A phase II trial of azacitidine with ipilimumab, nivolumab, or ipilimumab and nivolumab in previously untreated myelodysplastic syndrome

Patients with high-risk myelodysplastic syndrome (MDS) have dismal prognoses. The standard of care for patients with high-risk MDS who cannot undergo an allogeneic stem cell transplant (SCT) is continuous treatment with a hypomethylating agent.[1-3] However, hypomethylating agents do not elicit a response in at least half of patients with MDS.[4] Thus, innovative therapies are necessary. In this clinical trial, we found modest clinical activity of immunotherapy in combination with azacitidine and increased toxicity associated with dual checkpoint blockade.

Hypomethylating agents increase the expression of programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) in MDS cells.[5] PD-L1 is expressed on the surface of MDS cells resistant to hypomethylating agents, allowing for immune escape via T-cell evasion.[5] Hypomethylating agents also decrease the methylation of programmed cell death protein 1 (PD-1) promoters in $CD8^+$ T cells, increasing PD-1 expression.[6] Thus, combining PD-1 and CTLA4 blockade with hypomethylating agents may represent a successful treatment strategy for MDS.

Nivolumab and ipilimumab are fully human anti-PD-1 and anti-CTLA4 monoclonal antibodies, respectively. Whereas dual PD-1 and CTLA4 blockade is safe and effective in several tumor types,[7-9] the safety and efficacy of nivolumab and ipilimumab when administered with a hypomethylating agent in patients with MDS remain undefined.

This study was a sequential cohort, open-label, phase II trial involving previously untreated MDS (clinicaltrials.gov NCT02530463). Eligible patients were at least 18 years of age with treatment-naïve MDS according to the revised fourth edition of the World Health Organization criteria, with an Eastern Cooperative Oncology Group (ECOG) score of ≤2, adequate organ function, and less than 20% peripheral or bone marrow blasts. Patients received azacitidine-ipilimumab, azacitidine-nivolumab, or azacitidine-ipilimumab-nivolumab. We selected a sequential cohort design instead of a 3-arm randomized design to assess the safety of the doublet regimens before enrolling patients into the triplet cohort. The MD Anderson Institutional Review Board approved this study. All patients provided written informed consent according to the Declaration of Helsinki.

Patients received azacitidine 75 mg/m^2 intravenously (IV) on days 1-5 with nivolumab 3 mg/kg IV on days 6 and 20 in cohort 1; azacitidine 75 mg/m^2 IV on days 1-5 with ipilimumab 3 mg/kg IV on day 6 in cohort 2; azacitidine 75 mg/m^2 IV on days 1-5 with nivolumab 3 mg/kg IV and ipilimumab 1 mg/kg IV, both on day 6, in cohort 3. Patients continued the regimens until disease progression or intolerability.

The primary efficacy outcome was overall response, defined as complete response (CR), CR with limited count recovery (CR_L), or hematologic improvement according to the International Working Group 2023 criteria.[10] The study was monitored using the Bayesian design described by Thall and Sung.[11] Differences in mean values between groups were compared using the Mann-Whitney *U* test or Fisher's exact test. Overall survival (OS) was estimated using the Kaplan-Meier method and compared using the log-rank test. Hazard ratios (HR) were estimated using a Cox proportional hazards model. All reported *P* values were two-sided, with significance evaluated at the 0.05 alpha level. Statistical analyses were performed with GraphPad Prism 10.1.1 and R 4.4.1 for macOS. Data were stored in the ERIS MDS REDCap database designed for MD Anderson.[12,13]

We enrolled 66 patients from September 2015 to June 2021 in three cohorts: 33 (50%) patients in the azacitidine-ipilimumab cohort, 20 (30%) in the azacitidine-nivolumab cohort, and 13 (20%) in the azacitidine-ipilimumab-nivolumab cohort (Table 1). More patients had Revised International Prognostic Scoring System (IPSS-R) very poor-risk disease than any other risk category (47%; *P*=0.042). *TP53* mutations occurred significantly more frequently than did any other mutation (46%; *P*=0.003). The median Molecular International Prognostic Scoring System (IPSS-M) score was very high (1.59 [range, -1.55 to 4.08]). Overall, this trial included fit, older patients with high-risk cytogenetic and molecular characteristics.

The overall response rates (ORR) were 27% (95% CI: 14-44%) for azacitidine-ipilimumab, 55% (95% CI: 34-74%) for azacitidine-nivolumab, and 54% (95% CI: 29-77%) for azacitidine-ipilimumab-nivolumab (Table 2). CR was more frequent in the azacitidine-nivolumab cohort than in the azacitidine-ipilimumab cohort (40% *vs*. 7%; *P*=0.009). Among patients with 5% baseline blasts, the triplet approach of azacitidine-ipilimumab-nivolumab was associated with a better ORR compared to azacitidine-ipilimumab (55% *vs*. 17%; *P*=0.048).

We pooled the three cohorts and used a multiple logistic regression analysis to assess cytogenetic and molecular predictors of overall response in patients with ≥5% baseline blasts. Normal karyotypes were associated with an increased probability of response (odds ratio [OR], 9.39 [95% CI: 1.32-98.19]; *P*=0.036), and $DNMT3A^{mut}$ was associated with a reduced ORR (OR, 0.09 [95% CI: 0.004-0.638]; *P*=0.043). In contrast, when we performed the response

analysis in the overall cohort, irrespective of blast count, multiple logistic regression failed to identify significant cytogenetic or molecular predictors of overall response.

In a landmark analysis using the median time to SCT as the landmark, the median OS was 25.8 months for azacitidine-ipilimumab, 17.5 months for azacitidine-nivolumab, and 15.0 months for azacitidine-ipilimumab-nivolumab (*P*=0.224) (Figure 1A). A Cox proportional hazards model using SCT as a time-varying co-variate did not identify any significant differences in OS between treatment cohorts.

Table 1. Baseline characteristics of patients treated with azacitidine-ipilimumab, azacitidine-nivolumab, or azacitidine-ipilimumab-nivolumab.

Characteristic, N (%)	Azacitidine-ipilimumab N=33	Azacitidine-nivolumab N=20	Azacitidine-ipilimumab-nivolumab N=13
Female	13 (39.4)	4 (20.0)	11 (84.6)
Median age at diagnosis in years (range)	71 (45-86)	65 (39-83)	66 (46-72)
Median Charlson Comorbidity Index Score (range)	4 (0-10)	4 (0-6)	4 (0-7)
Median ECOG at diagnosis (range)	1 (0-2)	1 (0-2)	1 (0-1)
Diagnostic entity MDS MDS/MPN CMML	 28 (84.8) 0 5 (15.2)	 13 (65.0) 2 (10.0) 5 (25.0)	 13 (100) 0 0
Median hematologic parameters at diagnosis (range) Blasts, % WBC, x10^9/L ANC, x10^9/L Hemoglobin, g/dL Platelets, x10^9/L	 6 (1-18) 3.7 (0.6-46.1) 1.7 (0.1-24.0) 9.4 (5.2-16.1) 92 (7-647)	 9 (1-18) 4.9 (1.1-25.1) 1.8 (0-16.8) 9.7 (5.0-15.2) 53 (7-244)	 11 (1-19) 2.2 (1.1-7) 0.8 (0.2-3.2) 9.0 (6.1-13.3) 81 (18-209)
Median N of cycles (range)	4 (0-12)	6 (2-76)	3 (1-7)
Proceeded to allogeneic SCT	9 (27.3)	4 (20.0)	4 (30.8)
IPSS-R cytogenetic risk group Good Intermediate Poor Very poor	 7 (21.2) 7 (21.2) 7 (21.2) 12 (36.4)	 7 (35.0) 2 (10.0) 0 11 (55.0)	 2 (15.4) 1 (7.7) 2 (15.4) 8 (61.5)
Median IPSS-R risk classification	High	Very high	Very high
Median IPSS-R score (range)	5.5 (2-9.5)	7 (1-9.5)	7 (4-10)
IPSS-M risk group[a] Very low Low Moderate low Moderate high High Very high	 1 (8.3) 4 (12.1) 1 (8.3) 3 (9.1) 7 (21.2) 16 (48.5)	 0 2 (10.0) 1 (10.0) 2 (10.0) 4 (20.0) 11 (55.0)	 0 0 0 2 (15.4) 3 (23.1) 7 (53.8)
Median IPSS-M score (range)	1.54 (-1.55 to 4.08)	1.57 (-1.34 to 3.60)	1.66 (0.25 to 3.38)
Molecular aberrations *TP53* *ASXL1* *RUNX1* *DNMT3A* *TET2*	 14 (42.4) 7 (21.9) 5 (15.6) 4 (12.5) 4 (12.5)	 8 (40.0) 4 (20.0) 5 (25.0) 4 (20.0) 7 (35.0)	 8 (61.5) 0 2 (15.4) 4 (30.8) 2 (15.4)

[a]Molecular International Prognostic Scoring System (IPSS-M) risk group cohorts were based on availability of next-generation sequencing data at diagnosis. ANC: absolute neutrophil count; CMML: chronic myelomonocytic leukemia; ECOG: Eastern Cooperative Oncology Group; IPSS-R: Revised International Prognostic Scoring System; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; N: number; SCT: stem cell transplantation; WBC: white blood count.

The event-free survival was 12.1 months for azacitidine-ipilimumab, 13.7 months for azacitidine-nivolumab, and 11.9 months for azacitidine-ipilimumab-nivolumab (*P*=0.215). In a landmark subgroup analysis, azacitidine-nivolumab was associated with better OS compared to azacitidine-ipilimumab for patients with IPSS-R intermediate-risk MDS (not reached *vs*. 25.8 months; *P*=0.049) (Figure 1B). OS curves stratified by IPSS-R and IPSS-M risk are presented in *Online Supplementary Figure S2C* and *D*.

We noticed a disparity in post-transplant outcomes among treatment cohorts. Following SCT, OS was 49.6 months with azacitidine-ipilimumab and not reached with azacitidine-nivolumab with a median follow-up time of 88 months. In contrast, OS of patients in the triplet cohort who underwent SCT was significantly shorter at 13.7 months (doublet *vs.* triplet cohorts; *P*=0.008) (Figure 1D).

Next, we pooled the cohorts and used a multivariate Cox proportional hazards model with SCT as a time-dependent co-variate to assess the impact of baseline characteristics in addition to cytogenetic and molecular features on OS. Patients with monosomy 5 or loss of 5q had superior OS (HR, 0.24 [95% CI: 0.07-0.83]; *P*=0.024). Conversely, patients with *TP53*^{mut} MDS had inferior OS (HR, 19.46 [95% CI: 2.50-151.53]; *P*=0.005) (*Online Supplementary Figure S1*).

Azacitidine-ipilimumab-nivolumab had greater hematologic toxicity than did azacitidine-ipilimumab, including significantly higher rates of leukopenia (100% *vs*. 58%; *P*=0.004) and neutropenia (100% *vs*. 70%; *P*=0.022) (*Online Supplementary Table S1*). The rates of grade 3 rash and pneumonitis were higher in the triplet cohort than in the combined doublet cohorts (23% *vs*. 2%; *P*=0.021 and 15% *vs*. 4%; *P*=0.171, respectively). These findings translated to more days (d) hospitalized in the triplet cohort (27 d) compared to patients in the azacitidine-ipilimumab (8 d) or azacitidine-nivolumab (3 d) cohorts (*P*=0.002).

Overall, 29 (44%) patients had at least one immunotherapy-related adverse event (irAE). We saw no association between irAE incidence and the achievement of a response. Similarly, we observed no differences in OS between patients who did and did not experience an irAE (23.0 months *vs*. 16.2 months; *P*=0.924). However, we found that grade ≥2 pneumonitis was associated with worse OS (7.4 months *vs*. 22.7 months; *P*=0.025) (Figure 1F). Furthermore, we discovered that patients with pneumonitis of any grade were more likely to have *TP53*^{mut} MDS than patients without pneumonitis (86% *vs*. 43%; *P*=0.047).

Herein, we present data on the safety and efficacy of azacitidine-ipilimumab, azacitidine-nivolumab, and azacitidine-ipilimumab-nivolumab for patients with treatment-naïve MDS. We noted no differences in OS or event-free survival among the three treatment cohorts. However, in patients with IPSS-R intermediate-risk MDS, azacitidine-nivolumab produced better OS than did azacitidine-ipilimumab. Despite limited sample sizes, these findings imply that benefits of OS differ among discrete IPSS-R risk categories for patients undergoing immunotherapy-based approaches.

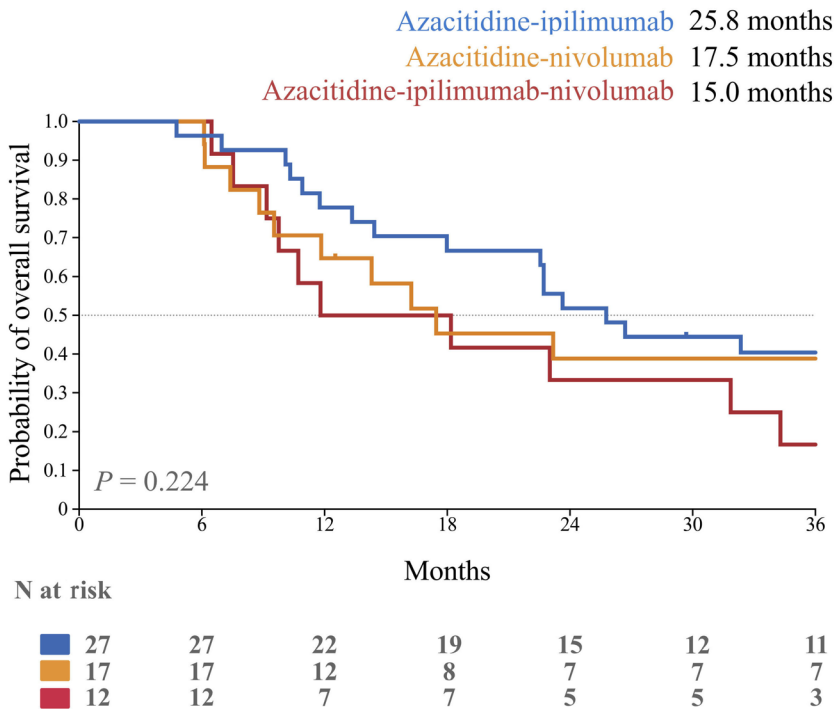
Post-transplant survival was significantly worse for the triplet cohort than for the doublet cohorts. However, our sample sizes are limited and our results should be interpreted with caution. Yet, our data suggest that azaciti-

Table 2. International Working Group 2023 response rates of patients treated with azacitidine-ipilimumab, azacitidine-nivolumab, or azacitidine-ipilimumab-nivolumab.

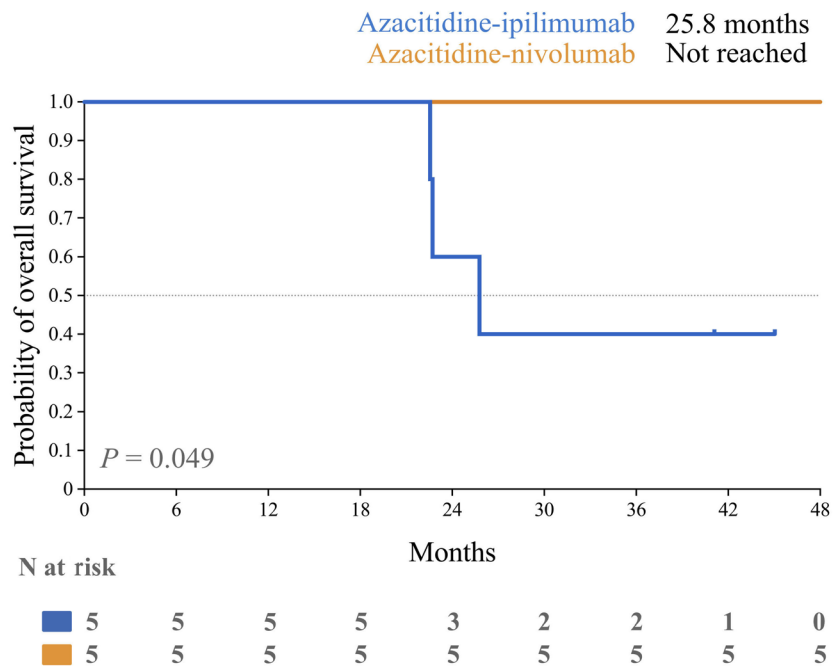
Response category, N (%)	Azacitidine-ipilimumab N=30[a]	Azacitidine-nivolumab N=20	Azacitidine-ipilimumab-nivolumab N=13
Complete remission	2 (6.7)	8 (40.0)	3 (23.1)
Complete remission with limited count recovery	2 (6.7)	0	3 (23.1)
Hematologic improvement	4 (13.3)	3 (15.0)	1 (7.7)
Marrow complete remission	9 (30.0)	4 (20.0)	3 (23.1)
No response	13 (43.3)	5 (25.0)	3 (23.1)
ORR[b]	8 (26.7)	11 (55.0)	7 (53.8)
Subgroup response categories			
IWG 2023 ORR, blasts ≥5%	3/18 (16.7)	6/15 (40.0)	6/11 (54.5)
IWG 2023 ORR by IPSS-M risk			
Very low or low	3/5 (60.0)	2/2 (100.0)	-
Moderate low or moderate high	2/4 (50.0)	2/3 (66.7)	1/2 (50.0)
High or very high	3/20 (15.0)	6/15 (40.0)	5/10 (50.0)
IWG 2023 ORR, *TP53*^{mut}	3/13 (23.1)	5/8 (62.5)	2/8 (25.0)

[a]Thirty of 33 patients were evaluable for response in the azacitidine-ipilimumab cohort. [b]The overall response rate (ORR) was determined using the International Working Group 2023 (IWG 2023) criteria. The ORR was calculated as the sum of patients with complete response (CR), CR with limited count recovery, and hematologic improvement. IPSS-M: Molecular International Prognostic Scoring System; N: number.

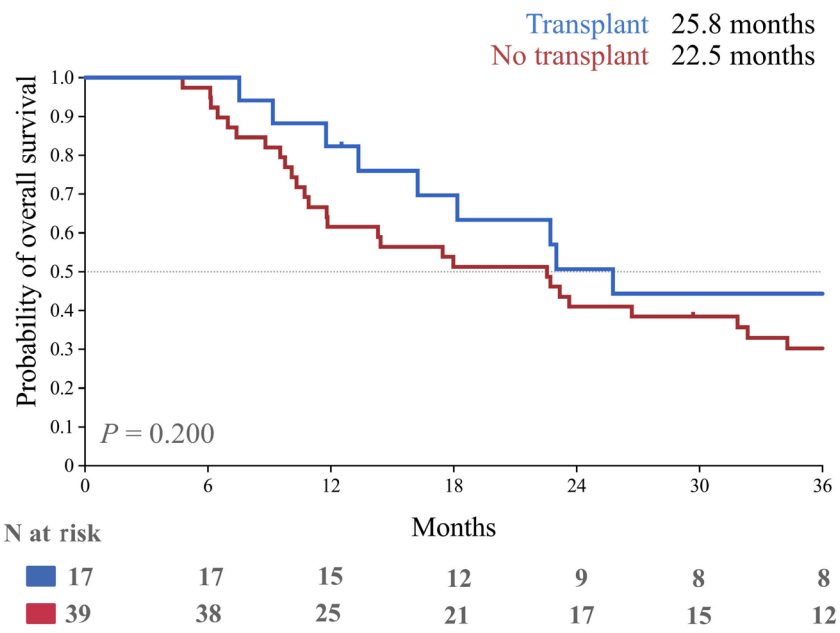
A **Overall survival**



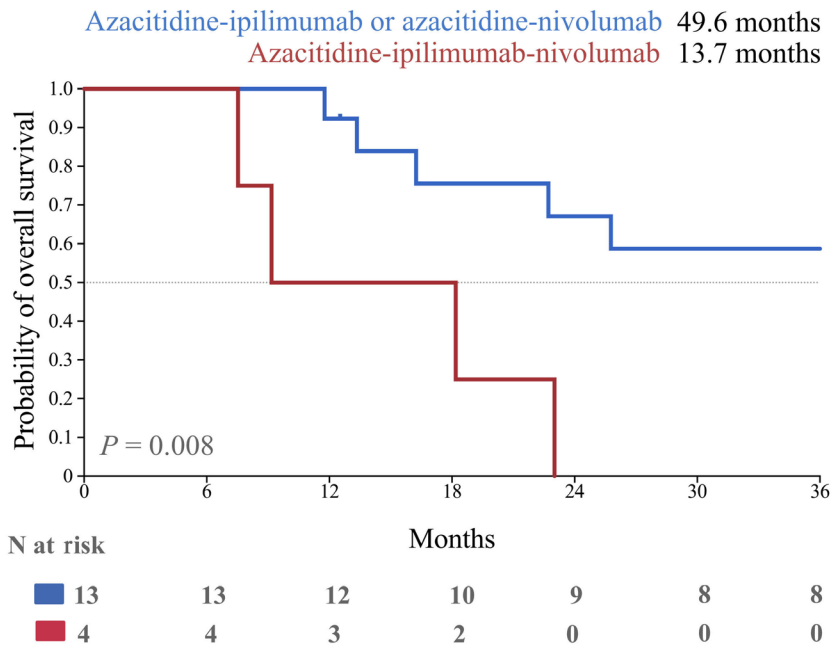
B **Overall survival, IPSS-R intermediate risk**



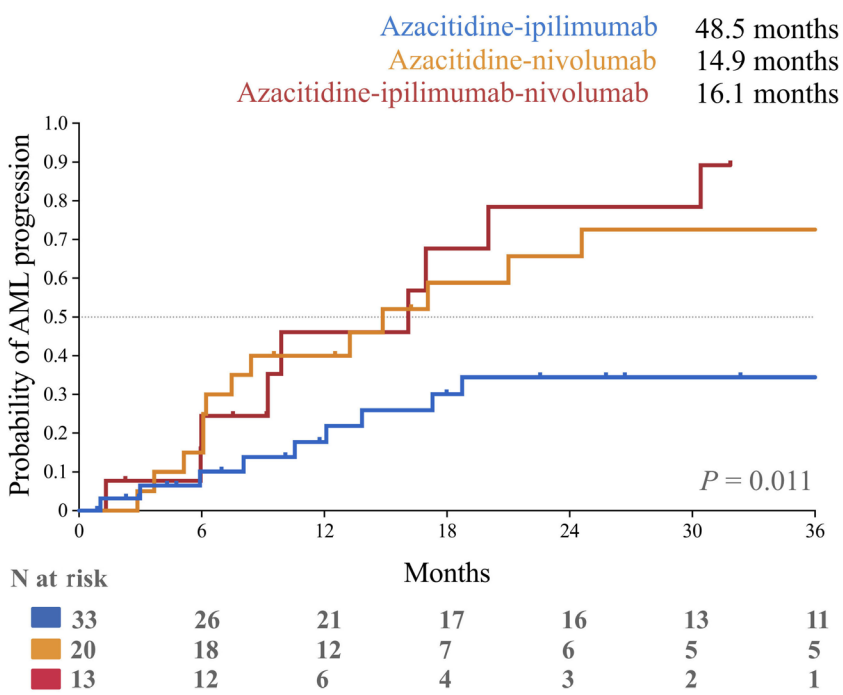
C **Overall survival**



D **Overall survival, post-transplant**



E **Time to AML progression**



F **Overall survival**

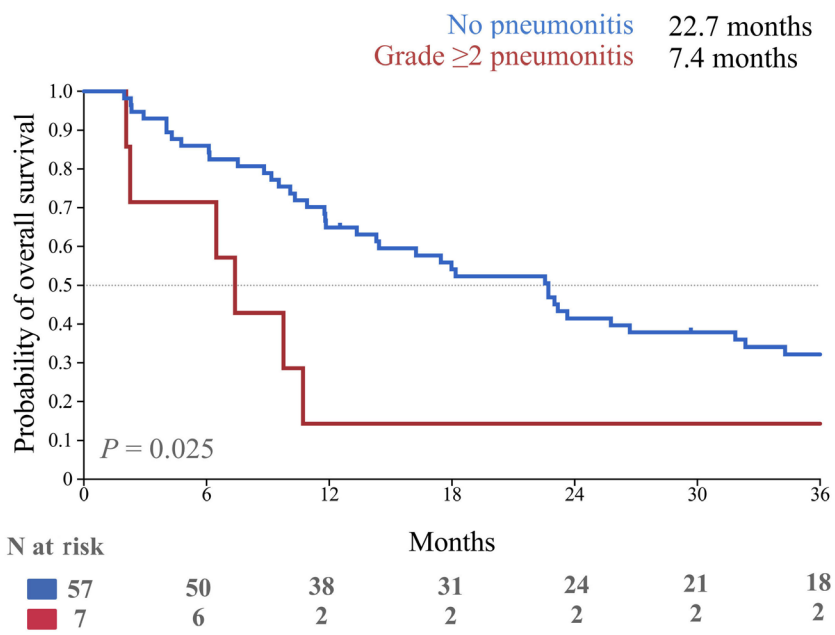


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Figure 1. Time-to-event analyses of azacitidine-ipilimumab, azacitidine-nivolumab, or azacitidine-ipilimumab-nivolumab. (A) Landmark analysis of overall survival (OS) of azacitidine-ipilimumab, azacitidine-nivolumab, or azacitidine-ipilimumab-nivolumab (*P*=0.224). (B) Landmark analysis of OS of patients with Revised International Prognostic Scoring System (IPSS-R) intermediate-risk disease treated with azacitidine-nivolumab *versus* azacitidine-ipilimumab (*P*=0.049). (C) Landmark analysis of OS of patients who did and did not undergo stem cell transplantation (SCT) from first-line therapy, regardless of immunotherapy cohort (*P*=0.200). (D) OS of patients who underwent SCT after receiving doublet therapy *versus* triplet therapy (*P*=0.008). (E) Progression to acute myeloid leukemia (AML) for all three cohorts. The probability of progression was significantly lower in the azacitidine-ipilimumab cohort (*P*=0.011). (F) OS of patients treated with azacitidine with immunotherapy who did and did not experience pneumonitis (*P*=0.025). N: number.

dine-ipilimumab-nivolumab is associated with a higher risk of post-transplant mortality than the doublet regimens. While post-transplant immune complications may play a role in our observations of inferior survival for the triplet approach, more research is needed to determine the safety of immunotherapy-based combinations preceding SCT.

The doublet combinations appeared to be well tolerated, with irAE incidence rates similar to those reported in a large meta-analysis of 7,936 patients treated with nivolumab or ipilimumab-nivolumab.[14] However, we emphasize the significantly increased toxicity associated with the triplet approach. Furthermore, in the overall study population, we discovered that patients with pneumonitis were enriched in mutated *TP53*, and that the incidence of pneumonitis was associated with shortened OS. Therefore, these findings raise awareness of the risk of irAE, which may be increased in patients with *TP53*^{mut} MDS.

In summary, azacitidine-nivolumab produced higher rates of CR and a non-significantly higher ORR in the overall study population than did the other two combinations. Azacitidine-nivolumab was also associated with a greater survival benefit for IPSS-R intermediate-risk MDS than azacitidine-ipilimumab. High-grade toxicities and hospitalization rates were considerably worse in the triplet cohort than in the doublet cohorts, and the triplet regimen appeared to be associated with increased post-transplant mortality. Therefore, azacitidine in combination with PD-1 or CTLA4 blockade had modest activity in MDS; the triplet approach failed to improve OS and was associated with increased toxicity.

Authors

Ian M. Bouligny,[1] Guillermo Montalban-Bravo,[1] Koji Sasaki,[1] Naval Daver,[1] Elias Jabbour,[1] Yesid Alvarado,[1] Courtney D. DiNardo,[1] Farhad Ravandi,[1] Gautam Borthakur,[1] Prithviraj Bose,[1] Naveen Pemmaraju,[1] Steven Kornblau,[1] Tapan Kadia,[1] Lucia Masarova,[1] Koichi Takahashi,[1] Michael Andreeff,[1] Alexandre Bazinet,[1] Hui Yang,[1] Rashmi Kanagal-Shamanna,[2] Chitra Hosing,[3] Sherry Pierce,[1] Meghan Meyer,[1] Xuelin Huang[4] and Guillermo Garcia-Manero[1]

[1]Department of Leukemia; [2]Department of Hematopathology; [3]Department of Stem Cell Transplantation and Cellular Therapy and [4]Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Correspondence:
G. GARCIA-MANERO - ggarciam@mdanderson.org

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Disclosures

No conflicts of interest to disclose.

Contributions

IMB designed the study database, analyzed the data, created the figures, and prepared the manuscript. GM-B, KS, ND, EJ, YA, CDDiN, FR, GB, NP, SK, TK, LM, KT and MA enrolled patients and revised the manuscript. AB collected data and revised the manuscript. HY and CH revised the manuscript. RK interpreted pathology samples and revised the manuscript. SP collected data and revised the manuscript. MM obtained patient consent, organized the trial, and revised the manuscript. XH helped analyze the data and revised the manuscript. GG-M oversaw the study, enrolled patients, and revised the manuscript. All figures, illustrations, and data in this manuscript were independently generated by the authors.

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Data-sharing statement

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

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