versus* 5 hemizygous deletions in 48 non-metastatic patients), CINSARC prognosis groups and metastatic occurrence. Previous studies have already pointed out the potential association of *CDKN2A* alterations or expression of $p16^{INK4a}$ to tumor progression (11-16, 43). Nevertheless, data remain controversial mainly due to lack of clear delineation of the targeted gene at the 9p21 locus. It is still unclear which gene of *CDKN2A, CDKN2B* or *MTAP* is driving the association to poor prognosis. At the genomic level, even if *CDKN2A* and *2B* appear to be systematically codeleted (37, 44, 45), two studies indicate that 9p21 deletions are likely to target the *MTAP* gene and not exclusively *CDKN2A* and *CDKN2B* (35, 46). Here, CGH and genomic qPCR analyses demonstrated that homozygous deletions specifically target *CDKN2A* and that the common region of deletion excludes *CDKN2B* and *MTAP.* Surprisingly, we did not find any harmful *CDKN2A* mutations in any of GISTs case tested. Schnieder-Stock and colleagues (14) reported 9 so-called mutations in a series of 43 GISTs. But two of them are identical and have been detected in a tumor and its recurrence, one is now referenced as a SNP, two are silent mutations and in one case no interpretable sequence was obtained. Considering all this, the authors evidenced only four *CDKN2A* mutations (4/43= 9%). According to these data we expected around five mutations in our study and we have identified three changes, two SNP and one silent change not referred so far. One explication for this discrepancy could be sampling bias, but it is of interest to note that, we detected twice more homozygous *CDKN2A* deletions than reported in the study of Schneider-Stock *et al* (7/63 vs 2/43). Following the idea that another exclusive alteration could explain aggressive tumors (CINSARC C2, AG2) without *p16* inactivation we identified two tumors without *RB1* functional copy and 12 significantly down-regulated due to loss of one *RB1 copy* (p value). We did not detect any truncating mutation in these tumors but we hypothesize that micro-deletions, that we did not identify because of the lowest resolution of the arrays, could account for this second inactivation, as in sarcomas (47). An exclusive occurrence of *p16* and *RB1* alterations is highly supported by the observation that none of the tumors with *CDKN2A* homozygous deletion harbors any *RB1* deletion and among the 29 GISTs with one of these deletions, only three cases harbor both deletions (table 1). Altogether, p16/RB1 pathway is inactivated or down regulated in 14/18 (78%) and in 3/48 (6%) patients with and without metastatic outcome, respectively, which clearly means that inactivation of p16/RB1 pathway is associated to metastatic development.

[0068] *CDKN2A* codes for two key tumor suppressor proteins, the $p16^{INK4a}$ and the $p14^{ARF}$, which are involved in the regulation of the cell cycle G1 and G2/M transition. Together, these proteins regulate two important cell cycle checkpoints, the p53 and the RB1 pathways for p14 and $p16^{INK4a}$, respectively. Loss of these genes can lead to replicate senescence, cell immortalization and tumor growth (48-51). Most of the CINSARC genes are under the transcriptional control of E2F, which is tightly regulated by RB1 interaction. Actually, RB1 sequestrates E2F which is delivered upon RB1 phosphorylation by CDK4 (Cyclin Dependent Kinase 4) and $p16^{INK4a}$ inhibits CDK4. Therefore, our results allow us to hypothesize that inactivation of the p16/RB1 pathway in GISTs, mainly by deletion, is likely to be the causative alteration that leads to the over-expression of genes involved in mitosis control. This deregulation triggers cell genome rearrangements until a combination is naturally selected and fixed. Thus, the resulting genome complexity and its related expression confer the tumor cell aggressiveness and metastatic potential. Although this hypothesis has to be experimentally validated in cellular and mouse models, it is supported by the expression analysis of the GISTs with or without functional p16/RB1 pathway which shows that 42/225 (19%) genes up regulated in GISTs without functional p16/RB1 pathway are common with CINSARC signature. Moreover these 225 genes are involved in the same pathways than those enriched in CINSARC and metastases signatures (Supplementary table 3).

[0069] Imatinib mesylate has been proven to target KIT-aberrant signaling inhibiting the proliferation and survival in GIST cells. Until 2009, imatinib therapy was restricted to disseminated or advanced disease at the time of diagnosis. Since then, adjuvant treatment has been approved and the necessity to apply selection criteria to identify patients susceptible to benefit from such management has emerged. Patient selection foreseen by FDA (Food and Drug Administration) and to a lesser extent by EMA (European Medicines Agency) is essentially based on the histological risk evaluation. Both AFIP (9) and NIH (8) histological-based staging systems are widely accepted as "gold standards" in determining tumor metastatic risk and to determine whether a GIST patient is eligible or not for adjuvant therapy with imatinib. Here we show that the CINSARC signature and *AURKA* expression outperform the AFIP classification (survival analysis according to AFIP classification is presented in fig 4), particularly when associated to the CGH genomic index. Of particular interest, the Genomic Index is able to distinguish good and poor prognosis patients in GISTs classified as intermediate-risk by these histopathological systems (which represent around 25% of diagnoses). More specifically, among the 16 AFIP intermediate-risk cases, four developed metastasis. These cases were classified as poor prognosis by GI (fig. 4 and Table 5). GI established in this study is therefore a very powerful tool to manage GIST patient more likely to benefit from therapy since CGH is a technique already used in the daily practice by a growing number of pathology departments and is applicable to formalin-fixed paraffin-embedded (FFPE) samples. To validate the clinical application of GI, we collect a larger cohort of FFPE GIST samples to perform CGH with DNA from FFPE blocks.

[0070] We thus propose two possible decisional methods either to enhance the AFIP or NIH grading systems or to replace these histopathological methods. Firstly, when using the AFIP or NIH classifications, intermediate-risk cases are problematic for therapeutic management and our results demonstrate that the use of CGH profiling can easily and rapidly solve such a problem. Secondly, our results suggest that the combined use of GI and AURKA expression offer a better selection of patients for imatinib therapy than the AFIP classification does. Both methods offer equally efficient treatments for patients with metastatic risk, but CINSARC/AURKA-based selection, which is totally investigator-independent, would diminish consistently the number of patients, without metastatic risk, who are falsely declared eligible for imatinib therapy.

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[0071]

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SEQUENCE LISTING

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INSERM
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REFERENCES CITED IN THE DESCRIPTION

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SIGNATURER AF KLINISK UDFALD I GASTROINTESTINALE STROMALE TUMORER OG FREMGANGSMÅDE TIL BEHANDLING AF GASTROINTESTINALE STROMALE TUMORER

Patentkrav

1. Fremgangsmåde til at *in vitro*-forudsige overlevelse og/eller metastatisk udfald af gastrointestinale stromale tumorer (GIST'er), **kendetegnet ved at** den omfatter målingen af niveauet, i en biologisk prøve af GIST fra en patient, af en pool af polypeptider eller polynukleotider bestående i Aurora kinase A (AURKA), hvor GIST er klassificeret i en gruppe med høj risiko for at udvikle metastaser inden for 5 år, dvs. med en risiko for at udvikle metastaser inden for 5 år på mere end 80%, når AURKA er opreguleret sammenlignet med en gruppe med ingen risiko for at udvikle metastaser inden for 5 år, hvor AURKA er nedreguleret og mindre end 9,13, hvor 9,13 er beregnet fra middelværdien af AURKA-ekspressionen af stratificerede tumorprøver.

2. Fremgangsmåde ifølge krav 1, hvor målingen af niveauet af poolen af polypeptider er en måling af ekspressions-niveauet af en pool af polynukleotider bestående i AURKA.

3. Fremgangsmåde ifølge et hvilket som helst af krav 1 til 2, omfattende beregningen af det genomiske indeks (GI), dvs. antallet og type af ændringer af GIST-genomet, ifølge følgende formlen:

$$GI = A^2 \times C,$$

hvor A er antallet af ændringer i GIST-genomet og C er antallet af involverede kromosomer i GIST.

4. Fremgangsmåde ifølge krav 3, hvor GIST er klassificeret i en gruppe af metastase- og sygdomsfri overlevelsesgruppe når AURKA er nedreguleret og GI er

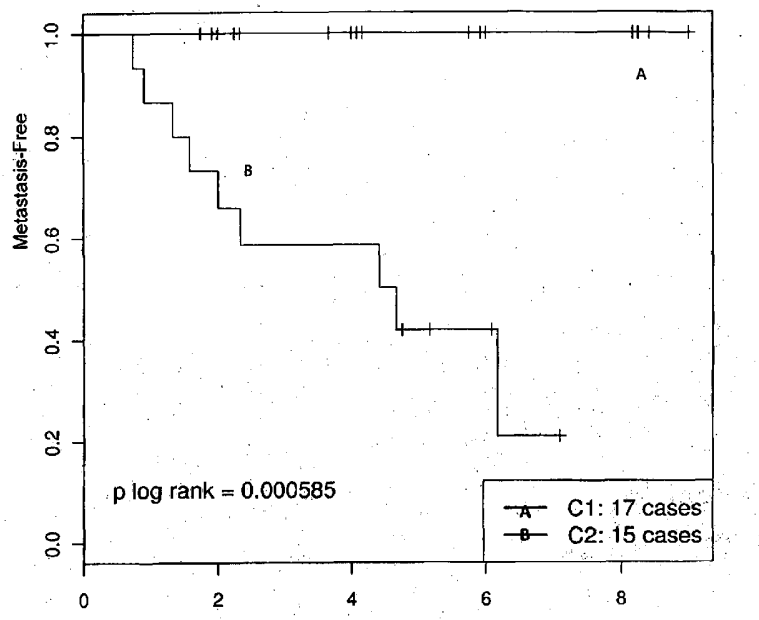
lig med eller mindre end 10.

5. Fremgangsmåde ifølge krav 3, hvor GIST er klassificeret i en gruppe med lav risiko for at udvikle metastaser inden for 5 år, dvs. med en risiko for at udvikle metastaser inden for 5 år lig med 0%, når AURKA-ekspression er lig med eller mindre end middelværdien af AURKA-ekspression og GI er lig med eller mindre end 10, idet middelværdien er middelværdien af AURKA-ekspression i flere GIST'er.

6. Fremgangsmåde ifølge krav 3, hvor GIST er klassificeret i en gruppe med høj risiko for at udvikle metastaser inden for 5 år, dvs. med en risiko for at udvikle metastaser inden for 5 år på mere end 75%, når AURKA-ekspression er over middelværdien af AURKA-ekspression og GI er over 10, idet middelværdien er middelværdien af AURKA-ekspression i flere GIST'er.

DRAWINGS

a)



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	Cumulated events	0	0	0	0	0	0
	MFS	1	1	1	1	1	1
C 2	Patients at risk	15	13	10	7	7	4
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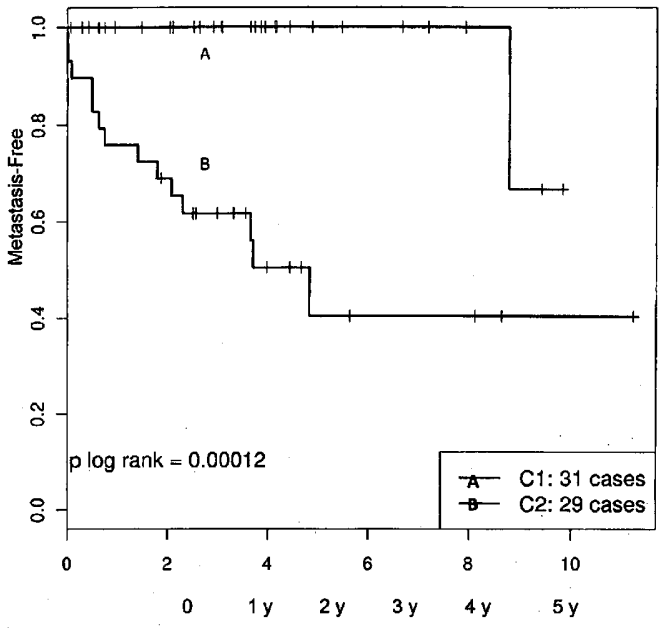
	MFS	1	0,87	0,66	0.59	0,59	0,42

Figure 1 (part 1)

b)



C1	Patients at risk	31	25	24	17	11	7
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C2	Patients at risk	29	22	19	13	8	4
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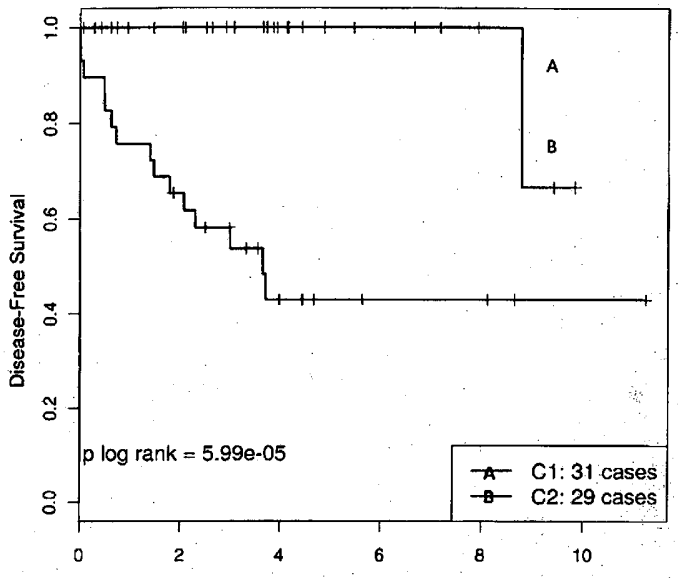
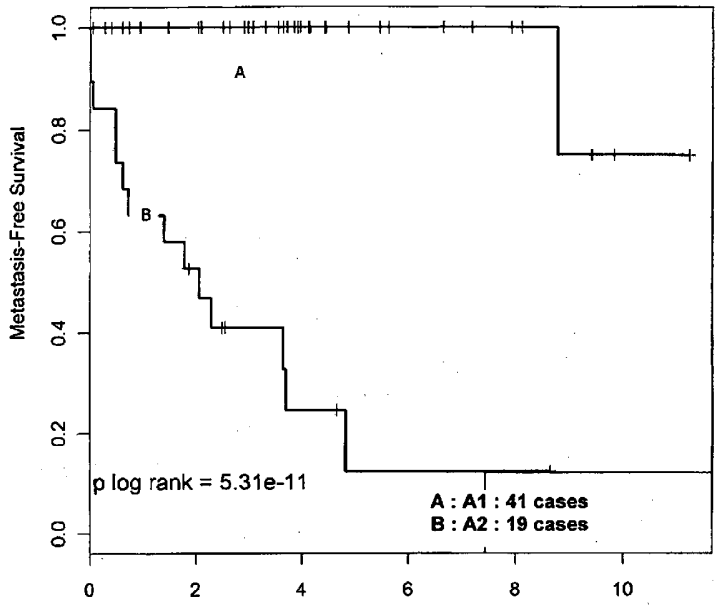
Figure 1 (part 2)



	0	1y	2y	3y	4y	5y
Patients at risk	31	25	24	17	11	7
Cumulated events	0	0	0	0	0	0
DFS	1	1	1	1	1	1
Patients at risk	29	22	18	12	7	4
Cumulated events	1	7	10	13	15	15
DFS	0,97	0,76	0,66	0,54	0,43	0,43

Figure 1 (part 3)

a)



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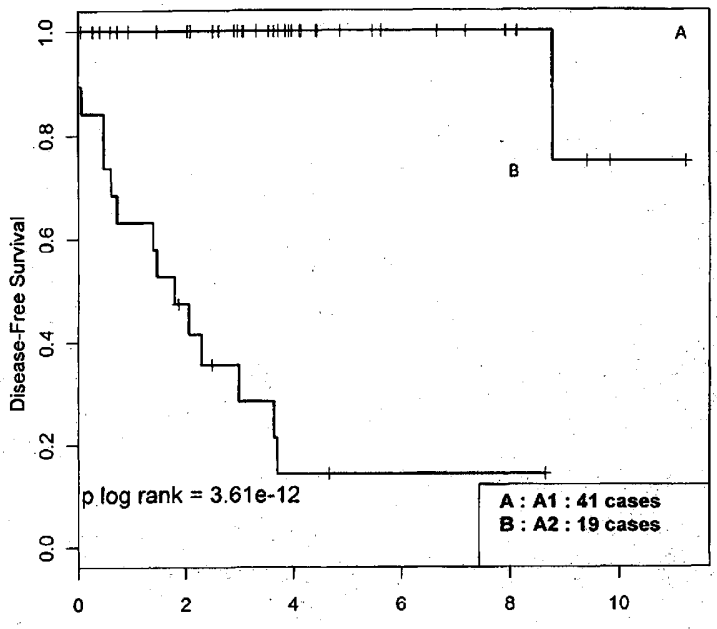
	Cumulated events	0	0	0	0	0	0
	MFS	1	1	1	1	1	1
A2	Patients at risk	19	12	9	5	3	1
	Cumulated events	1	7	9	11	13	14
	MFS	0,95	0,63	0,53	0,41	0,24	0,12

Figure 2 (part 1)



	0	1y	2y	3y	4y	5y
A: Patients at risk	41	35	34	25	16	10
Cumulated events	0	0	0	0	0	0
DFS	1	1	1	1	1	1
B: Patients at risk	19	12	8	4	2	1
Cumulated events	1	7	10	13	15	15
DFS	0,95	0,63	0,47	0,28	0,14	0,14

Figure 2 (part 2)

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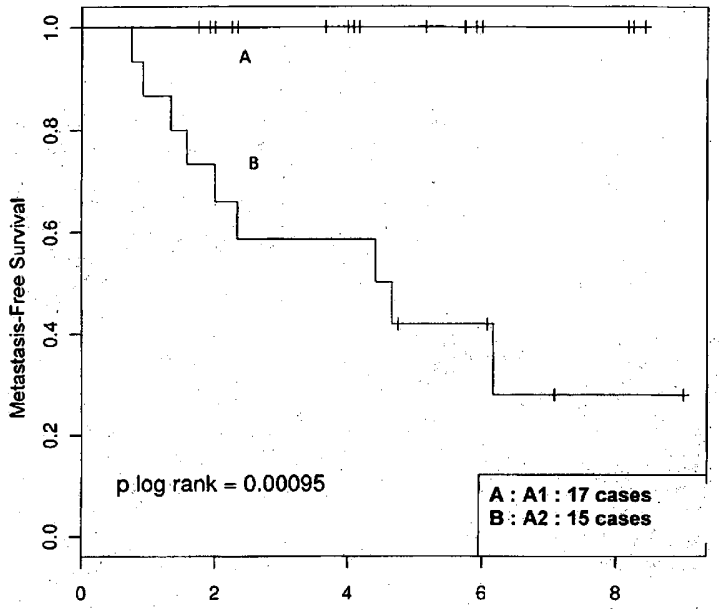

	0	1 y	2 y	3 y	4 y	5 y
A1: Patients at risk	17	17	14	11	10	7
Cumulated events	0	0	0	0	0	0
MFS	1	1	1	1	1	1
A2: Patients at risk	15	13	10	7	7	4
Cumulated events	0	2	5	6	6	8
MFS	1	0,87	0,66	0.59	0,59	0,42

Figure 2 (part 3)

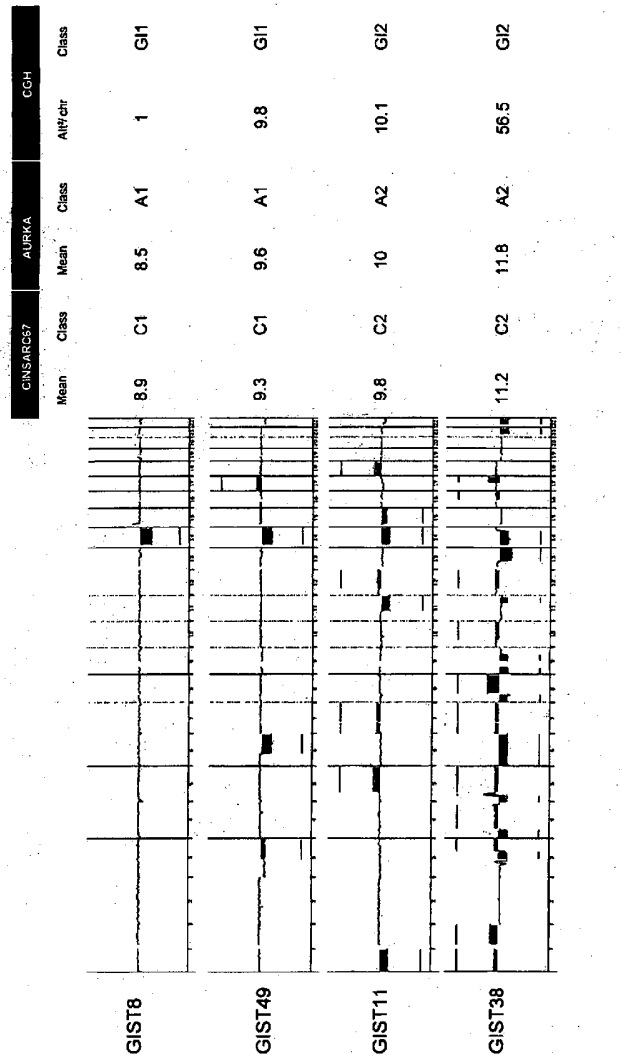
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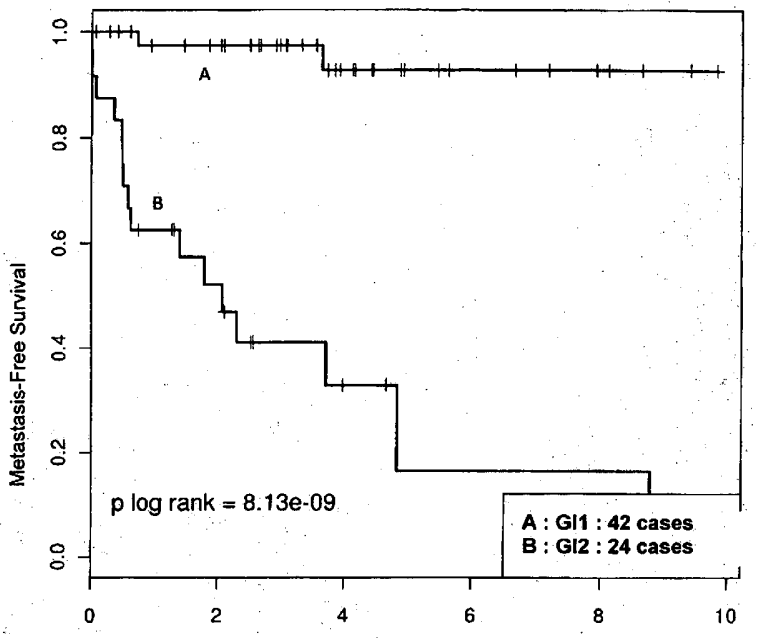
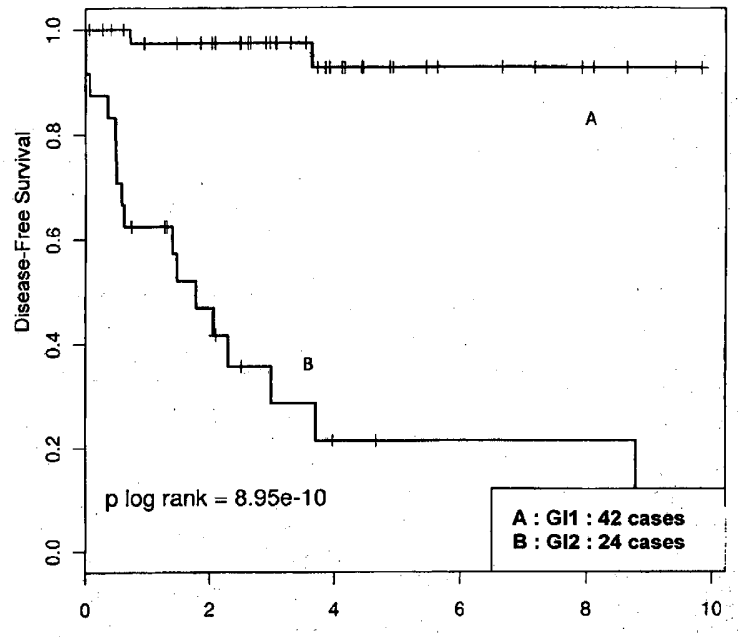
Figure 3

a)



		0	1 y	2 y	3 y	4 y	5 y
GI 1	A: Patients at risk	42	36	34	25	16	9
	Cumulated events	0	1	1	1	2	2
	MFS	1	0,97	0,97	0,97	0,93	0,93
GI 2	B: Patients at risk	24	14	10	5	3	1
	Cumulated events	1	9	11	13	14	15
	MFS	0,96	0,63	0,52	0,41	0,33	0,16

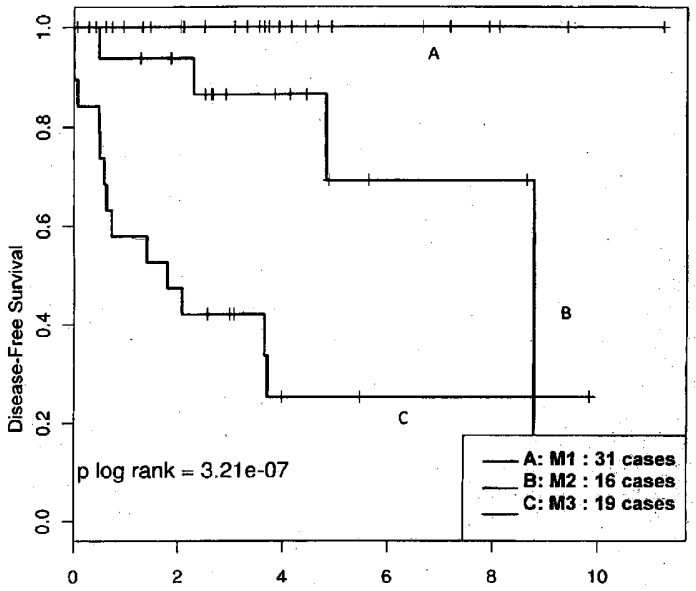
Figure 4 (part 1)



	0	1y	2y	3y	4y	5y
Patients at risk	42	36	34	25	16	9
	0	1	1	1	2	2
Cumulated events DFS	1	0,97	0,97	0,97	0,93	0,93
Patients at risk	24	14	9	4	2	1
	1	9	12	15	16	16
Cumulated events DFS	0,96	0,63	0,47	0,29	0,21	0,21

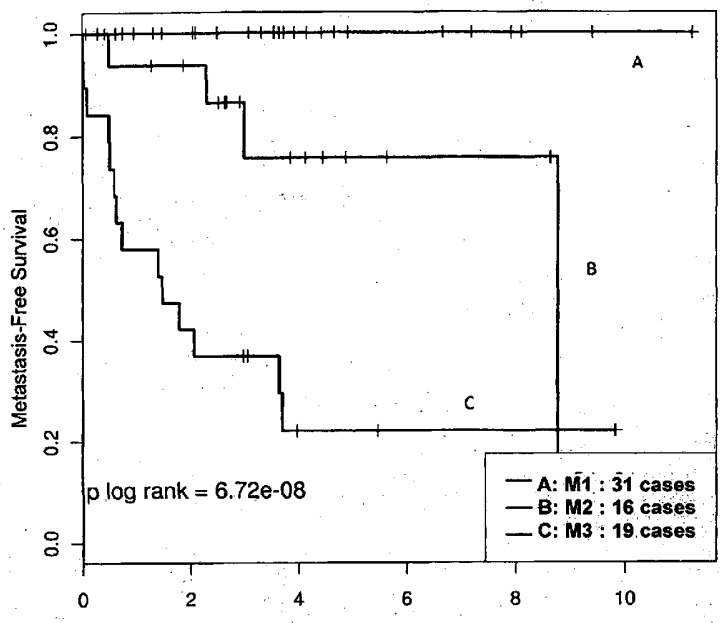
Figure 4 (part 2)

b)



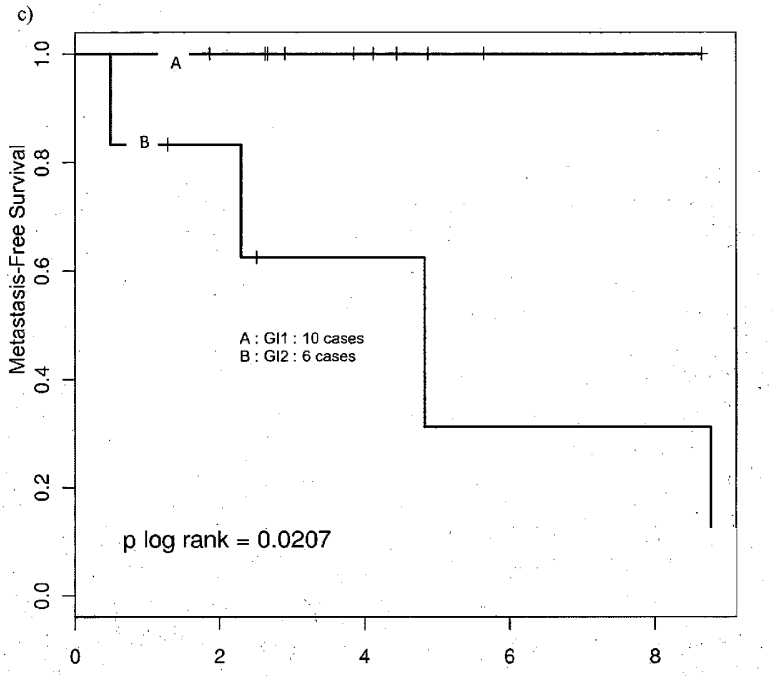
		0	1 y	2 y	3 y	4 y	5 y
M 1	Patients at risk	31	25	23	17	11	6
	Cumulated events	0	0	0	0	0	0
	MFS	1	1	1	1	1	1
M 2	Patients at risk	16	15	13	8	7	3
	Cumulated events	0	1	1	2	2	3
	MFS	1	0.94	0.94	0.87	0.87	0.69
M 3	Patients at risk	19	11	9	6	2	2
	Cumulated events	1	8	10	11	13	13
	MFS	0,95	0,58	0,47	0,42	0,25	0,25

Figure 4 (part 3)



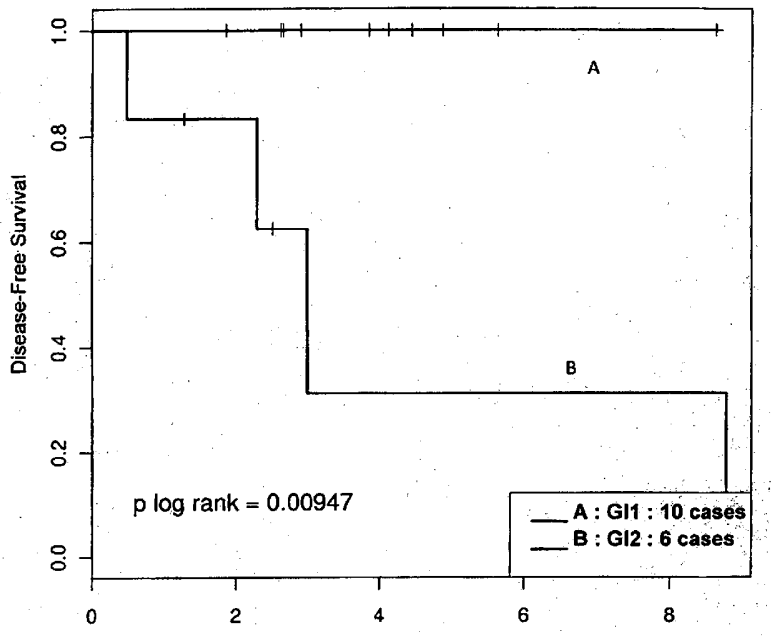
	0	1y	2y	3y	4y	5y
A: Patients at risk	31	25	23	17	11	6
Cumulated events	0	0	0	0	0	0
DFS	1	1	1	1	1	1
B: Patients at risk	16	15	13	7	6	3
Cumulated events	0	1	1	3	3	3
DFS	1	0.94	0.94	0.76	0.76	0.76
C: Patients at risk	19	11	8	6	2	2
Cumulated events	1	8	11	12	14	14
DFS	0,95	0,58	0,42	0,37	0,22	0,22

Figure 4 (part 4)



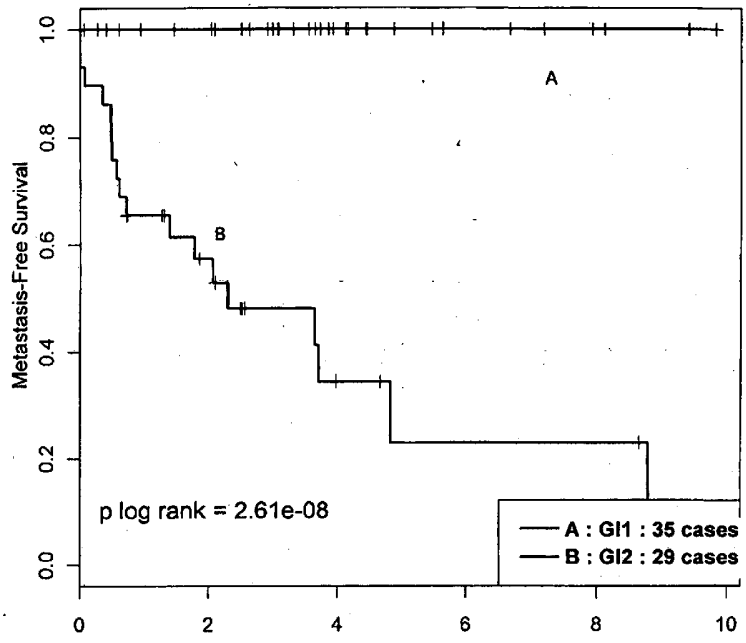
		0	1 y	2 y	3 y	4 y	5 y
GI1	Patients at risk	10	10	9	6	5	2
	Cumulated events	0	0	0	0	0	0
	MFS	1	1	1	1	1	1
GI2	Patients at risk	6	5	4	2	2	1
	Cumulated events	0	1	1	2	2	3
	MFS	1	0,83	0,83	0,63	0,63	0,31

Figure 4 (part 5)



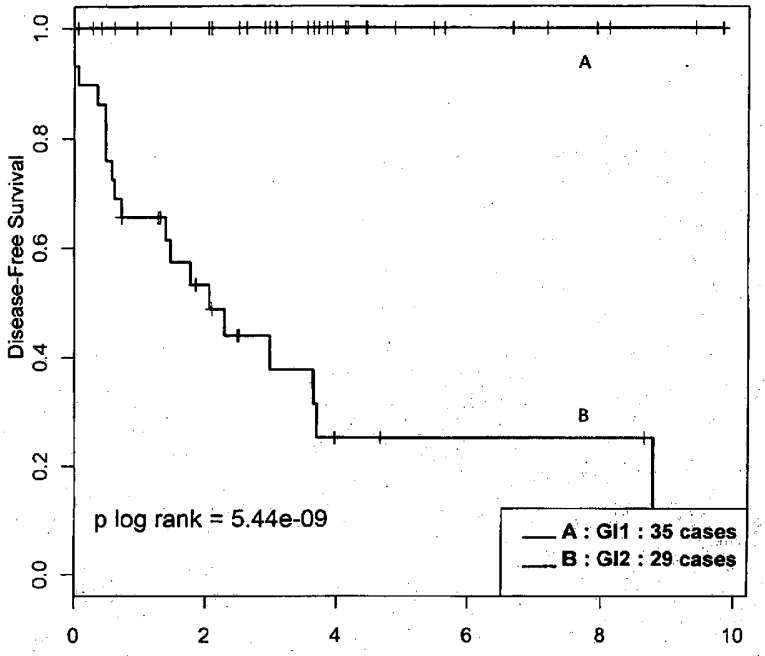
	0	1y	2y	3y	4y	5y
A: Patients at risk	10	10	9	6	5	2
	0	0	0	0	0	0
Cumulated events	1	1	1	1	1	1
DFS						
B: Patients at risk	6	5	4	1	1	1
	0	1	1	3	3	3
Cumulated events	1	0,83	0,83	0,31	0,31	0,31
DFS						

Figure 4 (part 6)



		0	1 y	2 y	3 y	4 y	5 y
A G1	Patients at risk	35	30	29	22	14	8
	Cumulated events	0	0	0	0	0	0
	MFS	1	1	1	1	1	1
A G2	Patients at risk	29	18	13	7	4	2
	Cumulated events	1	10	12	14	16	17
	MFS	0,97	0,66	0,57	0,48	0,34	0,23

Figure 5 (part 1)



	0	1y	2y	3y	4y	5y
A : Patients	35	30	29	22	14	8
at risk	0	0	0	0	0	0
Cumulated	1	1	1	1	1	1
events						
DFS						
B: Patients	29	18	12	6	3	2
at risk	1	10	13	16	18	18
Cumulated	0,97	0,66	0,53	0,38	0,25	0,25
events						
DFS						

Figure 5 (part 2)

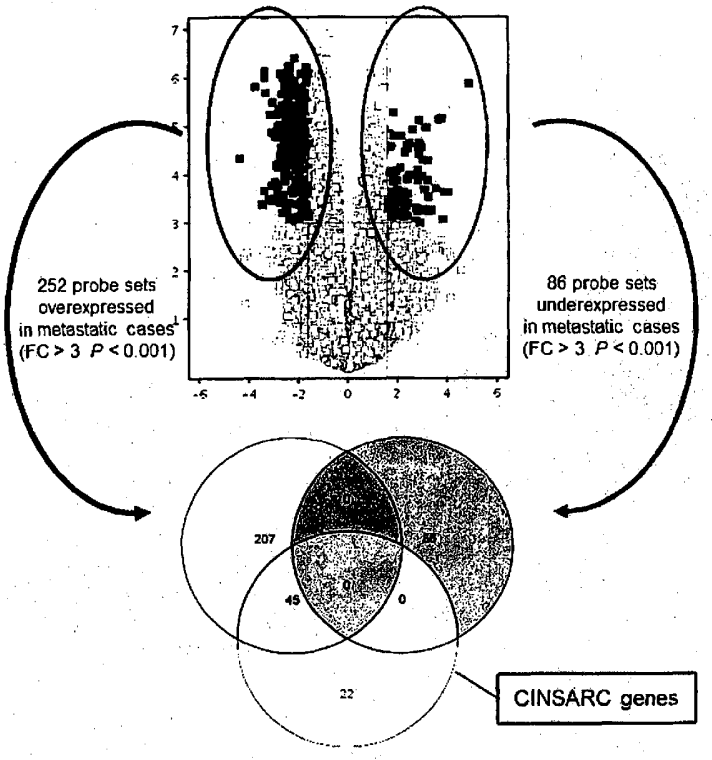

252 probe sets overexpressed in metastatic cases (FC > 3 P < 0.001)
86 probe sets underexpressed in metastatic cases (FC > 3 P < 0.001)
207
45
0
22
CINSARC genes

Figure 6

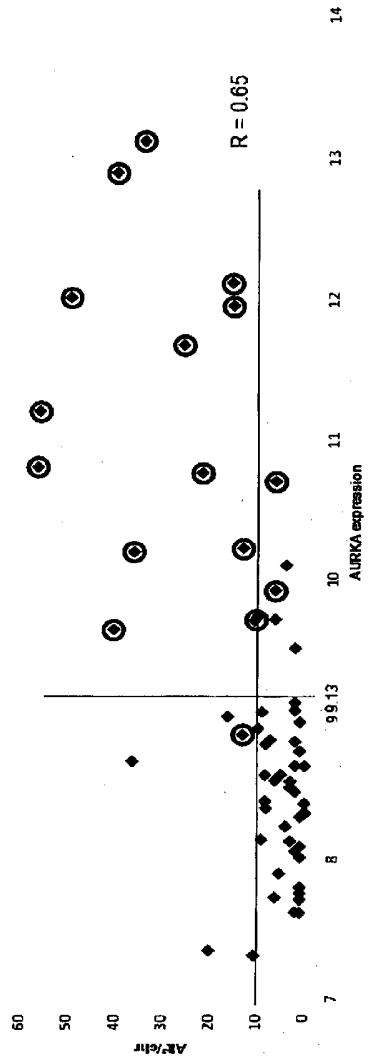

R = 0.65
AURKA expression
AR/cHr
14
13
12
11
10
9 9.13
8
7
60
50
40
30
20
10
0

Figure 7

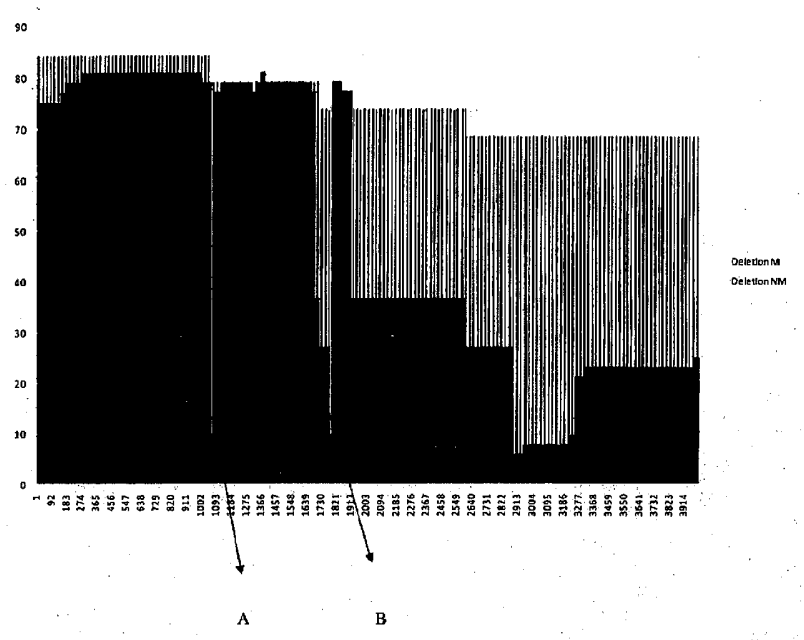


Figure 8 (part 1)

A:

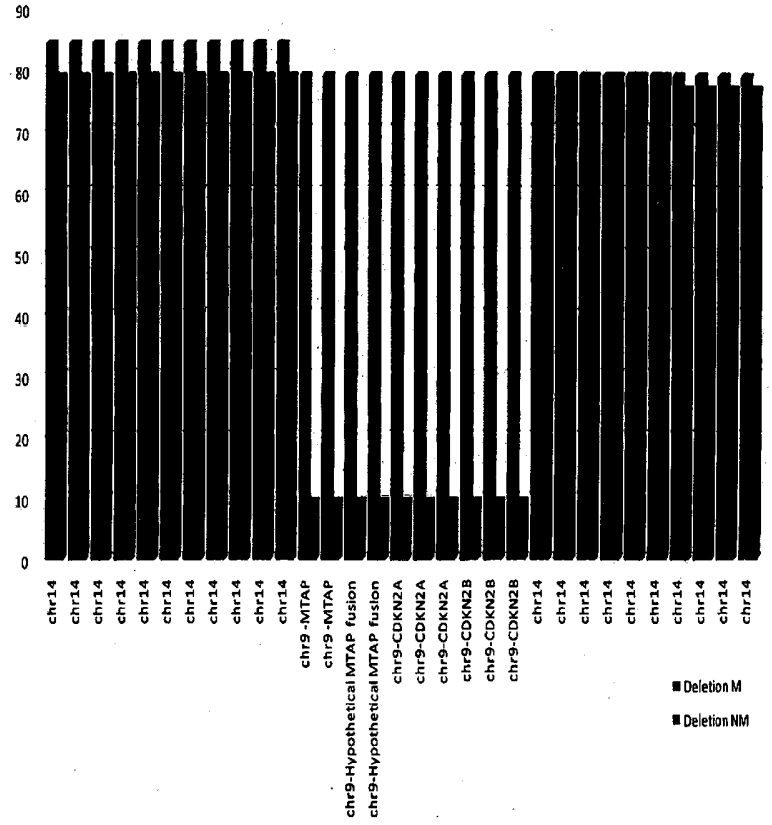


Figure 8 (part 2)

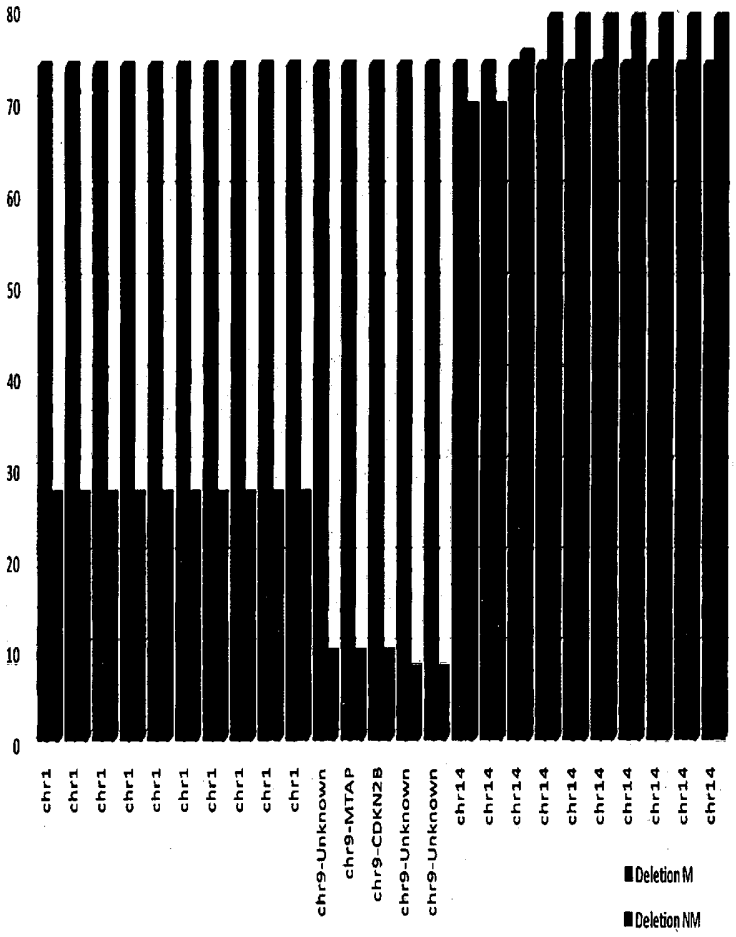


Figure 8 (part 3)

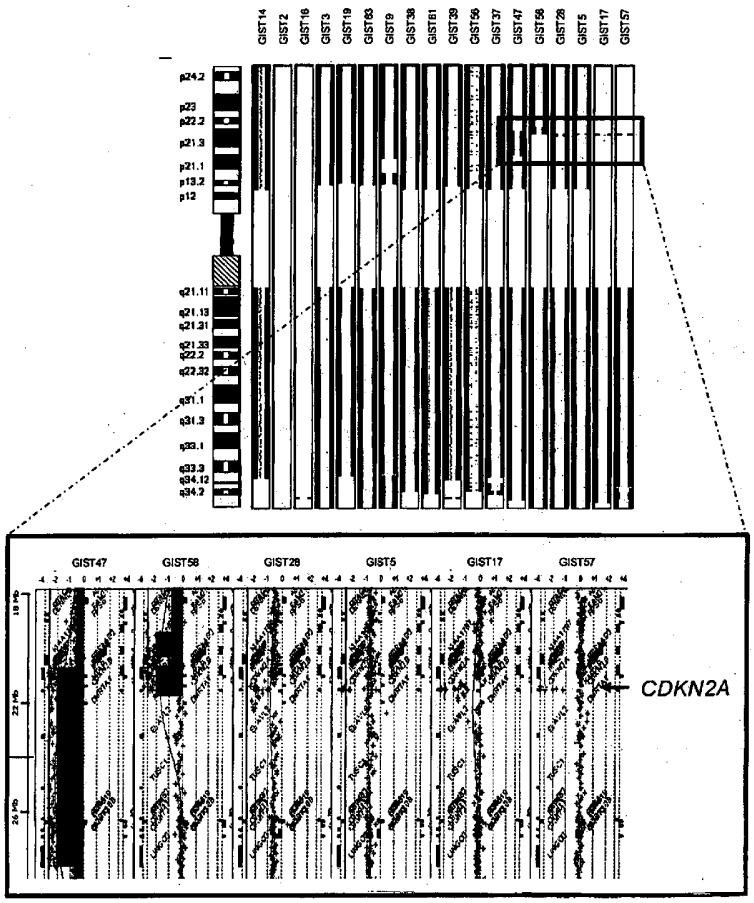

GIST14
GIST2
GIST16
GIST3
GIST19
GIST63
GIST9
GIST38
GIST61
GIST39
GIST56
GIST37
GIST47
GIST58
GIST28
GIST5
GIST17
GIST57
GIST47
GIST58
GIST28
GIST5
GIST17
GIST57
CDKN2A

Figure 9

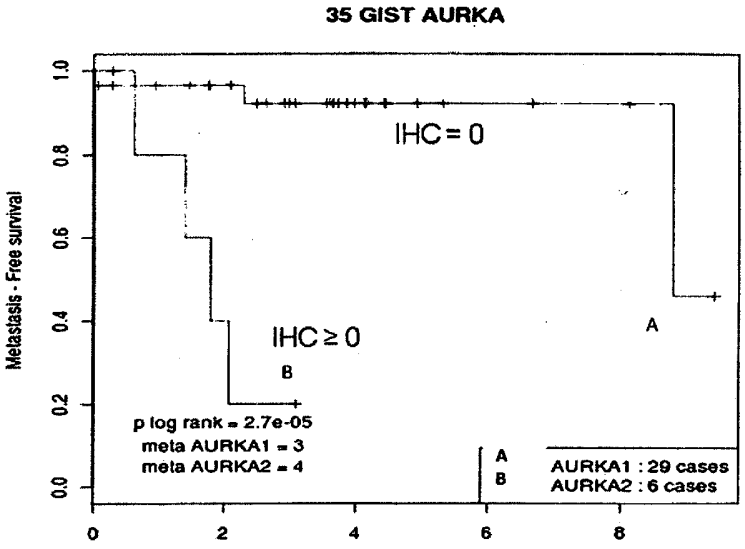

35 GIST AURKA
Metastasis - Free survival
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
IHC = 0
IHC ≥ 0
A
B
p log rank = 2.7e-05
meta AURKA1 = 3
meta AURKA2 = 4
A
B
AURKA1 : 29 cases
AURKA2 : 6 cases

Figure 10