Published in final edited form as:

Leukemia. 2016 March ; 30(3): 666–673. doi:10.1038/leu.2015.304.

Impact of *TP53* mutation variant allele frequency on phenotype and outcomes in myelodysplastic syndromes

DA Sallman¹, R Komrokji¹, C Vaupel², T Cluzeau^{1,3,4,5,6}, SM Geyer⁷, KL McGraw¹, NH Ali¹, J Lancet¹, MJ McGinniss², S Nahas², AE Smith⁸, A Kulasekararaj⁸, G Mufti⁸, A List¹, J Hall², E Padron¹

¹Department of Malignant Hematology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

²Genoptix, Inc., a Novartis company, Carlsbad, CA, USA

³Hematology Department, CHU of Nice, Nice, France

⁴Faculty of Medicine, University Nice Sophia Antipolis, Nice, France

⁵Mediterranean Center of Molecular Medicine, INSERM U1065, Nice, France

⁶French Group of Myelodysplasia, Paris, France

⁷Health Informatics Institute, University of South Florida, Tampa, FL, USA

⁸King's College London School of Medicine, Department of Haematological Medicine, Rayne Institute, King's College London School of Medicine, London, UK

Abstract

Although next-generation sequencing has allowed for the detection of somatic mutations in myelodysplastic syndromes (MDS), the clinical relevance of variant allele frequency (VAF) for the majority of mutations is unknown. We profiled *TP53* and 20 additional genes in our training set of 219 patients with MDS or secondary acute myeloid leukemia with findings confirmed in a validation cohort. When parsed by VAF, *TP53* VAF predicted for complex cytogenetics in both the training ($P = 0.001$) and validation set ($P < 0.0001$). MDS patients with a *TP53* VAF $> 40\%$ had a median overall survival (OS) of 124 days versus an OS that was not reached in patients with VAF $< 20\%$ (hazard ratio (HR), 3.52; $P = 0.01$) with validation in an independent cohort (HR, 4.94, $P = 0.01$). *TP53* VAF further stratified distinct prognostic groups independent of clinical prognostic scoring systems ($P = 0.0005$). In multivariate analysis, only a *TP53* VAF $> 40\%$ was an independent covariate (HR, 1.61; $P < 0.0001$). In addition, *SRSF2* VAF predicted for monocytosis

Correspondence: Dr E Padron, Department of Malignant Hematology, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612, USA. eric.padron@moffitt.org.

AUTHOR CONTRIBUTIONS

DS and EP designed the research, analyzed data and wrote the paper. MM, SN, KM, NA, AS and AK collected the data and gave final approval. RK, TC and SG analyzed data and gave the final approval. CV and JH collected the data, reviewed the paper and gave the final approval. JL, GM and AL reviewed the paper and gave the final approval.

CONFLICT OF INTEREST

CV, MM, SN and JH are employees of Genoptix, Inc., a Novartis company and own stock in the company. The other authors declare no conflict of interest.

Supplementary Information accompanies this paper on the Leukemia website (<http://www.nature.com/leu>)

($P = 0.003$), *RUNX1* VAF with thrombocytopenia ($P = 0.01$) and *SF3B1* with ringed sideroblasts ($P = 0.001$). Together, our study indicates that VAF should be incorporated in patient management and risk stratification in MDS.

INTRODUCTION

Myelodysplastic syndromes (MDS) represent a diverse group of hematopoietic stem cell malignancies characterized by ineffective hematopoiesis, dysplasia and a propensity for transformation to acute myeloid leukemia (AML). Central to the heterogeneity of MDS is the genetic diversity, which drives the clinical phenotype and prognosis of MDS patients. Targeted next-generation sequencing (NGS) of 111 genes in a large cohort of patients ($n = 738$) with MDS and MDS-myeloproliferative neoplasms (MDS-MPN) identified pathogenic mutations in 74%, with 43% of patients having two or three molecular and/or cytogenetic abnormalities.¹ Furthermore, striking genotype–phenotype associations were demonstrated for several recurrently mutated genes. For example, *SF3B1* and co-mutations of *SRSF2/TET2* are highly specific for refractory anemia with ringed sideroblasts (RS) and chronic myelomonocytic leukemia (CMML), respectively.^{1–4}

TP53 mutations have been reported in 5–10% of MDS patients, although enriched in those patients with deletion of 5q (del(5q)) or complex cytogenetics.^{5–7} Several reports have shown that mutations involving *ASXL1*, *ETV6*, *EZH2*, *RUNX1* and *TP53* portend an inferior overall survival (OS); and complement clinical scoring systems with improved prognostic discrimination.⁶ More recently, mutations of *TP53*, *DNMT3A* and/or *TET2* have been shown to predict inferior outcomes for patients undergoing allogeneic bone marrow (BM) transplantation.⁸ Although hypomethylating agents (HMA) remain the standard of care for higher risk MDS patients, *TP53* mutation has been associated with inferior survival in comparison with *TP53* wild-type (WT) patients treated with HMA despite no difference in response rates.^{9,10} Further, *TP53* mutation appears to be the critical driver of poor survival in patients with complex cytogenetics as patients with WT *TP53* and complex cytogenetics do not experience inferior outcomes in the context of treatment with HMA or BM transplantation.^{8–10}

NGS is being increasingly incorporated into clinical management decisions and the evaluation of patients with MDS and MDS-MPN. However, current results of NGS are reported and interpreted based solely on the presence or absence of mutations of interest without regard to the mutation allelic burden. Variant allele frequency (VAF) of somatic mutations has been used to reconstruct the clonal architecture in MDS and secondary AML and demonstrated to be intimately associated with the natural history and clinical course of these conditions, suggesting that the relative amount of mutation variants is actionable.¹¹ In addition, other myeloid malignancies such as MPN have been reported to have key phenotypic differences associated with the allelic burden of *JAK2 V617F*.¹² We therefore sought to investigate the clinical importance of VAF in highly recurrent gene mutations for which genotype–phenotype relationships have been characterized in MDS.

SUBJECTS AND METHODS

Patients

The training set was obtained from patients at Moffitt Cancer Center (MCC) who had NGS performed and a diagnosis of MDS, CMML, MDS/MPN, secondary AML or *de novo* AML with MDS features according to World Health Organization (WHO) criteria.¹³ Pathology review was performed at MCC. From May 2013 to October 2014, 219 patients were identified in the training set that met the above clinical criteria and had at least one pathogenic mutation. Clinical characteristics were abstracted from the date of mutational analysis. This study was approved by the MCC Scientific Review Committee and institutional review board.

The validation set for *TP53* mutation was obtained from MDS patients who had NGS performed at Genoptix with peripheral blood and cytogenetic information ($n = 150$). Genoptix-profiled patients with a *RUNX1* mutation and a CBC were identified to evaluate for association with thrombocytopenia ($n = 41$). The validation set also included co-mutated *TET2* and *SRSF2* or *ZRSR2* patients who had a CBC with differential for evaluation of monocytosis ($n = 78$ and 10 , respectively) and *SF3B1* mutated patients with pathologic quantitation of erythroid RS ($n = 35$).

Gene mutation analysis

Genomic DNA was extracted from mononuclear cells of BM aspirate or peripheral blood (Supplementary Table S1). Recent data have shown strong concordance (>95%) for both mutation identification and VAF between peripheral blood and BM samples.¹⁴ Selected exons and flanking sequences of 21 myeloid genes (*ASXL1*, *EZH2*, *ETV6*, *RUNX1*, *TP53*, *CBL*, *DNMT3A*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *MPL*, *NPM1*, *NRAS*, *PHF6*, *SETBP1*, *SF3B1*, *SRSF2*, *TET2*, *U2AF1* and *ZRSR2*) were amplified by multiplex PCR. Libraries were created (560 amplicons across 117 coding regions) using a Fluidigm Access Array system (Fluidigm Corporation, South San Francisco, CA, USA). Amplicon libraries were split to be sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) in duplicate. The lower limit of detection of this clinically validated assay was set at 5% mutant allele reads and the minimum depth of coverage was 500×. Alignment and variant calling were performed using NextGENe software (SoftGenetics, LLC, State College, PA, USA). Variant calls were then compared between the duplicate samples and annotated and filtered according to established guidelines using software that queried a variety of databases containing known germline and somatic variants (Supplementary Methods).¹⁵ Variant assessment and classifications were performed by board-certified molecular geneticists. Synonymous variants, noncoding variants more than six bases from splice junctions or germline polymorphisms at a population frequency of $\geq 1\%$ were excluded. VAF was derived by counting the number of variant reads divided by the number of reference reads detected for any given genomic position and reported as a percentage.

For validation of *TP53* VAF and survival analyses, an independent cohort ($n = 30$) was obtained from King's College Hospital, London, UK. In this cohort, *TP53* mutations were

analyzed using the Roche GS FLX sequencing platform (Roche, Indianapolis, IN, USA) as described previously.¹⁶

Statistical analysis

Survival analyses were evaluated utilizing the Kaplan–Meier method and curves compared via the log-rank test. OS was calculated from the date of mutation analysis to the date of death. Surviving patients were censored from the last date the patient was known to be alive. Multivariate Cox regression models were created to adjust for clinical and treatment characteristics with a backward selection algorithm incorporating variables with $P < 0.1$ on univariate analysis. Categorical and continuous variables were compared by Fisher's exact test and Mann–Whitney's test, respectively. Optimal cutpoints were evaluated using recursive partitioning algorithms in relation to OS using the R statistical package rpart. All tests were two-sided and considered statistically significant if $P < 0.05$.

RESULTS

Patient characteristics

The clinical characteristics of patients in the training set ($n = 219$) are summarized in Table 1 according to *TP53* gene mutation status. Overall, *TP53* WT was detected in 172 patients, whereas a *TP53* mutation (*TP53* MT) was detected in 47. The median follow-up in the training set was 12 months (range 6–23 months). The demographics were similar in the *TP53* MT and *TP53* WT cohorts with a median age of 70 years and male predominance. All patients with CMML were *TP53* WT, which is concordant with the previous observation that *TP53* mutation in CMML is a rare event.^{1,17} Patients with a *TP53* mutation, as expected, had higher risk features relative to the WT counterparts with respect to cytopenias, BM blasts and abnormal karyotype (91% in *TP53* MT patients versus 50% in *TP53* WT patients; $P < 0.0001$). Consequently, patients with *TP53* mutation had higher rates of secondary AML in comparison with *TP53* WT patients ($P = 0.03$). At a median follow-up of 12 months, 62% of *TP53* MT patients had died compared with 28% in *TP53* WT patients.

The mutation spectrum of the training set is shown in Figure 1a. Of note, 16% of patients ($n = 37$) had analysis of only *ASXL1*, *ETV6*, *EZH2*, *RUNX1* and *TP53*. The mutational spectrum of our cohort was enriched for higher risk mutations in comparison with previously published cohorts in regards to mutation of *ASXL1* (32%), *TP53* (21%), *DNMT3A* (15%) and *RUNX1* (14%).^{1,5–7} Consistent with a higher risk cohort, mutations of *SF3B1*, which are associated with improved OS in MDS, were present in 13% of patients, a fraction that is lower than previous studies.^{2,3,18} Missense mutations represented the most common aberration in *TP53* MT patients accounting for 85% overall ($n = 40$; Supplementary Table S2). As previously reported, *TP53* MT strongly predicted for the presence of complex karyotype, occurring in 75% of *TP53* MT patients versus only 6% of *TP53* WT patients (Figure 1b; $P < 0.0001$).^{6,7}

TP53 VAF and complex cytogenetics

To investigate the effect of *TP53* clonal burden on chromosomal complexity, we stratified our *TP53* MT patients from highest to lowest VAF and the presence of complex karyotype,

which demonstrated a striking relationship between VAF and frequency of karyotype complexity (Figure 1c). The median VAF was significantly higher in patients with complex karyotype than noncomplex patients (49% versus 14%; $P = 0.001$; Figure 2b). Next, we analyzed *TP53* MT patients stratified by those with a VAF >40% versus a VAF < 20%. An *a priori* VAF cutpoint of >40% was chosen because it is likely to enrich for mutants residing in a dominant clone as reported in multiple recent studies.^{18–21} To confirm that a VAF >40% is a suitable cut point that enriches for mutations within the dominant clone, we analyzed those *TP53* mutant patients with a VAF >40% who harbored additional mutations and confirmed that a clonal burden >40% was the highest VAF when compared with other acquired mutations.

In our training set, all *TP53* MT patients ($n = 21$) with a VAF >40% had a complex karyotype versus 54% of patients ($n = 7$) with a VAF < 20% ($P = 0.001$; Figure 2a). In addition, *TP53* MT patients with a VAF >40% infrequently had additional mutations versus patients with a lower VAF (20% versus 46%; $P = 0.07$; Supplementary Figure S1), a finding consistent with recent studies.^{7,8} In our validation set of 150 patients with a *TP53* mutation, a striking association of VAF with complex cytogenetics was again observed occurring in 78% of patients ($n = 46$) with a dominant clone and only 38% of patients ($n = 18$) with a VAF < 20% ($P < 0.0001$; Figure 2c). Patients with a complex karyotype in the validation set also had a significantly increased median VAF in comparison with noncomplex patients (40% versus 20%; $P < 0.0001$; Figure 2d). To further evaluate the association of *TP53* VAF and complex cytogenetics in our validation set, we further stratified VAF into three cohorts and again found a significant association between VAF and complex karyotype ($P = 0.0002$; Figure 2e).

***TP53* VAF and survival**

Because *TP53* mutation status is an important risk factor for OS in MDS, we next evaluated the clinical impact of *TP53* VAF on OS. As expected, *TP53* mutation predicated for an inferior OS confirming previously reported data (162 days versus not reached; hazard ratio (HR), 2.64; 95% confidence interval (CI) 2.00–6.31; $P < 0.0001$; Figure 3a).⁶ Patients with complex cytogenetics and *TP53* mutation had a median OS of 161 days versus 374 days in *TP53* WT patients with complex cytogenetics (HR, 2.08; 95% CI 0.80–4.34; $P = 0.16$; Supplementary Figure S2).⁸ In addition, *TP53* MT patients with additional molecular abnormalities had similar survival to those with isolated *TP53* mutation (Supplementary Figure S3).

We next examined the impact of VAF on the prognosis of patients with *TP53* mutation compared with binary mutational analysis alone. Patients with a *TP53* VAF >40% had a poor OS of only 124 days in contrast to a median OS that was not reached in patients with VAF < 20% (HR, 3.52; 95% CI 1.24–6.50; $P = 0.01$; Figure 3b). Furthermore, stratification of *TP53* VAF into three distinct categories (VAF >40%, VAF 20–40% and VAF < 20%) inversely correlated with inferior OS ($P = 0.02$; Figure 3c). External validation of an independent cohort of 30 *TP53* mutant patients from the King's College demonstrated that patients with a *TP53* VAF >40% had a median survival of 272 days compared with 1372 days with *TP53* VAF < 20% (HR 4.94, 95% CI 1.43–9.21; $P = 0.01$; Figure 3d), confirming

our initial findings. As a continuous measure in *TP53* mutant patients across both cohorts ($n = 77$), increasing *TP53* VAF was significantly associated with inferior OS ($P = 0.01$). Using a recursive partitioning algorithm to stratify our data relative to OS, an optimal VAF cutpoint was identified at 20% (Supplementary Figure S4). Specifically, median OS with *TP53* VAF $< 20\%$ was 685 days versus 183 days with VAF $\geq 20\%$ ($P = 0.0036$). Furthermore, there was no survival difference based on VAF in regards to the other most commonly mutated genes in the training set including *ASXL1*, *TET2*, *DNMT3A* and *RUNX1*.

Because binary output from genetic profiling refines MDS prognosis above that achieved with the international prognostic scoring system (IPSS) alone,⁶ we sought to determine whether *TP53* VAF would complement current risk models. Our analysis focused on *TP53* VAF in higher risk MDS because the majority of *TP53* MT patients had intermediate-2/high risk disease by the IPSS (74%, $n = 26$). In patients with intermediate-2 risk disease ($n = 12$), the presence of *TP53* mutation was predictive of an inferior survival with a median OS of 93 days versus 297 days in *TP53* WT patients (HR, 3.15; 95% CI 1.45–12.53; $P = 0.009$; Figure 4a). Interestingly, *TP53* VAF further refined poor prognosis in patients with intermediate-2 risk disease. Patients with a VAF $> 40\%$ had a median OS of 55 days compared with 154 days in those with VAF $< 40\%$, and 297 days in *TP53* WT patients ($P = 0.0005$; Figure 4b). These findings were also recapitulated in patients with IPSS-defined high risk disease ($n = 14$) when similarly stratified by *TP53* mutation status (HR, 2.56; 95% CI 1.14–7.54; $P = 0.03$; Figure 4c).⁶ When stratified by *TP53* VAF, high risk *TP53* MT patients demonstrated an inverse correlation between VAF and OS, which was not statistically significant ($P = 0.07$; Figure 4d).

Because our data suggest that analysis of *TP53* VAF refines the prognosis of MDS compared with binary mutational analysis, we developed a multivariate Cox model to determine the impact of VAF versus binary mutational analysis for prognosis (Table 2). In regards to disease-modifying therapy, there was no difference in utilization of HMA therapy or allogeneic BM transplantation based on *TP53* VAF (Supplementary Table S3). In univariate analysis, only a trend for significance in patients with a *TP53* VAF of 20–40% (HR, 2.13; 95% CI 0.91–9.32; $P = 0.07$) and no significance in patients with a VAF $< 20\%$ (HR, 1.18; 95% CI 0.40–3.57; $P = 0.75$) was identified with respect to OS. Further, in our final multivariable Cox model that incorporates age, sex, BM transplant status and IPSS, only the mutational status of *TP53* (HR, 1.47; 95% CI 1.16–1.86; $P = 0.002$) and *TP53* mutation VAF $> 40\%$ retained prognostic significance (HR, 1.61; 95% CI 1.25–2.08; $P < 0.0001$). The above analyses were also validated with incorporation of the revised-IPSS (RIPSS).

VAF is associated with phenotype penetrance across multiple genes

Because we have shown that *TP53* VAF correlates strongly with complex karyotype, we hypothesized that VAF could influence the genotype–phenotype association with additional gene mutations in MDS patients. Malcovati *et al.*¹⁸ previously reported the association of *SF3B1* VAF with an increase in BM percentage of RS. Given the lower proportion of *SF3B1* MT patients in our training set, we sought to investigate this previous finding in our validation set in patients with an absolute quantity of RS on the BM ($n = 35$). Although the distribution of *SF3B1* MT VAF was Gaussian rather than bimodal in our cohort, which

differs from the previously mentioned study, *SF3B1* VAF indeed strongly predicted for the percentage of RS with median RS of 60% in patients with a dominant clone versus 15% in patients with a VAF < 20% ($P = 0.001$; Supplementary Figure S5).

Co-mutation of *TET2* and *SRSF2* or *ZRSR2* has been found to be highly specific for CMML with a specificity of 98.4% in a cohort of 308 patients.⁴ Therefore, we investigated the impact of *SRSF2* and/or *ZRSR2* VAF on monocytosis in our data set. *SRSF2* MT VAF strongly predicted for the presence of monocytosis defined as a monocyte count of greater than 1000/dl, 83% ($n = 10$) with *SRSF2* VAF >40% versus 33% ($n = 5$) with *SRSF2* VAF < 20% ($n = 78$; $P = 0.003$; Figure 5a). Further, the *SRSF2* median VAF was significantly higher in patients with monocytosis (32% versus 18%; $P = 0.0003$; Figure 5b) and was also predictive of the degree of monocytosis (median monocyte count 1750 versus 550; $P = 0.004$; Figure 5c). Although limited numbers, *ZRSR2* VAF in *TET2/ZRSR2* MT patients did not correlate with the presence of monocytosis ($n = 10$; $P = 0.37$; Figure 5d).

Because severe thrombocytopenia has been reported to be associated with mutation of *RUNX1*,^{6,22} we next investigated whether *RUNX1* VAF could predict for the presence of thrombocytopenia (platelet count < $150 \times 10^9/l$) and/or its severity. Indeed, thrombocytopenia was influenced by the VAF of *RUNX1* mutation with a median *RUNX1* MT VAF of 31% in thrombocytopenic patients versus 12% in those without thrombocytopenia within our training set ($P = 0.01$; Figure 5e), which approached significance in the validation set (median VAF 24% versus 11%; $P = 0.09$; Figure 5f). Patients with a *RUNX1* MT VAF >40% had lower platelet counts than patients with a VAF < 20% in both the training set ($29 \times 10^9/l$ versus $48 \times 10^9/l$; $P = 0.53$; Figure 5g) and validation set ($54 \times 10^9/l$ versus $77 \times 10^9/l$; $P = 0.10$; Figure 5h), although this did not reach statistical significance.

DISCUSSION

The optimization of genomics in routine clinical MDS management remains an evolving challenge. Although the importance of VAF is implied across many genetic MDS studies, the clinical impact of VAF has been largely unexplored.^{1,18} Furthermore, there was no difference in leukemia-free survival when mutations involving *TET2*, *SF3B1*, *ASXL1*, *SRSF2*, *CBL*, *EZH2* and *RUNX1* were identified in the dominant clone versus a subclone.^{1,18}

To comprehensively investigate the clinical relevance of mutation VAF in MDS, we molecularly profiled 219 patients and characterized clinical variables at the time of mutation analysis. Because *TP53* mutation is a critical adverse risk factor in MDS,^{6,16,23–25} we focused our efforts on the influence of *TP53* VAF on outcome by leveraging a training set ($n = 47$), a validation set ($n = 150$) and an external independent cohort ($n = 30$) that collectively represents among the largest MDS cohorts assembled to date for the clinical evaluation of *TP53* mutation. Mutations of *TP53* have historically been associated with complex cytogenetics that was also evident in our study.^{23,24,26} However, we show that VAF strongly influences this association with the vast majority of patients with a *TP53* mutation in the dominant clone having a complex karyotype. Furthermore, *TP53* VAF was directly

proportional to the absolute number of cytogenetic abnormalities (Supplementary Figure S6). This has particular clinical relevance because of the inferior survival in patients with five or more chromosome abnormalities compared with patients with three to four abnormalities.²⁵ We also report that the mutation VAF is strongly associated with specific clinical phenotypes across multiple gene mutations, suggesting that allelic burden is likely to influence phenotype across the genetic spectrum of MDS. Specifically, *SRSF2* mutation in co-mutated *TET2/SRSF2* patients was strongly concordant with monocytosis. Furthermore, we also identified *RUNX1* VAF to predict for the presence and severity of thrombocytopenia and confirmed the association of *SF3B1* VAF with BM RS percentage. Together, these data indicate the importance of allelic burden in clinical phenotype in patients with MDS and perhaps other myeloid malignancies.

Although multivariable analyses validated the independent negative prognostic impact of *TP53* mutation,⁶ our Cox regression model highlights that this is dependent on its location within the clonal hierarchy as only patients with a VAF >40% had inferior survival. Notably, patients with *TP53* VAF of 20–40% had a trend for inferior survival in univariate analysis and no survival difference in *TP53* MT patients with a VAF < 20%, suggesting that VAF is a critical determinant of prognosis in MDS patients. Furthermore, we validated our survival findings in an independent cohort and identified a VAF threshold of 20% as the optimal cutoff to stratify survival. Although our data suggest that VAF of recurrent mutations influences clinical characteristics, we fully recognize that clonal hierarchy is a dynamic process. Notably, recent data identified that an increase in *TP53* VAF was greatest at the time of progression to higher risk disease or AML.²⁷ This highlights the potential importance of serial analysis, particularly in the examination of *TP53* VAF and suggests that *TP53* may be uniquely connected to adverse clinical trajectories.

Another distinct issue is that recent molecular characterization of large cohorts of patients with no known hematologic disease demonstrated that clonal hematopoiesis is a common event with age occurring in approximately 5–10% of patients over the age of 65.^{28–30} Although the overall risk of progression to overt hematologic malignancy was uncommon (1% per year), the risk was significantly higher in patients with a higher clonal burden with a 50-fold increase in patients with a VAF > 10%.²⁹ Although *TP53* mutations were overall a rare event in these studies, they did occur in 1–5% of patients profiled. Whether mutations with a low VAF truly impart the same prognosis should be explored.

The present study highlights the value of VAF across MDS mutations and strongly suggests that *TP53* VAF should be incorporated into routine clinical prognostication and treatment decisions of MDS. They further support a critical need for sequential investigation of patients with MDS to improve realtime prognostication and genetically inform treatment-related decisions such as BM transplantation and HMA therapy. International collaborative efforts are ongoing to definitively characterize the mutational spectrum of MDS and its influence on clinical phenotype and outcomes.²⁵

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

REFERENCES

1. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood* 2013; 122: 3616–3627. [PubMed: 24030381]
2. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature* 2011; 478: 64–69. [PubMed: 21909114]
3. Papaemmanuil E, Cazzola M, Boultonwood J, Malcovati L, Vyas P, Bowen D et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N Engl J Med* 2011; 365: 1384–1395. [PubMed: 21995386]
4. Malcovati L, Papaemmanuil E, Ambaglio I, Elena C, Galli A, Della Porta MG et al. Driver somatic mutations identify distinct disease entities within myeloid neoplasms with myelodysplasia. *Blood* 2014; 124: 1513–1521. [PubMed: 24970933]
5. Jonveaux P, Fenaux P, Quiquandon I, Pignon JM, Lai JL, Loucheux-Lefebvre MH et al. Mutations in the p53 gene in myelodysplastic syndromes. *Oncogene* 1991; 6: 2243–2247. [PubMed: 1766671]
6. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G et al. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med* 2011; 364: 2496–2506.
7. Kulasekararaj AG, Smith AE, Mian SA, Mohamedali AM, Krishnamurthy P, Lea NC et al. TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. *Br J Haematol* 2013; 160: 660–672. [PubMed: 23297687]
8. Bejar R, Stevenson KE, Caughey B, Lindsley RC, Mar BG, Stojanov P et al. Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation. *J Clin Oncol* 2014; 32: 2691–2698. [PubMed: 25092778]
9. Bejar R, Lord A, Stevenson K, Bar-Natan M, Perez-Ladaga A, Zaneveld J et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. *Blood* 2014; 124: 2705–2712. [PubMed: 25224413]
10. Bally C, Ades L, Renneville A, Sebert M, Eclache V, Preudhomme C et al. Prognostic value of TP53 gene mutations in myelodysplastic syndromes and acute myeloid leukemia treated with azacitidine. *Leuk Res* 2014; 38: 751–755. [PubMed: 24836762]
11. Walter MJ, Shen D, Ding L, Shao J, Koboldt DC, Chen K et al. Clonal architecture of secondary acute myeloid leukemia. *N Engl J Med* 2012; 366: 1090–1098. [PubMed: 22417201]
12. Passamonti F, Rumi E. Clinical relevance of JAK2 (V617F) mutant allele burden. *Haematologica* 2009; 94: 7–10. [PubMed: 19118374]
13. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 2009; 114: 937–951. [PubMed: 19357394]
14. Mohamedali AM, Gaken J, Ahmed M, Malik F, Smith AE, Best S et al. High concordance of genomic and cytogenetic aberrations between peripheral blood and bone marrow in myelodysplastic syndrome (MDS). *Leukemia* 2015; 29: 1928–1938. [PubMed: 25943179]
15. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17: 405–424. [PubMed: 25741868]
16. Jadersten M, Saft L, Smith A, Kulasekararaj A, Pomplun S, Gohring G et al. TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. *J Clin Oncol* 2011; 29: 1971–1979. [PubMed: 21519010]
17. Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M et al. Prognostic score including gene mutations in chronic myelomonocytic leukemia. *J Clin Oncol* 2013; 31: 2428–2436. [PubMed: 23690417]
18. Malcovati L, Papaemmanuil E, Bowen DT, Boultonwood J, Della Porta MG, Pascutto C et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. *Blood* 2011; 118: 6239–6246. [PubMed: 21998214]

19. Mian SA, Smith AE, Kulasekararaj AG, Kizilors A, Mohamedali AM, Lea NC et al. Spliceosome mutations exhibit specific associations with epigenetic modifiers and proto-oncogenes mutated in myelodysplastic syndrome. *Haematologica* 2013; 98: 1058–1066. [PubMed: 23300180]
20. Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. *Blood* 2013; 122: 4021–4034. [PubMed: 24136165]
21. Klcó JM, Spencer DH, Miller CA, Griffith M, Lamprecht TL, O’Laughlin M et al. Functional heterogeneity of genetically defined subclones in acute myeloid leukemia. *Cancer Cell* 2014; 25: 379–392. [PubMed: 24613412]
22. Chen CY, Lin LI, Tang JL, Ko BS, Tsay W, Chou WC et al. RUNX1 gene mutation in primary myelodysplastic syndrome--the mutation can be detected early at diagnosis or acquired during disease progression and is associated with poor outcome. *Br J Haematol* 2007; 139: 405–414. [PubMed: 17910630]
23. Kita-Sasai Y, Horiike S, Misawa S, Kaneko H, Kobayashi M, Nakao M et al. International prognostic scoring system and TP53 mutations are independent prognostic indicators for patients with myelodysplastic syndrome. *Br J Haematol* 2001; 115: 309–312. [PubMed: 11703325]
24. Horiike S, Kita-Sasai Y, Nakao M, Taniwaki M. Configuration of the TP53 gene as an independent prognostic parameter of myelodysplastic syndrome. *Leuk Lymphoma* 2003; 44: 915–922. [PubMed: 12854888]
25. Bejar R, Papaemmanuil E, Haferlach T, Garcia-Manero G, Maciejewski JP, Sekeres MA et al. TP53 mutation status divides MDS patients with complex karyotypes into distinct prognostic risk groups: Analysis of combined datasets from the International Working Group for MDS-Molecular Prognosis Committee. 56th ASH Annual Meeting and Exposition 6-9 December 2014, vol. 124: San Francisco, CA, pp 532–532.
26. Kaneko H, Misawa S, Horiike S, Nakai H, Kashima K. TP53 mutations emerge at early phase of myelodysplastic syndrome and are associated with complex chromosomal abnormalities. *Blood* 1995; 85: 2189–2193. [PubMed: 7718890]
27. Pellagatti A, Roy S, Genua CD, Burns A, McGraw K, Valletta S et al. Targeted re-sequencing analysis of 31 genes commonly mutated in myeloid disorders in serial samples from myelodysplastic syndrome patients showing disease progression. *Leukemia* 2016; 30: 248–250.
28. Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *New Engl J Med* 2014; 371: 2477–2487. [PubMed: 25426838]
29. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 2014; 371: 2488–2498. [PubMed: 25426837]
30. Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med* 2014; 20: 1472–1478. [PubMed: 25326804]

Figure 1.

Mutational spectrum across patients profiled at Moffitt Cancer Center. **(a)** A total of 219 patients were identified by next-generation sequencing for mutation in a 21-gene panel. Each column represents an individual patient and each colored box represents mutation of the gene listed to the left. The presence of complex cytogenetics is shown in black for each individual patient in the most inferior row. Bar graph on right shows distribution of each mutation. *TP53* VAF and number of mutations for each *TP53* mutated patient are depicted in the superior and inferior bar graphs, respectively. **(b)** Presence of complex cytogenetics in *TP53* mutant (*TP53* MT) and *TP53* wild-type (*TP53* WT) patients. **(c)** *TP53* VAF for the 47

TP53 MT patients along with presence of complex cytogenetics. Dashed bar highlights patients with *TP53* mutation with additional somatic mutations.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2.

TP53 VAF strongly predicts for complex cytogenetics in the training (**a, b**) and validation sets (**c–e**). (**a**) Presence of complex cytogenetics in patients with *TP53* mutation with VAF >40% versus < 20%. (**b**) Median *TP53* VAF in patients with and without complex cytogenetics in the training set. (**c**) Presence of complex cytogenetics in patients with *TP53* mutation with VAF >40% versus < 20%. (**d**) Median *TP53* VAF in patients with and without complex cytogenetics in the validation set. (**e**) Association of complex cytogenetics stratified across three VAF cohorts.

Figure 3.

OS by *TP53* mutation status and *TP53* VAF. (a) OS of patients stratified by *TP53* mutation status. OS of patients (b) stratified by *TP53* VAF >40% versus < 20% and (c) across three VAF cohorts. (d) In our validation cohort, OS of patients stratified by *TP53* VAF >40% versus < 20%.

Figure 4.

OS by *TP53* VAF and IPSS risk category. OS of intermediate-2 (int-2) patients (a) and high risk patients (c) stratified by *TP53* mutation status. (b) OS of int-2 patients and high risk patients (d) stratified by the mutational VAF of *TP53* in comparison with *TP53* WT patients.

Figure 5.

VAF is associated with phenotype penetrance across multiple genes. (a) Presence of monocytosis (monocyte count > 1000 cells/dl) in co-mutated *TET2*/*SRSF2* mutant patients stratified by *SRSF2* VAF. (b) Median *SRSF2* VAF in patients with co-mutated *TET2*/*SRSF2* with and without monocytosis. (c) Absolute monocyte count in *TET2*/*SRSF2* mutant patients with *SRSF2* VAF > 40% versus < 20%. (d) Median *ZRSR2* VAF in *TET2*/*ZRSR2* mutant patients with and without monocytosis. Median *RUNX1* VAF in patients with and without thrombocytopenia in the training (e) and validation (f) sets. Median platelet counts in patients with *RUNX1* mutation with VAF > 40% versus < 20% in the training (g) and validation (h) sets.

Table 1.

Baseline characteristics of the study population

Sample	Training set	TP53 wild-type cohort	TP53 mutated cohort
Sample size with outcome data	219	172	47
Median (range) follow-up	12 (6–23) months	12 (6–23) months	12 (6–19) months
<i>Demographics</i>			
<i>Sex</i>			
Male	145 (66%)	117 (68%)	28 (60%)
Female	74 (34%)	55 (32%)	19 (40%)
Age, mean $\pm$ s.d.	70 $\pm$ 10 years	72 $\pm$ 10 years	69 $\pm$ 11 years
<i>MDS classification</i>			
RA	5 (2%)	5 (2%)	0%
RARS	12 (4%)	11 (7%)	1 (2%)
RCMD	45 (21%)	38 (22%)	7 (15%)
RAEB-1	30 (14%)	24 (14%)	6 (13%)
RAEB-2	30 (14%)	21 (12%)	9 (19%)
5q-	8 (4%)	5 (3%)	3 (6%)
CMML-1	22 (10%)	22 (13%)	0%
CMML-2	6 (3%)	6 (4%)	0%
sAML	32 (15%)	20 (12%)	12 (26%)
<i>De novo</i> AML with MDS features	11 (5%)	3 (2%)	8 (17%)
MDS-U	3 (1%)	2 (1%)	1 (2%)
MDS-MPN	15 (7%)	15 (9%)	0%
<i>Blood counts (at time of mutation analysis)</i>			
Neutrophil count, mean $\pm$ s.d.	3.8 $\pm$ 8.1 $\times 10^9$ per liter	4.3 $\pm$ 9.0 $\times 10^9$ per liter	1.7 $\pm$ 2.2 $\times 10^9$ per liter ^a
Hemoglobin, mean $\pm$ s.d.	9.9 $\pm$ 5.9 g/dl	10.1 $\pm$ 6.5 g/dl	9.3 $\pm$ 1.6 g/dl
Platelet count, mean $\pm$ s.d.	132 $\pm$ 164 $\times 10^9$ per liter	145 $\pm$ 176 $\times 10^9$ per liter	83 $\pm$ 101 $\times 10^9$ per liter ^a
<i>Bone marrow (at time of mutation analysis)</i>			
Bone marrow blasts $\geq$ 5%	103 (48%)	68 (40%)	35 (75%) ^a
<i>Karyotype</i>			

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

	Training set	TP53 wild-type cohort	TP53 mutated cohort
Normal	109 (50%)	105 (61%)	4 (9%) ^a
Abnormal	110 (50%)	67 (39%)	43 (91%) ^a
Complex	45 (21%)	10 (6%)	35 (75%) ^a
Outcome			
Leukemia (transformation or sAML)	64 (29%)	43 (25%)	21 (45%) ^a
Died	77 (35%)	48 (28%)	29 (62%) ^a

Abbreviations: CMML, chronic myelomonocytic leukemia; MDS/MPN, myelodysplastic syndrome/myeloproliferative neoplasm; MDS-U, myelodysplastic syndrome unclassified; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RARS, refractory anemia with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; sAML, secondary acute myeloid leukemia.

^a $P < 0.05$ for comparison between TP53 mutated and TP53 wild-type cohorts.

Table 2.

Prognostic impact of *TP53* mutation and *TP53* VAF in the training set in univariate (log-rank tests) and multivariate^a (Cox models) analyses

Variable	Univariate			Multivariate		
	Median OS (days)	P	HR	95% CI	P	
<i>TP53</i> mutation present versus absent	162	NR	1.47	1.16–1.86	0.002	
<i>TP53</i> VAF >40% versus absent	124	NR	1.61	1.25–2.08	< 0.0001	
<i>TP53</i> VAF 20–40% versus absent	202	NR	0.98	0.58–1.67	0.94	
<i>TP53</i> VAF < 20% versus absent	NR	NR				

Abbreviations: HR, hazard ratio; OS, overall survival; NR, not reached; VAF, variant allele frequency.

^aMultivariate model was created from Cox regression model that included age, sex, bone marrow transplant status and International Prognostic Scoring System (IPSS) risk category, which included number of cytopenias, bone marrow blast percentage and cytogenetics.