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Mutational Profiling of Therapy-related Myelodysplastic Syndromes and Acute Myeloid Leukemia by Next Generation Sequencing, a Comparison with *de novo* Diseases

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Abstract

In this study we used a next generation sequencing-based approach to profile gene mutations in therapy-related myelodysplastic syndromes (t-MDS) and acute myeloid leukemia (t-AML); and compared these findings with *de novo* MDS/AML. Consecutive bone marrow samples of 498 patients, including 70 therapy-related (28 MDS and 42 AML) and 428 *de novo* (147 MDS and 281 AML) were analyzed using a modified-TruSeq Amplicon Cancer Panel (Illumina) covering mutation hotspots of 53 genes. Overall, mutation(s) were detected in 58.6% of t-MDS/AML and 56.8% of *de novo* MDS/AML. Of therapy-related cases, mutations were detected in 71.4% of t-AML versus 39.3% t-MDS (p=0.0127). *TP53* was the most common mutated gene in t-MDS (35.7%) as well as t-AML (33.3%), significantly higher than *de novo* MDS (17.7%) (p=0.0410) and *de novo* AML (12.8%) (p=0.0020). t-AML showed more frequent *PTPN11* but less *NPM1* and *FLT3* mutations than *de novo* AML. In summary, t-MDS/AML shows a mutation profile different from their *de novo* counterparts. *TP53* mutations are highly and similarly prevalent in t-MDS and t-AML but mutations in genes other than *TP53* were more frequent in t-AML than t-

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Disclosure/Conflict of Interest

Authors do not report conflict of interest.

Authors' contributions. C.Y.O. and S.A.W. designed the study; G.G-M., S.A.P. and H.M.K. provided patient information; C.Y.O., K.P.P., M.J.R., B.F., G.T., M.G., R.S., R.K-S., R.L. and S.A.W. collected and analyzed data; C.Y.O. and S.A.W. performed the statistical analysis; C.Y.O., K.P.P., K.H.Y., L.J.M., R.L. and S.A.W. wrote the manuscript and created the figures and tables; and all authors critically reviewed the manuscript and read and approved the final version of the manuscript.

MDS. The molecular genetic profiling further expands our understanding in this group of clinically aggressive yet heterogeneous myeloid neoplasms.

Keywords

Next generation sequencing; therapy-related; MDS; AML; karyotype and *TP53*

1. Introduction

Therapy-related myelodysplastic syndromes (t-MDS) and acute myeloid leukemia (t-AML) are a group of clinically aggressive diseases that occur after various cytotoxic chemotherapy regimens and/or radiation therapy administered previously for prior neoplastic, or rarely, non-neoplastic diseases [1]. Patients with t-MDS/AML commonly respond poorly to conventional therapies and show rapid disease progression [2, 3]. Chromosomal abnormalities, present in 40%–60% of patients with *de novo* MDS/AML [4], are observed in up to 80–90% t-MDS/AML patients [5, 6] with frequent high-risk cytogenetic abnormalities. The presence of frequent and high-risk cytogenetic abnormalities is thought to be one main reason for clinical aggressiveness of t-MDS/AML. However, in our recent study of 411 t-MDS/oligoblastic AML patients stratified by the Revised International Scoring System (IPSS-R), we showed that for a same IPSS-R score (a combination of blasts, severity of cytopenias and cytogenetic risk), patients with t-MDS/oligoblastic AML did significantly worse than their *de novo* counterparts. These differences were more pronounced in patients in the “low” and “very low” IPSS-R risk categories [7]. This observation indicates that genetic events in addition to chromosomal alterations may be involved in the pathogenesis and contribute to clinical aggressiveness of patients with t-MDS/AML.

In the past decade, massive parallel next generation sequencing (NGS) technology has been developed and more recently has been integrated into the routine laboratory work-up of MDS and AML cases at our institution. NGS assays allow the analysis of numerous genes in a quicker, more scalable fashion and the cost of NGS assays has dropped substantially in recent years. Application of NGS to samples from patients with *de novo* MDS [8] and AML [9] has expanded our understanding in these diseases. Using these data, several risk stratification models for *de novo* MDS and AML have been proposed [10–12]. In contrast, systematic mutational analysis of therapy-related (t) MDS and AML has only been performed in a small number of cases [13, 14].

In this study, our goal was to improve our understanding in molecular genetic events in t-MDS/AML and to contrast these findings with *de novo* MDS/AML. To achieve this goal we assessed the mutational profiles of a large number of t-MDS/AML cases using next generation sequencing methods. We also correlated the mutation results with cytogenetic data.

2. Materials and Methods

2.1 Patients

We studied a cohort of 498 patients with MDS/AML from October, 2012 through August, 2013 at The University of Texas MD Anderson Cancer Center. Clinical, hematological and cytogenetic data were collected for all patients. For patients with t-MDS/AML, primary malignant diseases were collected from the medical record. Brachytherapy, radioisotopes, and radiation therapy in patients in whom the field did not include active hematopoietic bone marrow were not considered as radiation therapy. All cases were collected consecutively and classified according to the World Health Organization (WHO) classification system. An informed consent was obtained from the patients or their guardians. This study was conducted in accord with the Declaration of Helsinki and was approved by the IRB at The University of Texas MD Anderson Cancer Center in Houston, Texas, USA.

2.2 Next generation sequencing

Genomic DNA (gDNA) was extracted from bone marrow aspirate or peripheral blood of each case using an Autopure extractor (Qiagen, Valencia, CA) and was quantified using a Qubit DNA BR assay kit (Life Technologies, Carlsbad, CA). The genomic library was prepared using 250 ng of DNA template and a commercially available 48-gene TruSeq Amplicon Cancer Panel (Illumina Inc., San Diego, CA) to which custom-designed probe pairs for 5 genes were added (Table 1).

The generated library was purified using AMPure magnetic beads (Agencourt, Brea, CA) and then subjected to next generation sequencing (NGS) using a MiSeq sequencer (Illumina Inc., San Diego, CA) [15]. In our clinical practice, a minimum quality score of AQ30 is required for a minimum of 75% of bases sequenced ensuring high quality sequencing results. In practice, >85% of the bases consistently show quality scores of >AQ30 and 90–95% bases show quality score of >AQ20. Using human genome build 19 (hg 19) as a reference, variant calling was performed with Illumina MiSeq Reporter Software 1.3.17. To visualize read alignment and confirm the variant calls, Integrative Genomics Viewer (IGV, Broad Institute, MA) was used [16]. For clinical reporting, a sequencing coverage of 250X (bi-directional true paired-end sequencing) and a variant frequency of 5% in the background of wild-type were used as cutoffs. To annotate sequence variant, custom-developed, in-house software (OncoSeek) was used to interface the data with IGV. OncoSeek has several functionalities including mapping of variants directly to COSMIC and dbSNP, automatic translation of variants and their genomic position to Human Genome Variation Society compliant nomenclature, identification of amplicons with suboptimal coverage for clinical reporting, and self-updating population analysis, which identified reference genome polymorphisms or sequencing artifacts [17]. Among the 53 genes in the panel, *NPM1* mutations and *FLT3* internal tandem duplication (ITD) were confirmed with fragment analysis using capillary electrophoresis. Pyrosequencing was performed to confirm mutations in *BRAF* codons 599 and 600, *JAK2* codon 617, *KRAS* codons 12, 13 and 61, *NRAS* codons 12, 13 and 61. Sanger sequencing was performed to confirm mutations in the remaining genes in the panel. Confirmation tests were generally triggered when the sequencing coverage was less than 250X or mutational frequency in major genes such as

DNMT3A, IDH1, IDH2, KRAS, NPM1 and *NRAS* was less than 5%. Fragment analysis for *FLT3*-ITD was performed in all cases [18].

2.3 Cytogenetic Analysis

Conventional cytogenetic analysis was performed using standard methods as previously described [19]. Twenty metaphases were analyzed and the results were reported using the current International System for Human Cytogenetic Nomenclature [20]. Only karyotypes with adequate metaphases for analysis were included, except in some cases where a lesser number of metaphases were available in which fluorescence *in situ* hybridization (FISH) was performed to confirm clonal cytogenetic abnormalities. For MDS patients, the cytogenetic risk was stratified according to the International Prognostic Scoring System (IPSS) [21] and for AML patients the risk was categorized by the revised cytogenetic classification proposed by the United Kingdom Medical Research Council (UKMRC) [22].

2.4 Statistical analysis

For continuous variables, data were reported as a median and range. For nominal variables, data were reported as the number of patients if not otherwise specified. Fisher's exact test was used for categorical variables and the Mann-Whitney U test for continuous variables. All differences with $p<0.05$ were considered to be statistically significant (two-tailed). GraphPad Prism 6.0 (La Jolla, CA, USA) was used for statistical analyses.

3. Results

3.1 Patient Characteristics

The study cohort consisted of 28 patients with t-MDS, 42 with t-AML, 147 with *de novo* MDS, and 281 with *de novo* AML. In the therapy-related patient group, the prior diseases were hematological malignancies (n=33); carcinoma (n=32), sarcoma (n=4), and medulloblastoma (n=1). The cytotoxic therapy used to treat these tumors included radiation therapy only (n=5), chemotherapy only (n=41), and combined chemoradiation therapy (n=24). The WHO categories of *de novo* MDS as well t-MDS are shown in Table 2.

De novo AML included 44 (16.7%) cases of AML with recurrent cytogenetic abnormalities, 127 (45.2%) cases of AML with myelodysplasia related changes (AML-MRC), and 110 (39.1%) cases of AML, not otherwise specified (AML, NOS). There were no differences in demographic and hematological findings between patients with t-MDS and *de novo* MDS or between patients with t-AML and *de novo* AML (Table 2). However, the distribution of cytogenetic risk was significantly different between patients with therapy-related versus *de novo* diseases. Patients with good, intermediate and poor risk cytogenetics represented 14.3%, 14.3% and 64.3% of cases in t-MDS. In contrast, 50.3% of patients had good risk, 19.0% intermediate risk, and 30.6% poor risk cytogenetics in *de novo* MDS (p=0.0005). Similarly, t-AML patients with favorable, intermediate and unfavorable risk cytogenetics were 4.8%, 26.2% and 66.7%, respectively in t-AML; in contrast with 9.3%, 54.8% and 33.5%, respectively, in *de novo* AML (p=0.0006).

3.2 Mutational profiles

In all patients, mutations were found involving 19 different genes in 284 (57.0%) patients. Of these 284 patients, 193 patients (68.0%) had mutations involving one single gene, 59 (20.8%) had mutations in two genes, 24 (8.5%) had mutations in 3 genes and 8 (2.8%) had mutations in 4 genes.

As a group, t-MDS/t-AML, mutations were detected in 41 (58.6%) patients, involving 13 genes. These rates were not significantly different from *de novo* MDS/AML that mutations were detected in 243 patients (56.8%) (p=0.7962) and involved 17 genes.

3.2.1 Comparing t-MDS with t-AML—In t-MDS, mutations were found in 11 of 28 (39.3%) patients and these mutations involved only 2 genes, *TP53* and *IDH1* (Figure 1A).

In contrast, mutations were detected in 30 of 42 (71.4%) t-AML patients and involved 13 different genes, significantly more frequent as well as more diverse than t-MDS (p=0.0127 and p=0.0040 respectively). The most frequently mutated gene in t-AML was *TP53* (35.7%). Other genes in t-AML in a decreasing frequency were: *PTPN11*, 11.9%; *IDH1*, 9.5%; *IDH2*, 9.5%; *NRAS*, 9.5%; *FLT3*, 7.1%; *DNMT3A*, 7.1%; and *KRAS*, 4.8% (Figure 1B). Mutations in *GNAS, KDR, KIT, NPM1* and *APC* were rare (<3.0%).

3.2.2 Comparing t-MDS/AML with de novo MDS/AML—In *de novo* MDS (n=147), mutations were found in 58 cases (39.5%) and mutation frequency was commensurate with the risk grade of MDS. For example, mutations were found in 24% low-grade MDS (RCUD, RARS, RCMD, and MDS with isolated deletion 5q) and 51% in high-grade MDS (RAEBs), respectively (p=0.0011). The overall mutation frequency was very similar to t-MDS (39.3%, not significant); however, mutations in *de novo* MDS involving 15 different genes, significantly more diverse than t-MDS (p=0.001) that only two genes were found mutated (p=0.0010). For individual mutations, the frequency of *TP53* mutations (17.7%) was significantly lower than t-MDS (p=0.0410). Other mutated genes in *de novo* MDS were: *NRAS*, 6.8%; *IDH1*, 4.8%; *DNMT3A*, 3.4%; *IDH2*, 3.4%; and *KRAS*, 2.7%. Mutations in *PTPN11*, *FLT3*, *BRAF, JAK2, EZH2, GNAQ, GNAS, MPL*, and *NPM1* were rarely (< 2.0%) detected (Figure 1A).

Mutation frequency was similar between *de novo* AML and t-AML (65.8% versus 71.4%, not significant). However, compared with *de novo* AML, t-AML showed a higher frequency of mutations in *TP53* (35.7% vs 12.8%, p=0.0020) and *PTPN11* (11.9% vs 2.1%, p=0.0075), whereas a lower frequency in mutations in *FLT3* (7.1% vs 21.7%, p=0.0357) and *NPM1* (2.5% vs 16.4%, p=0.0165). Compared to de novo MDS, mutations in *de novo* AML (n=281) were more frequently detected (65.8% versus 39.5%, p<0.0001), and involved 14 different genes, with *FLT3* (21.7%) being the most frequently mutated gene. Other mutated genes were: *NPM1*, 16.4%; *IDH2*, 15.7%; *TP53*, 12.8%; *NRAS*, 9.3%; *IDH1*, 8.5%; *DNMT3A*, 8.2%; *JAK2*, 3.9%; *KRAS*, 3.2%; and *PTPN11*, 2.1% (Figure 1B). Mutations in *KIT, GNAS, NOTCH1* and *EGFR* were rarely detected (< 2.0%). By the WHO group, mutations were less frequent in AML with recurrent cytogenetic abnormalities compared to AML-MRC and AML, NOS (44% vs. 70%, respectively, p=0.0009).

Co-mutations are shown by Circos plots using the common mutated genes in *de novo* AML, *de novo* MDS, t-MDS and t-AML, including *DNMT3A, FLT3, IDH1, IDH2, KRAS, NPM1, NRAS and TP53* (Figure 2). Differences in co-mutational patterns were observed between t-MDS/AML (Figure 2A) and *de novo* MDS/AML (Figure 2B). The common co-mutations in t-MDS/AML were *TP53* with *IDH1, NRAS and FLT3*; whereas, in *de novo* MDS/AML, the common co-mutations were *FLT3* with *NPM1, IDH2 and DNMT3A.*

3.3 Mutation profile and Karyotypical Abnormalities

Karyotype information was available in 26 t-MDS, 147 *de novo* MDS, 41 t-AML and 274 *de novo* AML. Cases with a normal, complex karyotype and all other abnormalities were shown in Figure 3.

t-MDS showed a higher risk karyotypic distribution than *de novo* MDS (p=0.0006); similarly, t-AML showed a high risk distribution of karyotypic abnormalities than de novo AML (p=0.0085). The mutation frequencies for cases with a normal karyotype, a non-complex abnormal karyotype and a complex karyotype in each subgroup are shown in Figure 2. Of 3 t-MDS patients with a normal karyotype (13.5%), one patient had an *IDH1* mutation. Of *de novo* MDS patients, 21 out of 63 cases with a normal karyotype were found to have mutations (33.3%). The most frequently mutated genes in a decreased frequency were *NRAS* (n=7), *DNMT3A* (n=4), *IDH1* (n=4), *IDH2* (n=2), *FLT3* (n=1), *BRAF* (n=1), *EZH2* (n=1), *GNAQ* (n=1), *GNAS* (n=1), *JAK2* (n=1) and *KRAS* (n=1). Of t-AML, 4 of the 7 patients with a normal karyotype harbored mutations, involving *IDH2* (n=3), *TP53* (n=1), *FLT3* (n=1), *DNMT3A* (n=1), *NPM1* (n=1) and *GNAS* (n=1); whereas, in *de novo* AML with a normal karyotype (n=91), mutations were detected in 57 cases (62.6%), involving *FLT3* (n=30), *NPM1* (n=26), *IDH2* (n=19), *DNMT3A* (n=12), *IDH1* (n=10), *NRAS* (n=5), *TP53* (n=4), *KRAS* (n=2), *PTPN11* (n=2), *GNAS* (n=1), *JAK2* (n=1) and *KIT* (n=1) in a decreased frequencies. *TP53* was the most frequently mutated gene in all subsets of cases with a complex karyotype, 50% in t-MDS, 60% in t-AML, 63.2% in *de novo* MDS, and 41.7% of in *de novo* AML. Of cases with a non-complex abnormal karyotype, one patient with t-MDS had *TP53* mutation; 13 patients (28.2%) with *de novo* MDS had mutations involving 10 genes. In t-AML, 13 patients (71.4%) had mutations, involving 8 genes; whereas, 75 patients with *de novo* AML (67.8%) had mutations involving 13 genes.

4. DISCUSSION

We assessed the bone marrow samples of 70 t-MDS/AML using a NGS of 53 selected gene panel and compared the data with 428 *de novo* MDS/AML patients. With this large dataset and the genes we tested, we showed that the mutation profiles of t-MDS/AML are conspicuously different from *de novo* MDS/AML (Figures 1 and 2). *TP53* was almost the only mutated gene in t-MDS (except for one case with *IDH1* mutation), and found in about 1/3 of our t-MDS cases. Similarly, *IDH1* mutations were rarely reported in t-MDS [23]. In contrast, we identified mutations involving 15 different genes in *de novo* MDS; and *TP53* gene mutation frequency in *de novo* MDS was significantly lower than t-MDS. Of note, the overall mutation frequency in our *de novo* MDS was ~40%, slightly lower than what reported by others [8, 24]. This lower frequency is likely attributable to the methods we used

as our gene panel focused on selected exons in 53 genes and did not include some genes known to be mutated in MDS. For example, *DNMT3A* and *EZH2* mutations were identified in <5% in our *de novo* MDS patients, slightly lower than 6–8% reported by others [8, 25–27], and this could be due to some regions not covered by our assay (Table 1). *TET2*, *ASXL1* and spliceosome genes (*U2AF35, SF3B1 and SRSF2)* were not included in our panel. These genes are reported frequently mutated in *de novo* MDS [24, 27, 28], but, infrequently in t-MDS [13].

In t-AML, like t-MDS, *TP53* was frequently mutated with a frequency significantly higher than *de novo* AML (p=0.0020). *PTPN11* mutations were seen in 11.9% of t-AML patients in this series, significantly higher than *de novo* AML (2.1%) patients. The *PTPN11* gene encodes a protein tyrosine phosphatase (SHP-2) in the RAS/MEK/ERK kinase pathway [29], and has been reported mutated in approximately 4–5% t-MDS/t-AML [14, 30]. *NPM1* mutations were detected in only 5% of t-AML cases in this study. In the literature, *NPM1* mutations have been reported in about 30% of all AML cases and 50–60% of cytogenetically normal AML [31–34]. In our *de novo* AML group, *NPM1* mutations were shown in 16.4% of all patients and 29.2% of AML with a normal karyotype. This slightly lower *NPM1* mutation frequency in our *de novo* AML group was likely due to higher risk patients in our cohort as a result of a tertiary referral cancer center. *FLT3* mutations were also significantly lower in t-AML, compared with *de novo* AML. These data are similar to that reported by Pedersen-Bjergaard and colleagues in their study of t-MDS/AML [14]. In addition, we identified 1 case of t-AML with mutations in *DNMT3A, NPM1*, and *FLT3.* This clustering of mutations has been shown in a small subset of *de novo* AML patients [34].

Due to a general aggressive clinical course related to t-MDS and AML, in the 2008 WHO classification, t-MDS and t-AML are combined under the umbrella term “therapy-related myeloid neoplasms (t-MN)” [1]. Recent studies have shown that t-MDS with a low blast count is less aggressive than t-AML [7, 35]. In this study, we showed that the frequency of *TP53* mutations were similarly high in both t-MDS and t-AML; however, mutations in genes other than *TP53* were significantly higher in t-AML than t-MDS. Our data also suggests that *TP53* mutation may be heavily involved in the early pathogenesis of myeloid neoplasms post cytotoxic exposure, but mutations in other genes likely provide proliferative advantage in cases of t-AML. These findings also illustrate that disease progression, such as from t-MDS to t-AML, is coupled with a step-wise molecular genetic evolution.

In summary, we showed that the mutational profiles of therapy-related MDS and AML differed substantially from their *de novo* MDS and AML counterparts. t-AML and t-MDS both harbored a high frequency of *TP53* mutations; and *PTPN11* mutations were more frequent, but *FLT3* and *NPM1* mutations were less frequent in t-AML than *de novo* AML. Within the therapy-related myeloid neoplasms, t-AML harbored more frequent mutations in genes other than *TP53*. Overall, *TP53* mutation*s* were strongly associated with a complex karyotype and the complexity degree of karyotypic abnormalities in t-MDS/AML as well as *de novo* MDS/AML. The information we provide here contribute to our further understanding of the molecular-genetic basis of therapy-related myeloid neoplasms, the similarity and difference between t-MDS and t-AML and how they differ from *de novo* counterparts.

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***Highlights (for review)**

Mutation profile of t-MDS/AML is different from their de novo counterparts.

Mutation profile is different between t-MDS and t-AML.

TP53 mutations are associated with a complex karyotype in both groups of MDS/AML.

Different molecular profiling may contribute to clinical heterogeneity of MDS/AML.

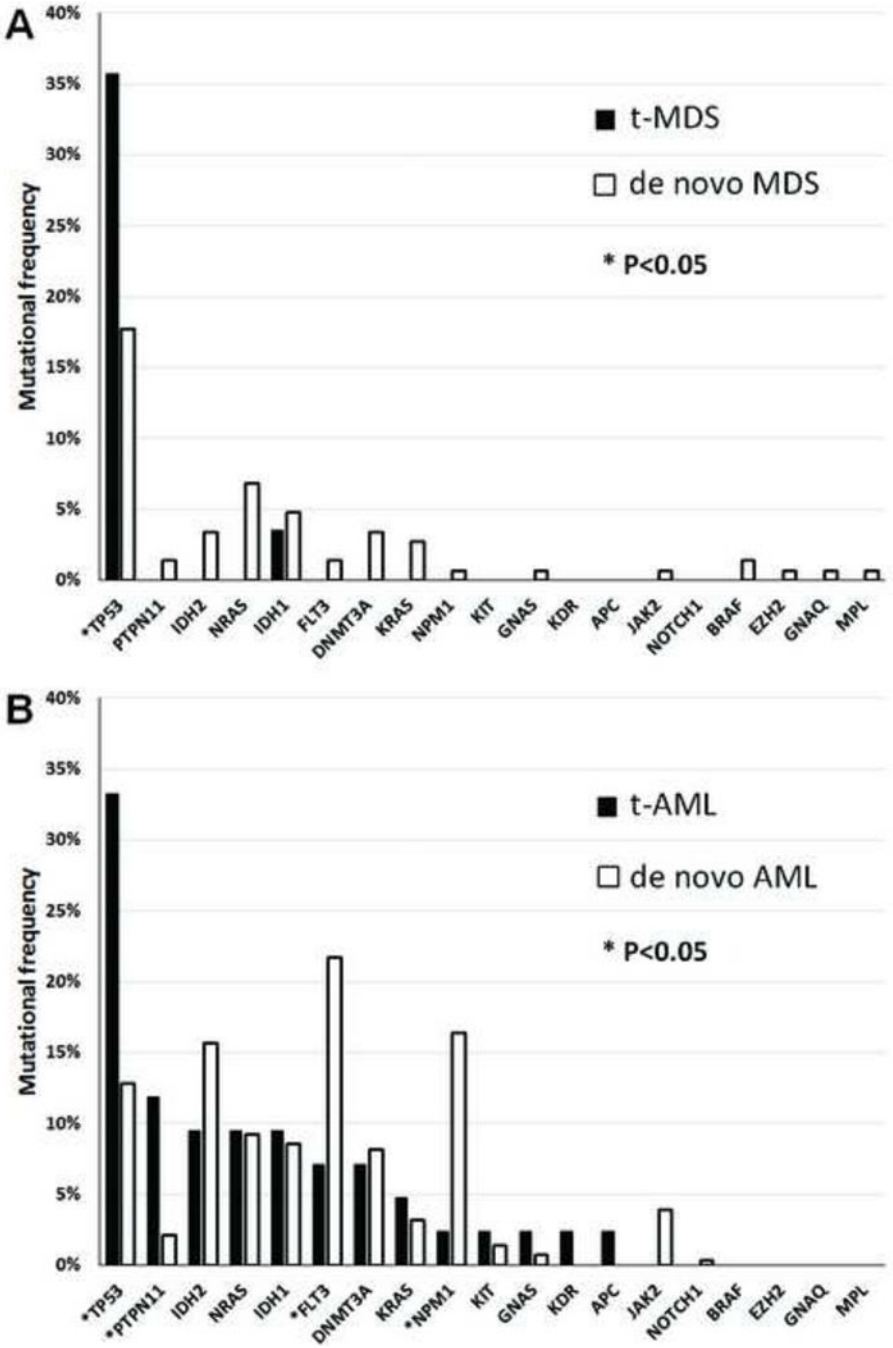

Figure 1.

Mutational profiles in myeloid neoplasms. 1A. mutational profile of therapy-related myelodysplastic syndromes (t-MDS) versus *de novo* MDS. 1B. mutational profile of therapy-related acute myeloid leukemia (t-AML) versus *de novo* AML. Asterisk denotes genes with significant difference between therapy-related versus *de novo* MDS/AML.

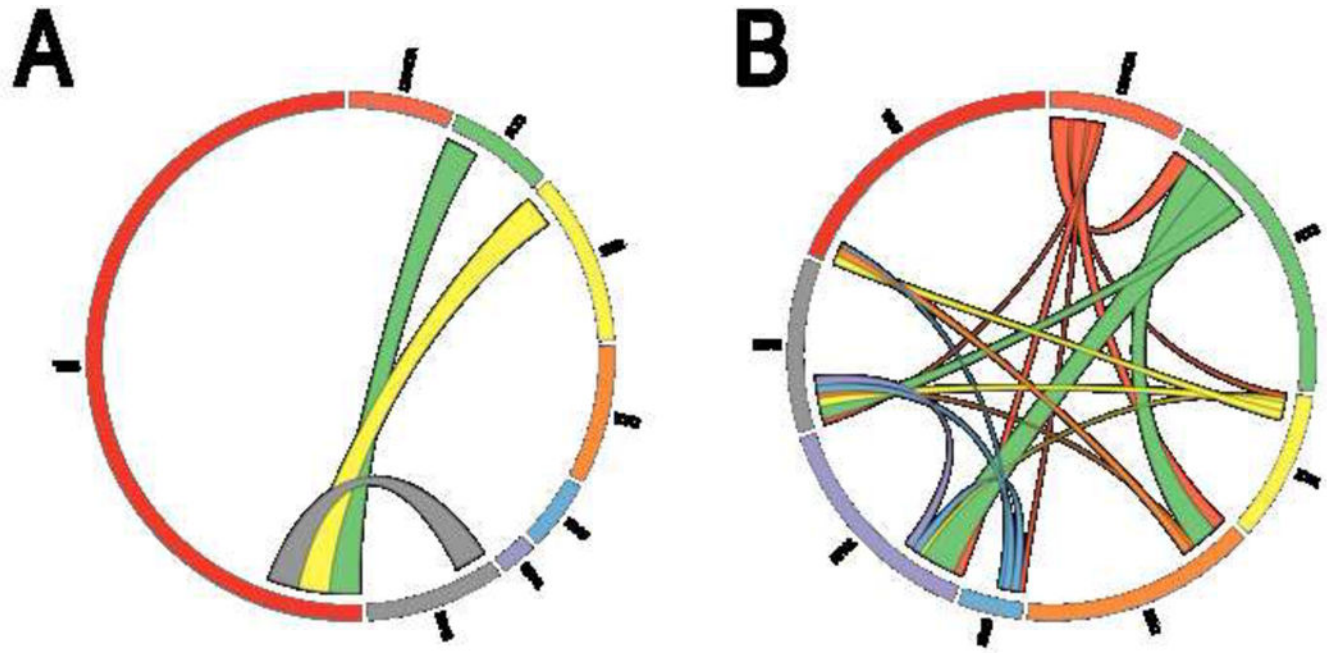

Figure 2.

Difference of mutational pattern between t-MDS/AML and de novo MDS/AML. 2A. Mutational pattern of t-MDS/AML in Circos plot. 2B. Mutational pattern of *de novo* MDS/AML in Circos plot. The thickness of connecting lines between two genes is proportional of the number of such cases. Areas without connecting lines denote cases with single gene mutation.

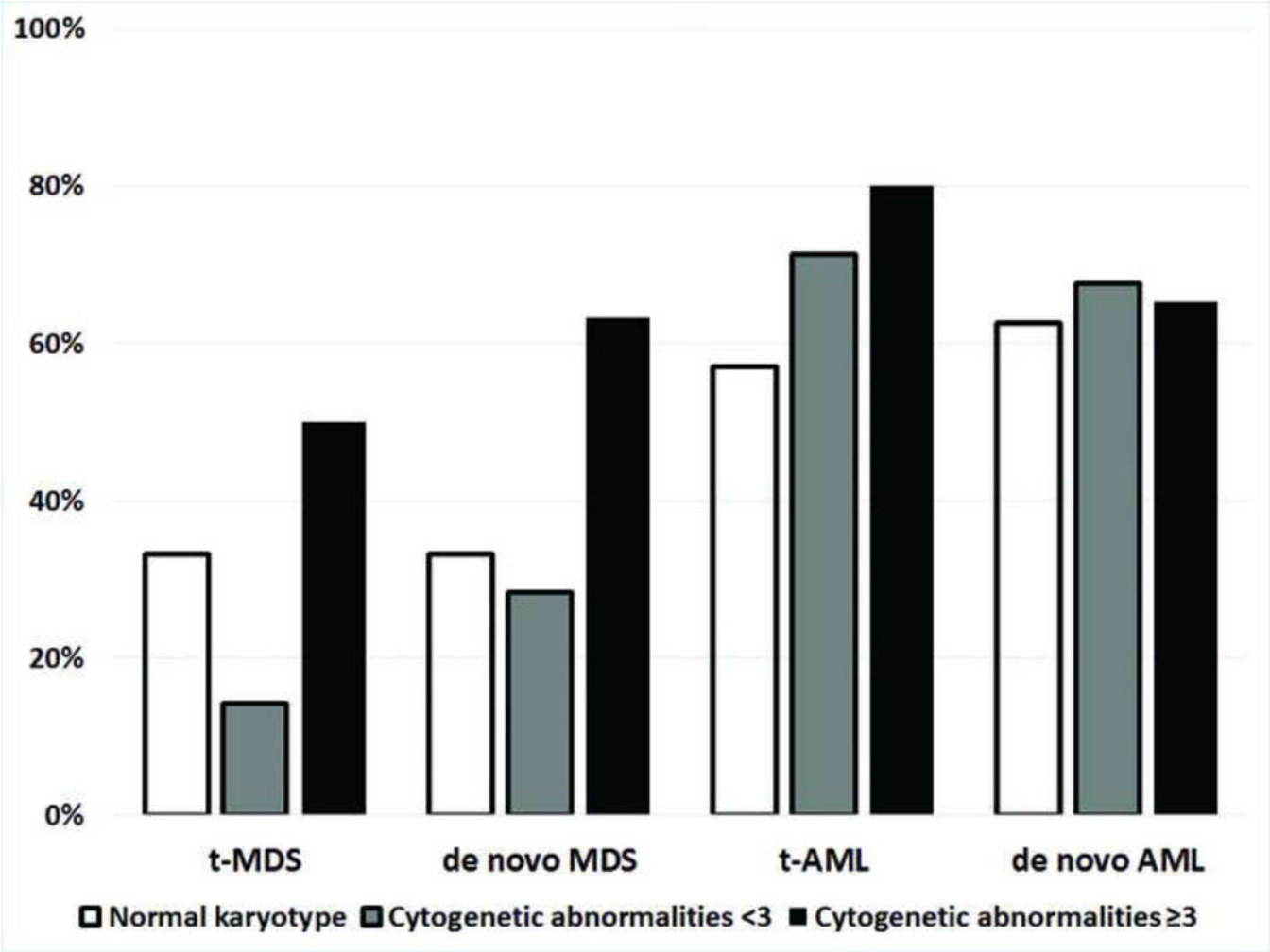

Figure 3.

Percentages of mutations in cases with a normal karyotype, an abnormal non-complex karyotype and a complex karyotype, in different disease subtypes. TP53 denotes mutations in *TP53* gene, and non-TP53 denotes mutations other than *TP53* gene.

Table 1

Complete list of 53 genes in our panel with exons/codons tested in each gene.

	Gene	Exons (codons) tested	Amplicon (number)
48 gene TruSeq Amplicon Cancer Panel (Illumina)	*ABL1*	4 (243–274), 5 (275–303), 6 (303–321), 6 (321–362), 7 (395–424)	5
	AKT1	3 (16–49)	1
	ALK	23 (1172–1175), 25 (1248–1275)	2
	APC	16 (875–918), 16 (1113–1153), 16 (1257–1297), 16 (1288–1328), 16 (1318–1357), 16 (1349–1386), 16 (1377–1416), 16 (1416–1456), 16 (1456–1494), 16 (1493–1530), 16 (1530–1575)	11
	ATM	8 (353–355), 9 (409–412), 12 (601–633), 17 (846–880), 26 (1308–1331), 34 (1678–1719), 35 (1741–1773), 36 (1792–1832), 39 (1940–1973), 50 (2441–2479), 54 (2665–2670), 55 (2694–2717), 56 (2725–2756), 59 (2889–2891), 61 (2946–2950), 63 (3007–3051)	16
	BRAF	11 (439–471), 15 (581–606)	2
	CDH1	3 (77–117), 8 (369–379), 9 (399–439)	3
	CDKN2A	2 (51–70) *	1
	CSF1R	7 (297–301), 22 (926–970)	2
	CTNNB1	3 (12–50)	1
	EGFR	3 (108–142), 7 (288–297), 15 (598–627), 18 (708–728), 19 (729–761), 20 (762–775), 20 (775–817), 21 (857–875)	8
	ERBB2	19 (754–769), 20 (772–818), 21 (839–883)	3
	ERBB4	3 (98–140), 4 (153–186), 6 (208–244), 7 (248–287), 8 (295–306), 9 (333–350), 15 (579–619), 23 (907–936)	8
	FBXW7	5 (243–278), 8 (375–394), 9 (429–471), 10 (473–508), 11 (549–583)	5
	FGFR1	4 (120–126), 7 (247–250)	2
	FGFR2	7 (250–273), 7 (273–311), 7 (302–313), 9 (362–382), 12 (521–550)	5
	FGFR3	7 (247–288), 9 (379–422), 14 (639–653), 15 (654–659), 18 (792–807) and 16 (692–723) *	6
	FLT3	11 (437–456), 14 (569–605), 16 (648–683), 20 (807–843)	4
	GNA11	4 (172–202), 5 (202–216), 6 (255–297), 7 (297–304), 7 (304–349), 7 (349–360) and 4 (159–172) *	7
	GNAQ	4 (159–202), 5 (202–210), 5 (210–245), 5 (241–245), 6 (246–263), 6 (263–297), 6 (291–297), 7 (297–324), 7 (324–360), 7 (355–360)	10
	GNAS	8 (200–220)	1
	HNF1A	3 (205–238), 4 (271–314)	2
	HRAS	2 (1–15), 3 (38–63)	2
	IDH1	4 (90–132)	1
	JAK2	14 (615–622)	1
	JAK3	13 (568–573), 16 (683–723)	2
	KDR	6 (220–248), 7 (267–276), 11 (471–476), 19 (872–874), 21 (946–985), 26 (1135–1146), 27 (1171–1211), 30 (1308–1352), 30 (1352–1357)	9

	Gene	Exons (codons) tested	Amplicon (number)
	KIT	2 (51–93), 9 (502–514), 10 (514–547), 10 (540–549), 11 (550–550), 11 (550–592), 13 (641–664), 14 (670–712), 15 (714–745), 17 (815–828), 18 (838–866)	11
	KRAS	2 (1–22), 3 (38–63), 4 (103–147)	3
	MET	2 (168–209), 2 (375–400), 14 (1008–1028), 16 (1110–1132), 19 (1247–1284)	5
	MLH1	12 (383–426)	1
	MPL	10 (514–522)	1
	NOTCH1	26 (1562–1601), 27 (1673–1679) and 34 (2467–2526) *	3
	NPM1	11 (283–295)	1
	NRAS	2 (1–18), 3 (38–62)	2
	PDGFRA	12 (552–592), 14 (659–668), 15 (673–717), 18 (823–854)	4
	PIK3CA	2 (83–118), 5 (345–353), 8 (418–445), 10 (538–555), 14 (701–729), 21 (988–1027), 21 (1027–1069)	7
	PTEN	1 (5–27), 3 (67–70), 6 (170–210), 7 (212–221), 7 (221–266), 8 (287–332), 8 (332–342)	7
	PTPN11	3 (59–104), 13 (501–533)	2
	RB1	4 (127–158), 6 (199–203), 11 (357–376), 17 (550–565), 18 (570–605), 20 (659–700), 21 (703–733), 22 (746–775)	8
	RET	10 (610–627), 11 (628–667), 13 (766–798), 15 (880–910), 16 (918–934)	5
	SMAD4	3 (119–142), 5 (167–208), 6 (243–263), 8 (310–319), 9 (329–373), 10 (385–424), 11 (443–480), 12 (496–535)	8
	SMARCB1	2 (39–78), 4 (156–167), 5 (199–210), 9 (381–386)	4
	SMO	3 (197–242), 5 (323–366), 6 (403–422), 11 (639–646) and 9 (533–551) *	5
	SRC	14 (530–537) *	1
	STK11	1 (36–77), 6 (261–288), 8 (332–370) and 4 (193–199), 5 (200–211) *	5
	TP53	2 (1–12), 4 (69–112), 5 (126–147), 5 (147–186), 5 (181–187), 6 (187–192), 6 (187–223), 6 (214–224), 7 (225–253), 8 (267–306), 10 (332–342)	11
	VHL	1 (88–114), 2 (129–155), 3 (157–200)	3
5 custom-designed gene panel	*DNMT3A*	23 (866–913)	1
	EZH2	16 (613–644)	1
	IDH2	4 (125–178)	1
	KLHL6	1 (1–13), 1 (13–73), 1 (73–98)	3
	XPO1	14 (501–522), 15 (523–539), 15 (539–575)	3

* Indeterminate amplicons, defined as those having total coverage depth below 250 reads. The remaining are adequately covered amplicons, defined as those having total coverage depth of greater than or equal to 250 reads, or for which orthogonal mutation analysis testing has been performed.

Table 2

Patient characteristics

	t-MDS (n=28)	*de novo* MDS (n=147)	p value
Age (years)	69 (37–81)	70 (14–91)	0.2061
Men:Women	17:11	106:41	0.2610
Hemoglobin (g/L)	96 (72–128)	97 (60–143)	0.5798
Mean corpuscular volume (femtoliter)	92 (79–109)	94 (70–120)	0.6973
White blood cell (× 109/L)	3.1 (0.5–24.9)	2.9 (0.1–14.3)	0.9497
Absolute neutrophil count (× 109/L)	1.2 (0.3–15.4)	1.4 (0–10.0)	0.9641
Platelet (× 109/L)	46.5 (17.0–257.0)	65.0 (3.0–614.0)	0.1423
BM blasts (%)	4.5 (0–18.0)	5.0 (0–19.0)	0.4926
BM cellularity (%)	40 (20–100)	60 (5–100)	0.2364
Cytogenetic risk, n (%)			0.0005
Good	4 (14.3%)	74 (50.3%)	
Intermediate	4 (14.3%)	28 (19.0%)	
Poor	18 (64.3%)	45 (30.6%)	
n/a	2 (7.1%)	0 (0.0%)	
WHO classification, n (%)			
RCUD	0	1 (0.7%)	
RARS	0	2 (1.4%)	
RCMD	12 (42.8%)	58 (39.5%)	
RAEB-1	8 (28.6%)	37 (25.2%)	
RAEB-2	8 (28.6%)	47 (32.0%)	
MDS with isolated del 5q	0	2 (1.4%)	

	t-AML (n=42)	*de novo* AML (n=281)	p value
Age (years)	66.5 (18.0–87.0)	65.0 (1.0–92.0)	0.3842
Men:Women	24:18	165:116	0.8678
Hemoglobin (g/L)	91 (60–118)	9.3 (56–154)	0.5081
Mean corpuscular volume (femtoliter)	91.5 (78.0–112.0)	91 (68–112)	0.6599
White blood cell (× 10^9/L)	2.3 (0.3–93.9)	3.5 (0.3–255.0)	0.0819
Platelet (× 10^9/L)	33.0 (7.0–394.0)	47.0 (3.0–1069.0)	0.1446
BM blasts (%)	31 (2–91)	39 (0–99)	0.1883
BM cellularity (%)	60 (5–100)	80 (5–100)	0.1693
UKMRC cytogenetic risk, n (%)			0.0006
Favorable	2 (4.8%)	26 (9.3%)	
Intermediate	11 (26.2%)	154 (54.8%)	
Unfavorable	28 (66.7%)	94 (33.5%)	
n/a	1 (2.4%)	7 (2.5%)	

BM, bone marrow; n/a, non-available; WHO, World Health Organization; UKMRC, United Kingdom Medical Research Council; RCUD; refractory cytopenia with unilineage dysplasia, RARS; refractory anemia with ring sideroblasts, RCMD; refractory cytopenia with multilineage dysplasia, RAEB; refractory anemia with excess blasts