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ORIGINAL ARTICLE

Chronic graft-versus-host disease could ameliorate the impact of adverse somatic mutations in patients with myelodysplastic syndromes and hematopoietic stem cell transplantation

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Abstract

Somatic mutations in patients with myelodysplastic syndromes (MDS) undergoing allogeneic hematopoietic stem cell transplantation (HSTC) are associated with adverse outcome, but the role of chronic graft-versus-host disease (cGVHD) in this subset of patients remains unknown. We analyzed bone marrow samples from 115 patients with MDS collected prior to HSCT using next-generation sequencing. Seventy-one patients (61%) had at least one mutated gene. We found that patients with a higher number of mutated genes (more than 2) had a worse outcome (2 years overall survival [OS] 54.8% vs. 31.1%, $p = 0.035$). The only two significant variables in the multivariate analysis for OS were *TET2* mutations ($p = 0.046$) and the development of cGVHD, considered as a time-dependent variable ($p < 0.001$), correlated with a worse and a better outcome, respectively. *TP53* mutations also demonstrated impact on the cumulative incidence of relapse (CIR) (1 year CIR 47.1% vs. 9.8%, $p = 0.006$) and were related with complex karyotype ($p = 0.003$). cGVHD improved the outcome even among patients with more than 2 mutated genes (1-year OS 88.9% at 1 year vs. 31.3%, $p = 0.02$) and patients with *TP53* mutations (1-year CIR 20% vs. 42.9%, $p = 0.553$). These results confirm that cGVHD could ameliorate the adverse impact of somatic mutations in patients with MDS with HSCT.

Keywords Myelodysplastic syndromes · Somatic mutations · *TP53* · Allogeneic hematopoietic stem cell transplantation · Chronic graft-versus-host disease

Juan Carlos Caballero and Mercedes Sánchez Barba contributed equally to this work.

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Introduction

Myelodysplastic syndromes (MDS) are clonal diseases defined by inefficient hematopoiesis, morphologic dysplasia, cytopenia, and increased risk of transformation into acute myeloid leukemia (AML). Approximately 50–60% of these patients have abnormalities by conventional cytogenetics (karyotype and fluorescence in situ hybridization) [1, 2]. Nevertheless, new techniques such as next-generation sequencing (NGS) methodology identify mutations in driver genes in up to 90% of patients [3–5]. Although some of these mutations have been associated with different disease characteristics and in some cases response to therapy [6–15], their role in the clinical practice is far from being completely defined. Nowadays, hematopoietic stem cell transplantation (HSCT) is the only potential curative therapy for these patients, due to the immune control mediated by the graft-versus-leukemia effect [16–19]. In this sense, the development of chronic graft-versus-host disease (cGVHD) has been reported as one of the most powerful antineoplastic mechanisms after HSCT, reducing relapse and improving survival [20–23]. Recent studies evaluating the impact of somatic mutations in patients with MDS receiving allogeneic HSCT suggest that mutations in *TP53*, *TET2*, *DNMT3A*, *RUNX1*, *ASXL1*, or *RAS* pathway genes and the number of mutations are associated with an adverse outcome [24–29]. However, the potential effect of GVHD among those patients with mutations remains unknown. To the best of our knowledge, this is the first study analyzing the impact of the development of GVHD in MDS patients with somatic mutations.

Material and methods

The results of HSCT in 115 patients with MDS from five centers in Spain, transplanted between years 1998 and 2015, were retrospectively analyzed. We selected available bone marrow samples collected prior to HSCT (median of 27 days before, range from 1 to 1075 days); in 7 cases, we used peripheral blood instead of bone marrow cells. Genomic DNA from mononucleated cells was screened for somatic mutations by NGS, using a TruSight Myeloid Sequencing Panel with a NextSeq platform (Illumina) that covers 54 genes for most patients and an Ampliseq 39 genes panel with an Ion Torrent platform (Thermo Fisher Scientific) in 16 patients, both including the most frequently mutated genes in myeloid malignancies (Table 1). Library preparation for each sample was performed following the manufacturer's instructions. Human genomic build 19 (hg19) was used as a reference. Alignment to the hg19 genome and variant detection was carried out using the Variant Studio Data Analysis and the Ion Reporter software, respectively. We excluded from the TruSight Myeloid Sequencing Panel the target regions with a minimum median coverage of 100 reads in at least 20% of the patients. The average coverage per patient was 10,662 reads (from 19,522 to 5109) and the Variant Allele Frequency (VAF) lower limit was 3%. Only those variants located in exonic or splicing regions that cause a change in the protein sequence were considered. Polymorphisms, variants with a minor allele frequency (MAF) in healthy people higher than 1%, were also excluded.

Table 1 Next-generation sequencing gene panels used in this study

TruSight Myeloid Sequencing Panel (Illumina): 54 genes

ABL1, ***ASXL1***, *ATRX*, ***BCOR***, *BCORL1*, ***BRAF***, *CALR*, ***CBL***, *CBLB*, *CBLC*, ***CDKN2A***, ***CEBPA***, *CSF3R*, *CUX1*, ***DNMT3A***, ***ETV6***, ***EZH2***, *FBXW7*, ***FLT3***, *GATA1*, *GATA2*, ***GNAS***, *HRAS*, ***IDH1***, ***IDH2***, *IKZF1*, ***JAK2***, *JAK3*, *KDM6A*, ***KIT***, ***KRAS***, *MLL*, ***MPL***, *MYD88*, *NOTCH1*, ***NPM1***, ***NRAS***, *PDGFRA*, ***PHF6***, *PTEN*, ***PTPN11***, ***RAD21***, ***RUNX1***, ***SETBP1***, ***SF3B1***, *SMC1A*, ***SMC3***, ***SRSF2***, ***STAG2***, ***TET2***, ***TP53***, ***U2AF1***, ***WT1***, ***ZRSR2***

AmpliSeq (Thermo Fisher Scientific): 39 genes

ASXL1, ***BCOR***, ***BRAF***, ***CBL***, ***CDKN2A***, ***CEBPA***, ***DNMT3A***, ***ETV6***, ***EZH2***, ***FLT3***, ***GNAS***, ***IDH1***, ***IDH2***, ***JAK2***, ***KIT***, ***KRAS***, *LUC7L2*, ***MPL***, ***NPM1***, *NF1*, ***NRAS***, ***PHF6***, ***PTPN11***, ***RAD21***, *RPS14*, ***RUNX1***, ***SETBP1***, ***SF3B1***, *SF1*, *SF3A1*, ***SMC3***, ***SPARC***, ***SRSF2***, ***STAG2***, ***TET2***, ***TP53***, ***U2AF1***, ***WT1***, ***ZRSR2***

Common genes are marked in bold

Overall survival (OS) was calculated from the date of HSCT to the date of death for any cause or last follow-up for living patients. Relapse was defined either as morphological persistence or recurrence of MDS (≥ 5% of bone marrow blast) and the presence of minimal residual disease (MRD) by flow cytometry after transplantation. Relapse-free survival (RFS) was calculated from the date of transplantation to the date of relapse, death or last follow-up for living patients, being relapse considered an uncensored event. Curves were performed for OS and RFS using the Kaplan-Meier method and compared using a log-rank test. Cox models were used to adjust for clinical and transplantation characteristics. cGVHD was considered only in those patients who were alive after day + 100. Cox regression model with cGVHD entered as a time-dependent covariate was used to determine the effect of cGVHD on survival. Multivariate models were constructed using statistically significant variables ($p < 0.05$) in the univariate analysis and other relevant variables, such as sex and age. Cumulative incidence of relapse (CIR) with or without death was calculated using competing risks and compared using the Gray test. p values were considered significant at the 0.05 level. All calculations were performed using SPSS 23.0, except CIR, that was calculated using Xlstat version 2014.5.03.

Results

Patients and disease characteristics

Median age was 53 years (p25–p75, 44.13–61.07), and 58% were male patients. Most patients presented as de novo MDS (79%) while 21% were secondary MDS. Diagnosis (WHO 2008) were RCUD in 4 patients (3.5%), RARS in 2 patients (1.8%), RCMD in 22 (19.5%), RAEB-1 in 28 (24.8%), RAEB-2 in 32 (28.3%), unclassifiable MDS in 12 (10.6%), CMML in 9 (8%), and AML 20–30% blast (RAEB-T in FAB classification) in 4 (3.5%). Revised International Prognosis Scoring System (R-IPSS) was available in 85 of 115 patients: very low risk in 2 (2.4%), low in 15 (17.6%), intermediate in 21 (24.7%), high in 22 (25.9%), and very high in 16 (18.8%); 9 patients with CMML (10.6%) were categorized separately. Forty-eight (41.7%) patients received azacitidine prior to transplant and 36 (31.3%) received chemotherapy. Among patients with known karyotype (101 of 115), 7 of them (6.9%) had a complex karyotype (CK). Fifty-four patients (47.8%) developed cGVHD after transplant, considering limited and extensive indistinctly; 43 (38.1%) patients did not develop cGVHD and 16 (14.2%) were not evaluable for cGVHD because they died before day + 100. Other characteristics related to the disease and the transplantation are shown in Table 2.

Mutational profile

Regarding the mutational status in pre-HSCT sample, 44 patients (38.3%) had no mutations. In the remaining 71 patients, we observed 1 mutated gene in 27 patients (23.5%), 2 mutated genes in 15 patients (13%), 3 mutated genes in 19 patients (16.5%), 4 mutated genes in 6 patients (5.2%), 5 mutated genes in 3 patients (2.6%), and 6 mutated genes in only 1 patient (0.9%). The most frequently mutated genes were *TP53* ($n = 15$, 13%), *SRSF2* ($n = 14$, 12.2%), *TET2* ($n = 13$, 11.3%), *DNMT3A* ($n = 9$, 7.8%), *RUNX1* ($n = 9$, 7.8%), *SF3B1* ($n = 9$, 7.8%), and *ASXL1* ($n = 8$, 7%). Figure 1 shows a summary of mutated genes and karyotype in our series. The mean number of mutated genes per patient was 1.38, with a median VAF of 35%.

We found a significant correlation between *TET2* mutations and IPSS, being more frequent in low and intermediate-1 IPSS patients (18.4% patients in low and intermediate-1 vs. 0% in intermediate-2 and high IPSS patients; $p = 0.004$), and between mutations in *SRSF2* and diagnosis of RAEB (15.6% of mutated patients in RAEB1–2 vs. 2.5% in not RAEB patients; $p = 0.048$). CMML was associated with mutations in *TET2* (44.4% of patients with CMML; $p = 0.005$), *SRSF2* (33.3%; $p = 0.02$), *KRAS* (22.2%; $p = 0.007$), and *EZH2* (22.2%; $p = 0.026$). We also observed a higher proportion of *TP53* mutations in CK patients (57.1%) as compared to patients without CK (only 8.5% of them had *TP53* mutations; $p = 0.003$).

Outcome

Overall survival and relapse-free survival

At the last follow-up, 48% of patients were alive and 46% disease free. Nineteen patients relapsed (16.5%). After a median follow-up of 2.02 years for living patients, OS was 48.1% (63.4% at 1 year, median OS 5.96 years, Fig. 2a) and RFS was 46.2% (59.7% at 1 year, median RFS 5.92 years).

Univariate analysis for OS included all mutated genes, number of mutations (≤ 2 mutated genes vs. > 2), age (continuous), sex, WHO 2008 classification, primary vs. secondary MDS, IPSS, R-IPSS, cytogenetic risk category, CK, modifying treatment, therapeutic response, disease status pre-HSCT, conditioning regimen, progenitor cell source, donor type, HLA matching, acute GVHD, and cGVHD as a time-dependent variable (considering only living patients after day + 100).

We observed that patients with ≤ 2 mutated genes (86/115, 74.5%) had a better prognosis than those with > 2 (29/115, 25.2%): 2 years OS was 64.5% (95% CI 53.72–75.28, median not reached, HR 1.875; $p = 0.035$) vs. 38.9% (95% CI 20.48–57.32, median 0.926 years) (Table 3 and Fig. 2b). The development of cGVHD (HR 0.101; $p < 0.001$), CK (HR 2.914; $p = 0.028$), and mutations in *TET2* (HR 2.824; $p = 0.005$) also showed significant impact on the outcome.

In the multivariate analysis, only cGVHD (HR 0.046, 95% CI 0.016–0.138; $p < 0.001$) and *TET2* mutations (HR 2.562, 95% CI 1.018–6.447; $p = 0.046$) retained the statistically significant influence on OS (Table 3).

Patients with ≤ 2 mutated genes had 2 years RFS of 60.5% (95% CI 49.32–71.67; median not reached, HR 2.04; $p = 0.014$) vs. 35.8% for patients with > 2 mutated genes (95% CI 17.77–53.83; median 0.921 years).

Cumulative incidence of relapse

In the whole series, CIR was 15% at 2 years (95% CI 9.5–23.6) (Fig. 3a). For patients with ≤ 2 mutated genes CIR was lower (13.6% at 2 years, 95% CI 7.6–24.6) as compared to patients with > 2 (25.3% at 2 years, 95% CI 13.6–47.1; $p = 0.007$) (Fig. 3b). Among mutated genes, only *TP53* was statistically related to higher CIR ($p = 0.006$, Fig. 3c).

Table 2 Demographic data, clinical characteristics, and transplant-related features

	≤ 2 mutated genes	> 2 mutated genes	*p* value
Number of patients (%)	86 (74.78)	29 (25.22)	
Age, years, median	51.64	56.57	0.115
Gender	86	29	0.198
Male	47 (54.70)	20 (69.00)	
Female	39 (35.30)	9 (31.00)	
WHO 2008 classification	84	29	0.338
RCUD	3 (3.60)	1 (3.40)	
RARS	2 (2.40)	0 (0.00)	
RCMD	12 (14.30)	10 (34.50)	
RAEB-1	22 (26.20)	6 (20.70)	
RAEB-2	26 (31.00)	6 (20.70)	
Unclassifiable MDS	9 (10.70)	3 (10.30)	
CMML	6 (7.10)	3 (10.30)	
AML (RAEB-T)	4 (4.80)	0 (0.00)	
Not available	2	0	
Complex karyotype	77	24	0.669
Yes	5 (6.50)	2 (8.30)	
No	72 (93.50)	22 (91.70)	
Not available	9	5	
Primary vs. secondary MDS	86	29	0.428
Primary	66 (76.70)	25 (86.20)	
Secondary	20 (23.30)	4 (13.80)	
Cytogenetic category	77	24	0.54
Very good	1 (1.30)	0 (0.00)	
Good	37 (48.10)	16 (66.70)	
Intermediate	20 (26.00)	5 (20.80)	
Poor	15 (19.50)	2 (8.30)	
Very poor	4 (5.20)	1 (4.20)	
Not available	9	5	
IPSS	71	23	0.323
Low	6 (8.50)	3 (13.00)	
Intermediate 1	20 (28.20)	10 (43.50)	
Intermediate 2	33 (46.50)	5 (21.70)	
High	6 (8.50)	2 (8.70)	
CMML	6 (8.50)	3 (13.00)	
Not available	15	6	
Revised IPSS (R-IPSS)	66	19	0.180
Very low–low-intermediate	27 (40.90)	11 (57.9)	
High–very high	33 (50.00)	5 (26.30)	
CMML	6 (9.10)	3 (15.80)	
Not available	20	10	
Treatment	86	29	0.151
Azacitidine	32 (37.20)	16 (55.20)	
Chemotherapy	31 (36.00)	5 (17.20)	
Others	3 (3.50)	0 (0.00)	
No treatment	20 (23.30)	8 (27.60)	
Status prior to HSCT	84	29	0.352
No treatment	17 (20.20)	7 (24.10)	
Complete response	29 (34.50)	6 (20.70)	

Table 2 (continued)

	≤ 2 mutated genes	> 2 mutated genes	*p* value
Partial response	14 (16.70)	5 (17.20)	
Stable disease	13 (15.50)	7 (24.10)	
Progression	10 (11.90)	2 (6.90)	
Aplasia without blasts	1 (1.20)	2 (6.90)	
Not available	2	0	
Progenitor cell source	86	29	0.161
Bone marrow	4 (4.70)	0 (0.00)	
Peripheral blood	71 (82.60)	28 (96.60)	
Umbilical cord	11 (12.80)	1 (3.40)	
Donor type	86	29	0.164
Related	39 (45.30)	17 (58.60)	
Not related	34 (39.50)	8 (27.60)	
Umbilical cord	10 (11.60)	1 (3.40)	
Haploidentical	3 (3.50)	3 (10.30)	
HLA matching	86	29	0.346
Identical	59 (68.60)	23 (79.30)	
Not identical	27 (31.40)	6 (20.70)	
Conditioning regimen	86	29	0.574
Myeloablative	33 (38.40)	11 (37.90)	
Reduced intensity	53 (61.60)	18 (62.10)	
Acute GVHD	86	29	0.378
Yes	54 (62.80)	21 (72.40)	
No	32 (37.20)	8 (27.60)	
Chronic GVHD	83	29	0.074
Yes	44 (53.01)	10 (34.50)	
No	26 (31.33)	16 (55.20)	
Not evaluable (death < day + 100)	13 (15.66)	3 (10.30)	
Not available	2	0	

TP53 mutations, complex karyotype, and cGVHD: impact on the outcome

TP53 mutations

In our study, patients with *TP53* mutations showed a trend to worse prognosis: 2 years OS 45%, median of 0.6 years vs. 59%, and median of 5.9 years (HR 1.49, 95% CI 0.695–3.191; $p = 0.3$). Moreover, 2 years RFS was 46.7% for *TP53*-mutated patients, with a median time to progression of 0.5 years vs. 54.6% for wild-type patients, median of 5.9 years to progression (HR for *TP53* mutations 1.389, 95% CI 0.652–2.961; $p = 0.4$). Interestingly, the presence of mutated *TP53* significantly was correlated with a higher CIR as compared to wild-type patients: 2 years CIR 41.7% (95% CI 22.5–77.1) vs. 12.9% (95% CI 7.4–22.6); $p = 0.006$ (Fig. 3c).

We also found that VAF in *TP53* mutations could be associated with a poorer outcome. Considering VAF as a continuous variable, we observed a significant impact on OS (HR 1.039; $p = 0.033$) and RFS (HR of 1.036; $p = 0.044$). Setting a cutoff on 10%, patients with VAF below 10% had a trend towards a better OS (58.3% at 1 year for VAF < 10% vs. 28.6% at 1 year for ≥ 10%; $p = 0.068$). However, the limited number of individuals with TP53 mutations in our study make difficult to find significant results.

TP53 and complex karyotype

In our series, 15 patients had *TP53* mutations and 7 patients CK. Four patients presented CK and *TP53* mutations together. When we compared OS in CK patients with and without *TP53* mutations, we found a significant difference ($p < 0.001$) with a worse OS for the group of CK patients with *TP53* mutations: 0% at 1 year vs. 62.5% for non-CK patients with *TP53* mutations, 66.7% for CK patients with wt *TP53* and 68.1% for non-CK patients with wt *TP53* (Supplementary Figure). In this regard, the impact on OS of *TP53* mutations should be explained in the context of its close association with CK.

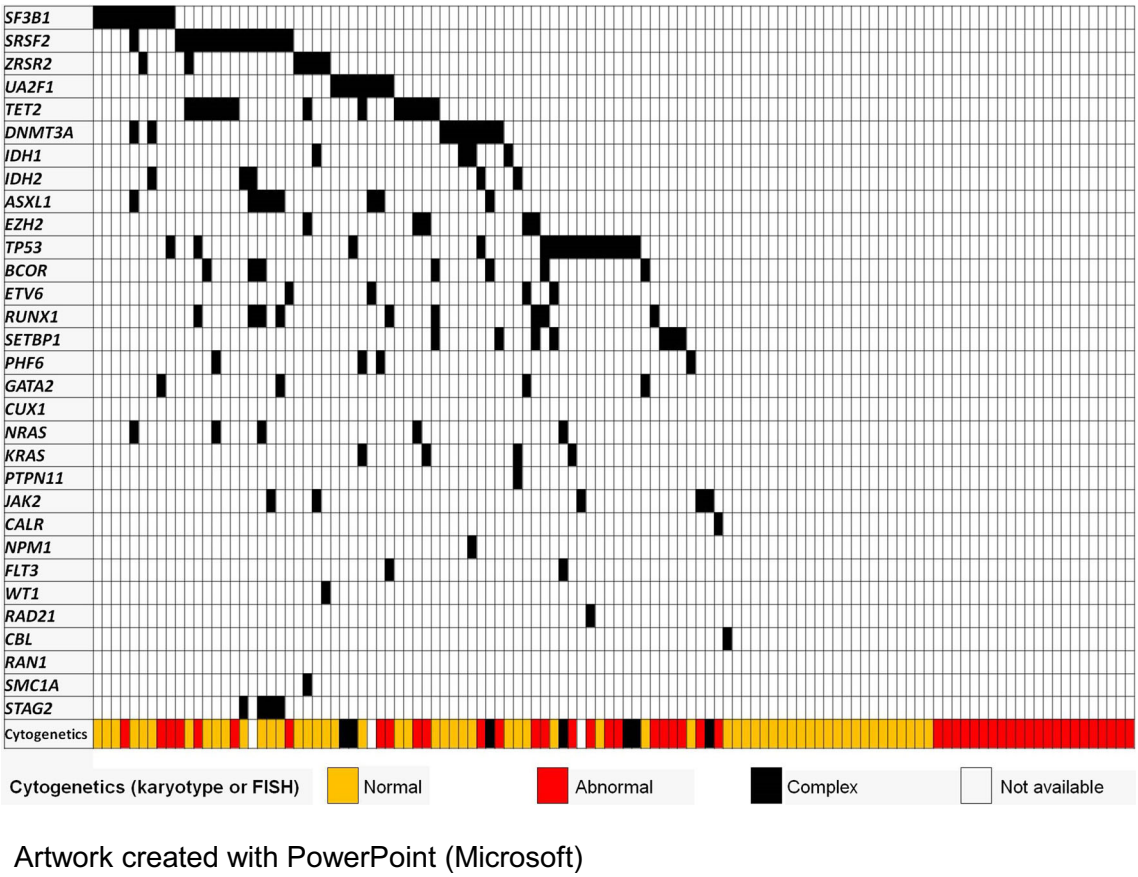


Fig. 1 Spectrum of somatic mutations and cytogenetics in 115 patients. Artwork created with PowerPoint (Microsoft)

Chronic graft-versus-host disease

In our study, cGVHD was the most powerful variable influencing prognosis, with a median OS for patients developing cGVHD not reached as compared to 1.268 years for those who did not develop cGVHD ($p < 0.001$). We explored the role of cGVHD in the different subsets of patients. According to the number of mutated genes, we found significant differences in patients with ≤ 2 or > 2 mutated genes, both in OS and CIR between patients who developed or no cGVHD. OS was 88.9% at 1 year and 77.8% at 2 years for patients with > 2 mutated genes who

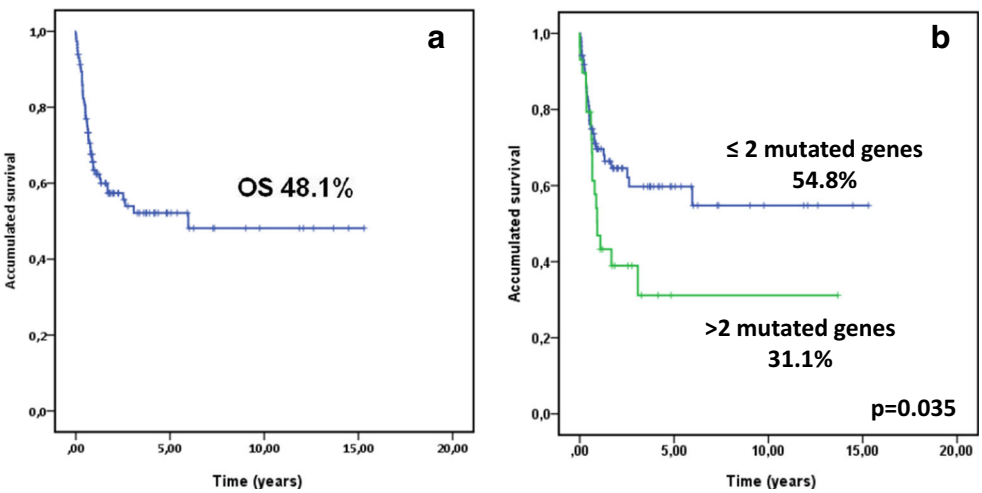


Fig. 2 Overall survival in the whole series (**a**) and according to the number of mutated genes, grouped in patients with ≤ 2 and > 2 mutated genes (**b**). Artwork created with SPSS (IBM)

Table 3 Univariate and multivariate analysis for OS

Variable	Univariate		Multivariate			
	HR	*p* value	HR	*p* value	95% CI	95% CI
Age (continuous)	1.023	0.094	1.004	0.808	0.975	1.033
Gender (male vs. female [ref])	1.625	0.112	1.196	0.608	0.603	2.375
WHO 2008 classification (RAEB vs. not RAEB [ref])	0.953	0.880				
CMML vs. not RAEB [ref]	2.195	0.100				
IPSS (intermediate 2-high vs. low-intermediate 1 [ref])	1.108	0.781				
R-IPSS (high-very high vs. very low-low-intermediate [ref])	0.917	0.833				
Karyotype (complex vs. non-complex [ref])	2.914	0.028	1.000	0.989	0.364	2.742
Secondary vs. primary MDS [ref]	2.123	0.085				
Treatment (yes vs. not [ref])	1.166	0.647				
Response (any response vs. no treatment [ref])	1.651	0.213				
No response/progression vs. no treatment [ref]	1.182	0.664				
Status prior HSCT (any response/stable disease vs. active disease without treatment [ref])	0.968	0.934				
No response/progression vs. active disease without treatment [ref]	1.312	0.502				
Donor cell source (peripheral blood vs. bone marrow [ref])	0.683	0.603				
Umbilical cord vs. bone marrow [ref]	2.331	0.280				
Donor type (related vs. haploidentical [ref])	1.488	0.698				
Not related vs. haploidentical [ref]	1.789	0.572				
Umbilical cord vs. haploidentical [ref]	5.084	0.123				
HLA matching (not-identical vs. identical [ref])	1.219	0.532				
Conditioning regimen (reduced intensity vs. myeloablative [ref])	1.234	0.486				
Acute GVHD (yes vs. no [ref])	1.048	0.877				
Chronic GVHD (yes vs. no [ref])	0.275	0.000				
Chronic GVHD time-dependent (yes vs. no [ref])	0.101	0.000	0.000	0.046	0.016	0.138
Mutated genes (group 2 vs. group 1 [ref])	1.875	0.035	1.436	0.381	0.645	3.196
TP53 (mutated vs. wild-type [ref])	1.49	0.305				
TET2 (mutated vs. wild-type [ref])	2.824	0.005	2.562	0.046	1.018	6.447

developed cGVHD as compared to 31.3% at 1 year and 25% at 2 years for those who did not develop cGVHD ($p = 0.02$) (Fig. 4a). Similarly, in the group of patients with ≤ 2 mutated genes, OS was superior for patients who developed cGVHD (85.6% and 82.6% at 1 and 2 years vs. 66.2% and 54% at 1 and 2 years, respectively; $p = 0.013$) (Fig. 4b).

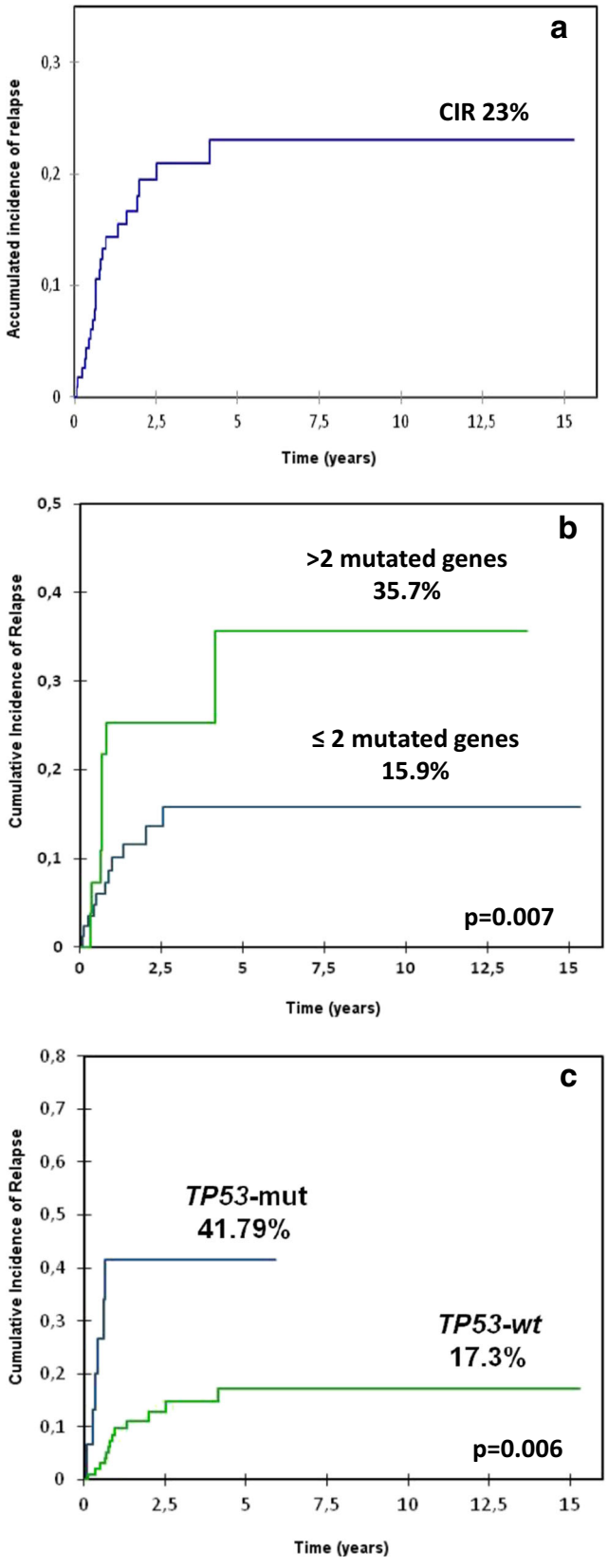
Two years CIR was 43.8% for patients with > 2 mutated genes without cGVHD vs. 0% for patients who developed cGVHD ($p = 0.064$). Among those with ≤ 2 mutated genes, 2 years CIR was 6.7% when developing cGVHD and 10.3% who did not develop cGVHD ($p = 0.9$).

Regarding cGVHD development and its impact on the outcome among the 15 patients with *TP53* mutations, 6 of them developed cGVHD and 2 were not evaluable because of early death (Table 4). Development of cGVHD improved the outcome in *TP53*-mutated patients, with a trend to a better OS (80% at 1 year vs. 28.6%; $p = 0.175$). Same results were observed for CIR that was lower in patients with cGVHD (20% vs. 42.9%; $p = 0.6$) (Fig. 4c). Patients with *TP53* mutations developing cGVHD had similar CIR to those with wt *TP53* (17.3%).

Discussion

Our results suggest that cGVHD in patients with MDS and adverse prognosis mutations pre-HSCT improves OS, RFS, and CIR, especially for those with somatic mutations in specific genes (i.e., *TP53*) and with a higher number of mutated genes. Additionally, in the line of previous studies, we observed the impact of pre-HSCT mutational profile on the transplant outcome.

Regarding mutational profile, our patients had only similar characteristics to other series described in *TET2* status (11% of mutated patients in our series and 13%, 15%, and 10% in Bejar, Lindsley, and Della Porta series, respectively) while other relevant genes with impact on the outcome were less frequent (only 13% with *TP53* mutations as compared to 21% and 19% in Bejar and Lindsley series, respectively).



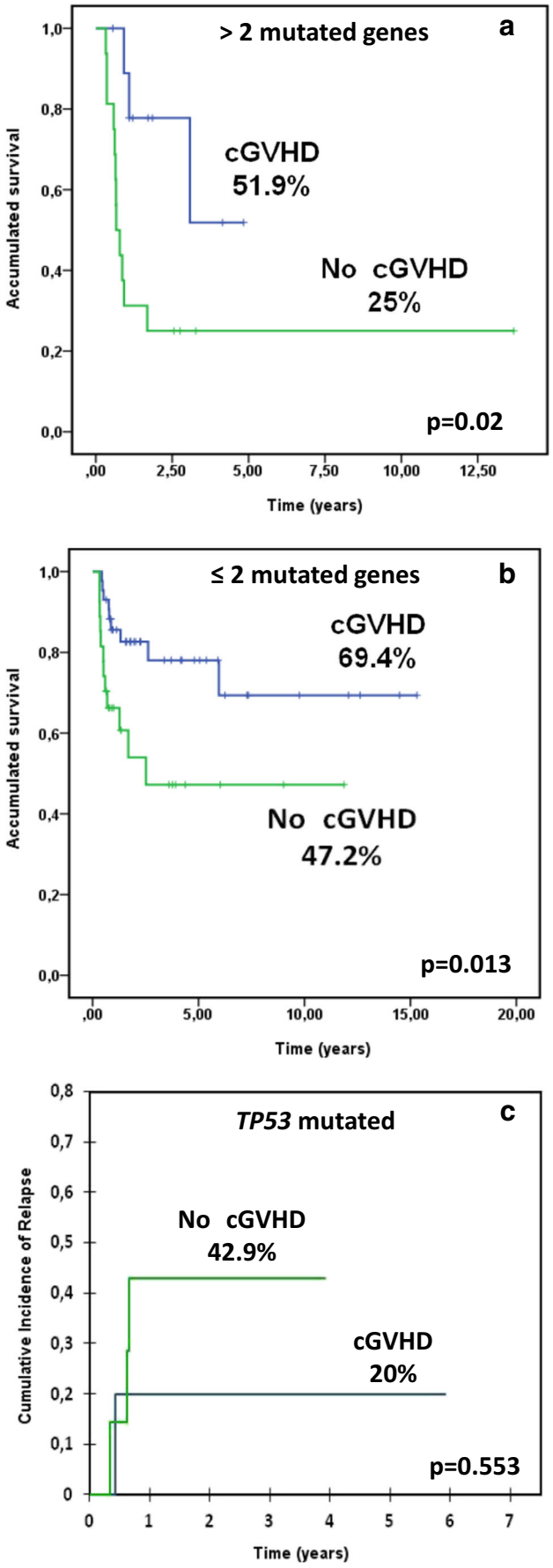
◀ **Fig. 3** Cumulative incidence of relapse in the whole series (**a**), according to the number of mutated genes grouped in patients with ≤ 2 and > 2 mutated genes (**b**), and according to TP53 mutational status (**c**). Artwork created with Excel (Microsoft)

These differences could be explained due to the distinct types of disease included in the studies (high-risk MDS, low-risk MDS, secondary AML) and the treatments received before HSCT: no treatment, hypomethylating agents (HMA), or chemotherapy. In our series, 60% of patients were high-risk MDS and two thirds received chemotherapy or HMA before transplant, which could probably have decreased and even also achieved molecular response before transplant.

When analyzing survival, presence of CK, absence of cGVHD, and adverse mutational profile significantly decreased survival in the univariate analysis; the more complex mutational profile (> 2 mutated genes) and mutations in *TET2* and *TP53* significantly impacted on the outcome, as other studies described previously [24–29]. Our data supports previous findings regarding the poor outcome of *TP53* mutations in the setting of HSCT in MDS [3, 6, 9, 10, 12, 15], especially when *TP53* mutations were associated with CK, as it has previously shown by Bejar and Yoshizato [24–29]. *TP53* mutations with CK are related to early post-HSCT mortality: all patients with this alteration in our series had died within the first year after transplant, while those with only one abnormality (either CK or *TP53* mutation) had an OS comparable to those without these abnormalities (1-year OS up to 60%). Our study reflects the need of new strategies for this subset of patients (CK and *TP53* mutations) in order to improve the results of HSCT. Some genes described in other series with impact on the outcome did not reach statistical significance in our study probably due to our limited number of patients (*ASXL1* mut 7%, *RUNX1* mut 7.8%, *DNMT3A* mut 7.8%) although a trend to poorer OS was observed in these mutated patients.

TET2 was the only mutated gene that retained its adverse influence in the multivariate analysis. Although some previous studies significantly associate *TET2* mutations with adverse prognosis in MDS patients undergoing HSCT [24, 25, 30], others did not observe any impact [26, 28, 29].

In our study, the development of cGVHD strongly improved OS (HR 0.046, $p < 0.001$). cGVHD has been shown to be related with a potent graft versus MDS, but to the best of our knowledge, no previous published data have analyzed the impact of cGVHD in patients with adverse genetics, as *TP53* mutations and complex karyotype. Our study shows that the development of cGVHD is able to, at least partially, overcome the deleterious effect of such adverse genetic factors. We observed that patients with > 2 mutated genes had an improved OS when cGVHD is developed (1- and 2-year OS of 88.9% and 77.8%, respectively, as compared to 31.3% (1 year) and 25% (2 years) for patients without cGVHD; $p = 0.02$). In the same way, the development of cGVHD could also overcome the poor prognosis of *TP53* mutations: those patients with



◀ **Fig. 4** Development of cGVHD improves outcome in patients with > 2 (**a**) and ≤ 2 mutated genes (**b**) and in patients with *TP53* mutations (**c**). Artwork created with SPSS (IBM) and Excel (Microsoft)

TP53 mutations developing cGVHD had a better survival (80% of survivors at 1 year) as compared to those who did not present cGVHD (28.6% at 1 year; $p = 0.175$). Interestingly, almost all patients with *TP53* mutations that developed cGVHD survived and did not relapse, which is why we probably did not find significant differences in OS between patients with and without *TP53* mutations in OS, but in CIR. Patients with *TP53* mutations and CK were not evaluable for cGVHD analysis because most of them died early after transplant (range from 28 to 185 days) and only one experienced cGVHD, although died at day + 157.

Impact of cGVHD on the outcome was clearly related to the graft-versus-leukemia effect, and it has been suggested in our study because CIR decreased among high risk features patients developing this complication after transplant. Patients with more than 2 mutated genes had better and close similar CIR than those with less than 2 mutated genes when they developed cGVHD. In the same way, those patients with *TP53* mutated gene had lower CIR when they developed cGVHD, and it was similar to CIR of those patients without *TP53* mutations (Figs. 3b and 4b). In this regard, we can conclude that the development of cGVHD ameliorates adverse prognostic features.

We also found that VAF > 10% in patients with *TP53* mutations could be associated with a poorer outcome, both OS and RFS. This suggestion has been previously reported, but not confirmed, in Della Porta and Lindsley series.[26, 28] Other studies show disappointing results regarding the influence of the VAF of *TP53* mutations in MDS prognosis [3, 30–32]. In our study, all patients with CK were in the subset of patients with VAF > 10% and it probably could have impacted the results, as well as the development of cGVHD (3/6 patients with VAF < 10% developed cGVHD and are alive, while those who did not develop cGVHD are dead; among those with VAF > 10%, 3/9 developed cGVHD and 2 are still alive, while 6 did not develop cGVHD and only 2 are still alive). Several studies have evidenced that tumor burden prior to transplant (measured by MRD techniques like flow cytometry, PCR, or even NGS) is related with the probability of relapse [33–37]. In this sense, treatments reaching a decrease in the VAF of the mutated genes before transplant could be effective in order to provide a better disease control after transplant.

Conclusions

This study confirms the negative impact of specific somatic mutations present before the transplant, but more important,

Table 4 *TP53*-mutated patients

	WHO 2008	R-IPSS	*TP53* VAF	Karyotype	Treatment	5q-	Status prior HSCT	Relapse	Chronic GVHD	Last status
1	RAEB-1	Very high	66	Complex	Azacitidine	No	Stable disease (not response)	Yes (+ 24)	Not evaluable	Death (+ 28)
2	RAEB-1	Very high	35.7	Complex	No treatment	No	Progression	No	No	Death (+ 185)
3	RCMD	Unknown	79.7	Complex	Chemotherapy	Yes	PR	Yes (+ 91)	No	Death (+ 119)
4	RAEB-1	Very high	51	Complex	No treatment	Yes	Active disease (no treatment)	Yes (+ 104)	Yes (+ 104)	Death (+ 157)
5	RAEB-1	High	35	Abnormal	No treatment	Yes	Active disease (no treatment)	Yes (+ 59)	Not evaluable	Death (+ 90)
6	RAEB-2	Very high	48.1	Abnormal	Azacitidine	No	CR	No	No	Alive (+ 1007)
7	RCMD	Very low	7.8	Abnormal	Azacitidine	Yes	Progression	No	No	Death (+ 189)
8	RCMD	Intermediate	2	Abnormal	Chemotherapy	No	Stable disease (not response)	Yes (+ 99)	No	Death (+ 227)
9	CMML	Not applicable	13.3	Abnormal	Azacitidine	No	CR	No	Yes (+ 225)	Alive (+ 1541)
10	U-MDS	Low	38.5	Abnormal	No treatment	No	Active disease (no treatment)	No	Yes (+ 393)	Alive (+ 1234)
11	RAEB-1	Unknown	20	Unknown	Chemotherapy	No	PR	No	No	Alive (+ 1427)
12	RAEB-2	Unknown	–	Unknown	Azacitidine	No	PR	Yes (+ 112)	No	Death (+ 242)
13	U-MDS	Unknown	3	Unknown	No treatment	No	CR	No	Yes (+ 147)	Alive (+ 203)
14	U-MDS	High	5	Normal	Others	No	Progression	No	Yes (+ 370)	Alive (+ 2159)
15	RAEB-1	High	4	Normal	Azacitidine	No	PR	No	Yes (+ 103)	Alive (+ 1777)

we show that the development of cGVHD could ameliorate this adverse impact, especially among *TP53*-mutated patients and those with > 2 mutated genes. The improved outcome of those patients with mutational adverse features developing cGVHD opens the possibility of post-transplant strategies to stimulate the graft-versus-leukemia effect or even association with pre-emptive strategies (i.e., HMA) in those in a high risk of relapse. Survival among patients with *TP53* and CK is extremely poor and cGVHD has still no evidence to reduce this poor outcome, so novel strategies for this subset of patients are clearly needed to improve outcome of these patients.

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Compliance with ethical standards

Ethical approval This research has been performed in accordance with the Declaration of Helsinki and has been approved by the ethics committee of the University Hospital of Salamanca, which has assigned the reference number 2014/07/110.

Conflict of interest The authors declare that they have no conflicts of interest.

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