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Impact of Copy Neutral Loss of Heterozygosity and Total Genome Aberrations on Survival in Myelodysplastic Syndrome

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Introduction

Myelodysplastic syndromes are a group of clonal hematopoietic stem cell disorders characterized by cytopenias, morphologic dysplasia, and an increased risk of acute myeloid leukemia(1). The incidence of myelodysplastic syndrome is, on average, 3–5/100,000 with increasing rates in older patients. Current standards and guidelines for myelodysplastic syndrome are primarily those referenced in the 2008 Edition of the WHO Classification of Haematopoietic and Lymphoid Tissue Textbook with updates published in 2016(2–4). Despite recent advances in diagnostic modalities, myelodysplastic syndrome continues to show variability in its clinical course for prognosis and response to treatment, indicating the need for further sub-classification(5–7). Difficulties in establishing dysplastic features especially in the early stages, accurate counting of blast percentages, as well as the lack of

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AUTHOR CONTRIBUTIONS STATEMENT:

Drs. Yeung and Fang are responsible for the overall content, study design, planning, funding, and coordination of this research and manuscript.

Mr. McElhone, with Drs. Yeung and Fang performed the chromosome genomic array testing analysis and cytogenetics data analysis.

Drs. Ng, and Chen performed the flow cytometry analysis.

Dr. Yeung performed the morphologic assessment.

Dr. Deeg performed the clinical reviews and provided senior oversight of the research project.

Dr. Storer performed all of the statistical data analysis.

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uniformity in cytogenetic and molecular analysis across institution are some of the reasons for variability(7).

Currently, the only curative treatment for myelodysplastic syndrome is allogeneic hematopoietic cell transplantation(8). The use of the International Prognostic Scoring System, now in a revised version, has aided physicians in identifying patients who may or may not benefit from hematopoietic cell transplantation(9). The new Revised International Prognostic Scoring System takes into consideration marrow blast percentage, peripheral blood counts and cytogenetic findings, of which the latter has the most profound impact on prognosis. However, 40–50% of myelodysplastic syndrome patients have a normal karyotype(10), therefore obscuring the prognostic value by the Revised International Prognostic Scoring System and indicating a need for new markers for improved diagnostics and prognosis.

Copy neutral loss of heterozygosity describes a phenomenon whereby one of two homologous chromosomal regions is lost but various mechanisms have ensured the presence of two identical copies of such region in the genome. As a result, the karyotype appears normal or “copy neutral”. Copy neutral loss of heterozygosity and microdeletions in myelodysplastic syndrome have been well-described in the 2008 and 2016 WHO Editions of myelodysplastic syndrome classification(11, 12). Single nucleotide polymorphism arrays are consistent and reliable in finding regions of copy neutral loss of heterozygosity and for review of the whole genome for copy numbers. Copy number aberrations can be detected more accurately by array testing than by routine karyotyping(13) and FISH. Total genomic aberration numbers, including both acquired copy neutral loss of heterozygosity and copy number aberrations, can be calculated from single nucleotide polymorphism array data. Potential mechanisms of copy neutral loss of heterozygosity include mitotic recombination, gene conversion, or trisomy rescue in somatic cells(11, 14, 15) serving as the second-hit in the Kanudson 2-hit tumorigenesis hypothesis. Copy neutral loss of heterozygosity can lead to duplication of an activating mutation in an oncogene, deletion or loss of function of a tumor suppressor gene, and duplication or deletion of a methylation allele that regulates gene expression(15). The presence of copy neutral loss of heterozygosity in the genome of myelodysplastic syndrome cells appear to portend a worse prognosis for the patient(16, 17). However, additional studies to evaluate the significance of copy neutral loss of heterozygosity in relation to morphologic features and the clinical course are needed.

Our goal in this study was to evaluate the significance of copy number aberrations and copy neutral loss of heterozygosity in myelodysplastic syndrome by correlating our findings with clinical characteristics, immunophenotypes, morphologic abnormalities, and outcome data. We hypothesize that chromosome genomic array testing studies will show that total genomic aberration numbers positively correlate with dysplastic features/lineage involvement and impacts survival.

Materials and Methods

Patients and biologic materials

Patients who were diagnosed with myelodysplastic syndrome (WHO 2008) and myelodysplastic syndrome/myeloproliferative neoplasms unclassifiable at the University of Washington Medical Center and/or Seattle Cancer Care Alliance and underwent chromosome genomic array testing were included in this study. All patients signed informed consents and the Fred Hutchinson Cancer Research Center Institutional Review Board approved the study. Pertinent clinical information was reviewed which included: presentation of disease, associated co-morbidities, select laboratory data, transplant parameters, relapse and survival.

Hematopathology review, flow cytometry, molecular diagnostic data

Pathology slides were reviewed by a hematopathologist and morphologic dysplastic features categorized according to those listed in the 2008 Edition of the WHO Classification of Haematopoietic and Lymphoid Tissue Textbook(18). Clinical chart reviews were performed by a clinical oncologist with expertise in myelodysplastic syndrome and, when data was available, Revised International Prognostic Scoring System scores were calculated. Relapse and survival data were retrospectively captured in May 2016.

Ten-color multiparameter flow cytometry was performed on bone marrow aspirates obtained as routine baseline assessment; details have been described previously(19, 20). Data were collected from samples with copy neutral loss of heterozygosity, and specific blast immunophenotype and other pertinent findings were recorded.

Molecular diagnostics

Molecular diagnostic testing with PCR-based single gene assays were performed as part of routine clinical management in a subset of these patients. The following genes were included: *JAK2, BCR/ABL1, FLT3, NPM1, CEBPA*.

Targeted gene panel next generation sequencing data were available in the form of Oncoplex reports (University of Washington, Genomics and Molecular Pathology) as part of the diagnostic workup. Specific methodology has been previously published(21). Specific myelodysplastic syndrome-related genes included in this 194-gene panel were: *TP53, RUNX1, ETV6, TET2, DNMT3A, ASXL1, EZH2, IDH1/2, SF3B1, SRSF2, U2AF1, ZRSR2, NRAS, CBL, JAK2, SETBP1 (For full gene list please see*: http://tests.labmed.washington.edu/UW-OncoPlex).

Conventional cytogenetics and fluorescence in situ hybridization (FISH)

Bone marrow aspirate samples from all patients were tested for cytogenetic abnormalities using standard culturing and G-banding technique at the Seattle Cancer Care Alliance. Karyotype designation was based on the International System for Human Cytogenetic Nomenclature(22). FISH was performed at the Seattle Cancer Care Alliance according to standard procedures. FISH probes were purchased from Abbott Molecular (Abbott Park, IL) and Cytocell-Revisedainbow Scientific (Windsor, CT).

Chromosomal genomic array testing

DNA was extracted from fresh bone marrow aspirates and from frozen marrow samples using Qiagen Puregene (Germantown, MD) according to the manufacturer's protocol. Genomic DNA microarray CytoScan HD, with probes for both copy number and single nucleotide polymorphisms, was purchased from Affymetrix (Santa Clara, CA). The criteria used to identify an aberration were: a minimum of 100 Kb and 25 probes for copy number aberrations and 10 Mb for terminal copy neutral loss of heterozygosity (13 Mb for interstitial copy neutral loss of heterozygosity). Total genomic aberrations were calculated based on total length of DNA in Mb of somatic aberration (copy neutral loss of heterozygosity and copy number aberrations).

Statistical analyses

Based on chromosome genomic array testing results, our study population was separated into three groups for statistical comparison: 1. patients with copy neutral loss of heterozygosity (with or without additional chromosome genomic array testing abnormalities), 2. patients with abnormal chromosome genomic array testing but no copy neutral loss of heterozygosity, and 3. patients who had a normal chromosome genomic array testing study.

In addition to chromosome genomic array testing results, other characteristics that were examined included: morphology, immunophenotype, mutation results, FISH, conventional cytogenetic data, and outcome parameters such as relapse and survival. Comparisons among chromosome genomic array testing groups were performed using the Kruskal-Wallis test. Linear regression analysis was used to assess trends in total genomic aberrations as a function of number of dysplastic lineages. Overall survival was estimated using the Kaplan-Meier method. Relapse was estimated using cumulative incidence estimates, with non-relapse mortality as a competing risk. Cox regression analysis was used to assess univariate prognostic factors for relapse and survival. Follow-up time as of May 25th, 2016 ranged from 790 to 2715 days.

Five patients had no follow-up after sample collection and are not included in the survival analyses. Five additional patients were known to have died without a precise date of death. Two of these were assigned death dates using the date of the clinic note stating that the patient was deceased; the other three were assigned death dates of 9 months after sample collection, which was the median time to death among patients with known dates.

Results

Population characteristics

We tested bone marrow samples from 68 patients with myelodysplastic syndrome and indeterminate myelodysplastic syndrome/myeloproliferative neoplasms, evaluated from November 2008 through March 2014 by chromosome genomic array testing. Clinical characteristics are summarized in Table 1. Clinical data were incomplete in 12 of 68 patients. For 58 patients, we could calculate the Revised International Prognostic Scoring System scores, confirming 37 patients were high risk and 21 patients with low risk disease.

Transplant data included in Table 1 reflects the patient characteristic at the time of sample collection. During follow-up, 11 patients relapsed and 27 patients died.

Chromosome genomic array testing data compared to cytogenetics by karyotype and FISH

Of 68 total patients, 38 (56%) had abnormal cytogenetics (by karyotype and FISH), 26 (38%) had normal cytogenetics (by karyotype and FISH), and in 4 patients (5.8%) karyotyping was unsuccessful. Table 2 summarizes the chromosome genomic array testing results in comparison to cytogenetic data. By chromosome genomic array testing, 50 of 68 patients showed an abnormal result (73%). Among these, 22 (32%) showed copy neutral loss of heterozygosity whereas 28 (41%) showed no copy neutral loss of heterozygosity but did have abnormal copy numbers (copy number aberrations). Eighteen patients (28%) had normal chromosome genomic array testing results.

Of the 26 samples with normal cytogenetics, 12 (46%) showed clonal abnormalities only detected by chromosome genomic array testing. Of the 38 samples with abnormal cytogenetics, chromosome genomic array testing provided additional information for copy neutral loss of heterozygosity abnormalities in 13 samples (33%) and small submicroscopic copy number aberrations in 10 samples (26%). In the 4 samples which failed to grow in culture, 2 (50%) showed isolated copy neutral loss of heterozygosity.

The most common regions of copy number aberrations were very similar to those described in myelodysplastic syndrome including del 5q, monosomy 7, trisomy 8, and del 20q (Fig 1A). Gain of 1q was also prevalent, as seen in 5 patients. The most frequently noted copy neutral losses of heterozygosity, in descending order, were copy neutral loss of heterozygosity of 9p (n=8), 11q and 17p (n=3 each), 4q, 11p, and 17q (n=2 each). Single cases of 5q, 7q and 14q copy neutral loss of heterozygosity were also observed. Furthermore, 8 samples with 9p copy neutral loss of heterozygosity showed myelofibrotic changes, and 5 showed unilineage dysplasia. Diagnoses included myelodysplastic syndrome with marrow fibrosis (n= 4), chronic myelomonocytic leukemia with marrow fibrosis (n= 1), and myelodysplastic syndrome secondary to polycythemia vera with cytogenetic clonal evolution (n= 3). All samples with 17p copy neutral loss of heterozygosity occurred in the context of 5q deletions (in addition to other chromosomal aberrations) with multilineage dysplasia. Five 9p copy neutral loss of heterozygosity cases had *JAK2* V617F mutations (Figure 1B), 2 were negative for *JAK2* mutation by Oncoplex NGS, and one could not be tested.

Chromosome genomic array testing and blast percentage (morphology and flow cytometry)

Morphology of the erythroid, myeloid and megakaryocyte lineages was evaluated, and dysplasia was called out when more than 10% of the lineage cells met the 2008 WHO criteria(18). Table 3 shows a significant correlation between total genomic aberrations of abnormal chromosome genomic array testing results and dysplastic morphology (P_{trend}=0.05 for abnormal chromosome genomic array testing samples with copy neutral loss of heterozygosity, and P_{trend}= 0.003 for all abnormal chromosome genomic array testing samples). In a separate analysis (data not shown), we noted that patients with abnormal

chromosome genomic array testing had higher blast percentages by flow cytometry, but this association did not reach statistical significance ($P = 0.07$). No significant association existed between abnormal immunophenotypic markers, including CD4, CD5, CD7, CD13, CD15, CD33, CD34, CD38, CD45, CD56, CD117, CD123, and HLA-DR, and chromosome genomic array testing results.

Clinical follow-up information to perform a survival analysis was available in 63 patients. When dividing this group of patients into three categories - those with normal chromosome genomic array testing, versus patients with abnormal chromosome genomic array testing with or without copy neutral loss of heterozygosity - the latter two categories had worse overall survival ($P = 0.04$) (Fig 2). The median overall survival for patients whose myelodysplastic syndrome features only copy neutral loss of heterozygosity was 24.9 months, when compared to patients whose myelodysplastic syndrome were abnormal by chromosome genomic array testing but did not show copy neutral loss of heterozygosity, the latter group of patients has a longer overall survival of 35.8 months. For patients whose chromosome genomic array testing were interpreted as 'normal' the median overall survival was not reached. We also assessed whether the *extent* of chromosome genomic array testing abnormalities mattered. Patients with total genomic aberrations above the median of 68.6 Mb (range, 0–592), had inferior overall survival compared to those below the median (mortality hazard ratio= 2.9, 95% CI, 1.3–6.8, $P = 0.01$) (Fig 3).

Because Cluzeau et al. previously reported that total genomic aberrations greater than 100 Mb was associated with worse survival among high-risk myelodysplastic syndrome patients(23), we specifically evaluated the high-risk patients in our cohort. Among 36 patients with Revised International Prognostic Scoring System> 3 with survival data available, 14 had total genomic aberrations < 100, and 22 had total genomic aberrations > 100.

Patients with total genomic aberrations > 100 Mb had worse overall survival than those with total genomic aberrations < 100 Mb (mortality hazard ratio = 3.0, 95% CI, 1.0–9.3, $P=0.05$) (Fig 4). There was no evidence of an impact of total genomic aberrations among the low-risk patients, but the sample size was small (n=21) and,, there were only 3 deaths in this category. The median total genomic aberrations in the low risk patients was 0.7 Mb (range, 0–305.7).

Thirty-seven of the 63 patients (59%) received transplants at a median of 117 days (range, 20–1030 days) after sample collection. The proportion of patients transplanted was similar among the groups defined above and adjustment for transplant as a time-dependent covariate had no material impact on the results.

There was no significant association of total genomic aberrations and relapse but sample size was small - only 11 patients relapsed. One patient had a normal chromosome genomic array testing study, 4 showed copy neutral loss of heterozygosity, and 6 showed an abnormal chromosome genomic array testing study without copy neutral loss of heterozygosity.

Among 9 patients with myelodysplastic syndrome, marrow samples were submitted for concurrent Oncoplex testing. Of these, no mutations were seen in 4 samples; one case had

low tumor burden and next generation sequencing was not performed; and mutations were present in the remaining 4 patients (Table 4). Mutations included the most commonly mutated genes associated with myelodysplastic syndrome such as *SF3B1, SRSF2, ASXL1*, and *TET2*. In one case with 4q copy neutral loss of heterozygosity, there was a *TET2* splicing variant occurring at 96% allelic frequency consistent with a homozygous abnormality due to copy neutral loss of heterozygosity.

Discussion

The first myelodysplastic syndrome case series utilizing array chromosomal genomic hybridization was published in 2006 by Paulsson et al(24). Publications since then have established the improved diagnostic yield of this approach as compared to conventional cytogenetics(25–28). New technology has also been added, including flow cytometry, chromosome genomic array, and next generation sequencing. However, the clinical utility of these modalities, especially in regards to their correlation with classical morphology-based diagnosis of myelodysplastic syndrome and with patient outcome, remains to be characterized. Here, we investigated sequential patients with myelodysplastic syndrome who underwent chromosome genomic array testing and we performed an in-depth retrospective analysis of chromosome genomic array testing correlation with pathologic and clinical characteristics to determine the impact of copy neutral loss of heterozygosity and total genomic aberrations on survival. Utilizing total genomic aberrations as a quantitative measure of cytogenomic abnormality, we compared morphology and immunophenotype to clinical outcomes and demonstrated important clinical utility of array testing in myelodysplastic syndrome.

The association between dysplastic features and increasing total genomic aberrations is a novel observation which suggests that the more morphologic dysplasia a marrow sample displays the more extensive the underlying genomic perturbation reflected by higher total genomic aberrations. Both copy number aberrations and copy neutral loss of heterozygosity contributed significantly to this correlation in the current study, highlighting the utility of the single nucleotide polymorphism-containing array platforms. When comparing morphologic findings with the quantitative measure of total genomic aberrations from chromosome genomic array testing (Table 3) a significant parallel trend was seen. Samples with fewer dysplastic lineage (0 or 1) showed lower total genomic aberrations while samples with more dysplastic lineages (2 or 3) tended to yield higher total genomic aberrations numbers (P_{trend} 0.003). However, when considering samples with copy neutral loss of heterozygosity alone a borderline trend was observed among the dysplastic lineages (P_{trend} 0.05). It may be that this result reflects lower grade myelodysplastic syndromes, such as that used by the 2008 WHO classification system which classifies myelodysplastic syndrome into unilineage versus those with multilineage dysplasia. Although our initial hypothesis of a linear relationship between total genomic aberrations and dysplastic lineage was not confirmed, there was a trend for 0, 1, or 2 (not for 3) dysplastic lineages and increasing total genomic aberrations. Immunophenotype analysis by flow cytometry showed no significant differences when we compared the patients with a normal chromosome genomic array testing study to those with an abnormal chromosome genomic array testing, with and without copy neutral loss of heterozygosity.

Our data suggest that chromosome genomic array testing can be an effective risk stratification tool. When considering the three groups of patients – abnormal chromosome genomic array testing with copy neutral loss of heterozygosity, abnormal chromosome genomic array testing without copy neutral loss of heterozygosity, and normal chromosome genomic array testing - a statistically significant survival difference was seen (see Fig 2). Furthermore, we have shown better survival in myelodysplastic syndrome patients with total genomic aberrations below the median (68.6 Mb) in our cohort (Fig 3). Of the high-risk subset based on Revised International Prognostic Scoring System scores, we demonstrated that patients with total genomic aberrations less than 100 Mb had a survival advantage compared to those with total genomic aberrations greater than 100 Mb (see Fig 4). Cluzeau et al. previously used a total genomic aberration number of 100 Mb to stratify prognostic groups among high-risk myelodysplastic syndrome patients treated with single agent azacitidine given as first line therapy(23). Ganster et al. examined a cohort of very-high-risk myelodysplastic syndrome patients treated with azacitidine and lenalidomide and showed that using a 200 Mb total genomic aberration cutoff would further stratify patients for overall survival(29). Taken together, a higher total genomic aberration number was associated with earlier death, although the total genomic aberrations cutoff differed between studies. Our data suggests there is a survival impact if total genomic aberrations is greater than 100MB and it could be incorporated into the next iteration of the IPPS score. However, prior to that a chromosome genomic array testing study with high total genomic aberrations may warrant prompt notification of the oncologist to allow closer monitoring/surveillance of the patient.

Our study further adds to the body of literature providing evidence that array testing improves diagnostic and prognostic yield in myelodysplastic syndrome. Volkert et al. showed that an additional 11% of myelodysplastic syndrome patients with normal karyotype had copy number aberrations when an array-based test was performed with conventional cytogenetics(26) however, this study did not use a single nucleotide polymorphism-containing array platform and therefore could not detect any copy neutral loss of heterozygosity. Several other studies have also shown that single nucleotide polymorphism arrays enhance the diagnostic yield of myeloid stem cell disorders, from a range of 39–47% by metaphase chromosomal analysis to 54–74% by single nucleotide polymorphism array(25, 30, 31). We demonstrated similar results in this study in that chromosome genomic array testing increased the abnormal detection rate for aberrancies from 57% to 73% when compared with karyotype. Of the subset with normal karyotype, chromosome genomic array testing detected abnormalities in 42% with both submicroscopic copy number aberration and somatic copy neutral loss of heterozygosity. Of the subset with unsuccessful karyotype testing, 100% showed informative chromosome genomic array testing results, underscoring the value of this assay in disease risk assessment.

Notably, in addition to the common abnormalities associated with myelodysplastic syndrome - including 5q deletions, monosomy 7, trisomy 8, and 20q deletions - gain of 1q and copy neutral loss of heterozygosity of several chromosome regions were prominent (Fig 1). In this cohort, copy neutral loss of heterozygosity of 9p was the most prevalent region afflicted by copy neutral loss of heterozygosity, followed closely by 11q, 17p, 4q, 11p, and 17q. Singh et al. reported frequent gain of 1q in patients with fibrosis and an association

with advancing disease(32). Of the 8 patients featuring 9p copy neutral loss of heterozygosity in our cohort, 50% showed myelofibrosis. Most of the copy neutral loss of heterozygosity regions we identified in this study are similar to those previously reported(33–39). These regions of copy neutral loss of heterozygosity are also areas where genes frequently containing myeloid disease associated mutations are located, such as *JAK2, CBL, TET2, EZH2*, and *TP53*.

Correlating specific regions of copy neutral loss of heterozygosity with molecular and morphology data yielded intriguing results. A patient with 4q copy neutral loss of heterozygosity encompassing *TET2* showed a splicing mutation with high allele frequency. One case with 9p copy neutral loss of heterozygosity also showed 11q copy neutral loss of heterozygosity encompassing the *CBL* gene and targeted gene panel next generation sequencing showed a *CBL* splicing mutation. 9p copy neutral loss of heterozygosity was observed more frequently in patients with unilineage dysplasia as compared to 17p copy neutral loss of heterozygosity, which was more common in patients with multilineage dysplasia. This observation suggests that 9p copy neutral loss of heterozygosity might be limited to specific semi-committed hematopoietic cells in myelodysplastic syndrome pathogenesis, whereas 17p copy neutral loss of heterozygosity could occur in more pluripotent hematopoietic stem cells(40, 41). A recent study of myelodysplastic syndrome patients with 17p copy neutral loss of heterozygosity indicated an association with complex karyotype and homozygous *TP53* mutations(42). Unfortunately, we could not sequence the 2 patients with copy neutral loss of heterozygosity of 17p in our cohort. Pairing the findings of copy neutral loss of heterozygosity with next generation sequencing allowed us to understand the role these mutations may play, since mutations seen in the copy neutral loss of heterozygosity regions with a high allelic fraction by next generation sequencing are supported to be homozygous and therefore more likely to play a role in the pathobiology of the disease.

Conclusions

Copy neutral loss of heterozygosity is an informative clonality marker and should be considered when making the diagnosis of myelodysplastic syndrome. Currently, these abnormalities can only be identified by chromosome genomic array testing or other single nucleotide polymorphism-based array methodologies. Chromosome genomic array testing may be helpful in establishing the diagnosis thus improving risk stratification(43) in samples with very low levels of morphologic abnormalities and no immunophenotypic abnormalities by flow cytometry. Our data indicate that the number of dysplastic lineages by morphology correlated with the total size of chromosome genomic array testing abnormalities. Our results have shown a significant survival advantage for myelodysplastic syndrome patients with lower total genomic aberrations, even when the analysis is performed among high-risk patient group. Conversely, an abnormal chromosome genomic array testing result is associated with adverse survival. Therefore, the presence of copy neutral loss of heterozygosity/copy number aberrations and total genomic aberration numbers obtained by chromosome genomic array testing analysis may provide clinically relevant prognostic information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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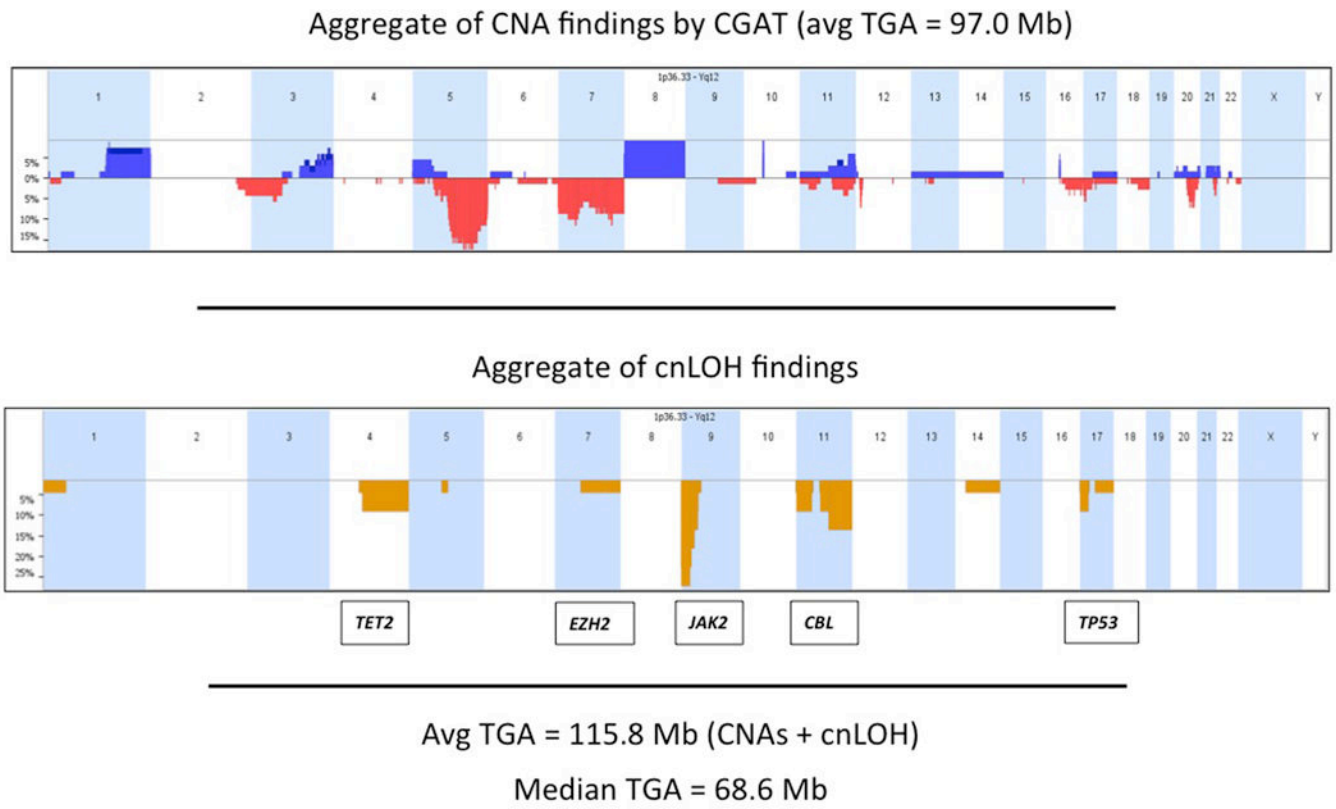

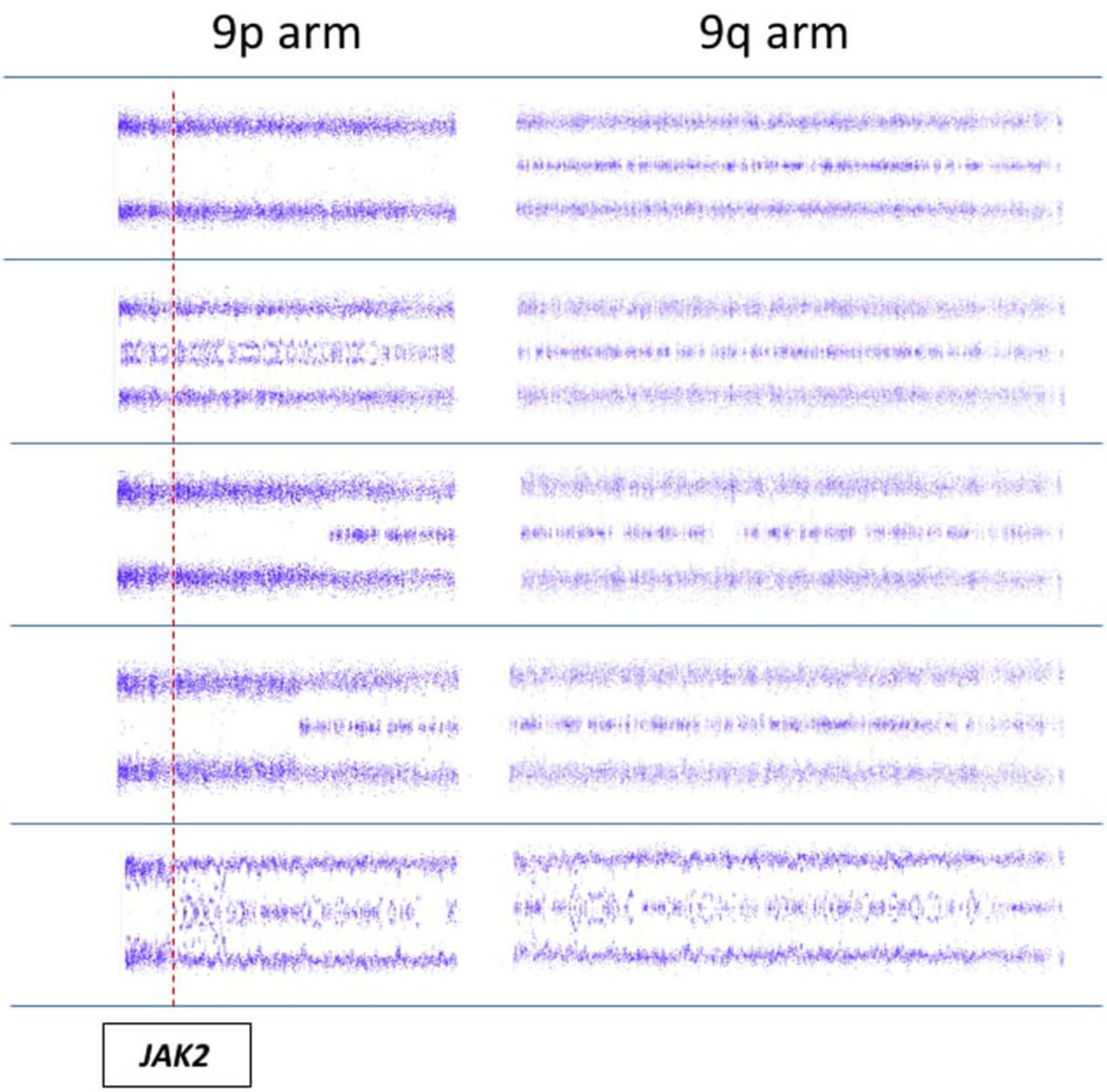

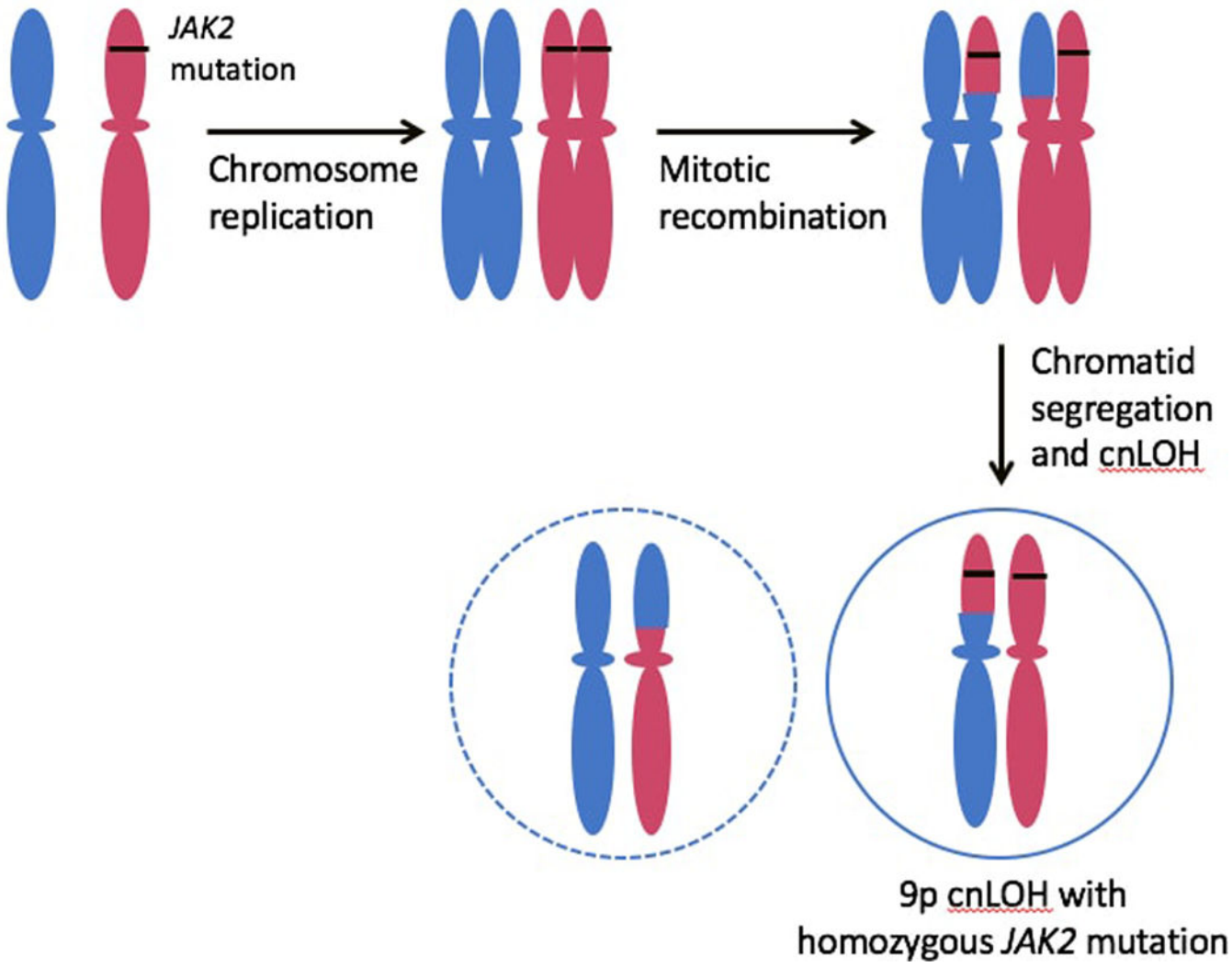

Figure 1. (A) The most common regions of copy number aberration (copy number aberrations) and copy neutral loss of heterozygosity from all patients in this study. (B) Allelic tracks of representative patients with 9p copy neutral loss of heterozygosity. (C) Diagram of one potential mechanism of 9p copy neutral loss of heterozygosity resulting from mitotic recombination in somatic cells of myelodysplastic syndrome/myeloproliferative neoplasms patients with *JAK2* mutation

A) Figure 1a shows a summary composite of the regions of chromosomal aberration in the 68 patients included in our cohort. In the top row are copy number aberrations, with blue representing areas of gains and red representing areas of losses, the bottom row is the single nucleotide polymorphisms track with golden areas representing areas of copy neutral loss of heterozygosity. B) Allelic tracks of patients with 9p copy neutral loss of heterozygosity depicting various size of the copy neutral loss of heterozygosity and the percentage of cells abnormal. The top panel shows copy neutral loss of heterozygosity of the entire short arm of chromosome 9 in 100% of cells. The second panel shows 9p copy neutral loss of heterozygosity in approximately 20% of cells. The bottom panel shows copy neutral loss of heterozygosity of the terminal 9p in 70–80% of cells in a post-transplant patient. The location of the *JAK2* gene is marked by the red dotted line. C) Diagram of one potential mechanism of 9p copy neutral loss of heterozygosity resulting from mitotic recombination

in somatic cells of myelodysplastic syndrome/myeloproliferative neoplasms patients with *JAK2* mutation. The normal chromosome 9 homologue is shown in blue and the *JAK2* mutated chromosome 9 homologue in red. The *JAK2* mutation is depicted by the black bar. Daughter cells with 9p copy neutral loss of heterozygosity and homozygous *JAK2* mutation would have the selective growth advantage in vivo. The difference in the crossover point of the mitotic recombination may lead to differences in the size of the copy neutral loss of heterozygosity seen in different patients, as shown in (B).

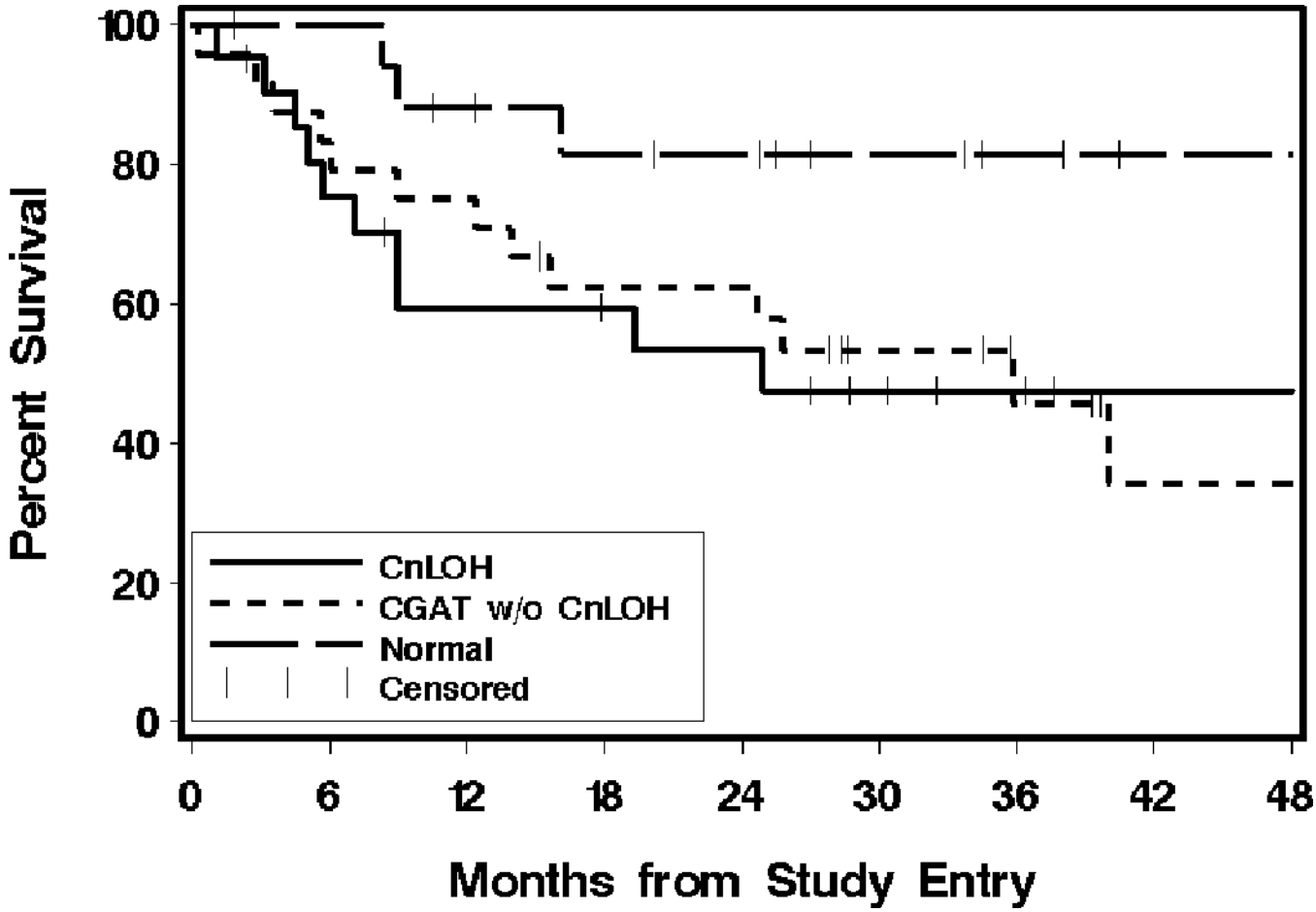

Figure 2.

Kaplan-Meier estimates show a statistically significant survival difference between patients with abnormal copy neutral loss of heterozygosity and patients with normal chromosome genomic array testing (overall P=0.04). The Individual comparisons were P=0.04 with copy neutral loss of heterozygosity, and P=0.05 without copy neutral loss of heterozygosity (using normal as the reference.)

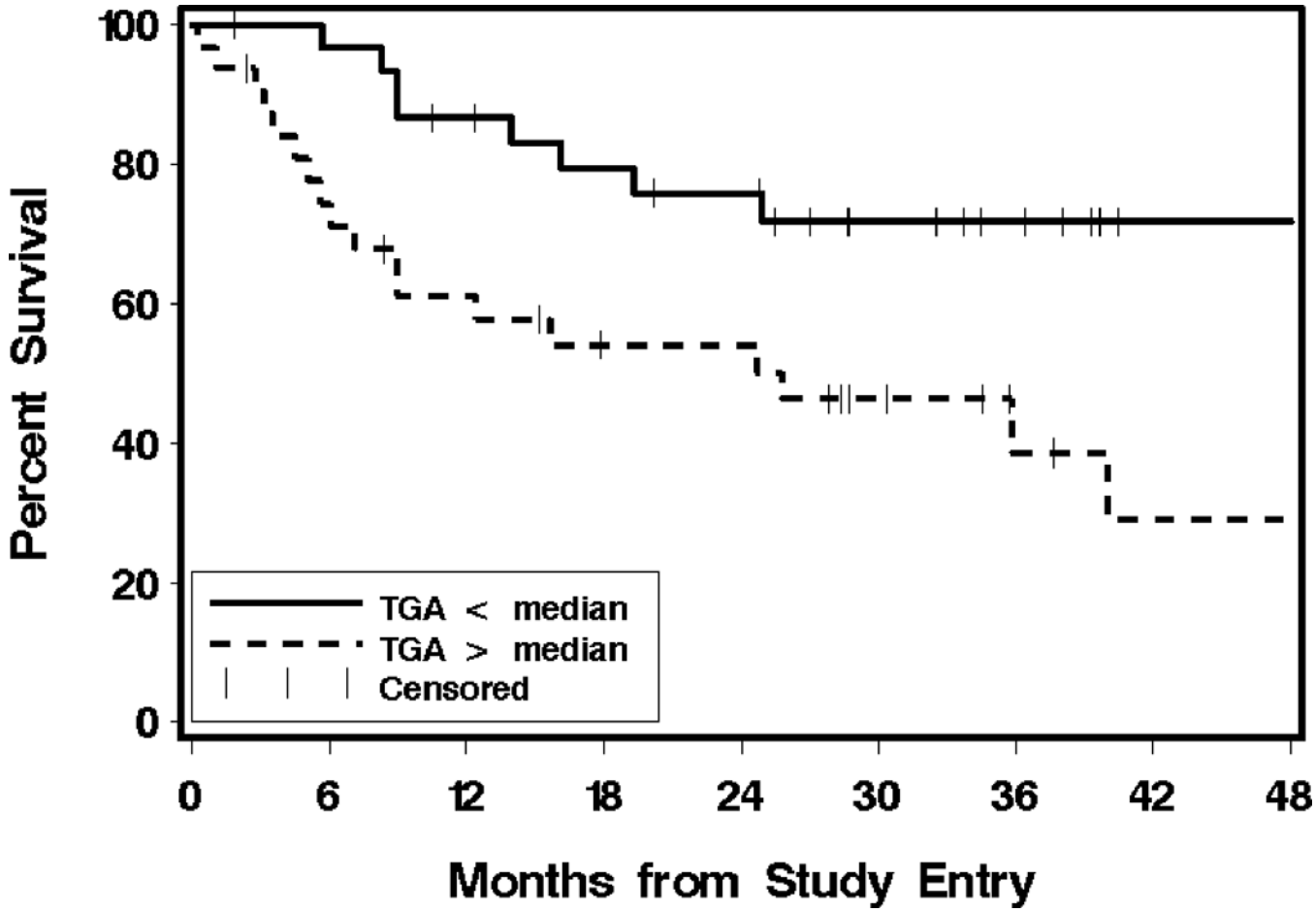

Figure 3.

Kaplan-Meier estimates of all patients showed a significant survival difference between patients with total genomic aberrations values above and below the median (mortality hazard ratio = 2.9, 95% CI, 1.3–6.8, *P*=0.01)

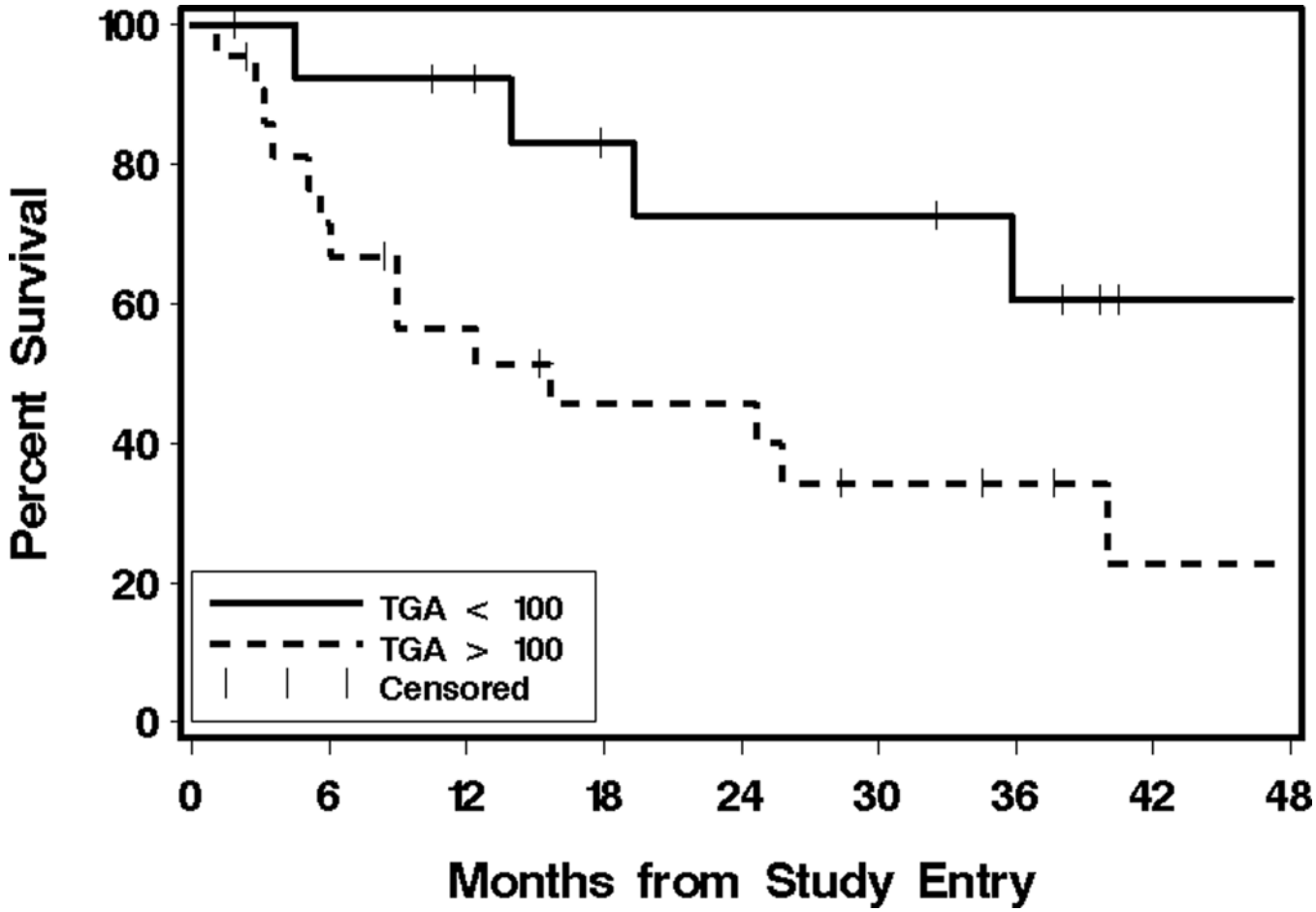

Figure 4.

Among patients with high risk myelodysplastic syndrome based on Revised International Prognostic Scoring System, Kaplan-Meier estimates showed a survival advantage for those with total genomic aberrations < 100 Mb (mortality hazard ratio = 3.0, 95% CI, 1.0–9.3, *P*=0.05)

Table 1

Clinical characteristics of patients who have undergone chromosome genomic array testing testing

Clinical Characteristics		
Age	Median	61
	Range	2–86
Gender	Male	46
	Female	22
Diagnosis	Suspected myelodysplastic syndrome	3
	myelodysplastic syndrome, unclassifiable *	25
	Refractory cytopenia with unilineage dysplasia	2
	Refractory cytopenia with multilineage dysplasia	7
	Refractory anemia with excess blasts-1	6
	Refractory anemia with excess blasts-2	8
	Myelodysplastic syndrome/chronic myelomonocytic leukemia	4
	myelodysplastic syndrome/myeloproliferative neoplasm, unclassifiable	8
	therapy related myelodysplastic syndrome	4
	Shwachman-diamond syndrome/myelodysplastic syndrome	1
IPSS-R	High risk (>3)	37
	Low risk (≤ 3)	21
Transplant	Not transplanted	34
	pre-transplant	29
	post-transplant	5
Clinical F/U	Relapse	11
	Death	27
	Lost to follow up	10
	Alive with follow up since 2014	32

* Patients who received original diagnoses and therapy at an outside hospital, including: 3 suspected myelodysplastic syndrome, 1 myelodysplastic syndrome with deletion 5q, 1 refractory anemia with unilineage dysplasia, 6 refractory cytopenia with multilineage dysplasia, 4 refractory anemia with excess blasts-1, 3 refractory anemia with excess blasts-2, 1 myelodysplastic syndrome, unclassifiable, 3 myelodysplastic/myeloproliferative neoplasms, unclassifiable, 1 therapy related myelodysplastic syndrome.

Table 2

Distribution of chromosome genomic array testing/copy neutral loss of heterozygosity results between patients with normal and abnormal cytogenetics

Cytogenetics (karyotype and FISH)	CGAT results	Samples/68
Normal		26
	Normal	14
	Abnormal with copy neutral loss of heterozygosity	7
	Abnormal with no copy neutral loss of heterozygosity	5
Abnormal		38
	Normal	2
	Abnormal with copy neutral loss of heterozygosity	13
	Abnormal with no copy neutral loss of heterozygosity	23
Failed		4
	Normal	2
	Abnormal with copy neutral loss of heterozygosity	2
	Abnormal with no copy neutral loss of heterozygosity	0

Table 3

Total total genomic aberrations according to number of dysplastic lineages

	Number of dysplastic lineages				P_{trend}
	0	**1**	**2**	**3**	
Normal chromosome genomic array testing Median (range), n= 18	0 (0 – 0.9 *)	0.2 (0 – 0.6 *)	0 (0 – 0.4 *)	0.6 (0 – 0.7 *)	0.94
Abnormal chromosome genomic array testing w/copy number aberrations Median (range), n= 28	20 (0.9 – 39)	117 (0.7 – 356)	182 (0 – 444)	160 (107 – 592)	0.03
Abnormal chromosome genomic array testing w/copy neutral loss of heterozygosity Median (range), n= 22	–	66 (24 – 343)	243 (15 – 318)	226 (77 – 565)	0.05
All Abnormal chromosome genomic array testing (copy number aberrations and copy neutral loss of heterozygosity) Median (range), n= 50	20 (0.9 – 39)	79 (0.7 – 356)	206 (0 – 444)	186 (77 – 592)	0.003

* These total genomic aberration numbers represents constitutional variants.

Table 4

Molecular testing results in patients with copy neutral loss of heterozygosity by chromosome genomic array testing studies

Dysplastic lineages	Key CGAT results	Mutations	Mutations identified by UW OncoPlex	Relapse
		Identified by single-gene test		
1	9p copy neutral loss of heterozygosity and multiple copy number aberrations	JAK2+, FLT3-, BCR/ABL-	Not available	
1	9p copy neutral loss of heterozygosity and 1q copy number aberration	JAK2+	Not available	
1	9p copy neutral loss of heterozygosity and 20q copy number aberration	JAK2+	Not available	Yes
1	9P copy neutral loss of heterozygosity, and multiple copy number aberrations	JAK2+	Not available	
1	1p copy neutral loss of heterozygosity and 12p copy number aberration	JAK2/MPL/CALR -	Not available	
1	4q copy neutral loss of heterozygosity and copy number aberrations of 1q, 18q	JAK2-	Low level 1q copy gain involving MCL1, DDR2, ABL2, MDM4	
			TET2 (splicing variant, VAF ~96%, suggests LOH), NM_001127208.2:c.3594+5G>A	
			CBL p.L493F, NM_005188.3:c.1477C>T	
			SF3B1 p.R625C, NM_012433:exon14:c.1873C>T	
			SRSF2 p.P95H, NM_003016.4:c.284C>A	
			ASXL1 p.G1306Wfs*23, NM_015338.5:c.3915dup	
1	Xp/q copy neutral loss of heterozygosity and copy number aberration, copy number aberration in 21	CEBPA-, FLT3-, NPM1-	Not available	
1	9p copy neutral loss of heterozygosity and 21q copy number aberration	CEBPA-, FLT3-, NPM1-	POSITIVE for PDGFRA and KIT amplification, PIK3R1 mutation (37bp insertion exon 9 NM_181523.2 hg19 chr5:67588990_67588991), FLT1 p.L452L; CRLF2 p.S16S	
1	11q copy neutral loss of heterozygosity and 1p copy number aberration		Not available	Yes
2	7q copy neutral loss of	JAK2+, BCR/ABL-	Not available	

Dysplastic lineages	Key CGAT results	Mutations	Mutations identified by UW OncoPlex	Relapse
		Identified by single-gene test		
	heterozygosity and 8p/q copy number aberration			
2	14q and 17q copy neutral loss of heterozygosity, multiple copy number aberrations	JAK2-	Not available	
2	11p copy neutral loss of heterozygosity		SF3B1 p.K700E, NM_012433:exon15:c.2098A>G	
			TET2 p.L182*, NM_001127208.2:c.543del	
			GRIN2A p.V820G, NM_000833.3:c.2459T>G	
2	5q copy neutral loss of heterozygosity		Not available	
2	5q copy neutral loss of heterozygosity and 4q copy number aberration		Not available	Yes
2	17q copy neutral loss of heterozygosity, multiple large copy number aberrations		Not available	
2	4q copy neutral loss of heterozygosity		Not available	
2	17p copy neutral loss of heterozygosity, multiple large copy number aberrations		Not available	
2	9p and 11q copy neutral loss of heterozygosity and 8 p/q copy number aberration		ASXL1 Exonic - deletion	
			TSC2 NM_000548.3:c.5050_5067+17del	
			NPM1 p.W288Cfs*12, NM_002520.6:c.860_863dup	
			TET2 p.P1894H, NM_001127208.2:c.5681C>A	
			CBL (splicing mutation), NM_005188.3:c.1096-7A>G	
			TET2 p.E1318G, NM_001127208.2:c.3953A>G	
			ASXL1 p.E635Rfs*15, NM_015338.5:c.1900_1922del	
3	9p copy neutral loss of heterozygosity	JAK2+	Not available	
3	11p copy neutral loss of heterozygosity	FLT3-	(9/11/13): no mutations, GATA2-: SBDS- (10/10/13); ELA2- (11/20/08); HAX1- (10/22/08)	
3	9p copy neutral loss of heterozygosity		Not available	
3	17p copy neutral loss of heterozygosity, multiple large copy number aberrations		Not available	Yes

Na = Not available; UW Oncoplex – see Methods; CNA = copy number aberration; cnLOH = copy neutral loss of heterozygosity