Higher-dose venetoclax with measurable residual disease-guided azacitidine discontinuation in newly diagnosed acute myeloid leukemia

Jonathan A. Gutman,[1] Amanda Winters,[2] Andrew Kent,[1] Maria Amaya,[1] Christine McMahon,[1] Clayton Smith,[1] Craig T. Jordan,[1] Brett Stevens,[1] Mohammad Minhajuddin,[1] Shanshan Pei,[1] Jeffrey Schowinsky,[3] Jennifer Tobin,[1] Kelly O'Brien,[1] Angela Falco,[1] Elizabeth Taylor,[1] Constance Brecl,[1] Katie Zhou,[1] Phuong Ho,[1] Connor Sohalski,[1] Jessica Dell-Martin,[1] Olivia Ondracek,[1] Diana Abbott[4] and Daniel A. Pollyea[1]

[1]Division of Hematology, Department of Medicine; [2]Center for Cancer and Blood Disorders, Department of Pediatrics; [3]Department of Pathology and [4]Center for Innovative Design and Analysis, Department of Biostatistics and Informatics, University of Colorado, Aurora, CO, USA

Correspondence: D.A. Pollyea
Daniel.pollyea@ucdenver.edu

Received: January 3, 2023.
Accepted: April 6, 2023.
Early view: April 13, 2023.

https://doi.org/10.3324/haematol.2023.282681

Abstract

Venetoclax+azacitidine is the standard of care for newly-diagnosed patients with acute myeloid leukemia (AML) for whom intensive chemotherapy is inappropriate. Efforts to optimize this regimen are necessary. We designed a clinical trial to investigate two hypotheses: i) higher doses of venetoclax are tolerable and more effective, and ii) azacitidine can be discontinued after deep remissions. Forty-two newly diagnosed AML patients were enrolled in the investigator-initiated High Dose Discontinuation Azacitidine+Venetoclax (HiDDAV) Study (*clinicaltrials gov. Identifier: NCT03466294*). Patients received one to three "induction" cycles of venetoclax 600 mg daily with azacitidine. Responders received MRD-positive or MRD-negative "maintenance" arms: azacitidine with 400 mg venetoclax or 400 mg venetoclax alone, respectively. The toxicity profile of HiDDAV was similar to 400 mg venetoclax. The overall response rate was 66.7%; the duration of response (DOR), event-free survival (EFS) and overall survival were 12.9, 7.8 and 9.8 months, respectively. The MRD negativity rate was 64.3% by flow cytometry and 25.0% when also measured by droplet digital polymerase chain recation. MRD-negative patients by flow cytometry had improved DOR and EFS; more stringent measures of MRD negativity were not associated with improved OS, DOR or EFS. Using MRD to guide azacitidine discontinuation did not lead to improved DOR, EFS or OS compared to patients who discontinued azacitidine without MRD guidance. Within the context of this study design, venetoclax doses >400 mg with azacitidine were well tolerated but not associated with discernible clinical improvement, and MRD may not assist in recommendations to discontinue azacitidine. Other strategies to optimize, and for some patients, de-intensify, venetoclax+azacitidine regimens are needed.

Introduction

Venetoclax with a backbone therapy (hypomethylating agent or low dose cytarabine) is the standard of care for a newly diagnosed patient with acute myeloid leukemia (AML) who is not a candidate for intensive induction chemotherapy. The complete remission (CR)/CR with incomplete hematologic recovery (CRi) rate of 66.4%, duration of response (DOR) of 17.5 months, event-free survival (EFS) of 9.8 months and overall survival (OS) of 14.7 months were all superior to azacitidine alone.[1] Measurable residual disease (MRD) negativity as measured by multiparameter flow cytometry was achieved in 41% of responders using this regimen, and these patients had superior outcomes.[2] During the phase I study with hypomethylating agents, venetoclax doses greater than 400 mg were administered without dose-limiting toxicity, and the maximum tolerated dose was not reached.[3] Furthermore, there were patients who achieved MRD-negative remissions in the >400 mg venetoclax cohorts.[4] We, therefore, hypothesized that higher doses of venetoclax could be safely administered, resulting in deeper remissions and better outcomes.

Patients who achieve a remission with venetoclax+hypomethylating agent are recommended to continue indefinite treatment with both therapies, although no prospective studies have been performed to evaluate the veracity of this approach. A retrospective study found that for patients who achieved long-term remissions, con-

tinued treatment had no benefit compared to discontinuation.[5] Long-term administration of hypomethylating agents can be challenging due to a multitude of factors, ranging from drug-related toxicity to logistical and quality-of-life obstacles.[6,7] Given the established evidence supporting the prognostic significance of MRD negativity in AML,[8] as well as the unique ability of venetoclax to target the leukemia stem cell population,[9-11] we hypothesized that patients who achieved MRD negativity could discontinue azacitidine and be effectively maintained on venetoclax as a single agent.

In order to test these hypotheses, we designed a single-institution, investigator-initiated, phase II study of newly diagnosed older AML patients. Patients were administered 600 mg of venetoclax with the standard dose and schedule of azacitidine. Those who achieved MRD negativity discontinued azacitidine and were maintained on venetoclax alone (High Dose Discontinuation Azacitidine+Venetoclax Study [HiDDAV], *clinicaltrials gov. Identifier: NCT03466294*). We report here the safety and efficacy outcomes for this clinical trial.

Methods

The protocol was approved by the Colorado Multiple Institutional Review Board and is included in the *Online Supplementary Appendix*; patients were treated in accordance with the Declaration of Helsinki. The primary endpoint was remission duration; at study initiation, the median remission duration for venetoclax with azacitidine in the M14-358 study was 8 months. The alternative hypothesis for this study was a 12-month median remission duration, requiring 42 subjects for 80% power to detect this difference with a significance of 0.10.[12] Patients were eligible if they had no prior therapy for non-acute promyelocytic AML, were ≥60 years, ineligible to undergo standard intensive induction chemotherapy due to age or comorbidities or declined to receive this treatment, had an Eastern Cooperative Oncology Group performance status[13] of ≤2, adequate organ function, were without central nervous system involvement and had a white blood cell count <25x10^9/L (hydroxyurea permitted to achieve this).

Patients were admitted to the hospital for "induction": dose escalation of venetoclax to 600 mg over 4 days, which was continued until day 28, and concomitant azacitidine 75 mg/m^2, delivered subcutaneously or intravenously on days 1-7 (Figure 1A). Bone marrow biopsies were performed on cycle 1 on days 8 and 28, with an MRD assessment on day 28. Patients without an overall response (CR/CRi/morphologic leukemia-free state [MLFS]) by day 28 either discontinued the study or, in the setting of a beneficial response short of MLFS (typically a scenario in which a significant decrease in the blast percentage occurred but did not reach <5%), repeated an "induction" cycle, without venetoclax dose escalation or mandatory hospitalization. Those who achieved an overall response and were MRD-positive repeated "induction", without venetoclax dose escalation or mandatory hospitalization. Up to three "induction" cycles were permitted for non-responders or MRD-positive responders, with bone marrow biopsies at the conclusion of each cycle. Patients without an overall response by the third induction cycle discontinued the study. If patients achieved an overall response but did not achieve MRD negativity after the third induction cycle, they moved to "MRD-positive maintenance": venetoclax 400 mg days 1-28 with azacitidine on days 1-7. Subsequent bone marrow biopsies were performed at the conclusion of each odd-numbered cycle until cycle nine, at which point bone marrow biopsies occurred after cycle 12 and then every six cycles. From the conclusion of the first "induction" cycle, and continuing with each response assessment, any patient who achieved "full MRD-negativity" (defined below) went to "MRD-negative maintenance": venetoclax 400 mg days 1-28 and discontinuation of azacitidine. These patients had bone marrow biopsies at the conclusion of every three cycles for the first year and every six cycles thereafter (Figure 1B). MRD-negative patients who had MRD recurrence, or morphologic disease relapse, could resume azacitidine.

Adverse events (AE) were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. Serious AE (SAE) were defined as AE that were life threatening, or resulted in death, hospitalization or prolongation of hospitalization. Response definitions were performed in accordance with the 2017 European Leukemia Network (ELN);[14] for patients who achieved MLFS or CRi, a 14-day treatment-free interval, with or without granulocyte colony-stimulating factor (GCSF), occurred, and "upgraded" responses based on blood count recovery were recorded.[15] A hematopathologist (JS) retrospectively reviewed all bone marrow biopsies to assign a French-American-British (FAB) category;[16] M0 and M1 were indistinguishable because cytochemical studies are no longer utilized.

In addition to cytogenetic testing, two MRD assessments were employed for patients who achieved CR/CRi/MLFS. First, a multiparameter flow cytometry (flow) approach, analyzing a "different from normal" aberrant immunophenotype[17] (Hematologics, Inc, Seattle WA) was utilized; MRD-negative by this modality was defined as no evidence of aberrant myeloid antigen expression or abnormal myeloblasts at a level of detection <0.1% in an adequate sample. In addition, droplet digital polymerase chain reaction (ddPCR) assays were used for MRD. This was accomplished through the application of an institutional baseline next-generation sequencing panel utilizing 49 genes (*Online Supplementary Table S1*); concurrent *FLT3* internal tandem

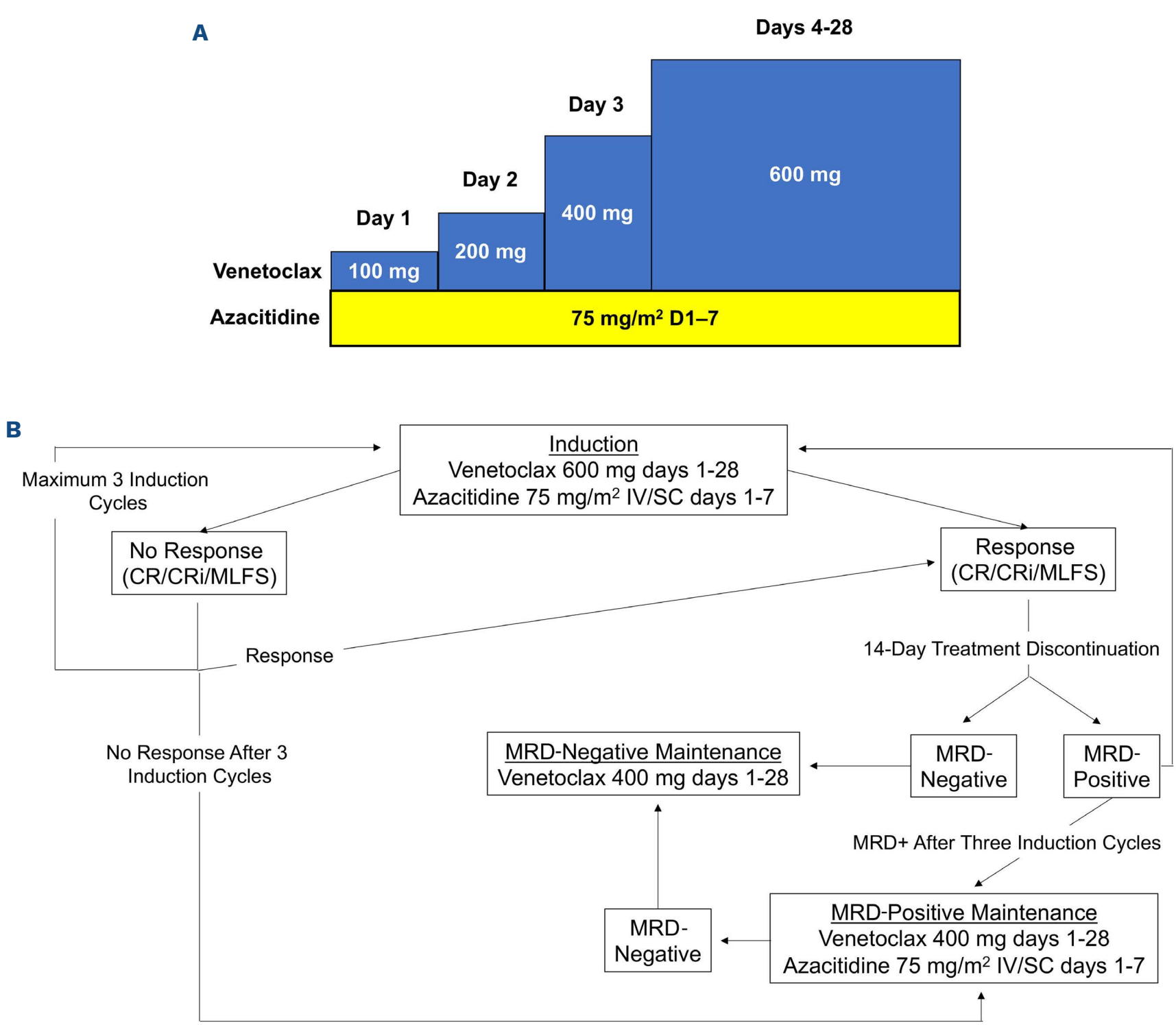

Figure 1. Logistical details related to patient management on the Higher Dose Venetoclax with Measurable Residual Disease-Guided Discontinuation of Azacitidine (HiDDAV) Study. (A) Venetoclax dose escalation during cycle 1. (B) Algorithm describing treatment for subjects. MRD: minimal residual disease; D: day; IV: intravenous; SC: subcutaneous; CR: complete remission; CRi: complete remission with incomplete hematologic recovery; MLFS: morphologic leukemia-free state.

duplications (ITD) was performed by PCR amplification and fragment analysis with a 5% lower limit of detection.[18] DNA from fingernails was sequenced and tested alongside the initial bone marrow sample in most patients to exclude germline variants. After gene mutation results were reported, ddPCR assays for as many somatic non-*DNMT3A/TET2/ASXL1* (DTA) mutations[19] as possible were applied, with a sensitivity of 0.02-0.1%. MRD negativity by this modality was defined as undetectable for all assayed gene mutations. Mutations with large insertions/deletions were unable to be followed by ddPCR. A patient was considered to be "fully MRD-negative" if they were MRD-negative by both flow and ddPCR if eligible for monitoring by ddPCR, or only by flow if not eligible for monitoring by ddPCR.

DOR was defined as time from first response to relapse, per ELN.[14] EFS was defined as time from study entry to death from any cause, relapse for responders and the day of treatment initiation for patients who were refractory.[20] OS was defined as the time from study entry to death from any cause. Duration of cytopenias in cycle 1 were defined as described elsewhere.[21]

SAS version 9.4 was used for statistical analyses. Median survival times and associated Hall-Wellner confidence bands were created using Kaplan-Meier product-limit estimates for DOR, EFS, and OS. In order to compare survival times based on MRD status, Mantel-Cox log-rank analysis with a significance threshold of 0.05 was used. Cox regression was used to compute hazard ratios (HR) to assess the effect of variables of interest on the time-to-event out-

comes. Cox regression survival analysis was analyzed both unadjusted for allogeneic stem cell transplantation (ASCT) status and using ASCT status as a time-dependent covariate. Additionally, Cox regression models assessed whether the relationship between survival and the variable of interest differed based on ASCT status; in instances in which the stratified model presented a different effect based on ASCT status, results for Cox regression are reported from the stratified model. Otherwise, the unadjusted Cox regression model results are the same as the modeled results that treat ASCT as a time-dependent covariate. In assessing the effect of variables of interest on response status, logistic regression models were run, and odds ratios (OR) with their 95% confidence intervals (CI) and associated *P* values are reported.

Results

Baseline data for the 42 patients who were enrolled are summarized in Table 1. Median follow up time is 42 months (95% CI: 29.3-42.5). Patients received a median of four total cycles of therapy (range, 0-40); 19 (45.2%) received more than one MRD-positive maintenance cycle (median 3; range, 1-18) and nine (21.4%) received more than one MRD-negative maintenance cycle (median 5; range, 1-39). All patients had at least one AE; 700 total AE were reported. Thirty-two patients (76%) had a SAE. Common AE (≥20%) are summarized in Table 2. The most frequently reported hematologic AE grade ≥3 included thrombocytopenia (52%), febrile neutropenia (50%), leukopenia (41%) and neutropenia (38%). Gastrointestinal AE of any grade were common and included constipation (55%), diarrhea (55%), nausea (52%) and emesis (33%). Notable SAE (grade ≥3) included febrile neutropenia (36%), pneumonitis (7%) and bacteremia (5%) (Table 2). In nearly all cycles (230/273, 91.6%), 28 days of venetoclax was administered, according to patient-reported diaries. Decreases in the duration or dose of venetoclax were never prescribed for mitigation of toxicity. Neither laboratory nor clinical tumor lysis syndrome were observed. No patients discontinued treatment due to AE. Mortality at 30 days occurred in five of 42 (11.9%) of patients, all of whom had refractory disease and died of AML.

Table 1. Baseline variables for the 42 patients enrolled in the Higher Dose Venetoclax with Measurable Residual Disease Guided Discontinuation of Azacitidine (HiDDAV) Study.

Variable	Value
Female, N (%)	21 (50)
Median age in years (range)	70 (60-88)
Median baseline bone marrow blast % (range)	50 (20-86)
Secondary AML, N (%) Evolved from prior MDS Treatment related AML	14 (33) 8 (19) 6 (14)
French American British Category, N (%) M5 M0/M1 Other	 3 (7) 33 (79) 6 (14)
ELN risk Group, N (%) Favorable Intermediate Adverse	 9 (21) 3 (7) 30 (72)
Gene mutations, N (%) *FLT3* ITD or TKD *NPM1* *ASXL1* *TP53* *RAS* Pathway *IDH1/2* *RUNX1*	 5 (12) 10 (24) 7 (17) 13 (31) 9 (21) 8 (19) 7 (17)
Required hydroxyurea to enroll, N (%)	10 (24)

AML: acute myeloid leukemia ELN: European Leukemia Network; ITD: internal tandem duplication; TKD: tyrosine kinase domain; MDS: myelodysplastic syndromes.

The overall response rate was 28 of 42 (66.7%); 26 (61.9%) had a CR and two (4.8%) had MLFS as best response (Table 3). GCSF was administered to 17 of 42 (40.5%) of patients; they received a median of two doses (range, 1-17) after cycle 1 (N=13), cycle 2 (N=7), cycle 3 (N=3), cycle 4 (N=3), cycle 5 (N=1), cycle 6 (N=1) and cycle 7 (N=1). During cycle 1, the median time to recover absolute neutrophil count (ANC) to >1.0x10^9/L was 39 days (range, 7-42), while the median time to recover platelets to >100x10^9/L was 26 (range, 7-37) days.

Of the responders, 18 of 28 (64.3%) achieved MRD negativity by flow at any point during their treatment course. Twenty-six of 28 (93%) were able to be monitored by ddPCR (2 patients had no baseline mutations); baseline mutations for all patients and those genes that were monitored are shown in the *Online Supplementary Table S2*. The percentage of all baseline non-DTA genes able to be monitored by ddPCR in responding patients was 70% (45/64). Of patients monitored by ddPCR, nine of 26 (35%) achieved MRD negativity. Full MRD negativity was achieved in seven of 28 (25%) (Table 3).

Forty-one patients came off study; 12 (29.3%) for death, four (9.8%) due to patient decision, four (9.8%) for refractory disease, 11 (26.8%) for relapsed disease and ten (24.3%) for ASCT. Eight (19%) remain alive. Individual patient outcomes are summarized in Figure 2.

Thirty-five (83.3%) patients had an interpretable cycle 1 day 8 bone marrow biopsy; 13 (37.1%) had blast clearance to <5% (CRi=1, MLFS=12). Eleven of 13 (84.6%) went on to achieve CR as best response. Fourteen patients without blast clearance on day 8 ultimately responded (N=13 CR, 1 MLFS).

The median DOR was 12.9 months (95% CI: 7.4-17.6).

Table 2. Adverse events that occurred with an incidence of ≥20% and serious adverse events that occurred in more than one patient for participants of the High Dose Discontinuation Azacitidine+Venetoclax (HiDDAV) Study.

Event	All Grades, N (%)	Grade ≥3, N (%)
All adverse events (% of patients)	700 (100)	182 (95)
Hematologic adverse events		
Thrombocytopenia	24 (57)	22 (52)
Febrile neutropenia	22 (52)	21 (50)
Anemia	19 (45)	1 (2)
Leukopenia	18 (43)	17 (41)
Neutropenia	18 (43)	16 (38)
Non-hematologic adverse events		
Constipation	23 (55)	0 (0)
Diarrhea	23 (55)	2 (5)
Nausea	22 (52)	3 (7)
Fatigue	15 (36)	6 (14)
Hypocalcemia	15 (36)	2 (5)
Emesis	14 (33)	2 (5)
Edema	12 (29)	0 (0)
Hypokalemia	12 (29)	0 (0)
Vertigo	12 (29)	0 (0)
Anorexia	11 (26)	2 (5)
Hypophosphatemia	11 (26)	7 (17)
Alkaline phosphatase increased	10 (24)	0 (0)
Aspartate aminotransferase increased	10 (24)	0 (0)
Headache	10 (24)	0 (0)
Dyspnea	10 (24)	3 (7)
Sore throat	10 (24)	0 (0)
Pruritis	10 (24)	0 (0)
Hypotension	10 (24)	0 (0)
Hypoalbuminemia	9 (21)	1 (2)
Hypoxia	9 (21)	5 (12)
Pleural effusion	9 (21)	5 (12)
Serious adverse events		
Febrile neutropenia	16 (38)	15 (36)
Pneumonitis	3 (7)	3 (7)
Bacteremia	3 (7)	2 (5)
Cholecystitis	2 (5)	1 (2)
Sepsis	2 (5)	2 (5)
Syncope	2 (5)	1 (2)
Acute kidney injury	2 (5)	2 (5)
Dyspnea	2 (5)	2 (5)

Median EFS was 7.8 months (95% CI: 2.5-11.2) and median OS was 9.8 months (95% CI: 6.6-14.9) (Figure 3). Patients who achieved MRD negativity by ddPCR, or full MRD negativity, did not have improved OS, EFS or DOR (*Online Supplementary Figure S1*). However, patients who were MRD-negative by flow had significantly improved EFS (median 18.7 *vs.* 8.2 months; *P*=0.047) and DOR (median 16.6 *vs.* 6.7 months; *P*=0.037), and an OS of 19.6 *vs.* 11.2 months, (*P*=0.16) (Figure 3). Because of the high rate of ASCT in this study (10/42, 24.3%), it was thought this factor may have overcome the impact of more stringent measures of MRD positivity. Therefore, MRD as defined by flow cytometry, ddPCR and both (full MRD negativity) were evaluated as predictors of DOR, EFS and OS after stratify-

Table 3. Responses and incidence of measurable residual disease.

Variable	N (%)
Overall response rate Complete remission Morphologic leukemia free state	28/42 (66.7) 26/42 (61.9) 2/42 (4.8)
MRD-negative by flow cytometry in patients with adequate samples	18/28 (64.3)
Evaluable by ddPCR MRD-negative by ddPCR	26/28 (92.9) 9/26 (34.6)
Full MRD negativity	7/28 (25.0)

MRD: measurable residual disease; ddPCR: digital droplet polymerase chain reaction.

ing patients by transplant status. Full MRD negativity, and MRD negativity by ddPCR, did not impact these outcomes in either the transplanted or non-transplanted group, while MRD negativity by flow may be predictive of better DOR, EFS and OS in the non-transplantation group only (*Online Supplementary Table S3*).

Azacitidine was discontinued in 11 patients; in seven patients this was protocol-mandated due to the achievement of full MRD negativity, and occurred after a median of two cycles (range, 1-6). Four patients chose to stop azacitidine, in violation of the protocol without having achieved MRD negativity, for convenience and/or perceived toxicity, after a median of four cycles (range, 4-6). Five of 11 patients who discontinued azacitidine progressed (N=3 with MRD negativity and N=2 without MRD negativity). At progression, all patients who had repeat cytogenetic and molecular testing showed evidence of clonal evolution (*Online Supplementary Table S4*). The median DOR, EFS and OS for those who stopped azacitidine with and without MRD guidance was 16.0 months (95% CI: 7.1- not reached [NR]) and 12.9 months (95% CI: 5.5-NR), 17.4 months (95% CI: 8.5-NR) and 14.3 months (95%CI: 6.6-NR), and 17.4 months (95% CI: 8.5-NR) and 18.8 months (95% CI: 6.6-NR), respectively (*Online Supplementary Table S5*).

Six patients who had discontinued azacitidine and continued venetoclax resumed azacitidine after a median of nine cycles (range, 5-20). In two cases this was done for relapse; in three it was done for conversion to MRD positivity from an MRD-negative state, and in one case it was due to patient's preference. In no cases did the re-institution of azacitidine result in a second remission or decrease in MRD.

Negative predictors for response included FAB M5 and *TP53* mutational status; male sex was the sole positive response predictor (*Online Supplementary Table S6*). Predictors for decreased DOR included *FLT3*, *FLT3* ITD, ELN adverse risk, the need for hydroxyurea to enroll in the study and no blast clearance on cycle 1 day 8, while *IDH*1/2 mutation and MRD negativity by flow cytometry were associated with improved DOR. Predictors for decreased EFS included FAB M5 and *TP53* mutation, while *IDH*1/2 mutations and ASCT were associated with longer EFS. For OS, FAB M5, *FLT3* ITD and *TP53* mutations were negative predictors while *IDH*1/2 mutations and ASCT were positive predictors (*Online Supplementary Table S7*).

Discussion

The HiDDAV study was conceived and conducted with the dual aims of determining i) whether venetoclax doses >400 mg might result in deeper remissions, and ii) whether MRD could safely guide azacitidine discontinuation. Primarily, we found that "induction" cycles of 600 mg venetoclax, administered for up to the first three cycles of therapy, had a safety profile similar to the definitive VIALE-A study using 400 mg of venetoclax.[1] While comparing toxicity between different studies must be done with caution, particularly for studies with different median ages (70 *vs.* 76-years old for HiDDAV *vs.* VIALE-A), grade ≥3 thrombocytopenia (52% *vs.* 45%), neutropenia (38% *vs.* 42%) and febrile neutropenia (50% *vs.* 42%) were similar for HiDDAV *versus* VIALE-A, respectively.[1] The 30-day mortality rates were 12% for HiDDAV and 7% for VIALE-A; however, all early mortality events in HiDDAV were from refractory AML. During cycle 1, time to recovery of ANC to >1.0x10^9/L and platelets to >100x10^9/L were similar to recently published results using 400 mg venetoclax (39 *vs.* 35 days and 26 *vs.* 25 days, for HiDDAV *vs.* comparator, respectively).[21]

Just as a comparison of toxicity between studies is fraught, so too are attempts to compare efficacy. However, when this is nonetheless done, there is no suggestion that the HiDDAV study had superior response rates (66.7% overall response rate *vs.* 66.4% CR+CRi rate for HiDDAV *vs.* VIALE-A), DOR (12.9 *vs.* 17.5 months for HiDDAV *vs.* VIALE-A) EFS (7.8 *vs.* 9.8 months for HiDDAV *vs.* VIALE-A) or OS (9.8 *vs.* 14.7 months for HiDDAV *vs.* VIALE-A[1]).

In this trial using 600 mg of venetoclax, MRD negativity by flow was numerically higher than previously reported with 400 mg, using a similar methodology (64% *vs.* 41%);[2] conclusions regarding a relationship between venetoclax dose and depth of response would require specific testing in a controlled setting. Consistent with Pratz *et al.*[2] MRD-negativity by flow in HiDDAV was also associated with superior clinical outcomes, here significantly with respect to DOR and EFS. Interestingly, this appeared to be only relevant in patients who did not proceed to ASCT (*Online Supplementary Table S3*), suggesting ASCT can overcome worse clinical outcomes associated with MRD in the post-venetoclax remission setting.[22]

MRD negativity by flow is not a panacea; MRD-negative patients using this modality still relapse.[2] We were, therefore, interested in investigating an independent and potentially

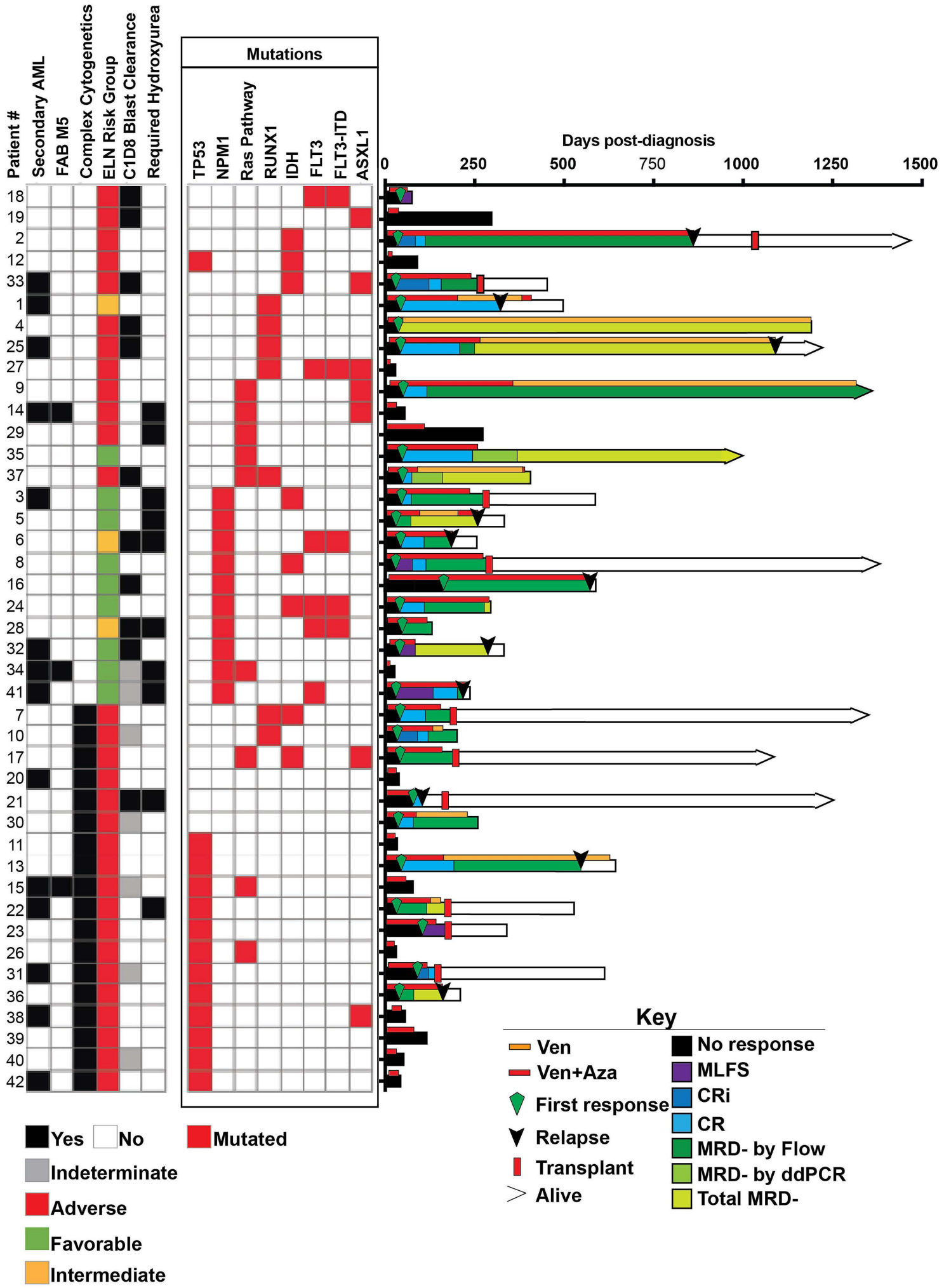

Figure 2. Select baseline characteristics and outcomes for each of the 42 patients treated. AML: acute myeloid leukemia; ITD: internal tandem duplications; ELN: European Leukemia Network; Ven: venetoclax; Aza: azacitidine; MRD: minimal residual disease; CR: complete remission; CRi: complete remission with incomplete hematologic recovery; MLFS: morphologic leukemia-free state.

complementary modality for MRD detection, and applied ddPCR to all responding patients with detectable baseline mutations. With this approach we had 70% coverage of all baseline non-DTA mutations. As far as we are aware, this represents the first time such comprehensive and prospective ddPCR MRD monitoring has been performed. How-

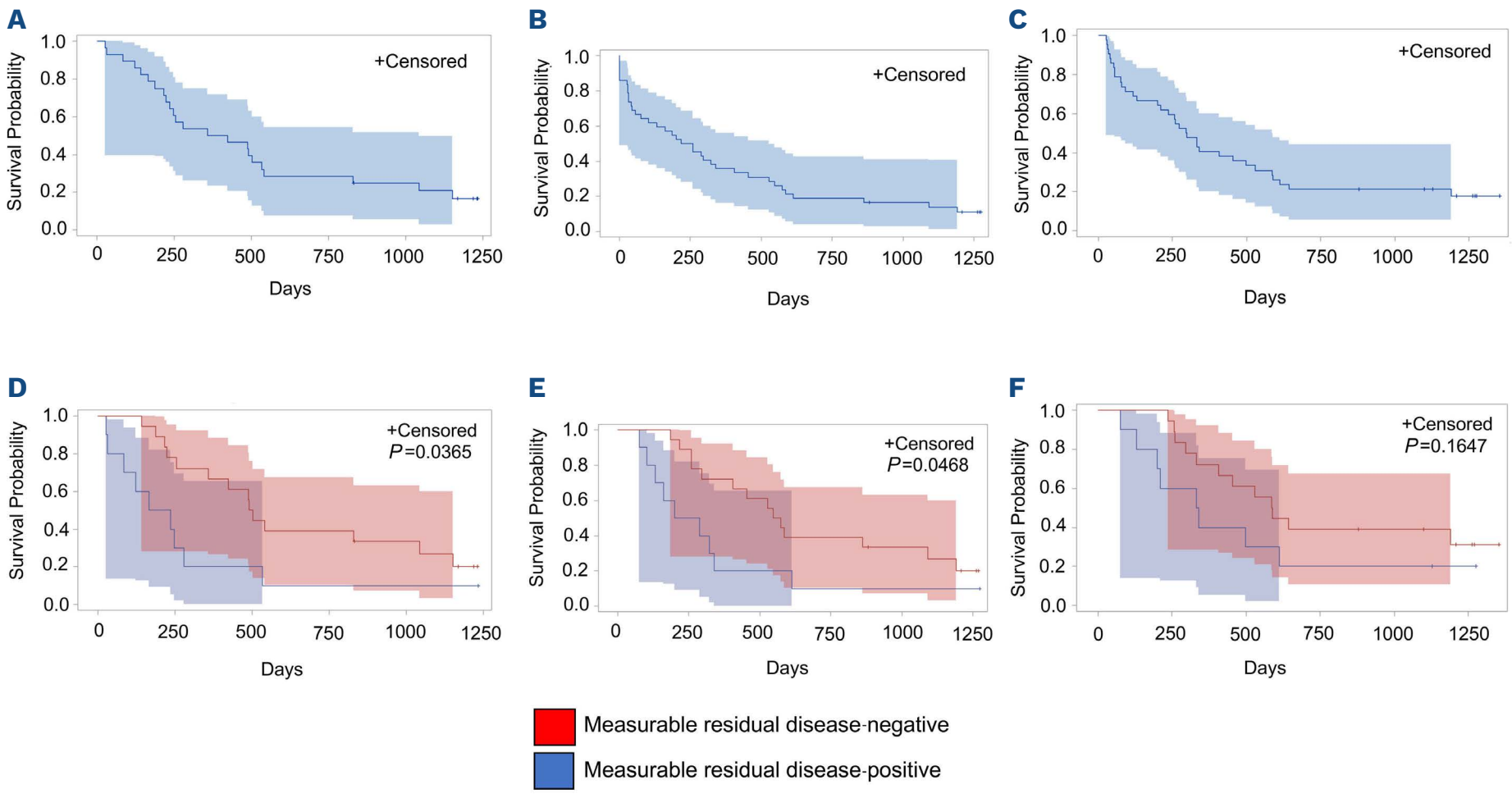

Figure 3. Time-to-event analyses for subjects on the Higher Dose Venetoclax with Measurable Residual Disease-Guided Discontinuation of Azacitidine (HiDDAV) Study. (A) Duration of response for all patients treated. (B) Event-free survival for all patients treated and (C) overall survival for all patients treated. (D) Responding patients stratified by measurable residual disease by flow cytometry status evaluating duration of response. (E) Event-free survival stratified by measurable residual disease-negative (red) *vs.* measurable residual disease-positive (blue). (F) Overall survival for measurable residual disease-negative (red) *vs.* measurable residual disease-positive (blue).

ever, in contrast to flow, MRD negativity by ddPCR was not associated with superior clinical outcomes, when considered alone or in combination with flow. As with the flow MRD assay, we considered whether the presumed deleterious impact of MRD positivity by ddPCR might have been overcome by ASCT, but when this MRD analysis was stratified by ASCT status, we saw no impact on clinical outcomes (*Online Supplementary Table S3*). For this more comprehensive approach to be predictive, coverage of all non-DTA mutations may need to be >70%. Alternatively, ddPCR may be overly stringent; if persistence of DTA mutations does not impact outcomes,[19] this might also be true of other mutations, or for certain mutations that remain detectable below a particular threshold. Finally, it may be that this modality would only show significance with a larger number of patients than were included in our analysis.

Most of the responding patients had full blood count recovery, even with the use of higher doses of venetoclax, and count recovery was not more delayed than recently reported in the setting of venetoclax 400 mg.[21] We believe this is due to routine use of breaks from treatment between cycles and the judicious use of GCSF, both of which are standard treatment principles with the use of venetoclax-based regimens.[23,24] In addition, responses to this regimen are typically rapid, but to date little is known about how quickly blast reduction in the bone marrow occurs. This study included cycle 1 day 8 bone marrow biopsies, and a significant percentage of patients had already achieved blast clearance at this early time point; indeed, lack of blast clearance at this time point was associated with a shorter DOR (*Online Supplementary Table S6*).

Indefinite therapy is a challenge for patients, and efforts to determine when or whether treatment discontinuation is possible are necessary. Our choice to discontinue venetoclax for patients in deep remission was informed by our experience in which long-term responders to venetoclax+azacitidine displayed frequent difficulty receiving indefinite infusions of azacitidine, but were able to manage the orally administered venetoclax. Other designs attempting to maintain patients with azacitidine, perhaps inspired by the data for maintenance oral azacitidine,[25] would be reasonable. From our study, we cannot conclude that MRD negativity should lead to a recommendation to discontinue azacitidine; perhaps a larger study would be able to reach this conclusion. A recent retrospective study found no benefit to indefinite treatment in long-term responders;[5] other formalized efforts to prospectively study de-escalation, based on MRD or other

factors, should be encouraged. Finally, in our study, after azacitidine was discontinued, we saw no evidence to suggest that its re-introduction could engender a re-response, or a decrease in MRD. Given the well-documented poor outcomes after progression on venetoclax regimens,[26-28] novel salvage therapies are needed when patients progress.

Findings from single institution studies can be difficult to extrapolate. Our newly diagnosed AML patients receiving venetoclax+azacitidine do not routinely receive antifungal prophylaxis due to our reported low rates of proven or probable invasive fungal infections in this population.[29] We are also likely outliers with respect to our aggressive approach to ASCT in venetoclax+azacitidine patients.[30] Our high ASCT rate explains the relatively low median total number of venetoclax+azacitidine cycles patients received (4 *vs.* 7 in VIALE-A[1]). We do not believe that "fitness" for induction chemotherapy has the same relevance it did in the pre-venetoclax era,[31,32] and so we allow patients ≥60 years old who are "fit" for intensive chemotherapy to choose between this treatment and venetoclax+azacitidine, a decision that is informed by biological risk factors and the likelihood of responding to either therapy;[33] the HiDDAV protocol specifically allowed for patients who were "unwilling" to receive intensive induction. The HiDDAV study, therefore, likely had younger and fitter patients than other similar studies.

In conclusion, venetoclax doses >400 mg with azacitidine were tolerated by this patient population with equivalent toxicity and time to blood count recovery compared with venetoclax 400 mg. Higher doses may result in deeper remissions; deeper remissions appear to have clinical benefit. However, without controlled data we would not recommend routine use of >400 mg venetoclax with azacitidine, as the clinical outcomes are not clearly superior to studies using 400 mg. Finally, MRD negativity cannot be used to recommend azacitidine discontinuation at this time; more efforts to optimize venetoclax-based regimens in AML are necessary.

Disclosures

DAP has served as an advisory board member and consultant for Abbvie and Genentech, and receives research funding from Abbvie.

Contributions

JAG, CSmith, MA, CM, KO, AF, CSohalski, JD-M and OO recruited and/or treated subjects and edited and approved the manuscript. AW, AK, JS, JT, KZ, PH and DA performed study-related analyses and edited and approved the manuscript. CTJ, BS, MM, SP, ET and CB edited and approved the manuscript. DAP recruited and treated subjects, performed study related analyses and wrote, edited and approved the manuscript.

Acknowledgments

The authors wish to acknowledge the courage and bravery of the patients and their families who participated in this study; without their hope and altruism, progress in this field would not be possible.

Funding

This research was supported by the Leukemia and Lymphoma Society's Scholar in Clinical Research Program, the Robert H. Allen MD Chair in Hematology Research and AbbVie.

Data-sharing statement

Data on individual patients will not be shared. Data from aggregate analyses may be shared upon request.

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