



Longitudinal study of recurrent metastatic melanoma cell lines underscores the individuality of cancer biology

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Key Words:	melanoma, metastasis, recurrence, personalized therapy

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Longitudinal study of recurrent metastatic melanoma cell lines underscores the individuality
of cancer biology

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12 Short title: Recurrent metastases retain specific traits
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17 Abbreviations: aCGH, array comparative genomic hybridization; ANOVA, analysis of variance; CN, copy
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19 number; CNA copy number alteration; FDR, false discovery rate; GX, gene expression; MDS,
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21 multidimensional scaling; RM ANOVA, repeated measures analysis of variance
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Abstract

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11 Recurrent metastatic melanoma provides a unique opportunity to analyze disease evolution in
12 metastatic cancer. Here, we followed 8 patients with an unusually prolonged history of metastatic
13 melanoma, who developed a total of 26 recurrences over several years. Cell lines derived from each
14 metastasis were analyzed by comparative genomic hybridization and global transcript analysis. We
15 observed that conserved, patient-specific characteristics remain stable in recurrent metastatic
16 melanoma even after years and several recurrences. Differences among individual patients exceeded
17 within-patient lesion variability, both at the DNA copy number ($p < 0.001$) and RNA gene expression level
18 ($p < 0.001$). Conserved patient-specific traits included expression of several cancer/testis antigens and the
19 c-kit proto-oncogene throughout multiple recurrences. Interestingly, subsequent recurrences of
20 different patients did not display consistent or convergent changes toward a more aggressive disease
21 phenotype. Finally, sequential recurrences of the same patient did not descend progressively from each
22 other, as irreversible mutations, such as homozygous deletions were frequently not inherited from
23 previous metastases. This study suggests that the late evolution of metastatic melanoma, which
24 dramatically turns an indolent disease into a lethal phase, is prone to preserve case-specific traits over
25 multiple recurrences and occurs through a series of random events that do not follow a consistent step-
26 wise process.
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6 Introduction
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10 Cancer progression is usually studied cross-sectionally, comparing lesions obtained from different
11 patients, excised at various stages. By combining these snapshots, the natural history of the disease can
12 be indirectly reconstructed. In contrast, the preferable longitudinal analysis of sequential lesions in the
13 same patients is usually not feasible, especially difficult to perform in rapidly progressing cancers, such
14 as melanoma, and particularly challenging when analyzing disease progression in metastases (Bonsing et
15 al, 1993; Kuukasjarvi et al, 1997; Navin et al, 2011).
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24 However, the limited number of such longitudinal studies leaves several questions open. First, cross-
25 sectional studies do not allow an estimate of the extent in which patient-specific traits remain stable
26 over time. Therefore, it is difficult to assess the stability of such patient-specific traits over time, which is
27 a question of basic importance in personalized cancer therapy (Gupta et al, 2009; Harbst et al, 2010;
28 Navin et al, 2010).
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35 In addition, with cross-sectional analyses it is impossible to test, whether late disease development
36 follows a pattern of sequential somatic microevolution, or subsequent metastases represent individual
37 buddings from a stable set of cancer progenitors creating independently established new metastatic
38 lesions (Sabatino et al, 2008; Wang et al, 2006).
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45 Finally, it is difficult to quantify whether sequential steps are involved in late stage progression, and
46 estimate whether consistent changes are required for the late progression of melanoma from a
47 metastatic phase that progresses slowly, to a rapid evolution in the declining phase of one patient's life.
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51 Studying longitudinally several recurrent melanoma metastases of a rare collection of eight
52 individuals who developed multiple recurrences over a period of years (see Table S1), we sought a
53 better understanding of the above questions. This study is a follow up from a previous longitudinal study
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3 of a single case (Sabatino et al, 2008; Wang et al, 2006) focusing on traits remaining stable and changes
4 repeated consistently among multiple developing recurrent metastases of several melanoma patients.
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8 To our best knowledge, these questions have not yet been analyzed by others.
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10 11 12 13 14 Results

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20 Long term metastatic melanoma is consistent with canonical melanoma genomics
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25 Since the cases with multiple recurrent metastases studied here differ behaviorally from classic
26 metastatic melanoma due to their unusually protracted course, we first evaluated whether the cell lines
27 derived from these unusual cases would differ markedly from typical cases of melanoma as published by
28 others.
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34 Array comparative genomic hybridization (aCGH) confirmed that the chromosomal distribution
35 of copy number (CN) alterations (CNAs) prominently observed here are in line with previous
36 observations (Fig. 1a) (Jonsson et al, 2007; Roschke et al, 2003; Spivey et al, 2012; Thompson et al,
37 1995). Also, at the individual gene level, most genes were affected by copy number gains and losses in
38 accordance with others' reports (Grafstrom et al, 2005; Jonsson et al, 2007; Okamoto et al, 1999; Pirker
39 et al, 2003; Shi et al, 2012) (Fig. 1b, see full data set in Table S2).
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48 Finally, similar to others' reports, we also found that a correlation between CN and gene
49 expression (GX) data is present, but limited in advanced cancer (Bacolod and Barany, 2010; Sabatino et
50 al, 2008; Spivey et al, 2012). Among 4,340 genes eligible for analysis, 2,766 correlated weakly (Pearson's
51 correlation $R < 0.3$, $p < 0.05$, false discovery rate (FDR) 0.05) and 272 strongly ($R < 0.5$, $p < 0.05$, FDR 0.01) in
52 CN and GX (see Figure S1).
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3 Taken together, this dataset was representative of typical characteristics of metastatic
4 melanoma genomics, as reported in the literature (Bacolod and Barany, 2010; Jonsson et al, 2007;
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6 Roschke et al, 2003; Sabatino et al, 2008; Spivey et al, 2012; Thompson et al, 1995).
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12 Advanced melanoma retains case-specific fingerprints after years of disease progression
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17 Following a rare case of metastatic melanoma that recurred several times over a decade, we
18 previously observed that in spite of the stochastic and selective forces affecting its genome, stable
19 characteristics prevailed to the point that recurrent lesions derived from this patient clustered away
20 from heterologous randomly collected cases (Sabatino et al, 2008; Wang et al, 2006). This patient-
21 specific stability, if shared by other cases of advanced melanoma, could have fundamental implications
22 for personalized cancer therapy. Thus, in this study, we first analyzed whether the previous observations
23 could be generalized to a larger set of patients.
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33 First we compared CNA and GX patterns on a global genomic scale among cell lines from the
34 eight patients with multiple recurrences. Multidimensional scaling (MDS), a computational method
35 enabling visualization of sample relatedness within large scale genetic data demonstrated that even
36 after years, recurrent metastases of a given patient remained closely related, keeping clear distance
37 from others' metastases (Fig. 2a and 2c). By comparing all metastases in all possible pairs (325 pairs
38 total), we found that MDS distances between subsequent metastases (estimates of sample relatedness)
39 of the same patient were significantly shorter than those between metastases of different patients (Fig.
40 2b and 2d). This finding held true whether CNA or GX data were compared.
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54 Stable patient-specific traits include genes of relevance to melanoma biology
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3 We next searched for genomic aberrations typically specific to a given patient. We found that
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5 stable case-specific CNAs occurred in chromosomes 1, 5, 13, and 19 (Fig. 3a One Way Analysis of
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7 variance (ANOVA), $p < 0.05$, $FDR < 0.001$, see Table S3 for details). Similarly, 925 genes were found to have
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9 stable, patient-specific expression; 61 among them could be categorized functionally as melanoma-
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11 related by the Ingenuity Pathway Analysis database (Fig. 3b, One-Way ANOVA, $p < 0.05$, $FDR < 0.05$). The
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13 latter included several genes with known tumorigenic properties supporting autonomous proliferation
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15 (KIT, MYC, CDK2, RBL2), controlling genomic stability (BRCA1), apoptosis and cell survival (TP53BP2,
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17 CASP8, TEP1), adhesion and motility (CDH1, ITGA4), invasiveness, matrix remodeling (MMP15, MMP19),
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19 angiogenesis (ANGPT1, EGF), modulation of anti-tumor immunity, (large clusters of major
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21 histocompatibility complex class I and II transcripts, the latter correlating with CIITA expression) and
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23 several melanoma antigens (MAGE-A1, -A4, -A9, -B2, -C2). This observation suggests that genes highly
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25 relevant to melanoma progression retain stable patient-specific expression levels over long periods of
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27 time (Fig. 3b).
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33 Notably among all possible patient-to-patient comparisons (28 pairwise comparisons involving 8
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35 patients), 37 genes demonstrated patient-specific expression pattern with significant differences among
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37 patients and an at least two-fold change in >70% of all pair wise comparisons. These included MAGE-A4,
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39 -B2, -C2, BAGE-2, and KIT (see Table S4). To further test these results, we analyzed KIT protein levels by
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41 flow cytometry in the investigated cell lines. Our analysis disclosed that although KIT expression is
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43 frequently affected by post-transcriptional regulation, KIT protein levels remain consistent throughout
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45 multiple recurrences of individual patients, and whenever expressed, correlate well with mRNA data
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47 (Fig. S2). Taken together, these observations suggest that genes relevant to melanoma immunology and
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49 melanoma cell biology are expressed stably within a given patient, and may, in turn, be responsible for
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51 behavioral differences among individual cancers.
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3 Lack of evidence for convergent evolution and consistent changes among patients over time
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8 Next, we asked whether subsequent metastases from different patients progressively converge
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10 to reach a terminal, potentially lethal “hyper-aggressive” status. This would imply that on average, early
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12 (e.g. first) metastases of individual patients would be more different, more distant from each other than
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14 late (e.g. the last) metastases of the same individuals. MDS genomic distances demonstrate that this is
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16 not the case (Fig. 4a and 4b), neither at the CN or at the GX level.
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19 To corroborate this finding, we next attempted to identify consistent CN or GX changes that
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21 might represent a recurrent theme in the transition from earlier to later metastases in a given patient.
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23 However, statistical analysis was unable to identify changes in CN alterations or GX patterns that
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25 constitute consistent trends in subsequent recurrences of metastatic melanoma, (Two-Way RM ANOVA
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27 $p < 0.05$ FDR 0.05). First, an analysis of all recurrent metastases inclusive of patient identity and lesion
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29 sequence revealed no consistent changes between subsequent metastases. Next, since patients with
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31 large numbers of recurrences dominate the analysis in such a pair wise comparison, we decreased or
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33 eliminated differences in per-patient sample sizes. To this end, we first replaced multiple synchronous
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35 metastases with a single averaged value for each parameter tested ($p < 0.05$ FDR 0.05). Also, in a
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37 separate analysis we limited the evaluated cases to 3 randomly selected samples per patient ($p < 0.05$
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39 FDR 0.05). No consistent changes were found by either analysis. Next, assuming that the first and last
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41 available lesions in a given patient were most distant genetically, we restricted the analysis to these
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43 extreme pairs; but again, a pair wise analysis including patient identity failed to identify statistically
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45 significant differences ($p < 0.05$ FDR 0.05). Finally, hypothesizing that the last, supposedly most advanced,
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47 fatal lesion in a given patient might be different from earlier ones, we compared the latter with the
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49 former ($p < 0.05$ FDR 0.05), again without observing consistent differences. Taken together, no consistent
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51 progression patterns could be observed between subsequent metastases, either at the DNA copy
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3 number or RNA gene expression level, regardless of the approach used for sample selection and
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6 grouping before statistical analysis.
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8 In line with this observation, comparison of the first metastasis from a given patient with his
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10 subsequent ones demonstrated that the latter are not necessarily drifting progressively further from the
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12 original one (Fig. 4c and 4d). Rather, the data suggest a stochastic drift among subsequent recurrent
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14 metastases.
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17 We also tested whether multiple cycles of phenotype switching between proposed invasive and
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19 proliferative phenotypes (Hoek et al, 2008) could explain a seemingly stochastic drifting of recurrent
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21 melanoma metastases. We found that this model may provide partial explanation for our observations,
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23 as key genes of the two phenotypes were expressed in an alternating fashion, and the two phenotypes
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25 seemed to change frequently back and forth through the recurrences of most (e.g. patients B, C, D, F, G),
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27 although certainly not all patients (e.g. patients A and E, Fig. S3).
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33 The fate of homozygous deletions does not support cumulative changes in the evolution of melanoma
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38 Since no step-wise evolutionary pattern could be discerned, we next asked whether recurrent
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40 metastases from the same patient descend sequentially from one another, i.e. if they acquire new
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42 mutations in a cumulative fashion. To this end, we followed the fate of common BRAF, NRAS mutations
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44 (Colombino et al, 2012) and homozygous deletions (-/-) in subsequent recurrent metastases.
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46 Unfortunately, BRAF and NRAS status turned out to be uninformative in this regard, because as
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48 frequently observed in melanoma, all recurrent melanomas analyzed were BRAFV600E mutated and
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50 NRAS wild type throughout (not shown). Next we analyzed the fate of homozygous deletions (-/-) that
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52 are thought to be irreversible since no known mechanisms for structural restoration of these alterations
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3 have been described. Based on this, we assumed that if subsequent recurrent metastases of the same
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5 patient show reversions of homozygous deletions, they cannot sequentially descend from each other.
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8 A total of 33 contiguous homozygous deletions were found affecting the CDKN2A/CDKN2B
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10 region, various interferon genes, B2M, major histocompatibility complex genes, etc. Out of these, 25
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12 deletions were eligible for analysis as they emerged in a metastasis for which there was at least a
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14 subsequent metastasis to evaluate (Fig. 5b). Out of 25 eligible homozygous deletions, 15 (60%) appeared
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16 to be reverted in a given patient's disease history, suggesting that in subsequent metastases of
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18 recurrent melanoma, new mutations are not acquired in a cumulative fashion, and hence, recurrent
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20 metastases do not descend from each other (Fig. 5b).
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26 Recurrent melanomas show hints of slower growth, but more frequent metastasis formation
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31 In initial MDS analyses, cancer cells from patients with recurrent long term metastatic disease
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33 were hardly discernible from those from sporadically excised, melanoma cases (Fig. 2a and 2c).
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35 Nevertheless, we identified a set of 177 genes differentially expressed between the two phenotypes,
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37 which is a very small number compared to patient-to-patient differences, 8 of which were melanoma-
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39 related. Interestingly, these genes hint to slower tumor growth (retained CDKN1A and ANAPC
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41 expression), higher sensitivity to immune- or therapy-mediated eradication (higher FAS but lower levels
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43 of MGMT expression), and higher pro-metastatic tendency (elevated levels of ALCAM, Fig. S4).
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49 Discussion

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54 This study analyzes a specific time point in the natural history of cancer when advanced disease
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56 of an indolent nature turns into an aggressive and lethal stage. We studied the genetic profile of
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3 melanoma cell lines derived from sequentially excised metastases in unusual cases when the metastatic
4 process followed a protracted course. Although the use of cell lines has significant limitations, we
5 observed that early passage cell lines maintain stable genetic traits in vitro that relate to the in vivo
6 phenotype of parental tumors (Spivey et al, 2012). Nevertheless, our samples clearly do not equal whole
7 tumors, and these cases may have represented a special subset of melanoma, as well. First, these
8 recurrent melanomas displayed CDK2NA, PTEN, and BRAF copy number aberrations more frequently
9 than average cases (Hodis et al, 2012; Krauthammer et al, 2012). In addition, all 26 metastases of the
10 analyzed 8 patients carried BRAF V600E, but displayed wild type NRAS. Conservation of BRAF mutation
11 status across metastases is in line with others' observations (Niessner et al, 2013). However, this
12 particular BRAF/NRAS pattern is typical for melanomas arising in intermittently sun-exposed areas
13 (Colombino et al, 2012), affects cell proliferation rate (Liu et al, 2007), prognosis (Long et al, 2011),
14 treatment of choice, and in this latter context, also BRAF copy numbers (Shi et al, 2012).

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31 Keeping these limitations in mind, our data suggest that key elements of the framework of
32 recurrent metastatic melanomas remain stable with time; since such stability was observed in 8 out of 8
33 patients, it possibly represents the rule rather than the exception. This is a remarkable finding
34 considering that at the same time, our data also support the accepted view of late stage cancer
35 evolution being a highly dynamic process, also shown recently by others (Gerlinger et al, 2012; Shah et
36 al, 2012) using indirect computational inference; however, this study uniquely provides direct evidence
37 by studying serially asynchronous metastases over a long period.

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47 Our findings suggest that individuality is maintained throughout a non-directional drift that does
48 not follow a clearly linear progression, with each metastatic signature stemming de novo from a stable
49 progenitor entity. Moreover, there was no sign of a convergent evolution in advanced late stage
50 melanoma toward the creation of a convergent lethal phenotype, and recurrent metastases did not
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3 seem to be each other's clonal descendants, or accumulate incremental changes, which is in line with
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6 others' recently published observations (Colombino et al, 2012).
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8 On the other hand, the observation that stable expression of cancer/testis antigens and the c-kit
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10 proto-oncogene across multiple recurrences of melanoma implies that, late stage melanoma is capable
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12 of displaying stable, case-specific differences directly affecting markers determining vulnerability to
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14 novel forms of immunological or small molecule biotherapy (Guo et al, 2011; Tyagi et al, 2005; Tyagi and
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16 Mirakhur, 2009).
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19 It remains to be clarified to what extent these observations are attributable to the effects of
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21 clonal heterogeneity (Gerlinger et al, 2012; Shah et al, 2012), circulating tumor cells (Maheswaran et al,
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23 2008; Yu et al, 2011) that may remain dormant for years and reset the evolutionary clock upon their
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25 reactivation, multiple events of phenotype switching (Eichhoff et al, 2010; Hoek et al, 2008), or
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27 persistent cancer stem cells opening multiple alternative ways to cancer evolution with each individual
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29 recurrence (La Porta, 2012; Shakhova and Sommer, 2012). Larger and more comprehensive studies
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31 involving genome-wide DNA sequencing, epigenetic and proteomic analyses, analyzing patients with
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33 average survival times, and resected whole tumors instead of cell lines, are strongly warranted to clarify
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35 these questions and confirm the applicability of our findings to usual cases of advanced melanoma.
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43 Materials and Methods
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49 Patients and samples
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3 Twenty-six recurrent melanoma metastases were surgically isolated from 8 patients experiencing
4 relapse after one or more successful treatment intervention(s) with no signs of residual disease.
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6 Recurrent metastases from different tissues appeared in periods spanning 10-148 months with 8-101
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8 months between recurrences (see Table S1 for all data regarding samples, patients, treatments and
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10 disease history). Patients received therapy and underwent surgery at the Surgery Branch of the National
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12 Cancer Institute, National Institutes of Health, USA, or at the Centro di Riferimento Oncologico (Italian
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14 National Cancer Institute) in Aviano, Italy. Patients were treated and samples obtained after signing
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16 informed consent, with the approval of each institute's review board, and in accordance with the
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18 Declaration of Helsinki Principles. From all lesions, stable cell cultures were established and maintained
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20 at the Department of Transfusion Medicine, Clinical Center, National Institutes of Health for at least
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22 eight passages. Patients experiencing recurrent metastases were labeled with capital letters; "A", "B",
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24 "C", etc., their subsequent metastases as "A/1", "A/2", "B/1", "B/2", etc., while synchronous metastases
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26 in a given patient were labeled as "A/1a", "A/1b" etc. All recurrent melanoma metastases analyzed
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28 appeared after a single primary tumor. Another 22 melanoma cell lines isolated and maintained as
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30 above were expanded from melanoma patients with rapid disease course, for whom only one
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32 metastasis was available. As no extended follow up was possible in these cases, the cell lines are
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34 considered representative of random time points in the natural course of metastatic melanoma. These
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36 cell lines were labeled with Arabic numbers, as "1", "2", "3", etc.
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49 DNA Isolation

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55 Total genomic DNA of cell lines was isolated using the QuickGene DNA whole blood kit S and a
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57 QuickGene-810 Nucleic Acid Isolation System (Fujifilm, Tokyo, Japan).
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7 HLA-Typing
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12 To exclude accidental cross-contamination of samples, low resolution HLA-typing was performed at the
13 HLA Laboratory, Laboratory Services Section, Department of Transfusion Medicine, Clinical Center,
14 National Institutes of Health.
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24 BRAF and NRAS genotyping
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30 PCR was performed from 50 ng genomic DNA using the HotStarTaq Master Mix Kit (Qiagen, Valencia,
31 CA) and the following primers: BRAF exon 15 forward: 5'-TCATAATGCTTGCTCTGATAGGA-3' BRAF exon
32 15 reverse: 5'-GGCCAAAATTTAATCAGTGGA-3', NRAS exon 2 forward: 5'-ATAGCATTGCATTCCTGTG-3'
33 NRAS exon 2 reverse: 5'-CACAAAGATCATCCTTTCAGAGA-3'. PCR products were labeled using a Big Dye
34 terminator kit v3.1 (Life Technologies, Carlsbad, CA). Sequencing was performed using a 3730 Genetic
35 Analyzer (Applied Biosystems) and analyzed by Sequencher software (Gene Codes, Ann Arbor, MI).
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48 Array Comparative Genome Hybridization (aCGH)
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54 All aCGH studies were performed using Agilent's oligo aCGH platform. Briefly, 1 µg of genomic DNA per
55 sample was directly labeled with a Genomic DNA Enzymatic Labeling Kit, prepared for hybridization with
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3 help of an Oligo aCGH Hybridization Kit, and hybridized to 105K Human Genome CGH 105A Oligo
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5 Microarrays. Arrays were washed with Oligo aCGH Wash Buffers and scanned in a High-Resolution
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7 Microarray Scanner (all from Agilent, Santa Clara, CA). Data were deposited in the GEO public database
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9 under GSE38187.
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12 13 14 15 16 RNA Isolation

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23 Total RNA was isolated using Qiagen's RNEasy Mini Kit, following standard protocol.
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29 Gene expression microarray

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35 For expression array studies, the Affymetrix Gene Array System was utilized. Briefly, 250 ng total RNA
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37 per sample was amplified using a WT expression kit. Next, cDNA was labeled with help of a GeneChip
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39 WT Terminal Label and Control Reactions kit. Samples were then prepared for hybridization using the
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41 GeneChip Hyb Wash and Stain Kit and loaded to Human Gene ST 1.0 Arrays. Arrays were washed, PE-
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43 labeled on a GeneChip Fluidics Station 450, and loaded into a GeneChip Scanner 3000 7G with
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45 autoloader for scanning (all from Affymetrix, Santa Clara, CA). Data were submitted to GEO and made
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47 publicly available under accession GSE38187.
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52 53 54 55 Microarray data analysis

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7 Agilent aCGH microarray data were imported into the Partek Genomics Suite software (Partek,
8 St. Louis, Missouri), quantile normalized and pre-processed using a built-in chromosomal segmentation
9 algorithm (Hawthorn et al, 2010). Individual chromosomal segments were defined as continuous regions
10 covered by at least 10 consecutive microarray probes, a significant ($p < 0.001$) and considerable (> 0.3
11 copies on average) difference between the CN of the given segment and neighboring segments,
12 accepting an error rate of less than ± 0.3 copies. Segmented genomes were subjected to
13 Multidimensional Scaling (MDS) to describe inter-sample relationships. Partek's One-Way and Two-Way
14 RM ANOVA analyses were performed on segment CNs to identify CNAs different between individual
15 patients, CNAs consistently changed in consecutive metastases of the same patient, and CNAs between
16 recurrent and random cancer samples. To avoid over-estimation of patient-to-patient differences in CNA
17 studies analyzing a mixed-gender group of patients, X and Y chromosome-related data were excluded
18 from all such analyses. Significant differences were identified with a nominal $p < 0.05$ and were corrected
19 with FDR of < 0.05 . Homozygous deletions (-/-) were identified as segments with $CN < 0.4$ at an error rate
20 of $< \pm 0.3$ copies.
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39 Affymetrix gene expression data were imported to Partek Genomic Suite, quantile normalized
40 and batch-corrected using Distance-Weighted-Discrimination, as described elsewhere (Benito et al,
41 2004). MDS, One-Way and Two-Way RM ANOVA analyses were performed as above. CN and GX data
42 were integrated and analyzed with help of Partek Genomic Suite. Genes whose expression levels were
43 found to be affected by CNAs were identified by computing Pearson's correlation between CN and GX
44 values. A Pearson's correlation of $R > 0.3$ with $p < 0.05$ and FDR 0.05 was accepted as proof for CNA-
45 affected gene expression.
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57 Flow cytometry

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7 Cells were harvested non-enzymatically using Cellstripper (Corning, Manassas, VA), and stained
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9 with LIVE/DEAD Kit (Life Technologies, Carlsbad, CA), anti-CD117(KIT)-APC (BD Biosciences, San Jose,
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11 CA), or isotype controls. Data analysis was performed using a MACSQuant Analyser (Miltenyi Biotec,
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13 Germany) and FlowJo (TreeStar).
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Conflict of interest

The authors state no conflict of interest.

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10 Figure 1
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15 Description and basic characterization of the analyzed sample set by integrated copy number and
16 gene expression analysis
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21 Panel a) shows frequency and spatial distribution of autosomal CN aberrations in the analyzed
22 melanoma sample set. Panel b) displays combined distribution analysis of CN gains and losses affecting
23 key melanoma genes, and also their distribution between various, disease-related biological functions,
24 as defined by the Ingenuity Pathway Analysis database. Selected key melanoma genes are labeled with
25 their respective HUGO gene symbols.
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35 Figure 2
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40 Comparison of the relative weights of within- versus between-patient differences in metastatic
41 melanoma
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47 Panel a) displays the whole complexity of DNA copy number data reduced to three dimensions (D1-3) by
48 Multidimensional Scaling (MDS). Metastases are symbolized by spheres. Recurrent metastases
49 belonging to the same patient (A-H) are color-coded; non-recurrent, random metastasis samples,
50 serving as controls, are grey. Panel b) shows distribution of MDS plot distances between individual
51 metastases representing the magnitude of actual genomic differences. Statistical comparisons of MDS
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3 distances (~genomic differences) between recurrent metastases belonging to the same, vs. different
4 patients are shown. P-values given are derived from a standard t-test considering all possible recurrent
5 metastasis pairs from the sample set. Panels c) and d) display similar information on whole genome RNA
6 expression data.
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14 Figure 3

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19 Identification of stable individual traits conserved in recurrent metastases of a given patient through
20 years of ongoing disease history
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26 On panel a), examples are shown for stable conserved copy number traits that remain characteristic for
27 a given case of recurrent melanoma (selected examples in yellow frames). Samples belonging to the
28 same patient are aligned horizontally and color coded (to the left). On panel b), conserved gene
29 expression patterns, characteristic for a given case, remain stable throughout multiple recurrences and
30 are shown using a standardized heatmap (selected examples in yellow frames). Samples are aligned
31 vertically and color coded (top). HUGO gene symbols of selected melanoma-related genes are shown to
32 the right.
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44 Figure 4

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49 Testing evolutionary convergence and sequential evolution in recurrent metastatic melanomas on a
50 global scale
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3 Panels a) and b) compare MDS-based distances (estimates of genomic difference) between first and last
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5 lesions of different patients experiencing multiple recurrences of melanoma. A standard t-test is applied
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8 to test whether late lesions are less different from each other than earlier ones, that is, if there is
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10 convergent evolution among individual cases of metastatic melanoma. Panels c) and d) analyze the
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12 question if subsequent recurrent recurrences (any n^{th} and $n+1^{\text{th}}$ lesion) would be more and more distant
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14 (~different) from the first diagnosed metastasis, implying incremental changes and thus sequential
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16 evolution of subsequent metastases.
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24 Figure 5

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28 Follow-up analysis of the stability of homozygous deletions in evolving recurrent metastatic
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30 melanomas
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35 Panel a) displays a histogram of the calculated DNA copy number values associated with every identified
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37 chromosome segment in the analyzed melanoma sample set. A blue circle marks segments accepted as
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39 homozygous deletions (-/-) considering the accuracy and statistical fidelity limits set for chromosomal
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41 segmentation. Panel b) displays the fate of these completely deleted segments in eight patients (A, B,
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43 etc.), experiencing several melanoma recurrences in a sequence (A/1, A/2, B/1, B/2, etc.), some of which
44
45 are multiple synchronous recurrences (A/2a, A/2b, etc.). Yellow frames indicate selected chromosomal
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47 regions that, although completely lost at one time point of disease history (-/- = blue), months or years
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49 later re-emerged in a recurrence of the same case of cancer (-/+ = grey or +/+ = red).
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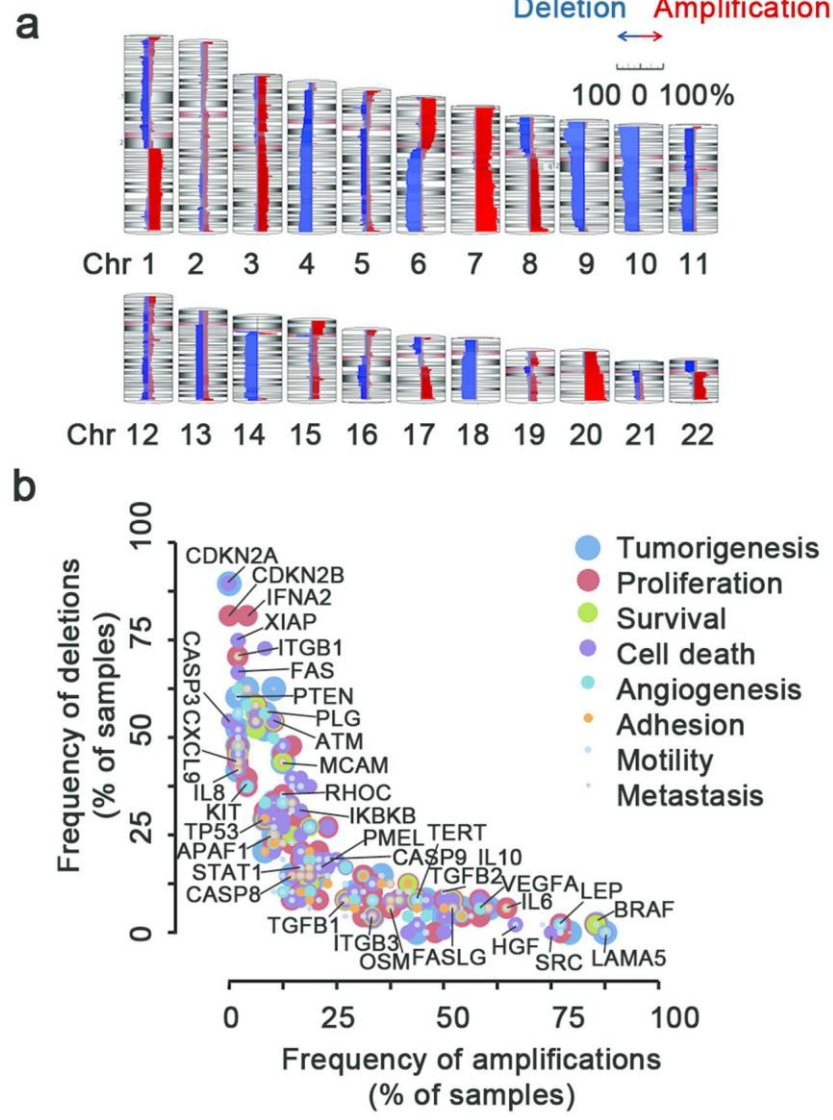
56 Supplementary Material
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Supplementary Tables (S1-S4) and Figures (S1-S4) have been submitted.

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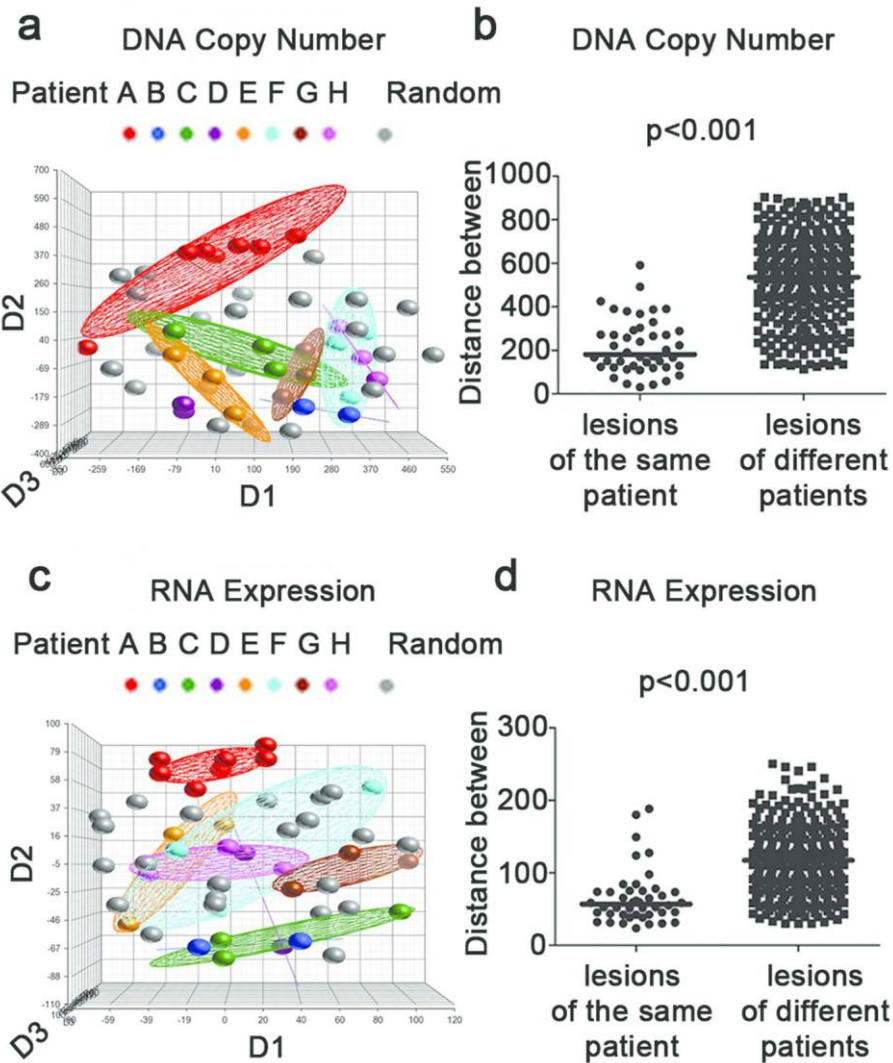
Figure 1



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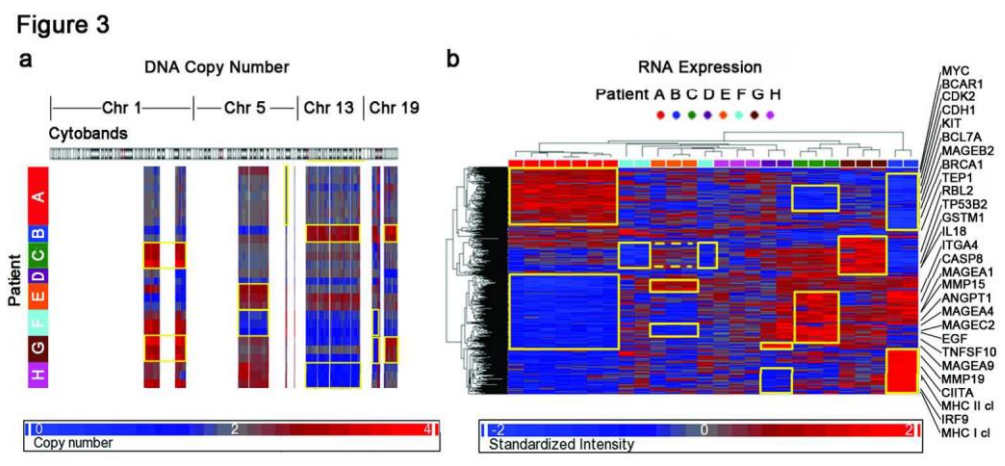
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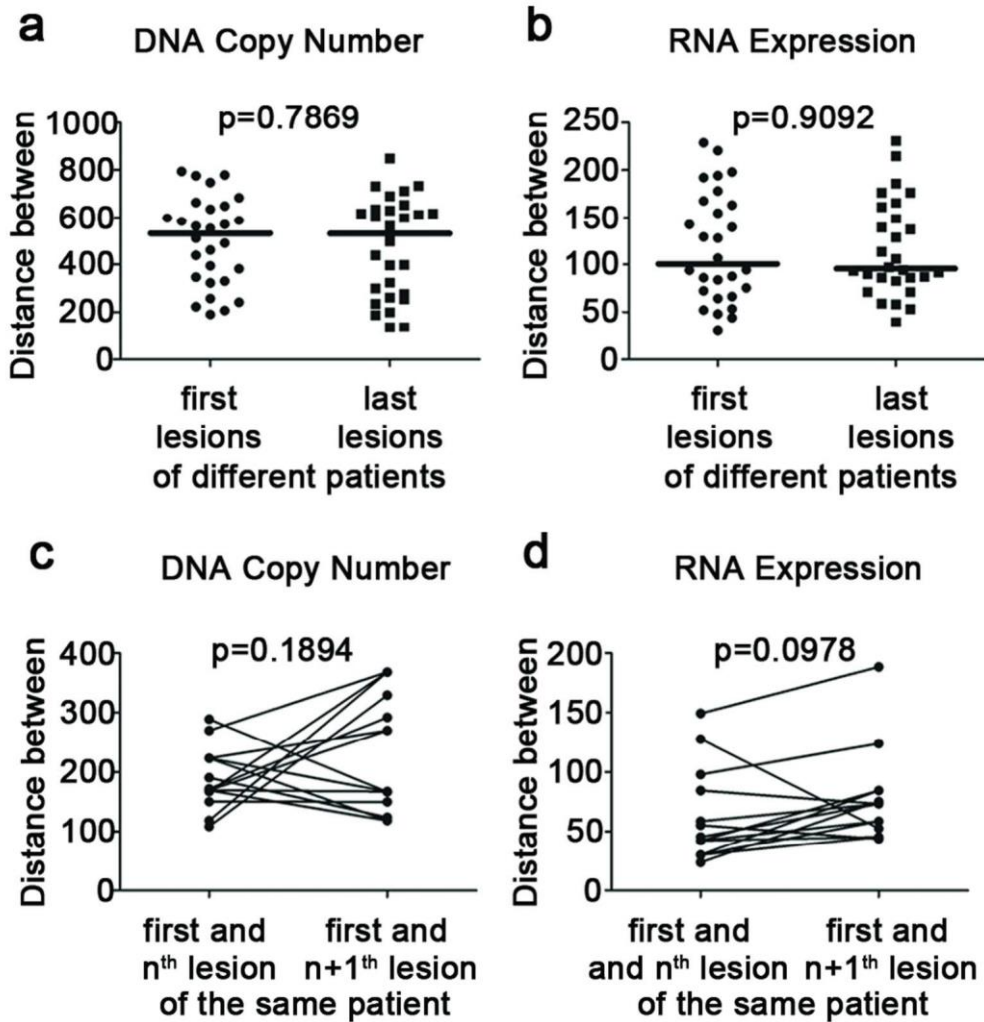
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Figure 4

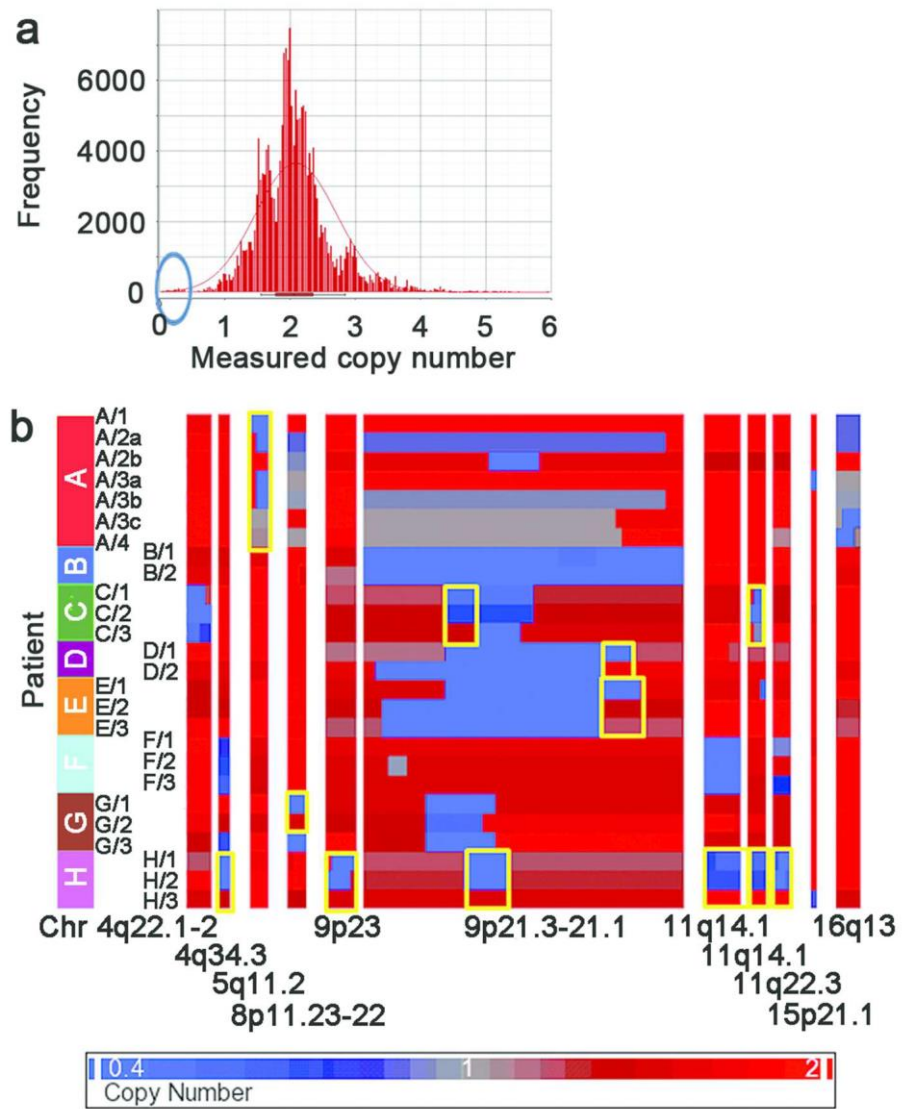


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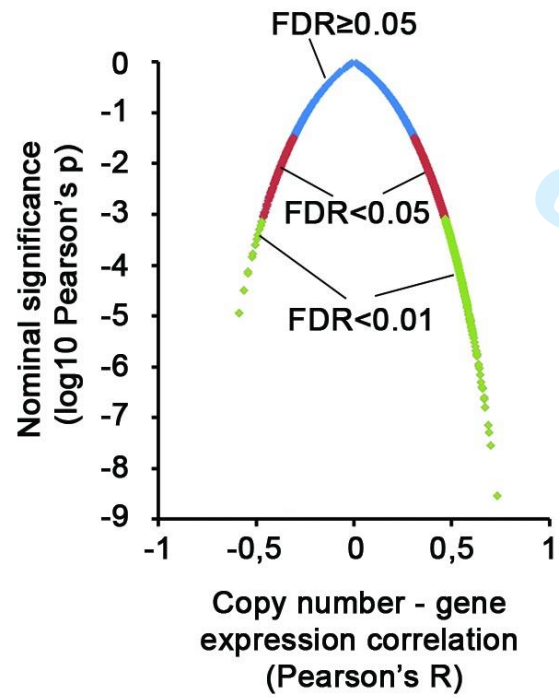
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Figure 5



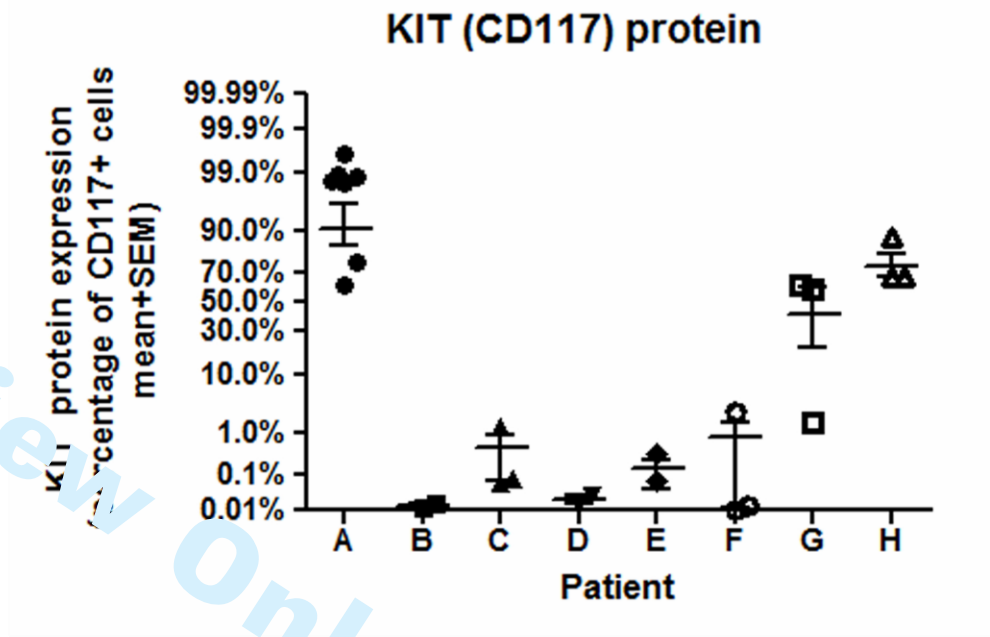
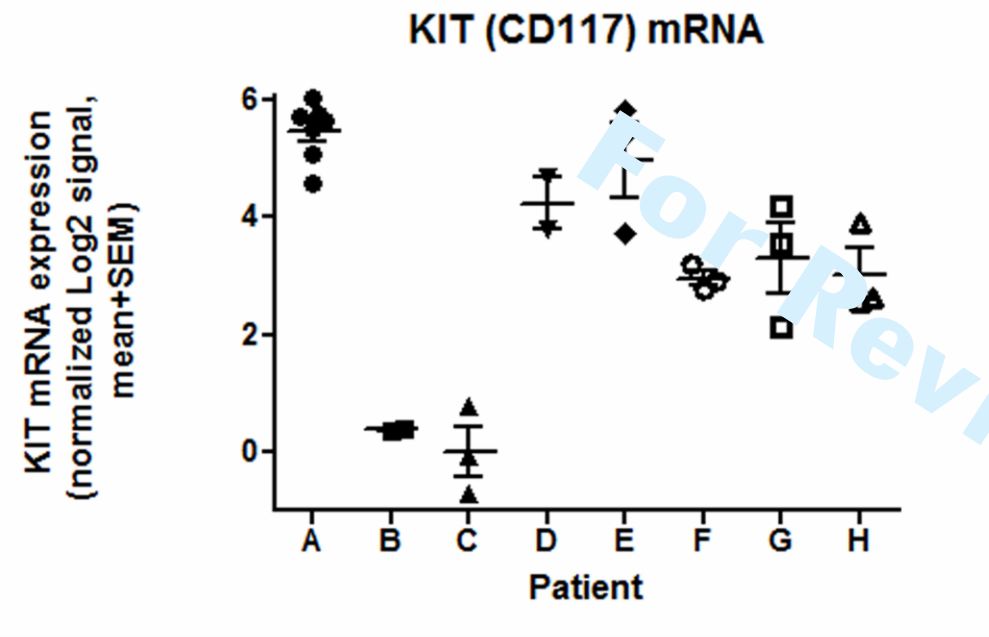
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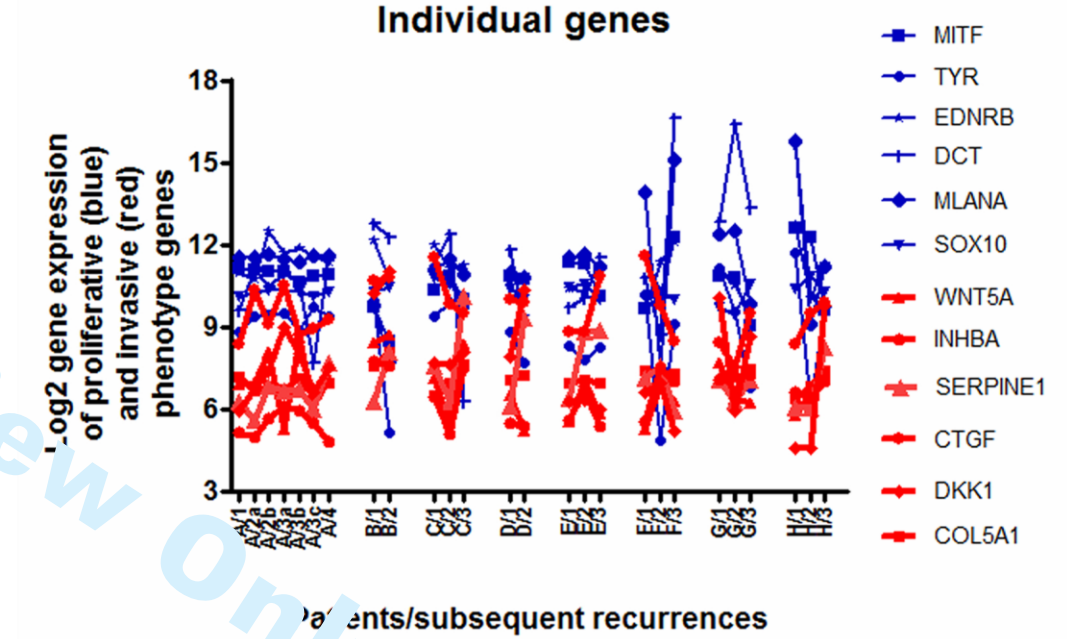
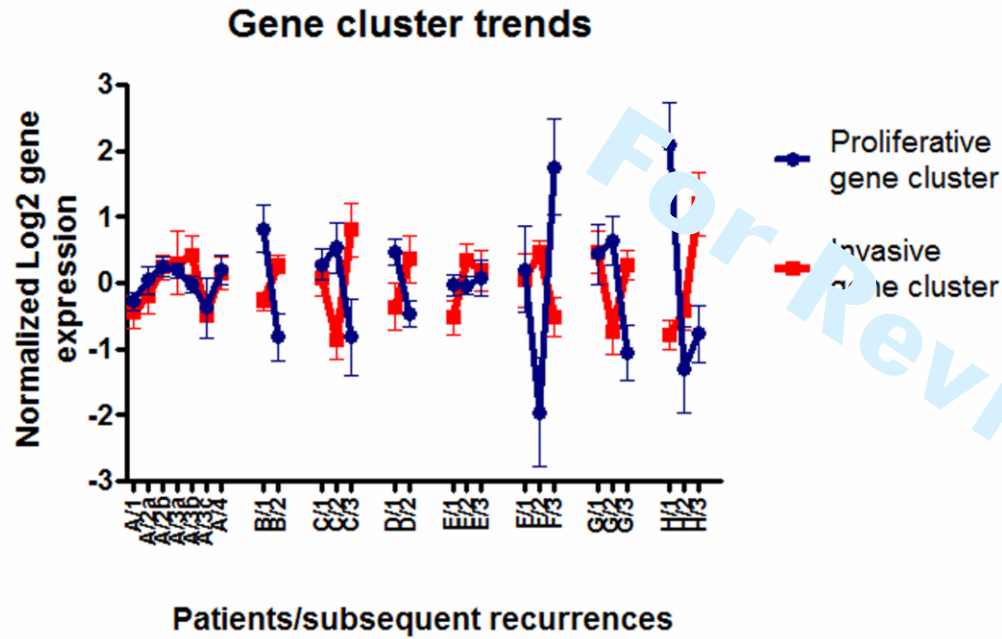


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GEO Microarray ID	Disease course	Patient ID	Lesion ID	Date of Primary	Site of primary	Applied therapy	Date of metastasis	Site of metastasis	Local vs. Distant	Date of death	Gender	Origin			
1	Recurrent	A	A/1	12/15/1998	cutaneous, right arm	TIL CELLS/IL2 HD, RADIATION (5000cGy, 3000cGy) DTIC/CDDP	6/21/1989	subcutaneous, soft palate	Distant	8/5/2002	F	Surgery Branch, NIH, Bethesda, USA			
2	Recurrent	A	A/2a				6/10/1992	uterine metastasis	Distant	8/5/2002	F	Surgery Branch, NIH, Bethesda, USA			
3	Recurrent	A	A/2b				6/10/1992	uterine metastasis	Distant	8/5/2002	F	Surgery Branch, NIH, Bethesda, USA			
4	Recurrent	A	A/3a				9/30/2000	subcutaneous, R flank	Distant	8/5/2002	F	Surgery Branch, NIH, Bethesda, USA			
5	Recurrent	A	A/3b				9/30/2000	subcutaneous, R flank	Distant	8/5/2002	F	Surgery Branch, NIH, Bethesda, USA			
6	Recurrent	A	A/3c				9/30/2000	subcutaneous, R flank	Distant	8/5/2002	F	Surgery Branch, NIH, Bethesda, USA			
7	Recurrent	A	A/4				8/28/2001	subcutaneous, R breast	Distant	8/5/2002	F	Surgery Branch, NIH, Bethesda, USA			
8	Recurrent	B	B/1				5/28/1985	cutaneous, left foot	SURGERY, DTIC, IL2	9/13/1999	abdominal subcutis	Distant	11/26/2000	F	CRO, Aviano, Italy
9	Recurrent	B	B/2	7/26/2000	cutis of the leg	Local				11/26/2000	F	CRO, Aviano, Italy			
10	Recurrent	C	C/1	7/12/1993	in transit cutis/subcutis of the leg	Local				2/1/1995	F	CRO, Aviano, Italy			
11	Recurrent	C	C/2	8/12/1991	cutaneous, right leg	SURGERY, anti-idiotype/IL-2	3/23/1994	inguinal lymph nodes	Local	2/1/1995	F	CRO, Aviano, Italy			
12	Recurrent	C	C/3				1/17/1995	cutis/subcutis of the arm	Distant	2/1/1995	F	CRO, Aviano, Italy			
13	Recurrent	D	D/1				9/28/1990	cutaneous, right arm	SURGERY, DTIC	5/15/1994	axillary lymph nodes	Local	9/3/1998	M	CRO, Aviano, Italy
14	Recurrent	D	D/2							12/23/1997	pancreas	Distant	9/3/1998	M	CRO, Aviano, Italy
15	Recurrent	E	E/1	8/30/1996	inguinal lymph nodes	Local				6/30/2004	M	CRO, Aviano, Italy			
16	Recurrent	E	E/2	9/6/1994	cutaneous, right leg	SURGERY, DTIC	6/19/1998	subcutis of the leg	Local	6/30/2004	M	CRO, Aviano, Italy			
17	Recurrent	E	E/3				4/20/1999	cutis/subcutis of the leg	Local	6/30/2004	M	CRO, Aviano, Italy			
18	Recurrent	F	F/1				7/7/1999	chest wall (subcutaneous)	Distant	4/25/2004	M	Surgery Branch, NIH, Bethesda, USA			
19	Recurrent	F	F/2	10/9/1995	cutaneous, left leg	GP100/MART-1 VACC, TYROS/GP100 VACC,IL2 HD, DTIC/CDDP, CYTOXAN/FLUDARABINE/TIL CELLS/IL2 HD, RADIATION, GAMMA KNIFE	6/21/2001	axilla	Distant	4/25/2004	M	Surgery Branch, NIH, Bethesda, USA			
20	Recurrent	F	F/3				3/6/2003	thigh (soft tissue - muscle)	Distant	4/25/2004	M	Surgery Branch, NIH, Bethesda, USA			
21	Recurrent	G	G/1	8/19/1997	cutaneous, right calf	IFN- α /MELPHALAN WITH ILP, DTIC/CISPLATIN/VELBAN/IL-2, gpGP100 VACC, IL2, CYTOXAN/FLUDARABINE/TIL CELLS/MART-1 VACC/IL2 HD, VEMURAFENIB	6/26/2001	pelvis	Distant	Alive	M	Surgery Branch, NIH, Bethesda, USA			
22	Recurrent	G	G/2				6/1/2002	thigh/buttock (subcutaneous)	Distant	Alive	M	Surgery Branch, NIH, Bethesda, USA			
23	Recurrent	G	G/3				1/14/2003	popliteal fossa	Local	Alive	M	Surgery Branch, NIH, Bethesda, USA			
24	Recurrent	H	H/1	12/9/1999	cutaneous, mid back	IFN α /GM-CSF/IFN γ VACC/ MART-1 VACC /GCSF, CYTOXAN/FLUDARABINE / TIL CELLS/IL2 HD/MART1 VACC, GCSF/DTIC/CDDP	1/21/2002	back (subcutaneous)	Local	10/4/2004	M	Surgery Branch, NIH, Bethesda, USA			
25	Recurrent	H	H/2				7/14/2003	shoulder (soft tissue - muscle)	Distant	10/4/2004	M	Surgery Branch, NIH, Bethesda, USA			
26	Recurrent	H	H/3				3/31/2004	axilla	Distant	10/4/2004	M	Surgery Branch, NIH, Bethesda, USA			
27	Random	1	1				N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA	
28	Random	2	2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
29	Random	3	3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
30	Random	4	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
31	Random	5	5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
32	Random	6	6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
33	Random	7	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
34	Random	8	8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
35	Random	9	9	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
36	Random	10	10	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
37	Random	11	11	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
38	Random	12	12	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
39	Random	13	13	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
40	Random	14	14	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
41	Random	15	15	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
42	Random	16	16	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
43	Random	17	17	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
44	Random	18	18	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
45	Random	19	19	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
46	Random	20	20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
47	Random	21	21	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				
48	Random	22	22	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Surgery Branch, NIH, Bethesda, USA				

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HUGO Gene Symbol	% of samples with deletions	% samples with amplifications	Entrez Gene Name	Location	Type(s)	Drug(s)	Tumorigenesis	Proliferation, Cell Cycle	Survival	Cell death, Apoptosis	Angiogenesis	Adhesion, Binding, Attachment, Detachment	Movement, Migration, Invasion	Metastasis
CDKN2A	90	0	cyclin-dependent kinase inhibitor 2A (menstrual cycle)	Nucleus	transcription regulator		+	-	-	+	-	-	-	-
CDKN2B	81	0	cyclin-dependent kinase inhibitor 2B (p16)	Nucleus	transcription regulator		-	+	-	-	-	-	-	-
IFNA2	81	4	interferon, alpha 2	Extracellular Space	cytokine		-	+	-	-	-	-	-	-
XIAP	75	2	X-linked inhibitor of apoptosis	Cytoplasm	other		-	-	-	+	-	-	-	-
SAT1	73	8	spermidine/spermine N1-acetyltransferase	Cytoplasm	enzyme		-	-	-	+	-	-	-	-
ITGB1	71	2	integrin, beta 1 (fibronectin receptor, beta 1)	Plasma Membrane	transmembrane receptor		-	+	-	-	-	+	+	-
FAS	67	2	Fas (TNF receptor superfamily, member 6)	Plasma Membrane	transmembrane receptor		-	-	-	+	-	-	-	-
PLAU	63	2	plasminogen activator, urokinase	Extracellular Space	peptidase		-	-	-	-	+	-	-	-
CXCL12	63	4	chemokine (C-X-C motif) ligand 12	Extracellular Space	cytokine		-	-	-	-	+	-	+	-
RET	63	4	ret proto-oncogene	Plasma Membrane	kinase	sunitinib	+	-	-	-	-	-	-	-
CD274	63	10	CD274 molecule	Plasma Membrane	other		-	-	-	-	-	-	-	-
PTEN	60	2	phosphatase and tensin homolog	Cytoplasm	phosphatase		+	-	-	-	-	-	-	+
MAPK8	58	4	mitogen-activated protein kinase 8	Cytoplasm	kinase	aplidine	-	-	-	-	+	-	-	-
FLNA	58	6	filamin A, alpha	Cytoplasm	other		-	-	+	-	-	-	-	+
L1CAM	58	6	L1 cell adhesion molecule	Plasma Membrane	other		-	-	-	-	-	+	+	-
POU3F2	58	6	POU class 3 homeobox 2	Nucleus	transcription regulator		-	+	-	-	-	-	-	-
SMPD2	58	6	sphingomyelin phosphodiesterase 2, neuronal	Cytoplasm	enzyme		-	-	-	-	-	+	-	-
DNMBP	56	2	dynamin binding protein	Cytoplasm	other		-	-	-	-	-	-	+	-
FGF8	56	2	fibroblast growth factor 8 (androgen-inducible)	Extracellular Space	growth factor		-	-	-	-	+	-	-	-
MGMT	56	6	O-6-methylguanine-DNA methyltransferase	Nucleus	enzyme	O6-benzylguanine	-	-	+	-	-	-	-	-
PM2AIP1	56	6	phorbol-12-myristate-13-acetate-induced protein 1	Cytoplasm	other		-	-	-	+	-	-	-	-
SMAD4	56	6	SMAD family member 4	Nucleus	transcription regulator		-	+	-	-	-	-	-	-
PLG	56	8	plasminogen	Extracellular Space	peptidase	tenecteplase	+	-	-	-	+	-	-	+
CASP3	54	0	caspase 3, apoptosis-related cysteine peptidase	Cytoplasm	peptidase	IDN-6556	-	-	-	+	-	-	-	-
BCL2	54	6	B-cell CLL/lymphoma 2	Cytoplasm	other	oblimersen	-	-	+	-	-	-	-	-
CDH2	54	6	cadherin 2, type 1, N-cadherin (neuronal)	Plasma Membrane	other		-	-	-	-	-	+	-	-
SMAD7	54	6	SMAD family member 7	Nucleus	transcription regulator		-	-	-	-	-	-	+	-
ATM	54	10	ataxia telangiectasia mutated	Nucleus	kinase		-	+	+	+	-	-	-	-
CTSL1	52	2	cathepsin L1	Cytoplasm	peptidase		+	-	-	-	-	-	+	-
GADD45G	52	2	growth arrest and DNA-damage-inducible beta	Nucleus	other		-	-	-	-	-	-	-	-
S1PR3	52	2	sphingosine-1-phosphate receptor 3	Plasma Membrane	G-protein-coupled receptor	finngolimod	-	-	-	-	-	-	+	-
BAG1	52	6	BCL2-associated athanogene	Cytoplasm	other		-	-	+	-	-	-	-	-
AKIRIN2	52	8	akirin 2	Nucleus	oth		-	+	-	-	-	-	-	-
LTBP2	52	2	latent transforming growth factor beta binding protein 2	Extracellular Space	other		-	-	-	-	-	-	+	-
CRYAB	50	10	crystallin, alpha B	Nucleus	other		-	-	-	-	+	-	-	-
IL18	50	10	interleukin 18 (interferon-gamma-inducible)	Extracellular Space	cytokine		-	-	-	-	+	-	-	-
BMP4	48	2	bone morphogenetic protein 4	Extracellular Space	growth factor		-	-	-	-	-	-	+	-
FGF2	48	2	fibroblast growth factor 2 (basic)	Extracellular Space	growth factor	66644	+	-	-	+	-	-	-	-
IL2	48	2	interleukin 2	Extracellular Space	cytokine		-	-	-	-	+	-	-	-
PRKACG	48	2	protein kinase, cAMP-dependent, catalytic	Cytoplasm	kinase		-	+	-	-	-	-	-	-
SPP1	48	2	secreted phosphoprotein 1	Extracellular Space	cytokine		-	+	-	+	-	-	+	-
TNC	48	2	tenascin C	Extracellular Space	other		-	+	-	-	-	-	-	-
BIRC2	48	13	baculoviral IAP repeat-containing 2	Cytoplasm	other		-	-	-	+	-	-	-	-
CASP4	48	13	caspase 4, apoptosis-related cysteine peptidase	Cytoplasm	peptidase		-	-	-	-	-	-	-	-
ZBTB16	48	13	zinc finger and BTB domain containing 16	Nucleus	transcription regulator		-	-	-	-	-	-	+	-
TFE3	48	15	transcription factor binding to IGHE enhancer	Nucleus	transcription regulator		-	-	-	-	-	-	-	-
AIMP1	46	2	aminoacyl tRNA synthetase complex-interacting motif 1	Extracellular Space	cytokine		-	-	-	-	-	-	-	-
ARHGAP5	46	2	Rho GTPase activating protein 5	Cytoplasm	enzyme		-	-	-	-	-	-	+	-
GSN	46	2	gelsolin	Extracellular Space	other		-	-	-	-	-	-	+	-
GZMB	46	2	granzyme B (granzyme 2, cytotoxic T-lymphocyte associated protein 10)	Cytoplasm	peptidase		-	-	-	-	-	-	-	-
RAPGEF2	46	2	Rap guanine nucleotide exchange factor 2	Cytoplasm	other		-	+	-	+	-	-	-	-
NOX4	46	13	NADPH oxidase 4	Cytoplasm	enzyme		+	+	-	-	-	-	-	-
CXCL1	44	2	chemokine (C-X-C motif) ligand 1 (melanocyte-stimulating factor)	Extracellular Space	cytokine		-	+	-	-	-	-	-	-
CXCL9	44	2	chemokine (C-X-C motif) ligand 9	Extracellular Space	cytokine		-	-	-	-	-	+	+	-
HSPA5	44	2	heat shock 70kDa protein 5 (glucose-regulated protein 78)	Cytoplasm	other		-	-	-	-	-	-	-	-
MCAM	44	13	melanoma cell adhesion molecule	Plasma Membrane	other		-	-	+	-	-	+	+	-
IL8	42	2	interleukin 8	Extracellular Space	cytokine		-	-	-	-	-	-	+	-
MMP14	42	2	matrix metalloproteinase 14 (membrane-type 1)	Extracellular Space	peptidase		-	-	-	-	-	-	+	-
STATH	42	2	statherin	Nucleus	other		-	-	-	-	-	+	-	-
SPTAN1	40	4	spectrin, alpha, non-erythrocytic 1 (alpha spectrin)	Plasma Membrane	other		-	+	-	-	-	-	-	-
CTSB	40	15	cathepsin B	Cytoplasm	peptidase		-	-	-	+	-	-	+	-
PBK	40	17	PDZ binding kinase	Cytoplasm	kinase		-	-	-	-	+	-	-	-
KDR	38	4	kinase insert domain receptor (a type III tyrosine kinase)	Plasma Membrane	kinase	AEE 788, dasatinib	-	-	-	-	+	-	-	-
KIT	38	4	v-kit Hardy-Zuckerman 4 feline sarcoma virus receptor	Plasma Membrane	kinase		-	+	-	-	-	-	-	-
REST	38	4	RE1-silencing transcription factor	Nucleus	transcription regulator		-	-	-	+	-	-	-	-
NRG1	38	15	neuregulin 1	Extracellular Space	growth factor		-	-	-	-	-	-	+	-
PACS2	38	17	phosphofurin acidic cluster sorting protein 2	Cytoplasm	other		-	-	-	+	-	-	-	-
PTK2B	38	17	PTK2B protein tyrosine kinase 2 beta	Cytoplasm	kinase		-	-	-	-	+	-	+	-
TNFRSF10B	38	19	tumor necrosis factor receptor superfamily member 10B	Plasma Membrane	transmembrane receptor	tigatuzumab	-	-	-	-	-	-	-	-
TNFRSF10C	38	19	tumor necrosis factor receptor superfamily member 10C	Plasma Membrane	transmembrane receptor		-	-	-	-	-	-	-	-
TNFRSF10D	38	19	tumor necrosis factor receptor superfamily member 10D	Plasma Membrane	transmembrane receptor		-	-	-	-	-	-	-	-
RHOC	35	13	ras homolog gene family, member C	Plasma Membrane	enzyme		-	-	-	-	-	-	+	-
TRIM33	35	13	tripartite motif containing 33	Nucleus	transcription regulator		-	+	-	-	-	-	-	-
HTATIP2	33	8	HIV-1 Tat interactive protein 2, 30kDa	Nucleus	transcription regulator		-	-	-	-	+	-	-	-
CSR3	33	10	cysteine and glycine-rich protein 3 (cardiac)	Nucleus	other		-	+	-	-	-	-	-	+
TEAD1	33	10	TEA domain family member 1 (SV40 transactivator)	Nucleus	transcription regulator		-	+	-	-	-	-	-	+
CYLD	33	13	cyllindromatosis (turban tumor syndrome)	Nucleus	transcription regulator		-	+	-	-	-	-	-	-
MMP2	33	13	matrix metalloproteinase 2 (gelatinase A)	Extracellular Space	peptidase		-	-	-	-	+	-	+	-
NOL3	33	13	nucleolar protein 3 (apoptosis repressor)	Nucleus	other		-	-	-	+	-	-	-	-
CDH1	33	15	cadherin 1, type 1, E-cadherin (epithelial)	Plasma Membrane	other		-	-	-	-	-	+	+	-
CAT	31	8	catalase	Cytoplasm	enzyme		-	+	-	-	-	-	-	-
WT1	31	8	Wilms tumor 1	Nucleus	transcription regulator		-	-	-	+	-	-	-	-
NGF	31	15	nerve growth factor (beta polypeptide)	Extracellular Space	growth factor		-	-	-	-	-	-	+	-
IKKB	31	17	inhibitor of kappa light polypeptide gene enhancer factor 3	Cytoplasm	kinase		-	-	-	+	-	-	-	-
CD44	29	8	CD44 molecule (Indian blood group)	Plasma Membrane	other		-	+	-	-	-	+	-	-

Pre-proof only

HUGO Gene Symbol	% of samples with deletions	% samples with amplifications	Entrez Gene Name	Location	Type(s)	Drug(s)	Tumorigenesis	Proliferation, Cell Cycle	Survival	Cell death, Apoptosis	Angiogenesis	Adhesion, Binding, Attachment, Detachment	Movement, Migration, Invasion	Metastasis
TP53	29	8	tumor protein p53	Nucleus	transcription regulator		+	-	+	+	-	-	-	-
F3	29	10	coagulation factor III (thromboplastin, tis	Plasma Membrane	transmembrane receptor		+	-	-	-	-	-	-	+
XAF1	29	10	XIAP associated factor 1	Nucleus	other		-	-	-	+	-	-	-	-
JUN	29	13	jun proto-oncogene	Nucleus	transcription regulator		-	-	-	-	-	-	-	-
ARRB1	29	17	arrestin, beta 1	Cytoplasm	other		-	+	-	-	-	-	-	-
VCAM1	27	10	vascular cell adhesion molecule 1	Plasma Membrane	other		-	-	-	-	-	-	+	-
GADD45A	27	13	growth arrest and DNA-damage-inducibl	Nucleus	other		-	-	-	+	-	-	-	-
JAK1	27	13	Janus kinase 1	Cytoplasm	kinase		-	+	-	-	-	-	-	-
CCND1	27	15	cyclin D1	Nucleus	other		-	+	-	-	-	-	-	-
CAPN1	27	19	calpain 1, (mu)/I large subunit	Cytoplasm	peptidase		-	-	-	-	-	-	+	-
FLT1	27	19	fnr3-related tyrosine kinase 1 (vascular en	Plasma Membrane	kinase	sumitrib,	-	-	-	-	+	-	-	-
HDAC1	27	19	histone deacetylase 1	Nucleus	transcription regulator	tributyryn,	-	-	-	-	+	-	-	-
RBBP4	27	19	retinoblastoma binding protein 4	Nucleus	enzyme		-	-	+	-	-	-	-	-
DCT	27	23	dopachrome tautomerase (dopachrome	Cytoplasm	enzyme		-	-	-	+	-	-	-	-
ING1	27	23	inhibitor of growth family, member 1	Nucleus	transcription regulator		-	+	-	-	-	-	-	-
APAF1	25	10	apoptotic peptidase activating factor 1	Cytoplasm	other		-	-	-	+	-	-	-	-
ITGA1	25	10	integrin, alpha 1	Plasma Membrane	other		-	-	-	-	+	-	-	-
ITGA2	25	10	integrin, alpha 2 (CD49B, alpha 2 subunit	Plasma Membrane	other		-	+	-	-	-	+	-	+
BANP	25	13	BTG3 associated nuclear protein	Nucleus	other		-	+	-	-	-	-	-	-
RP56KA1	25	15	ribosomal protein S6 kinase, 90kDa, poly	Cytoplasm	kinase		-	-	+	-	-	-	-	-
YBX1	25	17	Y box binding protein 1	Nucleus	transcription regulator		-	-	-	+	-	-	-	-
MAPK7	23	10	mitogen-activated protein kinase 7	Cytoplasm	kinase		-	-	-	-	+	-	-	-
VCAN	23	10	versican	Extracellular Space	other		-	+	-	-	-	+	-	-
BCAR1	23	13	breast cancer anti-estrogen resistance 1	Plasma Membrane	other		-	-	-	-	-	-	+	-
EFNA5	23	13	ephrin-A5	Plasma Membrane	kinase		-	-	-	-	-	+	-	-
CSF2	23	15	colony stimulating factor 2 (granulocy	Extracellular Space	cytokine		-	+	-	-	-	-	-	-
WASF2	23	15	WAS protein family, member 2	Cytoplasm	other		-	-	-	-	-	-	+	-
F2R	21	8	coagulation factor II (thrombin) receptor	Plasma Membrane	G-protein coupled receptor	chrysalin,	-	-	-	-	-	+	-	-
F2R	21	8	coagulation factor II (thrombin) receptor	Plasma Membrane	G-protein coupled receptor	chrysalin,	-	-	-	-	-	-	-	+
PAWR	21	10	PRKC, apoptosis, WT1, regulator	Nucleus	transcription regulator		-	-	-	-	-	-	-	-
CSNK1A1	21	17	casein kinase 1, alpha 1	Cytoplasm	kinase		-	-	-	+	-	-	-	-
SPARC	21	17	secreted protein, acidic, cysteine-rich (os	Extracellular Space	other		-	-	-	-	-	-	+	-
SCARB1	21	19	scavenger receptor class B, member 1	Plasma Membrane	transmembrane receptor		-	-	-	-	-	-	+	-
CASC1	21	21	cancer susceptibility candidate 1	Cytoplasm	other		-	+	-	-	-	-	-	-
RAPGEF3	19	13	Rap guanine nucleotide exchange factor	Nucleus	other		-	-	-	-	-	-	+	-
ATF1	19	17	activating transcription factor 1	Nucleus	transcription regul.		-	-	-	+	-	-	-	-
EGR1	19	17	early growth response 1	Nucleus	transcription regulat.		-	-	+	-	-	-	-	-
PHLDA1	19	17	pleckstrin homology-like domain, family	Cytoplasm	other		-	-	-	-	-	-	-	-
IFNG	19	19	interferon, gamma	Extracellular Space	cytokine		-	+	-	-	+	-	-	-
HSPB8	19	21	heat shock 22kDa protein 8	Cytoplasm	kinase		-	-	-	+	-	-	-	-
PXN	19	21	paxillin	Cytoplasm	other		-	-	-	-	-	-	+	-
CASP9	19	23	caspace 9, apoptosis-related cysteine pe	Cytoplasm	peptidase		-	-	-	+	-	-	-	-
DIABLO	19	23	diablo, IAP-binding mitochondrial protei	Cytoplasm	other		-	-	-	+	-	-	-	-
PEBP1	19	23	phosphatidylethanolamine binding prote	Cytoplasm	other		-	-	-	+	-	-	-	-
PEBP1	19	23	phosphatidylethanolamine binding prote	Cytoplasm	other		-	-	-	+	-	-	-	-
RAP1GAP	19	23	RAP1 GTPase activating protein	Cytoplasm	other		-	-	-	-	-	-	+	-
CDKN1B	19	25	cyclin-dependent kinase inhibitor 1B (p2	Nucleus	other		-	-	-	+	-	-	+	-
MX1	17	17	myxovirus (influenza virus) resistance 1,	Nucleus	enzyme		-	-	-	-	-	-	-	+
STAT1	17	17	signal transducer and activator of trans	Nucleus	transcription regulator		-	+	-	-	-	-	-	+
ITGA5	17	19	integrin, alpha 5 (fibronectin receptor, al	Plasma Membrane	other		-	-	-	-	-	-	+	-
COL4A3	17	21	collagen, type IV, alpha 3 (Goodpasture	Extracellular Space	other	collagenas	-	+	-	-	-	-	-	-
GSTM1	17	21	glutathione S-transferase mu 1	Cytoplasm	enzyme		-	+	-	+	-	-	-	-
PMEL	17	21	premelanosome protein	Plasma Membrane	enzyme		-	+	-	-	-	-	-	-
BCL2L10	17	23	BCL2-like 10 (apoptosis facilitator)	Cytoplasm	other		-	-	-	-	-	-	-	-
PAX3	17	23	paired box 3	Nucleus	transcription regulator		-	-	-	-	-	-	-	-
B4GALNT1	17	27	beta-1,4-N-acetyl-galactosaminyl transfe	Cytoplasm	enzyme		-	-	-	-	+	-	-	-
DDIT3	17	27	DNA-damage-inducible transcript 3	Nucleus	transcription regulator		-	-	-	-	-	-	-	-
CTBP1	15	13	C-terminal binding protein 1	Nucleus	enzyme		-	-	-	-	-	-	+	-
CASP8	15	15	caspace 8, apoptosis-related cysteine pe	Nucleus	peptidase		-	-	-	+	-	-	-	-
CFLAR	15	15	CASP8 and FADD-like apoptosis regulator	Cytoplasm	other		-	-	-	+	-	-	-	-
FN1	15	15	fibronectin 1	Plasma Membrane	enzyme		-	-	-	-	+	-	+	+
ITGA4	15	17	integrin, alpha 4 (antigen CD49D, alpha 4	Plasma Membrane	other	natalizuma	-	-	-	-	-	-	-	-
PLCL1	15	17	phospholipase C-like 1	Cytoplasm	enzyme		-	-	-	-	-	-	+	-
ATF2	15	19	activating transcription factor 2	Nucleus	transcription regulator		-	+	+	+	-	-	-	+
ITGA6	15	19	integrin, alpha 6	Plasma Membrane	other		-	-	-	-	-	+	-	-
ITGA7	15	19	integrin, alpha 7 (vitronectin receptor, al	Plasma Membrane	other	abciximab	+	-	+	+	-	-	+	+
COL18A1	15	21	collagen, type XVIII, alpha 1	Extracellular Space	other	collagenas	-	-	-	-	-	-	-	+
SHC4	15	23	SHC (Src homology 2 domain containin	Cytoplasm	other		-	-	-	-	-	-	+	-
LTBR	15	31	lymphotoxin beta receptor (TNFR superf	Plasma Membrane	transmembrane receptor		-	-	-	-	+	-	-	-
PPARG	15	31	peroxisome proliferator-activated recept	Nucleus	ligand-dependent nuclear receptor	pioglitazo	-	+	-	-	-	-	-	-
RAF1	15	31	v-raf-1 murine leukemia viral oncogene b	Cytoplasm	kinase	sorafenib	-	+	-	-	-	-	-	-
VWF	15	31	von Willebrand factor	Extracellular Space	other		-	-	-	-	-	+	-	-
WNT5A	15	35	wingless-type MMTV integration site fam	Extracellular Space	cytokine		-	+	-	-	-	-	+	-
DPP4	13	19	dipeptidyl-peptidase 4	Plasma Membrane	peptidase	saxagliptin	-	-	+	-	-	-	-	-
THBS1	13	23	thrombospondin 1	Extracellular Space	other		-	-	-	-	-	+	-	-
ADAM10	13	27	ADAM metalloproteinase domain 10	Plasma Membrane	peptidase		-	-	-	-	-	-	+	-
FLNB	13	33	filamin B, beta	Cytoplasm	other		-	-	-	+	-	-	-	-
SKI	13	33	v-ski sarcoma viral oncogene homolog (a	Nucleus	transcription regulator		-	+	-	-	-	-	+	-
LTF	13	35	lactotransferrin	Extracellular Space	peptidase		-	-	-	-	-	+	-	-
ITPR1	13	38	inositol 1,4,5-trisphosphate receptor, typ	Cytoplasm	ion channel		-	-	-	-	-	-	+	-
RPSA	13	38	ribosomal protein S6A	Cytoplasm	translation regulator		-	-	-	-	+	-	+	-
GAS6	13	42	growth arrest-specific 6	Extracellular Space	growth factor		-	+	+	-	-	-	-	-
ITGA9	13	42	integrin, alpha 9	Plasma Membrane	other		-	-	-	-	-	+	-	-
VTN	10	10	vitronectin	Extracellular Space	other		-	-	-	-	-	-	+	-
CCR7	10	13	chemokine (C-C motif) receptor 7	Plasma Membrane	G-protein coupled receptor		-	-	-	-	-	-	+	-

Previews Only

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HUGO Gene Symbol	% of samples with deletions	% samples with amplifications	Entrez Gene Name	Location	Type(s)	Drug(s)	Tumorigenesis	Proliferation, Cell Cycle	Survival	Cell death, Apoptosis	Angiogenesis	Adhesion, Binding, Attachment, Detachment	Movement, Migration, Invasion	Metastasis
ADAM15	4	54	ADAM metalloproteinase domain 15	Plasma Membrane	peptidase		-	-	-	-	-	+	-	-
DDR2	4	54	discoidin domain receptor tyrosine kinase	Plasma Membrane	kinase		-	+	-	-	-	-	-	-
EFNA1	4	54	ephrin-A1	Plasma Membrane	other		-	-	-	-	+	-	-	-
CD36	4	56	CD36 molecule (thrombospondin receptor)	Plasma Membrane	transmembrane receptor		-	-	-	-	-	-	+	-
CCND3	4	58	cyclin D3	Nucleus	other		-	+	-	-	-	-	-	-
PRKCA	2	44	protein kinase C, alpha	Cytoplasm	kinase	L-threo-sar	-	-	-	-	-	-	+	-
SPHK1	2	44	sphingosine kinase 1	Cytoplasm	kinase		-	-	-	+	-	-	+	-
HGF	2	67	hepatocyte growth factor (hepapoietin A)	Extracellular Space	growth factor		-	-	-	+	-	-	-	-
HGF	2	67	hepatocyte growth factor (hepapoietin A)	Extracellular Space	growth factor		-	-	-	-	-	-	+	-
NRCAM	2	73	neuronal cell adhesion molecule	Plasma Membrane	other		-	-	-	-	-	-	+	-
EPO	2	77	erythropoietin	Extracellular Space	cytokine		-	-	-	-	-	-	+	-
LEP	2	77	leptin	Extracellular Space	growth factor		-	+	-	-	-	-	-	-
SERPINE1	2	77	serpin peptidase inhibitor, clade E (nexin)	Extracellular Space	other	drotrecogin	-	-	-	-	+	-	-	-
BRAF	2	85	v-raf murine sarcoma viral oncogene homolog B1	Cytoplasm	enzyme	sorafenib	-	+	+	-	-	-	+	-
NOS3	2	85	nitric oxide synthase 3 (endothelial cell)	Cytoplasm	enzyme	GW 27362	+	-	-	-	-	-	-	+
PKMYT1	0	42	protein kinase, membrane associated tyrosine kinase-like motif domain 1	Cytoplasm	kinase		-	-	-	+	-	-	-	-
BIRC5	0	44	baculoviral IAP repeat containing 5	Cytoplasm	other		+	-	-	-	-	-	-	-
TIMP2	0	46	TIMP metalloproteinase inhibitor 2	Extracellular Space	other		-	-	-	-	-	-	+	-
FASN	0	48	fatty acid synthase	Cytoplasm	enzyme		-	+	-	-	-	-	-	-
HGS	0	48	hepatocyte growth factor-regulated tyrosine kinase	Cytoplasm	other		-	+	-	-	-	-	-	-
P4HB	0	50	prolyl 4-hydroxylase, beta polypeptide	Cytoplasm	enzyme		-	-	-	+	-	-	-	-
SRC	0	75	v-src sarcoma (Schmidt-Rupprecht) viral oncogene homolog 2	Cytoplasm	kinase	dasatinib	-	-	-	+	-	-	-	-
CD40	0	77	CD40 molecule, TNF receptor superfamily member 5	Plasma Membrane	transmembrane receptor	SGN-40 (ar	-	+	-	-	-	-	-	-
MMP9	0	77	matrix metalloproteinase 9 (matrilysin)	Extracellular Space	peptidase		-	-	-	-	-	-	+	-
CSE1L	0	79	CSE1 chromosome segregation 1-like protein	Cytoplasm	transporter		+	-	-	-	-	-	-	+
BIRC7	0	88	baculoviral IAP repeat containing 7	Cytoplasm	other		-	-	-	+	-	-	-	-
LAMA5	0	88	laminin, alpha 5	Extracellular Space	other		+	-	-	+	+	-	+	+

For Review Only

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Table with columns: Chromosome, cytoband, Start, Stop, Nominal p-value, FDR, length (bps), Total Amplifications, Amplification Average Copy Number, Samples with Amplification, Total Deletions, Deletion Average Copy Number, Samples with Deletion, Total Aberrations, Total Unchanged

HUGO Gene Symbol	Nominal p-value	FDR	Patient specific in % of comparisons	Gene description	RefSeq	Affymetrix TC ID	GO biological process
CDH3	2.84E-10	8.20E-06	71	NM_001793 // CDH3 // cadherin 3, type 1, P-cadherin (placental) // 16q22.1 // 10	NM_001793	7996819	NM_001793 // GO:007155 // cell adhesion // traceable author statement // NM_0
KIT	1.29E-08	4.65E-05	75	NM_000222 // KIT // v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolo	NM_000222	8095110	NM_000222 // GO:002318 // myeloid progenitor cell differentiation // inferred f
CYGB	5.29E-07	6.88E-04	71	NM_134268 // CYGB // cytoglobin // 17q25.3 // 114757 // ENST00000293230 // CYGB	NM_134268	8018754	NM_134268 // GO:0006810 // transport // inferred from electronic annotation //
NNMT	1.10E-06	1.01E-03	75	NM_006169 // NNMT // nicotinamide N-methyltransferase // 11q23.1 // 4837 // ENS	NM_006169	7943998	---
NAA11	1.21E-06	1.01E-03	82	NM_032693 // NAA11 // N(alpha)-acetyltransferase 11, NaA catalytic subunit // 4	NM_032693	8101253	NM_032693 // GO:0008152 // metabolic process // inferred from electronic annotat
BAGE2	2.49E-06	1.44E-03	75	NM_182482 // BAGE2 // B melanoma antigen family, member 2 // 21p // 85319 // NM	NM_182482	8069487	---
TPTE	3.42E-06	1.73E-03	75	NM_199261 // TPTE // transmembrane phosphatase with tensin homology // 21p11 //	NM_199261	8069472	NM_199261 // GO:0006470 // protein amino acid dephosphorylation // inferred from
	1.22E-05	3.58E-03	71	---	---	8045525	---
GNAL	1.46E-05	3.97E-03	71	NM_182978 // GNAL // guanine nucleotide binding protein (G protein), alpha activ	NM_182978	8020164	NM_182978 // GO:0007165 // signal transduction // inferred from electronic annot
TUBA8	1.93E-05	4.56E-03	79	NM_018943 // TUBA8 // tubulin, alpha 8 // 22q11.1 // 51807 // ENST00000330423 /	NM_018943	8071147	NM_018943 // GO:0007018 // microtubule-based movement // inferred from electroni
MAGEC2	2.12E-05	4.75E-03	75	NM_016249 // MAGEC2 // melanoma antigen family C, 2 // Xq27 // 51438 // ENST000	NM_016249	8175562	---
TF	2.18E-05	4.80E-03	79	NM_001063 // TF // transferrin // 3q22.1 // 7018 // ENST00000264998 // TF // tr	NM_001063	8082797	NM_001063 // GO:0006811 // ion transport // inferred from electronic annotation
RNASE1	2.34E-05	5.04E-03	71	NM_198232 // RNASE1 // ribonuclease, RNase A family, 1 (pancreatic) // 14q11.2 /	NM_198232	7977615	---
HORMAD1	2.58E-05	5.11E-03	82	NM_032132 // HORMAD1 // HORMA domain containing 1 // 1q21.3 // 84072 // ENST000	NM_032132	7919787	NM_032132 // GO:0007067 // mitosis // inferred from electronic annotation // N
CFI	2.82E-05	5.11E-03	79	NM_000204 // CFI // complement factor 1 // 4q25 // 3426 // ENST00000394634 // C	NM_000204	8102328	NM_000204 // GO:0006508 // proteolysis // inferred from electronic annotation /
MAGEB2	3.47E-05	5.59E-03	71	NM_007364 // MAGEB2 // melanoma antigen family B, 2 // Xp21.3 // 4113 // ENST00	NM_007364	8166611	---
MAGEA4	3.60E-05	5.69E-03	75	NM_00101548 // MAGEA4 // melanoma antigen family A, 4 // Xq28 // 4103 // NM_00	NM_00101548	8170531	NM_00101548 // GO:0008150 // biological process // no biological data available
RGS5	5.74E-05	7.47E-03	71	NM_003617 // RGS5 // regulator of G-protein signaling 5 // 1q23.1 // 8490 // EN	NM_003617	7921916	NM_003617 // GO:0008277 // regulation of G-protein coupled receptor protein sign
ITGA4	1.11E-04	1.06E-02	75	NM_000885 // ITGA4 // integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4	NM_000885	8046695	NM_000885 // GO:0001974 // blood vessel remodeling // inferred from electronic a
TMSF1	1.13E-04	1.07E-02	75	NM_014220 // TMSF1 // transmembrane 4 L six family member 1 // 3q21-q25 // 4071	NM_014220	8091411	NM_014220 // GO:0008150 // biological process // no biological data available /
TD02	1.24E-04	1.13E-02	75	NM_005651 // TD02 // tryptophan 2,3-dioxygenase // 4q31-q32 // 6999 // ENST00000	NM_005651	8097991	NM_005651 // GO:0019441 // tryptophan catabolic process to kynurenine // inferre
SERPINA3	1.45E-04	1.23E-02	82	NM_001085 // SERPINA3 // serpin peptidase inhibitor, clade A (alpha-1 antiprotei	NM_001085	7976496	NM_001085 // GO:0006953 // acute-phase response // inferred from electronic anno
ARHGEF6	1.88E-04	1.46E-02	71	NM_004840 // ARHGEF6 // Ras guanine nucleotide exchange factor (GEF) 6 //	NM_004840	8175393	NM_004840 // GO:0006915 // apoptosis // not recorded // NM_004840 // GO:000691
DKK3	2.64E-04	1.81E-02	71	NM_015881 // DKK3 // Dickkopf homolog 3 (Xenopus laevis) // 11p15.2 // 27122 //	NM_015881	7946661	NM_015881 // GO:0007275 // multicellular organismal development // inferred from
APCDD1	2.82E-04	1.85E-02	75	NM_153000 // APCDD1 // adenomatous polyposis coli down-regulated 1 // 18p11.22	NM_153000	8020141	---
LOC100133469	3.14E-04	1.98E-02	71	NR_027457 // LOC100133469 // hypothetical protein // 100133469	NR_027457	7977447	---
DSCR8	3.63E-04	2.16E-02	75	NR_026839 // DSCR8 // Down syndrome critical region gene 8 // 21q22.2 // 84677 /	NR_026839	8068570	NR_026839 // GO:0008150 // biological process // no biological data available /
SLAMF7	4.81E-04	2.55E-02	75	NM_021181 // SLAMF7 // SLAM family member 7 // 1q24.1 // 57823 // ENST000	NM_021181	7906613	NM_021181 // GO:007155 // cell adhesion // non-traceable author statement //
VAMP8	8.46E-04	3.49E-02	75	NM_003761 // VAMP8 // vesicle-associated membrane protein 8 (endobrevin) // 2p12	NM_003761	8043197	NM_003761 // GO:0006461 // protein complex assembly // inferred from electronic
MGP	8.57E-04	3.51E-02	86	NM_000900 // MGP // matrix Gla protein // 12p1.1 // 123 // 56 // ENST000000228	NM_000900	7961514	NM_000900 // GO:0001502 // cartilage condensation // traceable author statement
CD200	8.74E-04	3.55E-02	71	NM_001004196 // CD200 // CD200 molecule // 3q12-q13 // 15 // NM_001004196 // CD2	NM_001004196	8081657	---
CD302	8.76E-04	3.55E-02	75	NM_014880 // CD302 // CD302 molecule // 2q24.2 // 9936 // ENST00000259053 // CD	NM_014880	8056102	---
FLRT3	1.10E-03	4.05E-02	75	NM_198391 // FLRT3 // fibronectin leucine rich transmembrane protein 3 // 7 //	NM_198391	8065071	NM_198391 // GO:0007155 // cell adhesion // inferred from electronic annotation
C1S	1.17E-03	4.16E-02	75	NM_201442 // C1S // complement component 1, s subcomponent // 12p12 // 16 // N	NM_201442	7953603	NM_201442 // GO:0006508 // proteolysis // inferred from electronic annotation /
MME	1.26E-03	4.33E-02	79	NM_007288 // MME // membrane metallo-endopeptidase // 3q25.1-q25.2 // 431 //	NM_007288	8083494	NM_007288 // GO:0006508 // proteolysis // inferred from electronic annotation /
Cyorf15B	1.26E-03	4.33E-02	71	NM_032576 // Cyorf15B // chromosome Y open reading frame 15B // Yq11.222 // 8466	NM_032576	8176709	---
SLITRK6	1.40E-03	4.63E-02	75	NM_032229 // SLITRK6 // SLIT and NTRK-like family, member 6 // 13q31.1 // 84189	NM_032229	7972239	NM_032229 // GO:0007409 // axonogenesis // inferred from electronic annotation

