

A multivariate analysis of the relationship between response and survival among patients with higher-risk myelodysplastic syndromes treated within azacitidine or conventional care regimens in the randomized AZA-001 trial

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Online Supplementary Design and Methods

Patients aged 18 years or over with higher-risk MDS (FAB-defined refractory anemia with excess blasts [RAEB], RAEB in transformation [RAEB-t], or chronic myelomonocytic leukemia (CMML) and an IPSS risk of intermediate-2 or high), Eastern Cooperative Oncology Group performance status (ECOG PS) of 0-2, and estimated life expectancy of 3 months or over were eligible. Patients who had received prior azacitidine treatment, were to receive allogeneic-stem cell transplantation, or had therapy-related MDS were excluded.

Study enrollment and monitoring were conducted by site investigators, with standardized evaluation of morphological data by central pathology review. Before randomization, patients were pre-selected by their local investigator to receive 1 of 3 conventional care (CCR): 1) supportive care (blood product transfusions, antibiotics, and G-CSF for neutropenic infection [not prophylaxis]); 2) low-dose ara-C (LDAC) 20 mg/m²/day subcutaneously (SC) x 14 days/28 days (delayed for blood count recovery) for at least 4 cycles; or 3) cytarabine-based (7+3) intensive chemotherapy. After CCR pre-selection for each patient, patients were randomized to azacitidine 75 mg/m²/day SC x 7 consecutive days/28 days or to their pre-selected CCR. All patients received supportive care. Per protocol, azacitidine and CCR were continued until study end (12 months after the last patient was randomized) or until discontinuation due to unacceptable toxicity or disease progression.

Hematologic evaluations were performed weekly during the first 2 treatment cycles, then bimonthly from cycle 3 to discontinuation. Bone marrow assessments were to be performed every 16 weeks, but could be done at any time at investigator discretion if he or she wanted to confirm a CR, disease progression, or other clinical status. Patient responses to treatment throughout the trial were reviewed by an independent review committee (IRC) of international MDS experts who were blinded to treatment assignment (SDG; AFL; Arnold Ganser, MD, Department Hematology, Hemostasis and Oncology, Hannover Medical School, Hannover, Germany; and Raymond Lowenthal, MD, University of Tasmania, Royal Hobart Hospital, Hobart, Tasmania).

Statistical analyses

A multivariate Cox regression analysis with response as a time-varying covariate was used to evaluate the relationship between response and overall survival (OS) over time. CR, PR, HI, stable disease, and disease progression were defined by IWG 2000 criteria for MDS and programmatically adjudicated.⁵ Importantly, for the purposes of this analysis, Stable Disease is defined as no evidence of progression without achievement of HI. This analysis included all IRC-adjudicated clinical assessments for all patients throughout the AZA-001 trial.

Response was categorized into 3 groups: Overall Response, which comprised any response of HI, PR, or CR; Stable Disease (with no HI); and Other (any other clinical state, i.e. disease progression or early discontinuation). Response was evaluated in the model as a time-varying covariate, such that a patient's response classification in the model changed each time the patient's clinical response changed. For example, an individual who started the study with stable disease, then achieved HI at Day 84, then achieved PR at day 195 and remained that way for the remainder of the study and ended the study alive at 581 days, would enter the model as Stable Disease for Days 0-83, HI (i.e. Overall Response) for days 84-194, and PR (Overall Response) for Days 195-581, with an end point of alive for all time periods.

Overall survival was estimated using a Cox proportional hazard model stratified by FAB and IPSS classification, with treatment as a factor in the model. Time-varying covariates of Overall Response and Stable Disease, and terms for Overall Response-by-treatment and Stable Disease-by-treatment, were added to the model and evaluated. The best Cox model was chosen using the Akaike Information Criterion (AIC),² which is a goodness-of-fit test, and by statistical significance of the covariates. HR and associated 95% confidence intervals (CI) are reported from this model; HR and *P* value for each individual factor in the model was adjusted for the presence of all other factors. An HR 1 below represents a decreased risk of death associated with that covariate. A sensitivity analysis was conducted to evaluate the relationship between HI and OS by determining the influence CR or PR as assigned by the local investigator on the response-survival relationships in the multi-

Online Supplementary Table 1. Baseline disease characteristics: patients with stable disease as best response at 6 months

Parameter	AZA (n=49)	CCR (n=41)	Parameter	AZA (n=49)	CCR (n=41)
BM Blasts, n (%)			No. of RBC transfusions		
≥5, ≤10	11 (22)	8 (20)	Mean ± SD	1.3 ± 2.1	1.0 ± 1.4
>10, ≤20	27 (55)	22 (54)	No. of platelet transfusions		
>20, ≤30	11(22)	9 (22)	Mean ± SD	0.5 ± 1.7	0
>30	0	1 (2)	Hgb g/L		
Missing	0	1 (2)	Median (range)	100 (69 – 142)	100 (72 – 150)
Cytogenetics¹, n (%)			Platelet		
IPSS Good	21 (43)	25 (61)	Median (range)	68 (7 – 546)	107 (23 – 958)
Intermediate	16 (33)	7 (17)	ANC g/L		
Poor	10 (20)	8 (20)	Median (range)	1.0 (0.2 – 38)	1.7 (0.1 – 6)
Missing	2 (4)	1 (2)	Transfusion dependent, n (%)		
Cytopenias², n (%)			RBC	20(41)	16 (39)
1	3 (6)	7 (17)	Platelets	5 (10)	0
2	17 (35)	23 (56)			
3	29(59)	9 (22)			
Missing	0	2 (5)			
<p>AZA = azacitidine, CCR = conventional care regimens ¹p=0.016 for abnormal cytogenetics (normal vs abnormal for azacitidine vs CCR) in the multivariate logistic model. ²p=0.007 for number of cytopenias (more cytopenias in the azacitidine group) in the multivariate logistic model.</p>					

variate analysis. (Investigator-reported hematologic responses of IWG 2000-defined CR and PR were reported in the primary analysis of AZA-001 data³ and are distinct from the IRC-adjudicated responses used in the current multivariate analysis.) For this sensitivity analysis, the relationship between OS and Overall Response was determined for 2 patient groups: those who had an investigator-reported PR or CR at any time during the study, and those who did not have an investigator-reported CR or PR at any

time during the study (i.e. HI was the patient's best response on-study). Similarly, the relationship between OS and Stable Disease was assessed in the group of patients who had a PR or CR at some point in the study, and in the group of patients who never had an investigator-reported PR or CR during the study.

Further sensitivity analyses added additional co-variables to the Cox regression model, including base-line ECOG PS, lactate dehydrogenase (LDH), and hemoglobin (Hgb);

number of red blood cell (RBC) transfusions in the 56 days before randomization; and presence or absence of -7/del(7q) abnormality.

To corroborate results of the time-varying multivariate Cox regression analysis, landmark analyses (i.e. “snapshot” assessments) were performed assessing the relationship between OS and patient status (Overall Response or Stable Disease) at each specific landmark. Landmark analyses were used to avoid biases inherent in classifying patients by their best response achieved during the study. The landmarks (3, 6, and 9 months) were prospectively determined based on clinically significant benchmarks: the reported median number of azacitidine cycles required to attain an initial response (3 cycles¹³), the recommended azacitidine dosing schedule (4-6 cycles¹⁴), and data showing a survival advantage with azacitidine vs CCR at a median of 9 treatment cycles.³ Only patients alive and on-study at the particular landmark were included in these analyses.

Median OS and 2-year OS rates were estimated using Kaplan-Meier (K-M) methods. OS estimates for patients with Stable Disease in landmark analyses included only patients who had Stable Disease as a best response at that time point; i.e. patients with Stable Disease who had not achieved an Overall Response (HI, CR, or PR) before the landmark measurement. HRs and 95% CIs were reported from a Cox proportional hazard model stratified for FAB classification and IPSS risk. The effect of treatment on OS was evaluated within patient response groups using a 2-sided logrank test, also stratified by FAB classification and IPSS risk.

To assess the effect of continued azacitidine or CCR treatment on response, the proportion of patients with Stable Disease as their best response at three months who went on to achieve an Overall Response at six months and the proportion of patients with Stable Disease as their best response at 6 months who went on to achieve an Overall Response at nine months, are reported.

Exploratory logistical regression analyses were employed to address two questions: 1) Of all patients with Stable Disease as a best response at 6 months, did those who received azacitidine have different clinical characteristics at

baseline from patients who received CCR?; and 2) to inform treatment decisions, were patients who achieved an Overall Response distinguishable at baseline from patients who maintained Stable Disease during the 9-month period? To address the first question, clinical features at baseline were compared between azacitidine patients and CCR patients who had Stable Disease as their best response at six months. To address the second question, clinical features at baseline of patients who maintained Stable Disease as a best response at three, six, and nine months were compared with those of patients who achieved Overall Response during the 9-month period. For both logistical regression analyses, baseline covariates in the model were age, sex, race, monosomy 7 (yes/no), FAB classification, ECOG PS, IPSS risk, number of RBC transfusions, baseline RBC transfusion dependence (yes/no), number of platelet transfusions, cytogenetics (normal/abnormal), bone marrow blasts %, years since diagnosis, number of cytopenias, cytogenetic abnormalities, DNA methylation level, and LDH, Hgb, platelet, white blood cell (WBC), and absolute neutrophil count (ANC) levels. Covariates were evaluated in a univariate regression model and variables with statistical significance less than 0.25 were included in a multivariate model. The best multivariate model was selected by fitting all possible models using SAS 9.1 and selecting the best model using the AIC.

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