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Favorable outcomes of *DDX41*-mutated myelodysplastic syndrome and low blast count acute myeloid leukemia treated with azacitidine ± lenalidomide

Myelodysplastic neoplasms (MDS) are clonal myeloid neoplasms with an increased risk of progression to acute myeloid leukemia (AML). For patients unfit for hematopoietic cell transplantation (HCT), hypomethylating agents, such as azacitidine (AZA), represent the main therapeutic option for patients with higher-risk MDS, with an overall response rate (ORR) of 51% and median overall survival 25 months [1, 2]. Lenalidomide is an immunomodulatory drug that is effective in lower-risk MDS with isolated del(5q) [3], but may also have activity in non-del(5q) MDS [4].

Germline *DDX41* mutations are found in approximately 5% of patients with MDS and AML, representing the most common myeloid neoplasms associated with germline predisposition [5–7]. To date, it is unclear if *DDX41*-mutated MDS should be treated differently than the standard treatment, such as AZA [8, 9]. While *DDX41* (5q35.3) is outside the minimal deleted region (q31–q33) of most MDS cases with del(5q), it is variably deleted in 25% of patients [10]. Thus, there is considerable interest if *DDX41*-mutated MDS might also respond favorably to lenalidomide [10–13].

The Australasian Leukaemia and Lymphoma Group (ALLG) MDS4 phase II trial (ACTRN12610000271000) randomized 160 patients with higher-risk MDS, chronic myelomonocytic leukemia (CMML), and low blast AML to either AZA or combination AZA with lenalidomide (LEN, from cycle 3 onward) [14]. While the combination of LEN and AZA was tolerable, overall there was no improvement in clinical benefit, response rates, or overall survival in patients compared to treatment with AZA alone [14]. We performed a post-hoc genomic analysis to correlate genomic lesions in this trial cohort with clinical outcomes.

This study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki. A unique molecular index-based QIAseq targeted DNA panel (QIAGEN) was performed with a sensitivity of 0.5% variant allele frequency (VAF) [15]. HumanCytoSNP-12 BeadChip array (SNP-A) was previously reported [14]. The primary endpoint of the study was an ongoing clinical benefit (alive and progression-free) at 12 months. Kaplan—Meier overall survival was measured from the date of treatment commencement. R statistical software version 4.2 was used for analysis.

A total of 66 patients had baseline DNA available for testing: 36 on AZA alone and 30 on AZA+LEN. Baseline characteristics are summarized in Figure 1. The median age was 71.6 years (range 52–87). IPSS-R risk groups were very low or low ($n = 20$), intermediate ($n = 18$), high or very high ($n = 25$), and not available ($n = 3$ due to missing/failed karyotype). Disease subtypes by WHO 2008 were MDS ($n = 52$), CMML ($n = 9$), and AML ($n = 5$). SNP-A was abnormal in 47% of patients, including 18% with complex (≥3) copy number changes. The most common mutations were *ASXL1* (48.5%), *TET2* (47%), and *RUNX1* (30%). Median follow-up among survivors was 49 months and median overall survival was 33.2 months (95% CI, 21.7 to NE).

DDX41 variants were found in six patients with all patients harboring ≥2 *DDX41* variants (including the Arg525His variant in three). The germline versus somatic origin of these variants could not be proven; however, all six patients had one *DDX41* variant at approximately 50% VAF with a second lower VAF *DDX41* detected (Table S1). All patients with *DDX41* variants had a normal karyotype and SNP-A (5/5, 100%) in comparison to 48% (26/55) *DDX41* wildtype patients among evaluable patients. Additionally, five patients had monosomy 5 by G-banded karyotyping (expected to delete the *DDX41* locus) but all were associated with complex karyotype and these five patients died after median 4.9 months (range 0.6—21.6). The most common co-mutation in *DDX41* mutant group was *ASXL1* (4/6 patients) (Table S2). One patient with a *DDX41* mutation (low blast AML) died before receiving therapy due to disease progression. The remaining five patients, aged 64–76 years, received AZA ($n = 4$) or AZA+LEN ($n = 1$) and achieved complete remission (CR, $n = 1$), marrow CR ($n = 1$), hematologic improvement (HI, $n = 1$), and stable disease ($n = 2$), and remarkably all remained alive at last follow-up of median 53 months (range 47–58; Figure 2), despite having intermediate ($n = 3$) and high ($n = 2$) R-IPSS. No patients underwent HCT.

Mutations in *TP53*, *U2AF1*, and *EZH2*, and abnormal SNP-A profile, including complex and abnormal 5q/7q/17p, were associated with inferior outcomes consistent with previous studies (Figures S1A and S2). Additional molecular subgroup analyses did not identify any significant interaction with the treatment arms (Figure S1B).

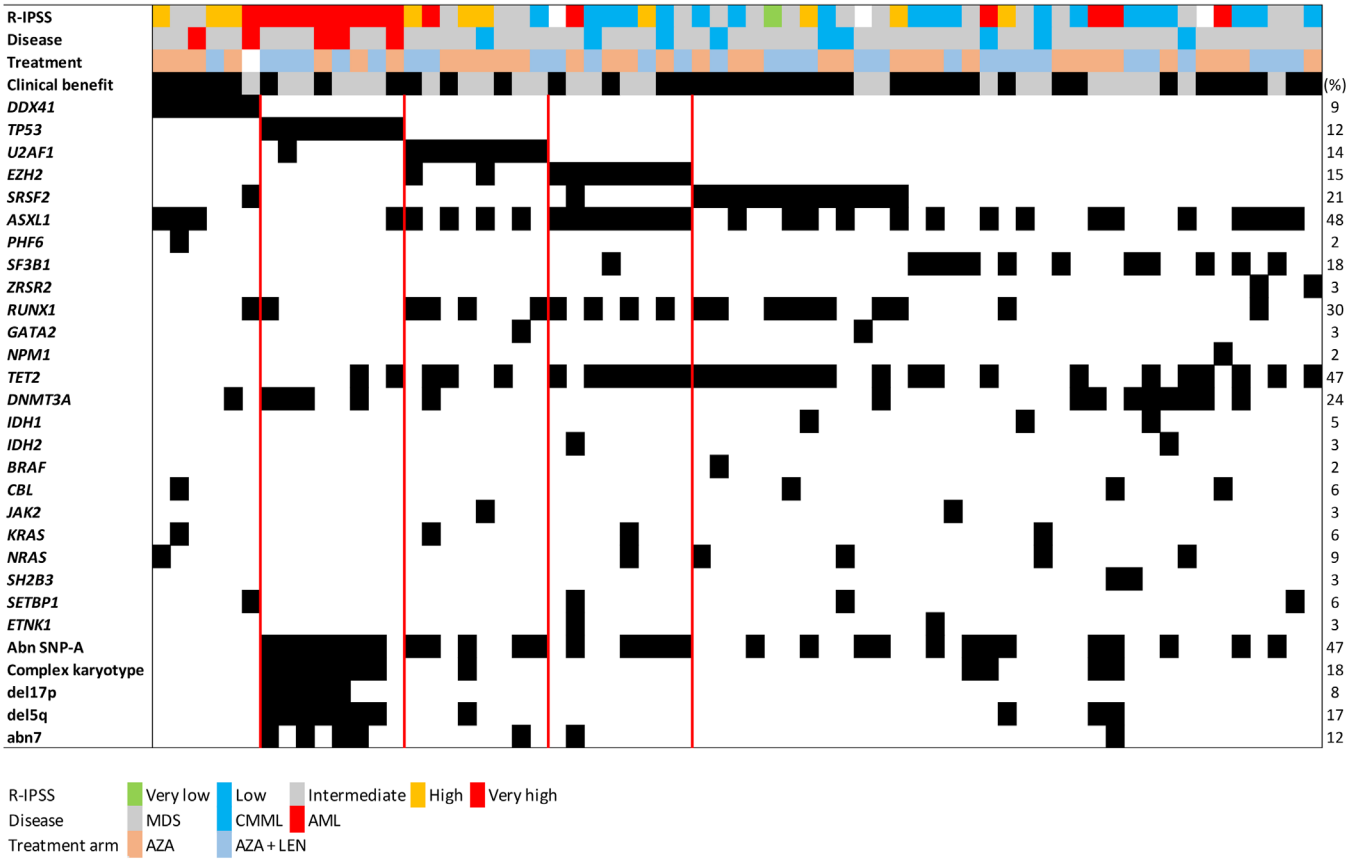

FIGURE 1 Molecular landscape and the associated clinical features of the 66 patients from the ALLG MDS4 study included in this post-hoc genomic correlative analysis.

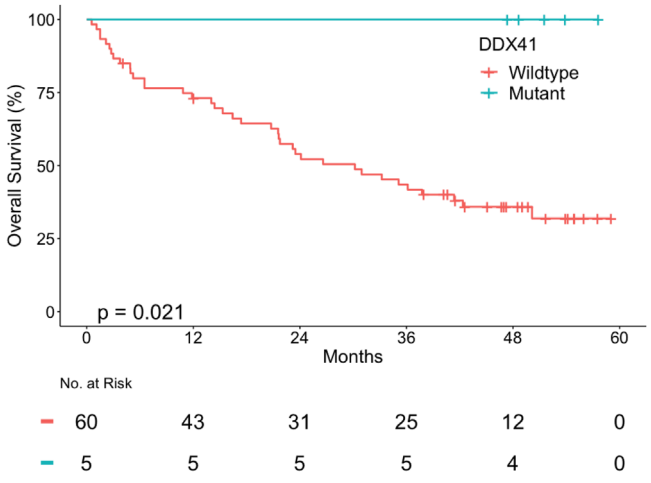

FIGURE 2 Kaplan–Meier overall survival according to *DDX41* mutation status.

Mutations were serially assessed on therapy in 45 patients after two (n = 38) to four cycles (n = 7) of therapy. The majority (67%) of the 166 mutations observed at baseline remained stable (< 10% VAF change). With the caveat of short interval between testing, no patient achieved complete molecular clearance. Fourteen (31%) patients had newly acquired mutations (n = 18 variants) or > 10% VAF increase (n = 2 variants). Twenty-six (58%) patients had mutation clearance of ≥1 variant (n = 20/166 [12%] variants) or > 10% VAF reduction (n = 33 [20%] variants). Of note, eight patients had mixed changes in VAF. Patients with mutation clearance/reduction had a trend toward better overall survival (Figure S3). Two patients with *DDX41*-mutated disease were assessed after two cycles of AZA with no significant change in the somatic mutations. One patient with *DDX41*-mutated MDS with increased blasts-1 achieved marrow CR after four cycles of AZA with approximately a 50% decrease in somatic *DDX41*, *NRAS*, and *ASXL1* mutations (Figure S4).

Our cohort demonstrated excellent outcomes among patients with *DDX41*-mutated myeloid neoplasms treated with AZA. Earlier retrospective studies suggested that patients with *DDX41*-mutated myeloid neoplasms might respond favorably to lenalidomide [10, 12, 13]. Sebert et al. studied 11 patients with *DDX41*-mutated MDS/AML who received AZA with 73% ORR and prolonged response duration (median 2.5 years); note seven patients underwent HCT [8]. A pooled analysis of additional patients (total 33 patients) showed similar ORR of 70% to AZA [9]. The small number (n = 5) in our trial cohort prevents any conclusion to be drawn regarding the impact of the addition of lenalidomide (n = 1) on *DDX41*-mutated MDS in comparison to AZA alone (n = 4). However, until further data become available, the management of *DDX41*-mutated MDS should follow the current standard of care.

In summary, our correlative molecular analysis of the ALLG MDS4 trial of AZA versus AZA+LEN in MDS/AML has identified that patients with *DDX41* mutations are associated with favorable outcomes with either AZA or AZA+LEN supporting the use of this therapy in these patients. This observation and subgroup of *DDX41*-mutated patients warrants further study in randomized trials.

AUTHOR CONTRIBUTIONS

PB and WSS designed the study. IST, MW, YZY, and PB performed the molecular analyses. JFS and MK contributed essential clinical data. IST and PB wrote the manuscript. All authors reviewed and approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

FUNDING INFORMATION

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL STATEMENT

This study was approved by the Northern Sydney Local Health District Human Research Ethics Committee (2019/ETH08385).

PATIENT CONSENT STATEMENT

All patients provided written informed consent to the ALLG MDS4 study and the storage and use of blood and tissue samples for research.

CLINICAL TRIAL REGISTRATION (INCLUDING TRIAL NUMBER)

anzctr.org.au (ACTRN12610000271000).

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Disclaimer

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SUPPORTING INFORMATION

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