

Mutational Profile and Karyotypic Abnormalities of a Cohort of Clinical Trial Patients With Higher-risk Myelodysplastic Syndromes (MDS) Following Failure of Hypomethylating Agents (HMAs): Impact on Response to Rigosertib Therapy

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BACKGROUND

Diverse cytogenetic abnormalities and specific aberrations in RNA splicing, cell-signaling, transcription regulation and tumor suppressor genes are increasingly being applied for the prognostic stratification of MDS pts at diagnosis. Despite these advances, treatment options are limited to hypomethylating agent (HMA) therapy and lenalidomide; the survival advantage of these agents is established, but most pts eventually relapse. The prognosis for pts in whom HMA therapy has failed is grim, with a median overall survival (OS) of 4.3 to 5.6 months.^{1,2} The clonal architecture and evolution of molecular changes have been chronicled in newly diagnosed MDS pts, but the assessment of these abnormalities in pts who have failed or relapsed after HMAs is limited.

OBJECTIVES

- To document for the first time the very high incidence of these molecular changes in higher-risk MDS patients after failure of HMAs
- To assess the relationship between the genetic and cytogenetic abnormalities and response to rigosertib
- To correlate the results of cytogenetic abnormalities in pts with HMA failure with response to rigosertib (RIG) in the context of a clinical trial comparing RIG with best supportive care (BSC).

METHODS

Genomic DNA was isolated from single microscopic slides from 175 pts from Study 04-21 and subjected to sequence analysis of a "myeloid panel" composed of 24 selected loci known to be frequently mutated in patients with MDS and AML. Standardized cytogenetic investigations were performed using G banding and were centrally reviewed. Whenever possible, 25 metaphases were analysed. Description of chromosome aberrations and clone definition followed the International System for Cytogenetic Nomenclature. FISH for deletion 5q was included. Depending on the aberrations detected during karyotyping, further probes were applied. A complex karyotype was defined as ≥ 3 aberrations within 1 clone.

RESULTS

Adequate DNA samples were obtained from 111 (63%) of 175 patients. All but 12 of the 111 samples carried at least 1 mutation (89%), with 17 of the 24 myeloid mutations detected. The most frequently mutated loci were SRSF2 (28%), mutations were detected at multiple coding regions of the protein), TP53 (23%), ASXL1 (19%), SF3B1 (14%), TET2 (1ASXL1 (19%), SF3B1 (14%), TET2 (14%), U2AF1 (12%), RUNX1 (11%), and DNMT3A (10%). Mutations were found in RUNX1 (5 samples); 4 samples each carried a mutation in ETV6 (4), EZH2 and N- and K-ras. All but 1 of the mutations were represented at $>10\%$ of the alleles, with a range of 9.2-94%. Sixty-two percent of mutations detected in rigosertib patients who did not respond to initial HMA therapy ("primary HMA failure," 61% of the study population) carried single or multiple mutations. The effect of single and multiple mutations on OS is summarized by IPSS-R class in Figure 1 and by karyotype class in Figure 2. Patients carrying mutations in TP53, ASXL1, and SRSF2 showed a trend toward increased survival benefit of rigosertib therapy. It is noteworthy that pts with monosomy 7 and trisomy 8 mutations demonstrated a survival benefit with rigosertib therapy compared to BSC (monosomy 7: HR=0.24, p=0.0033; trisomy 8: HR=0.34, p=0.035). The significance of individual and combined mutations, in the context of "founder" and "subclonal" lesions is being evaluated further.

Table 1. Mutations by Revised IPSS Score

Myeloid Gene Mutation*	IPSS-R Score					Total
	Low	Intermediate	High	Very high	Unknown	
Single	0	5	15	25	5	50
Multiple	0	1	15	23	10	49
None	0	3	5	3	1	12
% with mutation	0	67	86	94	94	89
% without mutation	100	33	14	6	6	22
Analyzable samples	100	9	35	51	16	111
Not analyzed	1	19	58	83	27	188
Total	1	28	93	134	43	299

* A panel of 24 mutations were screened for in paraffin section derived DNA

Figure 1. Mutation Frequency in IPSS-R Classes

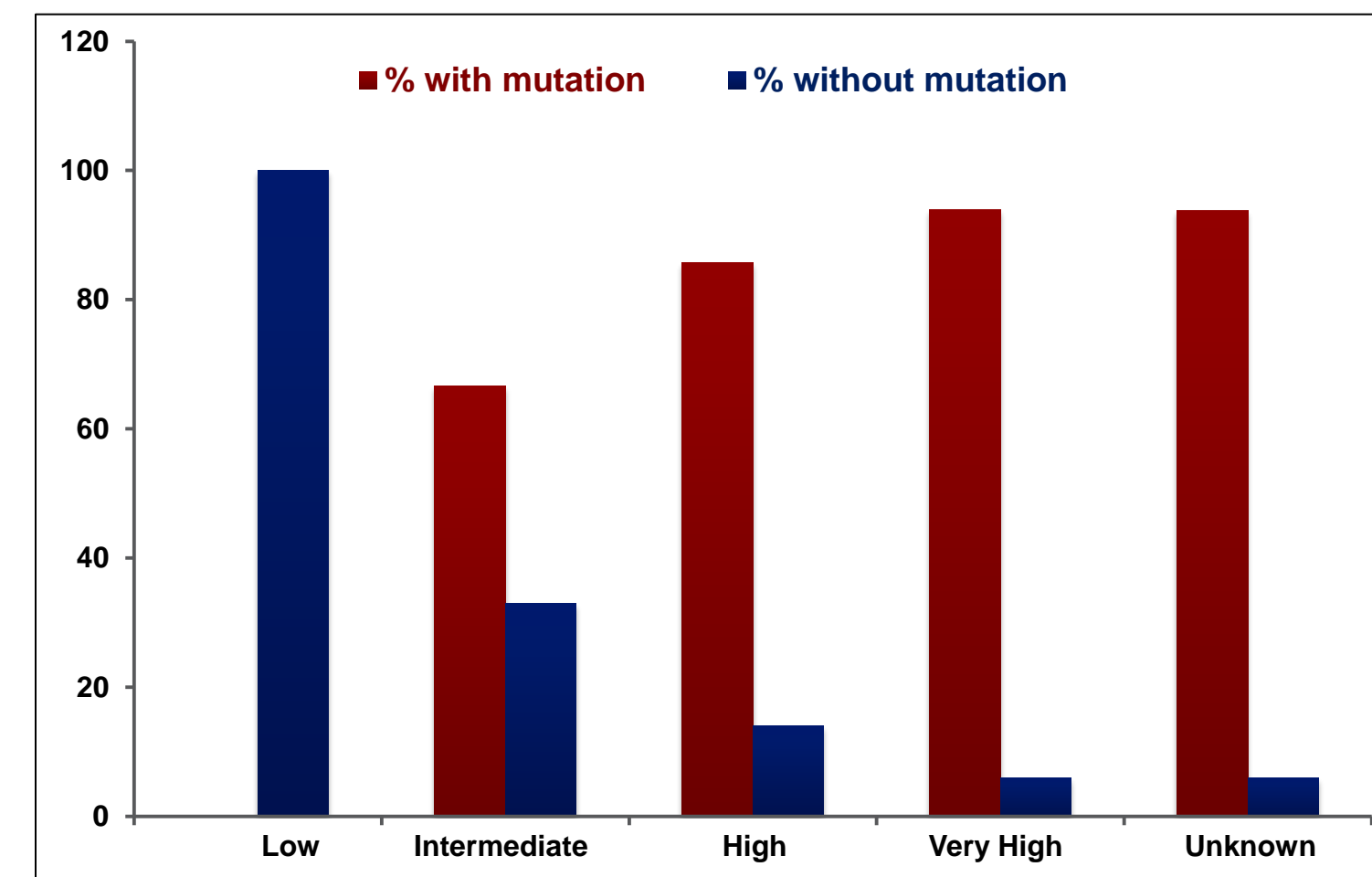


Figure 2. Mutation Frequency in Karyotype Classes

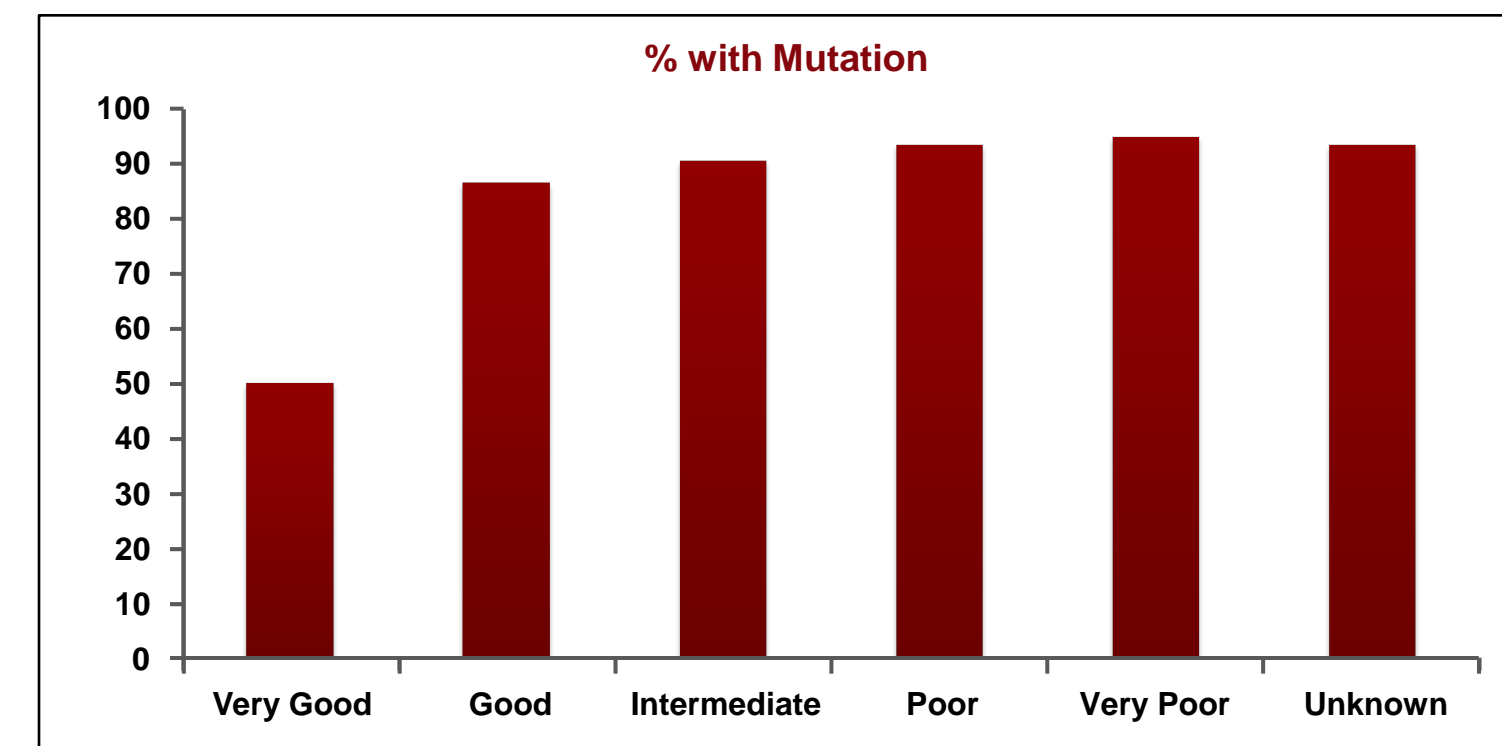


Table 2. Monosomy 7 and Trisomy 8

	Monosomy 7	Trisomy 8
Total Analyzed	12	11
Single Mutation	5	4
Multiple Mutation	7	7
No Mutation	0	0
Percent positive	100	100
Unknown	17	19
Total Patients	29	30

Figure 3. Overall Survival by Karyotype/Mutation

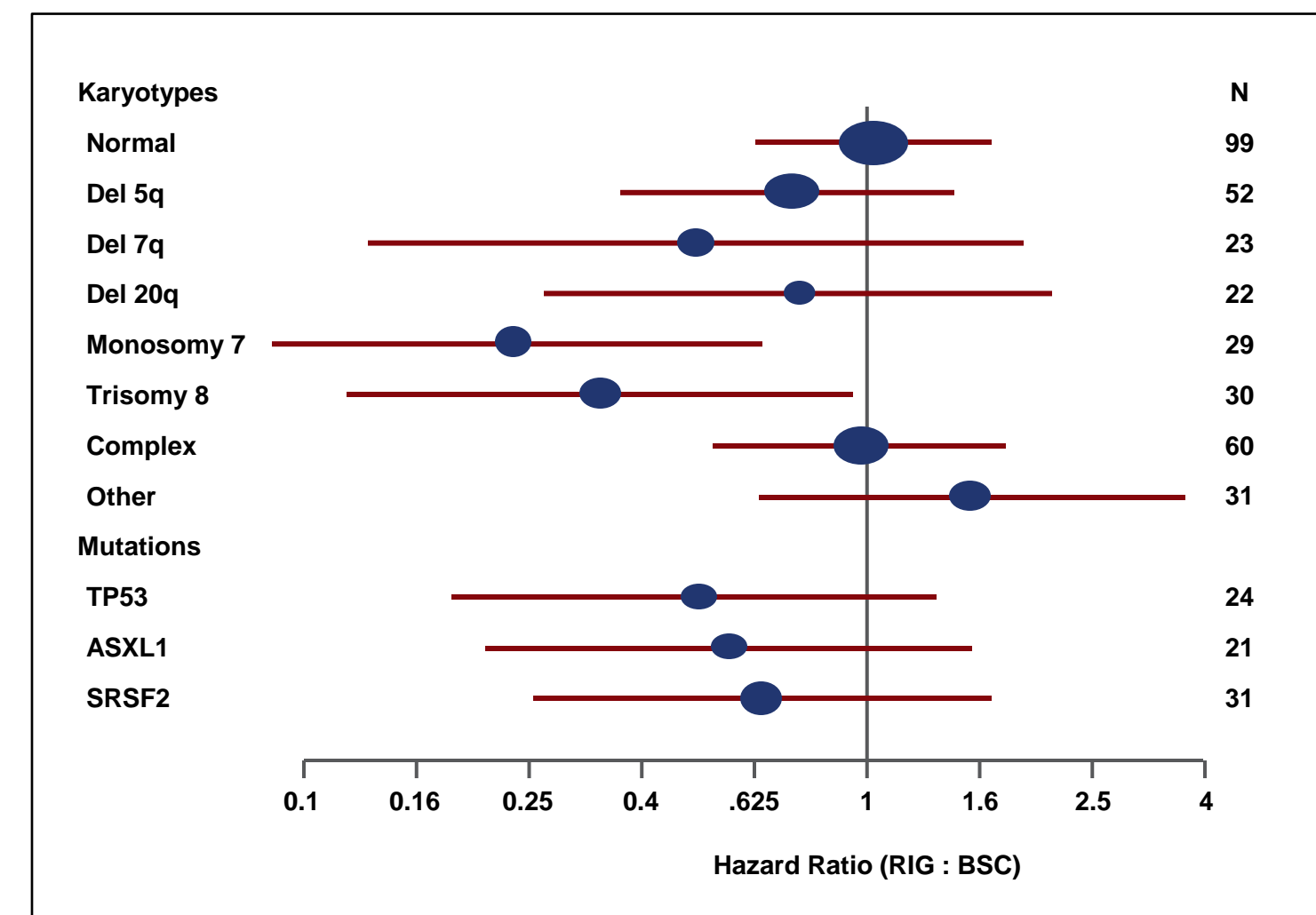


Figure 4. Impact of Cytogenetics on Survival Outcome

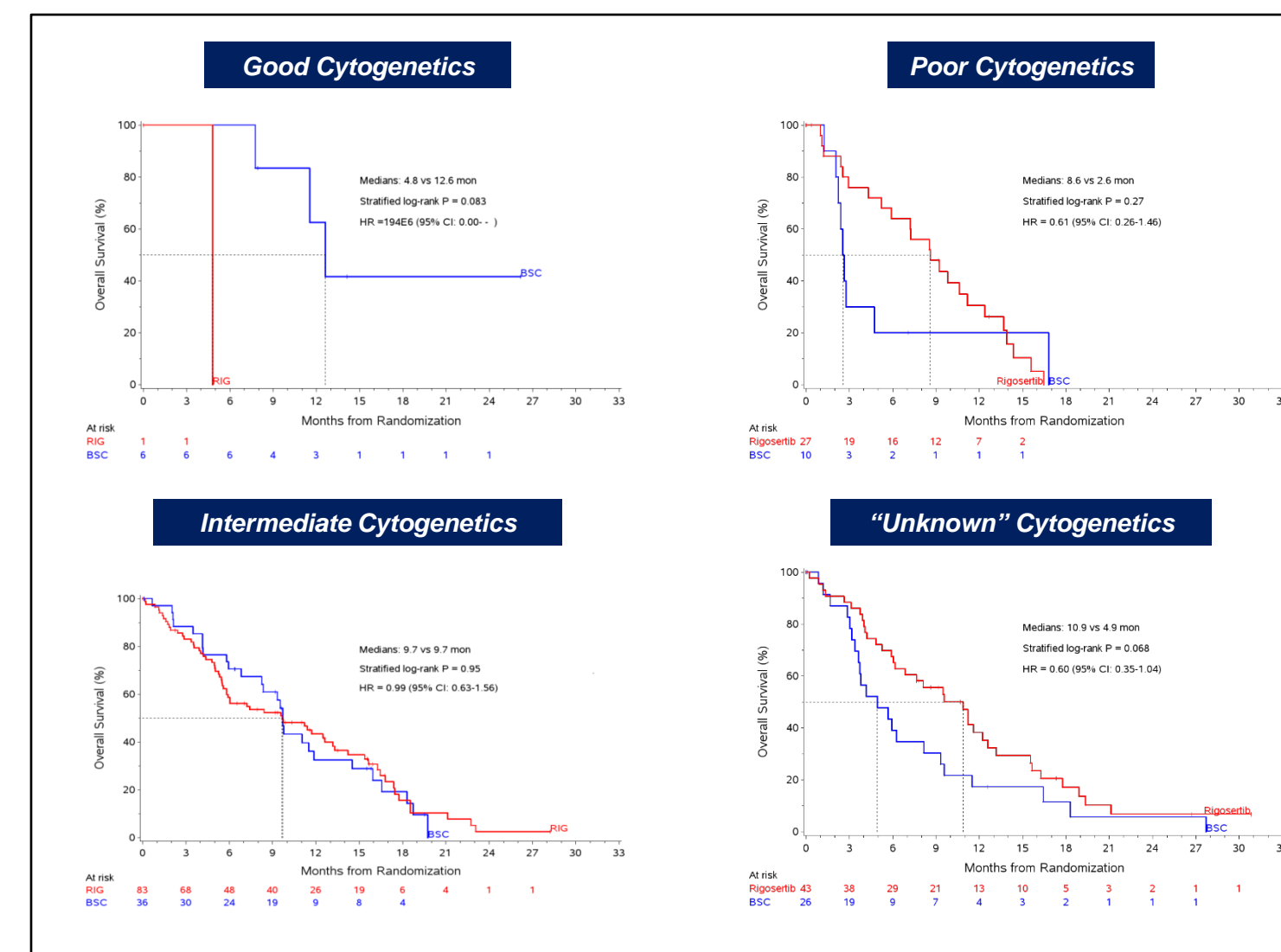
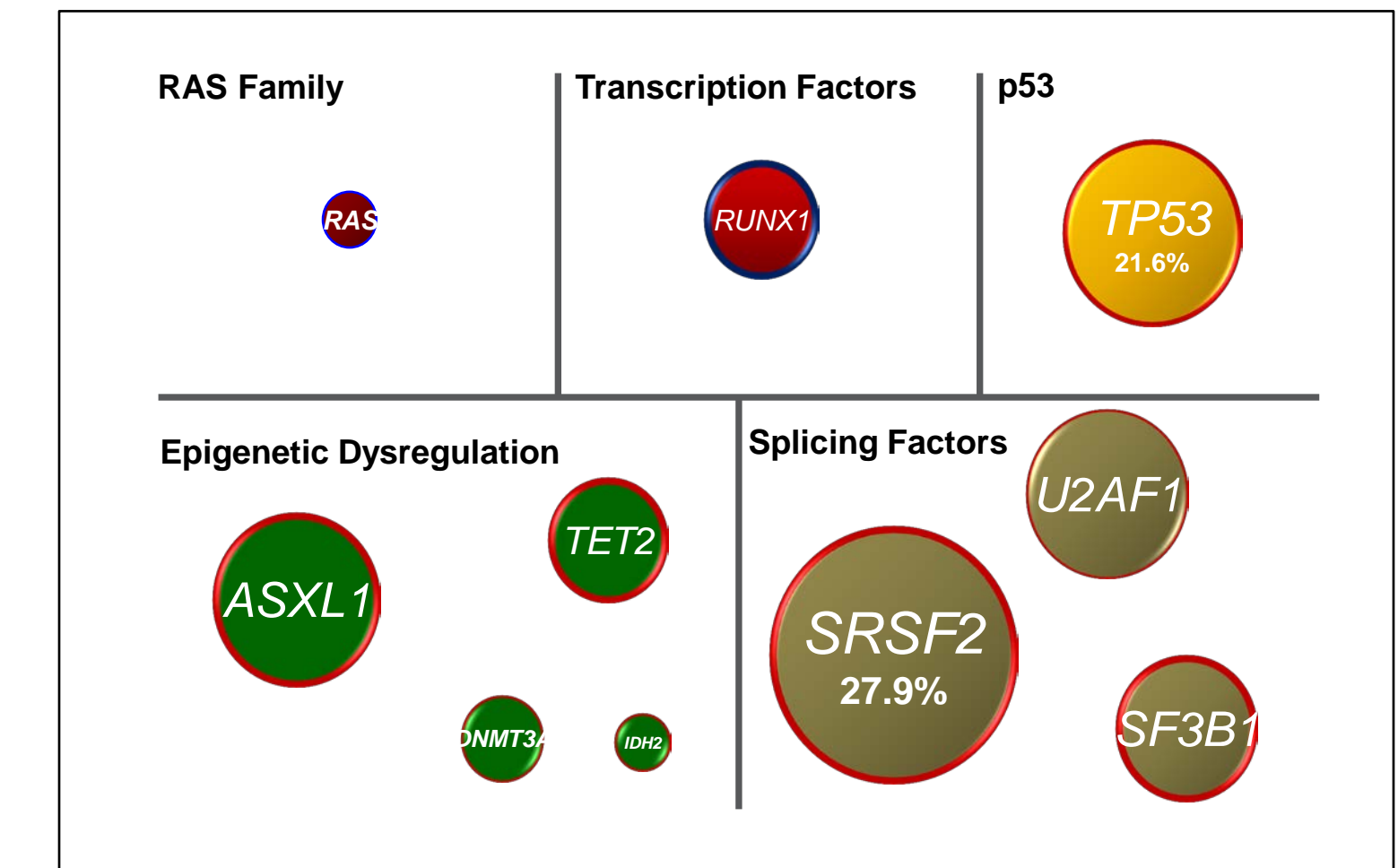


Figure 5. Mutation Frequency in Karyotype Classes



CONCLUSION

Genetic heterogeneity of MDS is evident in the reported diversity of karyotypic and mutational alterations in bone marrow of patients. Here we note that more than 80% of post-HMA MDS patients carry mutation in target genes. The prevalence of these mutations also correlates to the severity of the disease as defined by karyotype or IPSS-R criteria. Further, the karyotypes with poor prognosis and patients with high prognostic risk score exhibited better survival in the ONTIME clinical trial. Notably, patients with certain karyotypes, including monosomy 7 and trisomy 8 experienced enhanced survival benefit. These results have important implications on designing therapeutic approaches and trials for MDS patients after failure of HMAs.

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