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Impact of *PPM1D* mutations in patients with myelodysplastic syndrome and deletion of chromosome 5q

To the Editor:

Deletion of chromosome 5q occurs in 15%–20% of MDS patients and is associated with favorable prognosis if present as a single aberration or with only one additional cytogenetic aberration. The *TP53* mutations, reported in 5%–10% of MDS, are enriched in del(5q) MDS (~20%), therapy-related MDS and MDS with complex karyotype and are associated with high-risk disease, AML transformation, treatment resistance and poor outcome. (1,2) Recently, Bernard et al. showed that the number of *TP53* aberrations is prognostic for death and leukemic transformation. (3) The *PPM1D* mutations are found in clonal hematopoiesis of indeterminate potential (CHIP) and appear more frequent in therapy-related MDS compared to de novo MDS (15% vs 3%). (4,5) Activating *PPM1D* mutations are considered to act similarly to *TP53* loss-of-function mutations. Loss of the C-terminal localization domain of PPM1D activates PPM1D and inhibits p53 activation. (6) However, the prevalence of *PPM1D* mutations, their impact and role in lenalidomide (LEN) resistance and disease progression in MDS with del(5q) still remains unknown.

We performed a retrospective analysis of 234 patients ≥18 years old, with WHO 2016 defined del(5q) MDS (n = 175, 74.8%) or other MDS with del(5q) (n = 38, 16.2%) or sAML with del(5q) (n = 21, 9%) (supplementary methods). Patients with del(5q) alone or with one additional chromosomal abnormality except monosomy 7 or del (7q) and a blast count of <5% in bone marrow (BM) and < 1% in peripheral blood (PB) comprised the group of WHO 2016 defined del (5q) MDS. All other cases of MDS with del(5q) and complex karyotype, chromosome 7 abnormalities or blasts >5% in the BM or > 1% in PB were included in the group of other MDS with del(5q). Overall survival information was available for 216 of the 234 patients, specifically for 164 patients with WHO 2016 defined del(5q) MDS, 31 patients with other MDS with del(5q) and 21 patients with sAML with del5(q). There were 65 patients with WHO 2016 defined del (5q) MDS were treated with LEN and had information about treatment response available (Figure S1(A)).

Del(5q) was the sole cytogenetic abnormality in 202 patients (86.3%). Ten patients (4.3%) harbored del(5q) and at least one additional chromosomal abnormality and 22 (9.4%) had a complex karyotype. The median age was 72.2 years (range 35–93). As expected, there was a female predominance (72.2%). Forty-four percent of the patients were transfusion-dependent at the time of diagnosis; 68 patients (29%) progressed to AML, and 19 (8.1%) underwent allogeneic hematopoietic cell transplantation (HCT) (Table S1).

At time of diagnosis *PPM1D* mutations were detected in 13 of 234 (5.6%) MDS del(5q) patients, 11 of which had mutations in the hotspot region between amino acids 427 and 542 (Figure S1(B); supplementary methods and Table S2 for sequencing details). The mutation frequency was 6.3% (11 of 175) in patients with WHO 2016 defined del(5q) MDS, 5.3% (2 of 38) in patients with other MDS with del(5q) and 0% (0 of 21) in sAML from MDS with del(5q). One of the 13 *PPM1D*-mutated patients harbored a trisomy 8 in addition to del (5q), and two had a complex karyotype. Three *PPM1D*-mutated patients had a *TP53* co-mutation (23%), including the two patients with complex karyotype and one with WHO 2016 defined del (5q) MDS. The *PPM1D* mutations co-occurred with *CSNK1A1*, *SF3B1*, *ETV6*, *KIT*, *ASXL1*, *TET2 and DNMT3A* mutations. Three of the 13 (23%) *PPM1D*-mutated patients had no additional mutations. Also, *TP53* mutations were found in 35 of 234 (15%) patients. Twelve of the 35 (34%) *TP53*-mutated patients had a complex karyotype.

We next investigated the prognostic impact of *PPM1D* mutations in 164 WHO 2016 defined del(5q) MDS patients (Tables S1 and S3). This cohort included 11 *PPM1D*-mutated patients, 16 *PPM1D*-wildtype/*TP53*-mutated patients and 137 *PPM1D-/TP53*-wildtype patients. All *TP53* mutations were monoallelic in this group (supplementary methods). The *PPM1D* mutated patients were numerically older compared to *PPM1D/TP53* wildtype patients (78.3 vs 71 years, p = .31). After a median follow up of 2.6 years, two of 11 (18.2%) *PPM1D*-mutated patients transformed to AML. The AML transformation rate was 6.3% for *TP53*-mutated/*PPM1D*-wildtype patients and 20.4% for *PPM1D-/TP53*-wildtype patients (Table S3). None of the 11 *PPM1D*-mutated patients and one of the 16 *PPM1D*-wildtype/*TP53*-mutated patients underwent HCT. The 2-year OS was 100% for *PPM1D*-mutated patients (n = 11) and *PPM1Dwt/TP53mut* patients (n = 16), and 85% for *PPM1D-/TP53*-wildtype patients (n = 137) with WHO 2016 defined del (5q) (Figure 1(A)). For multivariate analysis four variables were considered based on univariate analysis (age, sex, IPSS risk group, *PPM1D* mutation status). Only age and IPSS risk group were independent predictors of OS (Table S4).

We then investigated the prognostic effect of *PPM1D* mutations in 52 patients with other MDS with del (5q) (n = 31) and sAML (n = 21) with del(5q) (Table S1). Note, *PPM1D* was mutated in two of 52 patients, both showing a concurrent *TP53* mutation and complex karyotype. Thus, we could not evaluate the prognostic effect of *PPM1D* independently of a complex karyotype and a *TP53* mutation. Nine patients had a monoallelic and six patients a biallelic *TP53* aberration. Overall survival was shorter for the *TP53mut monoallelic ± PPM1Dmut* patients (n = 9) and significantly shorter for the *TP53mut biallelic ± PPM1Dmut* patients (n = 6) compared to *TP53*-wildtype and *PPM1D*-wildtype patients (n = 37; 2-y-OS 11% vs 0% vs 53%, respectively, Figure 1(B)).

To evaluate the hematologic response to LEN in WHO 2016 defined del(5q) MDS we analyzed 65 LEN treated patients (Tables S1 and S5). Nine of 65 (13.9%) patients were *TP53* (n = 5, 7.7%) or *PPM1D*-mutated (n = 4, 6.2%). Of 65 patients with WHO 2016 defined del(5q) MDS who were treated with LEN, 54 achieved hematologic response (83.1%) and 11 (16.9%) did not. Treatment response was independent of *PPM1D* (p = .35) (Figure 1(C)) or *TP53* (p = .15) mutation status (Figure 1(D)). After a median follow up of 3.1 years, 40 of the 65 (61.5%) LEN treated patients became refractory or progressed to AML (Figure S2(A)). The median time to AML progression was 2.6 years. The rate of LEN resistance or disease progression was independent of the *PPM1D* (p = .62, Figure S2(B)) or *TP53* (p = .38) mutation status (Figure S2(C)).

Lastly, we investigated clonal evolution under LEN treatment. Follow-up samples were available after LEN treatment for 22 patients with MDS with del(5q) (19 of 22 with WHO 2016 defined del(5q) MDS) (Table S1), who either achieved a complete remission (n = 5) or developed resistance to LEN, which was followed by MDS progression (n = 7) or AML transformation (n = 10). All samples were screened at diagnosis, time of LEN resistance and/or time of AML transformation by NGS (supplementary methods and Table S6). Of the five patients achieving complete hematological remission four patients displayed no mutations, while one patient was *PPM1D*-mutated and *ASXL1*-mutated prior LEN. After 76 months on LEN, the VAF decreased from 27.6% to 4.8% for *PPM1D* and from 12.1% to 1.1% for *ASXL1* in this patient. Of the 17 patients with LEN resistance or MDS/AML progression, two patients (11.8%) carried mutations in *PPM1D* and three patients (17.6%) in *TP53* prior to LEN treatment (p = .64). At the time of LEN resistance or MDS/AML progression, we observed three (17.6%) *PPM1D*-mutated and eight (47.1%) *TP53*-mutated patients (p = .03) (Figure 1(E),(F)). The one novel *PPM1D* and the five novel *TP53* mutations were not detected in the diagnostic sample at a median sequencing depth of 2528 reads (range 1393–12583 reads) and a median limit of detection of 0.72% (range 0.56%–1.77%). Two of eight *TP53*-mutated patients co-expressed *PPM1D* mutations. The prevalence of *PPM1D*-mutated and/or *TP53*-mutated patients increased from 29.4% prior LEN treatment to 52.9% (p = .09) at the time of LEN resistance/progression (Figure 1(G)). At the time of LEN resistance or AML progression, the VAF of *PPM1D* mutations increased from 10.2% to 23.3% and of *TP53* mutations from 5.9% to 23.2% (Figure S2(D),(E)). This corresponds to a 2.5% and 3% increase of the VAF per year in *PPM1D*-mutated and *TP53*-mutated patients, respectively. Novel *ETV6*, *RUNX1*, *WT1*, *U2AF1*, *SF3B1* and *SRSF2* mutations were observed in patients with LEN resistance or MDS/AML progression (Figure S3(A)–(I)).

In summary, we found a 5.6% and 15% prevalence of *PPM1D* and *TP53* mutations prior to LEN treatment, respectively in 234 MDS/sAML patients with del(5q). All patients with WHO 2016 defined del(5q) MDS harbored a *TP53* monallelic state. *PPM1D* and monoallelic *TP53* mutations had no prognostic impact in MDS patients with WHO 2016 defined del(5q), while *TP53* mutations, especially when biallelic, predicted poor OS in patients with sAML and other MDS with del(5q). Furthermore, neither the hematologic response to LEN nor MDS and AML progression risk was affected by *PPM1D* and *TP53* mutation status in patients with WHO 2016 defined del(5q) MDS, although this analysis is preliminary due to the limited number of patients bearing these mutations. Lastly, we found that LEN resistance and disease progression were associated with the acquisition of novel *TP53* and *PPM1D* mutations and a VAF increase suggesting that hematopoietic clones with these mutations are less inhibited by the selective pressure of LEN than *PPM1D* and *TP53* wildtype clones and therefore expand over time. Future studies need to investigate whether sequential genetic analysis for the detection of clonal evolution is useful to identify patients at risk of adverse outcomes and to choose an appropriate treatment to prevent transformation to AML.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PATIENT CONSENT STATEMENT

Written informed consent from patients was obtained according to the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

V.P and M.H designed the research; V.P, M.M., A.K.,R.G., R.S, C.K, P.K, J.S, S.K, M.H. performed the research; M.M, J.K.,A.M, G.G, C.F, C.G, K.S., A.G. C.T., U.G., T.S., G.K, C.K., B.S., N.K, D.H., K.D., W.S., P.V., A.G, F.T., T.H., U.P. contributed patient samples and clinical data; V.P, M.M., R.G, M.H. analyzed the data; V.P and M.H wrote the manuscript. All authors read and agreed to the final version of the manuscript.

DATA AVAILABILITY STATEMENT

All data are available from the corresponding author and in the supplementary data file.

ETHICS STATEMENT

The study was approved by the review board of Hannover Medical School (ethical vote 5558/2010).

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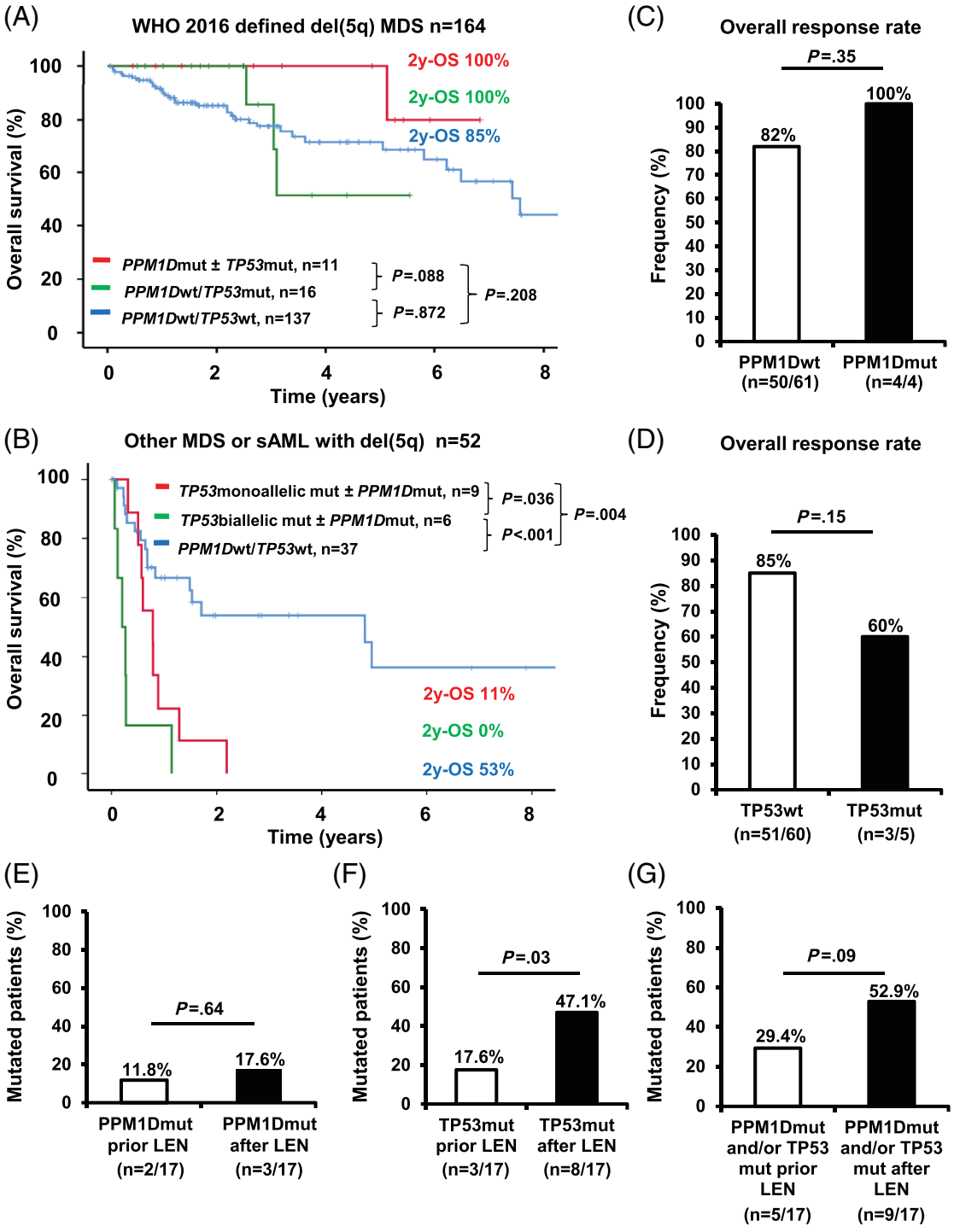

FIGURE 1 Impact of *PPM1D* and *TP53* mutational status on prognosis of patients with MDS or sAML and del(5q), and hematologic response to lenalidomide in WHO 2016 defined del(5q) MDS patients. (A), Overall survival according to *PPM1D* and *TP53* mutation status considering 164 patients with lower risk WHO 2016 defined del(5q) MDS and available survival information. (B), Overall survival according to *TP53* mutation status considering 52 patients with MDS with del(5q) (n = 31) and patients with sAML with del(5q) (n = 21) and available survival information. (C), Response rate to LEN of *PPM1D* mutated in comparison to *PPM1D* wildtype patients. (D), Response rate of *TP53* mutated in comparison to *TP53* wildtype patients to LEN treatment. (E), Percentage of *PPM1D* mutated patients prior LEN treatment (n = 2 of 17) and at the time of resistance or disease progression (n = 3 of 17). (F), Percentage of *TP53* mutated patients prior LEN treatment (n = 3 of 17) and at the time of resistance or disease progression (n = 8 of 17). (G), Percentage of *PPM1D* and/or *TP53* mutated patients prior LEN treatment (n = 5 of 17) and at the time of resistance or disease progression (n = 9 of 17)

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Mutational and immunogenetic landscape of HCV-associated B-cell lymphoproliferative disorders

To The Editor:

Besides robust epidemiological evidences, the direct link between HCV and B-cell lymphoproliferative disorders (LPDs) has been sustained by clinical studies that showed lymphoma regression after HCV eradication.[1,2] However, data regarding molecular characteristics of HCV-associated LPDs are still limited so far. The main purpose of our study was to explore the mutational profile of 27 patients with previously untreated HCV-associated low-grade LPDs by means of an extensive NGS genes panel.

Seven and twenty patients were diagnosed and managed at the Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy and at the Reference Center for Mixed Cryoglobulinemia, University "La Sapienza", Rome, Italy, respectively. For all patients, either peripheral blood (PB) (n = 19) or bone marrow (BM) (n = 6) samples or formalin-fixed paraffin-embedded (FFPE) tissue (n = 2) obtained at the time of LPD diagnosis were available (online supplemental methods). Clinical and virological data were retrospectively collected. The study was approved by the Ethics Committees of the Fondazione IRCCS Policlinico San Matteo, Pavia, Italy and of Sapienza University, Rome, Italy.

Immunoglobulin heavy variable (IGHV) and light variable chain (IGLV) genes rearrangements were assessed using the IGH Somatic Hypermutation Assay v2.0 kit (Invivoscribe, San Diego, California) or according to the BIOMED-2 guidelines. All IGH, IGK (κ light chain) and IGL (λ light chain) rearrangements were analyzed using the IMGT databases and the IMGT/V-QUEST tool to identify CDR3 AA sequences. Heavy chain CDR3 (HCDR3) and light chain CDR3 (LCDR3) stereotypy and homology to anti-HCV E2 antibodies and rheumatoid factors (RF) were searched, as previously described in