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Prognostic Mutations in Myelodysplastic Syndrome after Stem-Cell Transplantation

R.C. Lindsley, **W. Saber**, **B.G. Mar**, **R. Redd**, **T. Wang**, **M.D. Haagenson**, **P.V. Grauman**, **Z.-H. Hu**, **S.R. Spellman**, **S.J. Lee**, **M.R. Verneris**, **K. Hsu**, **K. Fleischhauer**, **C. Cutler**, **J.H. Antin**, **D. Neuberg**, and **B.L. Ebert**

Abstract

BACKGROUND—Genetic mutations drive the pathogenesis of the myelodysplastic syndrome (MDS) and are closely associated with clinical phenotype. Therefore, genetic mutations may predict clinical outcomes after allogeneic hematopoietic stem-cell transplantation.

METHODS—We performed targeted mutational analysis on samples obtained before transplantation from 1514 patients with MDS who were enrolled in the Center for International Blood and Marrow Transplant Research Repository between 2005 and 2014. We evaluated the association of mutations with transplantation outcomes, including overall survival, relapse, and death without relapse.

RESULTS—*TP53* mutations were present in 19% of the patients and were associated with shorter survival and a shorter time to relapse than was the absence of *TP53* mutations, after adjustment for significant clinical variables (P<0.001 for both comparisons). Among patients 40 years of age or older who did not have *TP53* mutations, the presence of RAS pathway mutations was associated with shorter survival than was the absence of RAS pathway mutations (P= 0.004), owing to a high risk of relapse, and the presence of *JAK2* mutations was associated with shorter survival than was the absence of *JAK2* mutations (P = 0.001), owing to a high risk of death without relapse. The adverse prognostic effect of *TP53* mutations was similar in patients who received reduced-intensity conditioning regimens and those who received myeloablative conditioning regimens. By contrast, the adverse effect of RAS pathway mutations on the risk of relapse, as compared with the absence of RAS pathway mutations, was evident only with reduced-intensity conditioning (P<0.001). In young adults, 4% of the patients had compound heterozygous mutations in the Shwachman–Diamond syndrome–associated *SBDS* gene with concurrent *TP53* mutations and a poor prognosis. Mutations in the p53 regulator *PPM1D* were more common among patients with therapy-related MDS than those with primary MDS (15% vs. 3%, P<0.001).

CONCLUSIONS—Genetic profiling revealed that molecular subgroups of patients undergoing allogeneic hematopoietic stem-cell transplantation for MDS may inform prognostic stratification

Address reprint requests to Dr. Ebert at Brigham and Women's Hospital, 1 Blackfan Circle, Karp Research Bldg. 5.211, Boston, MA 02115, or at bebert@partners.org.

The authors' full names, academic degrees, and affiliations are listed in the Appendix.

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and the selection of conditioning regimen. (Funded by the Edward P. Evans Foundation and others.)

The myelodysplastic syndrome (MDS) is clinically and biologically heterogeneous. In children and young adults, MDS can arise in the context of congenital mutations that cause bone marrow failure syndromes or inherited predisposition to myeloid cancers.[1] Therapy-related MDS develops as a late complication in patients with previous exposure to chemotherapy, radiation therapy, or both.[2] In most patients, however, primary MDS arises in the absence of an identified exposure, prodromal bone marrow failure syndrome, or inherited predisposition.

Although allogeneic hematopoietic stem-cell transplantation is the only curative therapy for MDS, mortality after transplantation is high, with deaths attributable to relapsed disease and to transplant-related complications. Predicting which patients are most likely to benefit from transplantation is thus a central challenge. Improvements in the identification of patients who are most likely to have a relapse and those who are most at risk for death without relapse could lead to the refinement of conditioning regimens and relapse-prevention strategies. The genetic lesions that drive the pathogenesis of MDS predict overall survival and many aspects of clinical phenotype and may contribute to the outcome of patients after hematopoietic stem-cell transplantation.[3–5]

Current prognostic models regarding transplantation in patients with MDS incorporate a number of factors that are related to the characteristics of the patient, the disease, and the transplant itself but not to molecular genetic characteristics.[6–8] Recently, somatic mutations have been linked to survival outcomes after transplantation, but previous studies have not comprehensively evaluated the effect of clinical and transplantation variables.[3,9] Here we report a comprehensive analysis of genes related to MDS and bone marrow failure in a broadly representative cohort of patients of all ages with rigorously defined MDS who underwent allogeneic stem-cell transplantation.

METHODS

PATIENTS

All the patients with MDS who were enrolled in the Center for International Blood and Marrow Transplant Research (CIBMTR) repository and research database and for whom comprehensive report form–level data had been collected between 2005 and 2014 were considered for inclusion in this study. Patients were not included if the percentage of blasts in the bone marrow or blood was 20% or more or if they had received a diagnosis of chronic myelomonocytic leukemia or overlap myelodysplastic–myeloproliferative neoplasms. A total of 1520 of 2990 eligible patients had banked samples available for analysis, and 1514 samples met the technical requirements for analysis. Patients were included from 130 of the 177 transplantation centers that perform allogeneic hematopoietic stem-cell transplantation for MDS in participating countries (Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). All the samples were frozen whole blood, which had been archived immediately before the administration of the preparative conditioning regimen.

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The clinical characteristics of the cohort are listed in Tables S2, S3, and S4 in the Supplementary Appendix. All the patients provided written informed consent to participate in the CIBMTR research database and repository. The study was conducted with the approval of the institutional review board at the Dana–Farber Cancer Institute.

GENETIC STUDIES

Genetic profiling included the targeted sequencing of 129 genes, which had been selected on the basis of their known or suspected involvement in the pathogenesis of myeloid cancers or inherited or acquired bone marrow failure syndromes. Annotation of the mutations was blinded with regard to clinical characteristics, and the genetic analysis was locked before being merged with clinical data. Detailed sequencing information is provided in the Methods section in the Supplementary Appendix.

STATISTICAL ANALYSIS

Overall survival was defined as the time from transplantation until death from any cause or until censoring at the time that the patient was last known to be alive. The analysis was performed with the use of a Cox proportional-hazards model. P values for likelihood-ratio tests were reported for covariates in univariate models, and P values for Wald tests were reported for covariates in multivariable models. Death without relapse was defined as any death while the patient was in clinical remission, with relapse as a competing risk, and was assessed with the use of Gray's test. For relapse, death without relapse was considered to be a competing risk. Multivariable modeling for both relapse and death without relapse was performed with the use of the Fine–Gray model. Recursive partitioning was used to generate a hierarchical model that integrated nonmodifiable clinical and genetic characteristics. Adjusted P values were calculated with the use of the Benjamini–Hochberg correction. Additional details are provided in the Methods section in the Supplementary Appendix.

RESULTS

GENETIC CHARACTERISTICS AND SPECTRUM OF MUTATIONS

We identified at least 1 mutation in 1196 of 1514 patients (79%) who had undergone hematopoietic stem-cell transplantation, with a median of 2 driver mutations per patient (range, 0 to 15). Mutations that are associated with higher-risk MDS according to the International Prognostic Scoring System, including *TP53* and *DNMT3A*, were more prevalent in our cohort than in published cohorts of patients with MDS who had not undergone transplantation, whereas mutations that are associated with lower-risk MDS, such as *SF3B1*, were less prevalent — findings that were consistent with the clinical practice to prioritize higher-risk cases for transplantation (Fig. 1A).[4,5] Complete details are provided in Table S8 in the Supplementary Appendix.

CLINICAL AND GENETIC DETERMINANTS OF OUTCOMES

To identify the mutations associated with overall survival, we evaluated the 32 genes that were mutated in at least 20 patients in the study cohort. Genes that were mutated less frequently were subject to descriptive analysis. Certain mutations were significantly associated, as compared with the absence of the mutation, with shorter overall survival,

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including *TP53* (hazard ratio for death, 1.96; 95% confidence interval [CI], 1.69 to 2.28; adjusted P<0.001) (Fig. 1B), *PPM1D* (hazard ratio, 1.64; 95% CI, 1.27 to 2.12; adjusted P = 0.002), and *JAK2* (hazard ratio, 1.77; 95% CI, 1.21 to 2.58; adjusted P = 0.03). No mutations were associated with prolonged survival.

Nongenetic factors that were related to the patient, disease, or the transplant itself have previously been shown to influence transplantation outcomes, including overall survival, relapse, and death without relapse. As expected, the variables that were significantly associated with shorter survival in univariate analyses included patient-related factors such as a recipient age of 40 years or more (hazard ratio vs. an age of <40 years, 2.05; 95% CI, 1.66 to 2.53; P<0.001) and a Karnofsky performance-status score less than 90 (on a scale from 0 to 100, on which 100 indicates fully active and asymptomatic, and lower numbers indicate greater disability; hazard ratio vs. a score of ≥90, 1.51; 95% CI, 1.29 to 1.77; P<0.001); transplant-related factors such as HLA mismatch (hazard ratio vs. HLA match, 1.25; 95% CI, 1.07 to 1.47; P = 0.006); and disease-related components of standard prognostic risk models for MDS, such as an elevated percentage of bone marrow blasts (>5%), severe thrombocytopenia ($<50\times10^9$ platelets per liter), and complex or monosomal karyotype. MDS that was linked to predisposing conditions, such as inherited bone marrow failure syndromes, aplastic anemia, or paroxysmal nocturnal hemoglobinuria, was associated with longer survival (hazard ratio vs. the absence of such conditions, 0.74; 95% CI, 0.57 to 0.96; P=0.02) (Table 1). Complete lists of univariate clinical and genetic associations with overall survival are provided in Tables S9 through S12 in the Supplementary Appendix.

In multivariable analyses, only mutations in *TP53* were independently associated with shorter survival (hazard ratio for death vs. no *TP53* mutation, 1.71; 95% CI, 1.45 to 2.02; P<0.001) (Fig. 1C). *TP53* mutations were also associated with a shorter time to relapse (hazard ratio vs. no *TP53* mutation, 2.03; 95% CI, 1.60 to 2.58; P<0.001), as were RAS pathway mutations (*NRAS*, *KRAS*, *PTPN11*, *CBL*, *NF1*, *RIT1*, *FLT3*, and *KIT*) (hazard ratio vs. no RAS pathway mutation, 1.56; 95% CI, 1.18 to 2.05; P=0.002). *JAK2* V617F mutations were associated with a higher rate of death without relapse (hazard ratio vs. no *JAK2* V617F mutation, 2.10; 95% CI, 1.36 to 3.24; P<0.001) but not a higher rate of relapse (hazard ratio, 0.68; 95% CI, 0.35 to 1.33; P = 0.26). The results of the multivariable Cox model for overall survival and the Fine–Gray model for the rates of relapse and death without relapse, along with adjusted covariates for each model, are provided in Tables S13, S14, and S15 in the Supplementary Appendix.

GENETIC CHARACTERISTICS OF *TP53* MUTATIONS

Since the presence of *TP53* mutations was the most powerful predictor of survival, we analyzed whether the consequences of such mutations depended on the particular mutation type, genetic context, or abundance within the tumor. Among 423 *TP53* mutations found in 289 patients, the median variant allele fraction (i.e., the fraction of alleles in the sample that were mutated) was 10% (range, 2 to 86). However, a *TP53* variant allele fraction of 10% or higher was not significantly associated with survival (hazard ratio for death vs. a variant allele fraction of <10%, 1.28; 95% CI, 0.98 to 1.66; P = 0.07). Similarly, the presence of

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multiple *TP53* mutations was not significantly associated with survival (hazard ratio vs. single *TP53* mutations, 1.20; 95% CI, 0.91 to 1.61; P = 0.18).

The majority of *TP53* mutations were missense substitutions affecting the DNA binding domain that can confer a precise alteration in TP53 function.[10] However, among patients with *TP53* mutations, 45 patients with only truncating *TP53* mutations (16%) had shorter survival than patients who had missense mutations (hazard ratio for death, 1.61; 95% CI, 1.20 to 2.67; P = 0.005), which indicated a distinct biologic activity of loss-of-function *TP53* mutations. Complete genetic details regarding the *TP53* mutations are provided in Figures S4, S5, and S6 in the Supplementary Appendix.

PPM1D AND *TP53* MUTATIONS AND A DISTINCT PATHWAY OF THERAPY-RELATED MDS

Patients with therapy-related MDS represented 21% of the study cohort (311 patients) and had significantly shorter survival than patients with primary MDS (hazard ratio for death, 1.34; 95% CI, 1.15 to 1.57; P<0.001). We found that *TP53* was mutated significantly more frequently among patients with therapy-related MDS than among those with primary MDS (38% vs. 14%, adjusted P<0.001); similar results were observed for the TP53 regulator *PPM1D* (15% vs. 3%, adjusted P<0.001) (Fig. 2A, and Table S16 in the Supplementary Appendix).

Mutations in *PPM1D* or *TP53* were present in 46% of the patients with therapy-related MDS and were mutated concurrently more frequently than would be expected by chance (adjusted P<0.001) (Fig. S17 in the Supplementary Appendix). As compared with patients with neither *PPM1D* nor *TP53* mutations (14% of whom had therapy-related MDS), patients with *PPM1D* mutations alone (51%) had a similarly elevated frequency of therapy-related MDS as those with *TP53* mutations alone (39%) or those with concurrent *PPM1D* and *TP53* mutations (54%) (P<0.001 for all comparisons) (Fig. 2B). The presence of *TP53* mutations has been strongly associated with complex chromosomal abnormalities.[11] Although PPM1D is a phosphatase that regulates TP53 activity, patients who had *PPM1D* mutations without concurrent *TP53* mutations had a similar frequency of complex karyotype as patients with neither mutation (3% and 2%, respectively) (Fig. 2C).

Among patients with therapy-related MDS, the presence of *TP53* mutations was associated with shorter overall survival (hazard ratio for death with vs. without *TP53* mutations, 1.63; 95% CI, 1.22 to 2.20; P<0.001). Among patients without *TP53* mutations, survival was similar among patients with therapy-related MDS and those with primary MDS (hazard ratio, 1.10; 95% CI, 0.89 to 1.35; P = 0.37). Despite being highly associated with therapy-related MDS, *PPM1D* mutations were not associated with an adverse prognosis in patients who had therapy-related MDS without *TP53* mutations (hazard ratio for death with vs. without *PPM1D* mutations, 1.26; 95% CI, 0.71 to 2.24; P= 0.39).

GENETIC DETERMINANTS OF OUTCOME IN CHILDREN AND YOUNG ADULTS

Patients younger than 40 years of age made up 16% of the cohort (241 of 1514 patients), which enabled us to identify age-related pathways of MDS pathogenesis and to evaluate their clinical effect. We first determined whether particular mutations were more common in children and young adults (<40 years of age) or in older adults (≥40 years of age). Mutations

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in five genes, *TET2*, *DNMT3A*, *SRSF2*, *SF3B1*, and *PPM1D*, were significantly more common in older adults than in children and young adults (Fig. 3A, and Table S17 in the Supplementary Appendix). Three mutations were significantly more common in younger patients than in older ones: *GATA2* (11% vs. 2%, adjusted P<0.001), *PIGA* (5% vs. <1%, adjusted P<0.001), and compound heterozygous mutations in the Shwachman–Diamond syndrome–associated *SBDS* gene (2% vs. <1%, adjusted P<0.001). *PIGA* mutations are found in patients with MDS as well as in those with paroxysmal nocturnal hemoglobinuria and aplastic anemia.[5,12] *GATA2* and *SBDS* mutations are associated with germline bone marrow failure syndromes that predispose patients to the development of myeloid cancers.[13,14]

Patients younger than 40 years of age who had compound heterozygous *SBDS* mutations had remarkably shorter survival than did those without such variants (median survival, 1.2 years vs. not reached; P=0.009). However, patients with *GATA2* or *PIGA* mutations had a favorable prognosis that was typical of younger patients in the study cohort (Fig. 3B).

The Shwachman–Diamond syndrome is a rare congenital bone marrow failure syndrome that is caused by compound heterozygous germline *SBDS* mutations. Persons with single germline *SBDS* mutations are asymptomatic carriers. Although only two of the seven patients with biallelic *SBDS* mutations in our cohort had clinically diagnosed Shwachman–Diamond syndrome, several characteristics suggest that the biallelic *SBDS* mutations that were identified in our study were germline: *SBDS* variant allele fractions were consistent with germline heterozygous mutations (median variant allele fraction, 42%; 95% CI, 37 to 47), and all the mutations that we identified have been found previously in patients with clinically confirmed Shwachman–Diamond syndrome.[15,16] Patients with biallelic *SBDS* mutations were significantly and uniformly younger (median age, 25.1 years; 95% CI, 18.2 to 38.2) than those with a single mutated *SBDS* allele (median age, 59.0 years; 95% CI, 48.6 to 64.4) or with wild-type *SBDS* (median age, 59.3 years; 95% CI, 58.7 to 60.3). Patients with biallelic *SBDS* mutations were in a significantly lower height percentile than those with a single mutated *SBDS* allele (P = 0.02) (Fig. S16 in the Supplementary Appendix).

All the patients with biallelic *SBDS* mutations had somatic *TP53* mutations (median variant allele fraction, 16%; 95% CI, 8 to 20), a finding that was consistent with their poor survival. *TP53* mutations were significantly more frequent among patients with biallelic *SBDS* mutations than among those with one *SBDS* mutation (P = 0.01) or no *SBDS* mutation (P<0.001) (Fig. S16 in the Supplementary Appendix). Taken together, these data suggest that the genetically defined Shwachman–Diamond syndrome may be underdiagnosed and that somatic acquisition of *TP53* mutations mediates the progression to MDS.

INTEGRATION OF CLINICAL AND GENETIC VARIABLES

As an alternate method of analysis, we used recursive partitioning to identify six distinct subgroups of patients with MDS on the basis of the association of clinical and genetic variables with overall survival (Fig. 4). Consistent with our multivariable model, the presence of *TP53* mutations was the most important prognostic variable, since it identified a subgroup of patients with diverse clinical characteristics that were unified by poor survival

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and a high risk of relapse. No further stratification of the patients with *TP53* mutations was identified by the algorithm.

Among the 1011 patients 40 years of age or older who had MDS without *TP53* mutation, we identified two subgroups with poor survival. Patients with a RAS pathway mutation had shorter survival than those without RAS pathway mutation (median, 0.9 vs. 2.2 years; P = 0.004), and the shorter survival was associated with a higher risk of relapse (P = 0.001). By contrast, patients with *JAK2* mutations had shorter survival than those without *JAK2* mutations (median, 0.5 vs. 2.3 years; P = 0.001), but they had a strikingly high risk of death without relapse (P<0.001). The majority of patients 40 years of age or older without *TP53* mutations had neither *JAK2* nor RAS pathway mutations, and this subgroup had a median survival of 2.3 years.

Among 214 patients younger than 40 years of age without *TP53* mutations, we identified two subgroups on the basis of evaluation of high-risk clinical features. Young patients with at least one high-risk feature (therapy-related MDS, platelet count of $<30\times10^9$ per liter at the time of transplantation, or a percentage of bone marrow blasts of ≥15% at diagnosis) had a median overall survival of 2.6 years, which was associated with a high risk of death without relapse, as compared with patients with no high-risk features (P<0.001). Among patients with no high-risk features, the median survival was not reached, and the survival rate at 3 years was 82%.

EFFECT OF CONDITIONING INTENSITY ON OUTCOMES

The intensity of the conditioning regimen before transplantation has the potential to influence the risks of relapse and of death without relapse. Whereas myeloablative conditioning is generally associated with a lower risk of relapse and higher rate of death without relapse, reduced-intensity conditioning is associated with a higher risk of relapse and a lower rate of death without relapse.[17]

Among patients with *TP53* mutations, the median survival was similar with myeloablative conditioning and with reduced-intensity conditioning (7.5 months and 9.2 months, respectively; P = 0.19), and there was no significant difference in the cumulative incidence of relapse, which suggests that high-intensity conditioning regimens do not influence the relapse-driven survival disadvantage of *TP53*-mutated MDS (Fig. S14 in the Supplementary Appendix). By contrast, we observed a higher risk of relapse among patients with RAS pathway–mutated MDS only in the context of reduced-intensity conditioning, with a 1-year cumulative incidence of relapse of 42% among patients with RAS pathway mutations versus 20% among those without RAS pathway mutations (P<0.001). This effect of RAS pathway mutations on relapse was not observed with myeloablative conditioning; the 1-year cumulative incidence of relapse was similar among patients with RAS pathway mutations and those without such mutations (22% and 15%, respectively; P = 0.31) (Fig. S14 in the Supplementary Appendix).

The higher cumulative incidence of death without relapse among patients with *JAK2* mutations than among those without *JAK2* mutations was similar regardless of the intensity of the conditioning regimen. Among patients who received a reduced-intensity conditioning

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regimen, 1-year non-relapse-related mortality was 53% among those with *JAK2* mutations versus 25% among those without *JAK2* mutations (P = 0.007). Among patients who received a myeloablative conditioning regimen, 1-year nonrelapse-related mortality was 50% among those with *JAK2* mutations versus 28% among those without *JAK2* mutations (P=0.003) (Fig. S14 and Tables S19 and S20 in the Supplementary Appendix). Overall, these findings indicate that high-intensity conditioning regimens may benefit patients with RAS pathway mutations but not those with *TP53* or *JAK2* mutations.

DISCUSSION

Assessment of gene mutations may help predict survival among patients with MDS undergoing allogeneic stem-cell transplantation,[3,9] but previous studies have not evaluated a large number of genetic risks or the extent to which genetic risk interacts with clinical variables. In this study, we paired genetic analysis with comprehensive clinical annotation in a large, registry-based cohort. We found that *TP53* mutations were the most powerful predictor of survival after transplantation among patients with MDS, independent of clinical factors such as age, Karnofsky performance-status score, and hematologic variables. The effect of *TP53* mutations on the risks of relapse and death was not attenuated by myeloablative conditioning regimens, a finding that is consistent with clinical and experimental evidence showing *TP53* mutation–mediated chemoresistance.[11,18,19] Our data suggest that the escalation of the intensity of the conditioning regimen in order to improve outcomes in patients with *TP53* mutated MDS will not be successful.

Mutations that drive activated RAS–mitogen-activated protein kinase (MAPK) signaling are late events in myeloid leukemogenesis that are linked to the leukemic transformation of MDS and to an adverse prognosis in patients who have not undergone transplantation.[20–22] We found that among patients with MDS who were 40 years of age or older, those with detectable RAS pathway mutations at the time of transplantation had a significantly elevated risk of early relapse that may be overcome by myeloablative conditioning. RAS pathway mutations may thus reflect the presence of low-volume but biologically transformed disease that, without adequate cytoreduction before transplantation, outpaces the development of effective graft-versus-leukemia activity. By contrast, *JAK2* mutations were associated with a higher rate of death without relapse but not a higher risk of relapse, regardless of conditioning intensity. The mechanism of these effects of *JAK2* mutations is unknown. In this context, early death without relapse may be driven by potentiated Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signaling in persistent *JAK2* mutated cells and therefore may be susceptible to targeting by pharmacologic JAK2 inhibition.

We observed highly recurrent *PPM1D* mutations in patients with MDS and found that such mutations, along with *TP53* mutations, were strongly associated with previous exposure to leukemogenic therapies. *PPM1D* encodes a serine–threonine protein phosphatase that regulates the cellular response to environmental stress, in part by means of inhibition of TP53 activity,[23,24] which suggests that *TP53* and *PPM1D* mutations represent convergent mechanisms of clonal survival in the context of leukemogenic exposures. Our results are consistent with the association between *PPM1D* mutations and asymptomatic clonal

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hematopoiesis after chemotherapy[22–27] and provide strong genetic evidence of the role of *PPM1D* mutations in the pathogenesis of therapy-related MDS.

Shwachman–Diamond syndrome–associated compound heterozygous mutations in *SBDS* were unexpectedly common in young adults, representing 4% of the patients with MDS who were 18 to 40 years of age, and were associated with a poor prognosis. Our results are consistent with the varied clinical presentation of patients with the Shwachman–Diamond syndrome and indicate that clinical criteria alone may be inadequate to identify all diagnoses of the Shwachman–Diamond syndrome.[19] Moreover, the close connection between putative germline *SBDS* mutations and somatic *TP53* mutations indicates a biologic synergy between *SBDS* and *TP53* mutations in the clonal MDS transformation of genetically defined Shwachman–Diamond syndrome. These results may influence donor selection and suggest that early transplantation in patients with the Shwachman–Diamond syndrome should be considered, since transplantation after MDS transformation may not offer long-term benefit.

Our data call into question the benefit of conventional myeloablative conditioning over reduced-intensity approaches in patients with *TP53*-mutated MDS. These patients, who have an exceptionally high risk of relapse-related death after transplantation, should be considered for investigative approaches to conditioning or new relapse-prevention strategies after transplantation. Patients with RAS pathway–mutated MDS, among whom the risk of early relapse is elevated only when reduced-intensity regimens are used for conditioning, may benefit from myeloablative conditioning when the safety profile is acceptable. By contrast, strategies that are focused on minimizing toxic effects would be useful in older patients with *JAK2* mutations, who have poor survival that is driven by a significantly elevated risk of death without relapse. In conclusion, our findings show that analysis of mutations in patients with MDS at the time of transplantation can predict outcomes and identify subgroups of patients who will derive the most benefit from particular conditioning regimens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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APPENDIX

The authors' full names and academic degrees are as follows: R. Coleman Lindsley, M.D., Ph.D., Wael Saber, M.D., Brenton G. Mar, M.D., Ph.D., Robert Redd, M.S., Tao Wang, Ph.D., Michael D. Haagenson, M.S., Peter V. Grauman, B.A., Zhen-Huan Hu, M.P.H., Stephen R. Spellman, Ph.D., Stephanie J. Lee, M.D., Michael R. Verneris, M.D., Katharine Hsu, M.D., Ph.D., Katharina Fleischhauer, M.D., Corey Cutler, M.D., M.P.H., Joseph H. Antin, M.D., Donna Neuberg, Sc.D., and Benjamin L. Ebert, M.D., Ph.D.

The authors' affiliations are as follows: the Department of Medical Oncology, Division of Hematological Malignancies (R.C.L., C.C., J.H.A.), and the Departments of Pediatric Oncology (B.G.M.) and Biostatistics and Computational Biology (R.R., D.N.), Dana–Farber Cancer Institute, and the Division of Hematology, Brigham and Women's Hospital, Harvard Medical School (P.V.G., B.L.E.) — all in Boston; the Center for International Blood and Marrow Transplant Research, Medical College of Wisconsin, Milwaukee (W.S., T.W., Z.-H.H., S.J.L.); the Center for International Blood and Marrow Transplant Research, National Marrow Donor Program–Be the Match (M.D.H., S.R.S.), and the Pediatric Blood and Marrow Transplantation Center, University of Minnesota (M.R.V.) — both in Minneapolis; the Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle (S.J.L.); Memorial Sloan Kettering Cancer Center, New York (K.H.); and the Institute for Experimental Cellular Therapy, University Hospital Essen, Essen, Germany (K.F.).

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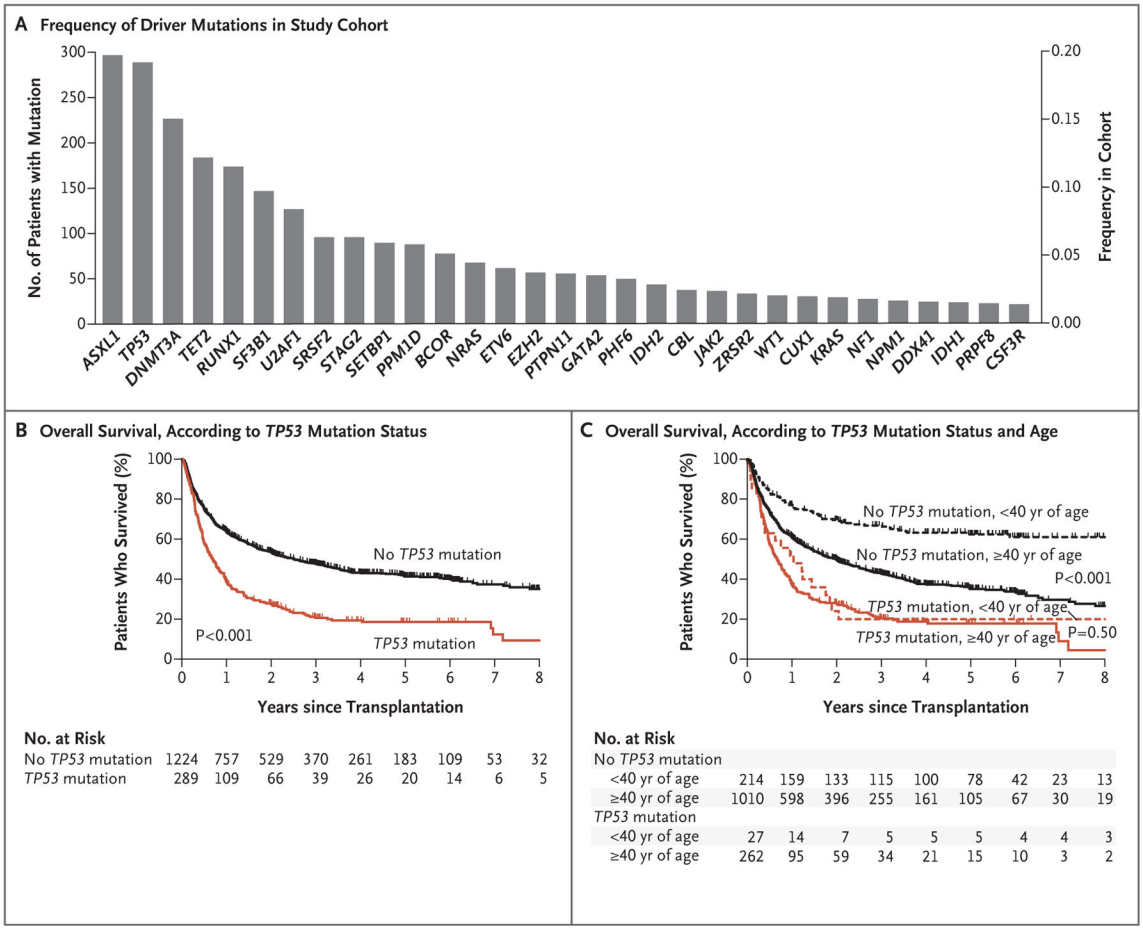

Figure 1. Frequency of Driver Mutations and Age-Independent Association of *TP53* Mutations with Poor Overall Survival

Panel A shows the frequency of driver mutations in the study cohort; included are the 32 genes that were evaluated in the multivariable models. A complete list of mutation frequencies is provided in Table S7 in the Supplementary Appendix. Panel B shows Kaplan–Meier curves for overall survival according to *TP53* mutation status. Tick marks indicate censored data. Panel C shows Kaplan–Meier curves for overall survival among patients with *TP53* mutations (red) or without *TP53* mutations (black), according to age. Dashed lines represent patients younger than 40 years of age, and solid lines patients 40 years of age or older. Among patients without *TP53* mutations, younger patients had longer survival than older patients (hazard ratio for death, 0.54; 95% confidence interval [CI], 0.45 to 0.65; P<0.001). Among patients with *TP53* mutations, survival was similar among younger patients and older patients (hazard ratio, 0.86; 95% CI, 0.56 to 1.31; P = 0.50).

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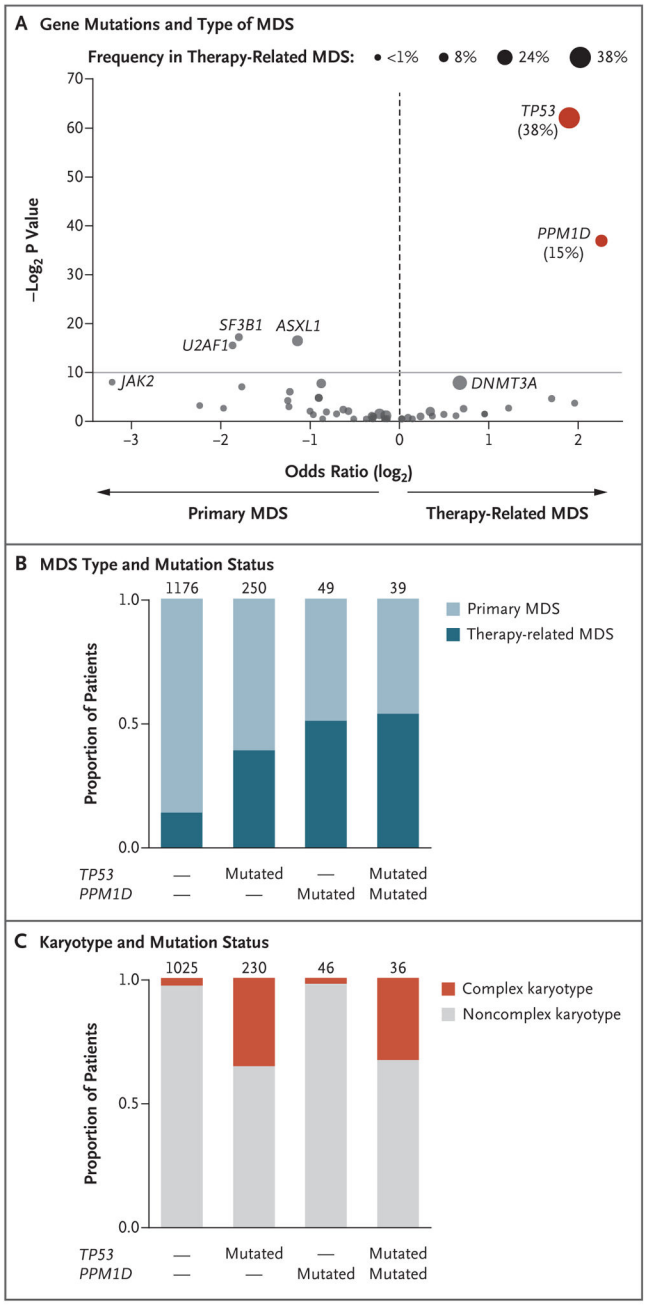

Figure 2. *PPM1D* and *TP53* Mutations Associated with Therapy-Related MDS

Panel A shows the association between gene mutations and therapy-related MDS or primary MDS. A volcano plot was constructed by plotting the negative log of the P value on the y axis; the x axis shows the magnitude of association ($\log_2$ odds ratio), and the y axis the $-\log_2$ P value. The gray line represents the threshold of significance determined by correcting for multiple hypothesis testing. Each circle represents a mutated gene, and the size of each circle corresponds to the frequency of the mutation among patients with therapy-related MDS, as indicated. Genes in the upper right quadrant were significantly associated with therapy-related MDS (red), and genes in the upper left quadrant with

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primary MDS. Panel B shows the proportion of patients with primary MDS or therapy-related MDS according to *TP53* and *PPM1D* mutation status. Panel C shows the proportion of patients with complex karyotype (>3 alterations) or noncomplex karyotype (≤3 alterations) according to *TP53* and *PPM1D* mutation status. The numbers above the bars in Panels B and C show the total number of patients in each group.

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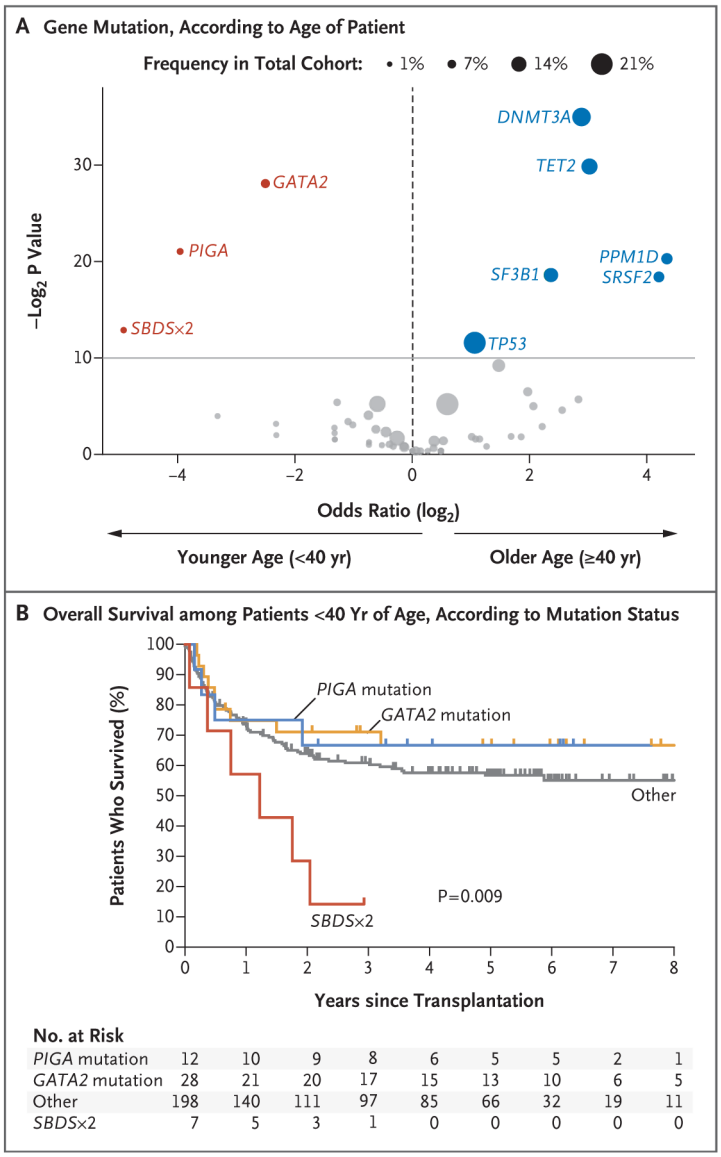

Figure 3. Biallelic *SBDS* Mutations in Young Adult Patients and Association with Poor Survival

Panel A shows the association between mutated genes and the age of the patient. Each circle represents a mutated gene, and the size of each circle corresponds to the frequency of the mutation in the total cohort, as indicated. Genes in the upper right quadrant were significantly associated with an age of 40 years or more (blue), and genes in the upper left quadrant with an age of less than 40 years (red). Panel B shows Kaplan–Meier curves for overall survival among patients younger than 40 years of age according to *PIGA*, *GATA2*, or *SBDS* mutation status. Data for patients without mutations in these genes are shown in gray. Patients with biallelic *SBDS* mutations (*SBDS*×2, red) had shorter survival than those without *SBDS* mutation (P = 0.009). Tick marks indicate censored data.

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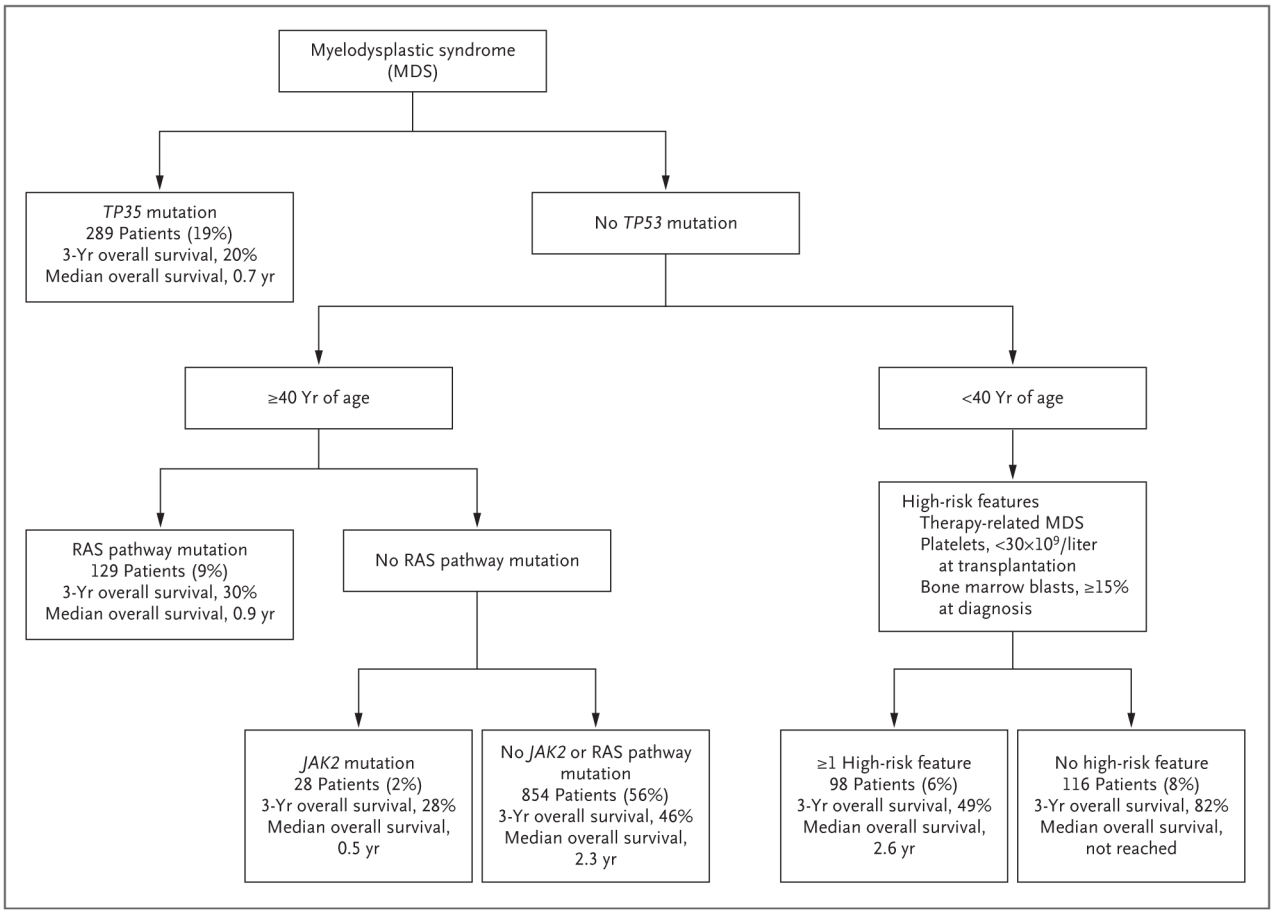

Figure 4. Models for Overall Survival, Including Clinical and Genetic Variables and Effect of Conditioning Intensity

Shown is a hierarchical prognostic model for overall survival that was based on recursive partitioning analysis.

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Table 1

Characteristics of the Patients and Univariate Associations with Overall Survival.*

Variable	Patients (N = 1514)	Hazard Ratio for Death (95% CI)	P Value
	no. (%)		
Patient-related variable			
Age at transplantation			<0.001
0–39 yr	241 (16)	1.00	
≥40 yr	1273 (84)	2.05 (1.66–2.53)	
Karnofsky performance-status score†			<0.001
90–100	817 (54)	1.00	
<90	419 (28)	1.51 (1.29–1.77)	
Missing data	278 (18)	—	
Disease-related variable			
IPSS-R cytogenetic risk group before transplantation			<0.001
Good or very good	579 (38)	1.00	
Intermediate	269 (18)	0.73 (0.60–0.89)	0.002
Poor	287 (19)	0.89 (0.74–1.08)	0.24
Very poor	125 (8)	1.76 (1.41–2.20)	<0.001
Missing data	254 (17)	—	
Bone marrow blasts before transplantation‡			0.03
0–2%	377 (25)	1.00	
3–5%	269 (18)	1.21 (0.98–1.48)	0.07
6–9%	238 (16)	1.23 (1.00–1.52)	0.06
10–19%	289 (19)	1.34 (1.10–1.63)	0.003
Missing data	341 (23)	—	
Platelet count before transplantation			0.01
≥100×10^9/liter	547 (36)	1.00	
50–99×10^9/liter	344 (23)	1.01 (0.84–1.21)	>0.99
<50×10^9/liter	538 (36)	1.24 (1.06–1.45)	0.006
Missing data	85 (6)	—	
Type of MDS			<0.001
Primary MDS	1203 (79)	1.00	
Therapy-related MDS	311 (21)	1.34 (1.15–1.57)	
Transplantation-related variable			
Conditioning regimen			0.005
Myeloablative	789 (52)	1.00	
Reduced intensity	582 (38)	1.13 (0.98–1.30)	0.10

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Variable	Patients (N = 1514)	Hazard Ratio for Death (95% CI)	P Value
	no. (%)		
Nonmyeloablative	130 (9)	1.45 (1.16–1.82)	0.001
Missing data	13 (1)	—	
Graft type			0.60
Bone marrow	221 (15)	1.00	
Peripheral-blood stem cell	1114 (74)	1.05 (0.87–1.27)	0.60
Cord blood	168 (11)	1.13 (0.87–1.47)	0.35
Missing data	11 (1)	—	

*P values for individual categories within variables were calculated with the use of the Wald test. The P value for each variable was calculated with the use of the log-rank test. Percentages may not sum to 100 because of rounding. The full characteristics of the cohort, including enumeration of missing data for all variables, are provided in Tables S2, S3, and S4 in the Supplementary Appendix. The complete analysis of univariate association of clinical characteristics is provided in Tables S10, S11, and S12 in the Supplementary Appendix. IPSS-R denotes Revised International Prognostic Scoring System, and MDS myelodysplastic syndrome.

†Karnofsky performance-status scores range from 0 to 100, with higher scores indicating better function.

‡Bone marrow blasts were assessed within 30 days before the initiation of the conditioning therapy that was used before transplantation.