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ORIGINAL REPORT

Randomized Phase II Study of Azacitidine Alone or in Combination With Lenalidomide or With Vorinostat in Higher-Risk Myelodysplastic Syndromes and Chronic Myelomonocytic Leukemia: North American Intergroup Study SWOG S1117

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ABSTRACT

Purpose

Azacitidine is standard, first-line therapy in higher-risk myelodysplastic syndromes (MDS). Whether azacitidine-based combinations with lenalidomide or vorinostat produce superior overall response rates (ORRs) to azacitidine is not known.

Patients and Methods

North American Intergroup Study S1117 is a phase II/III trial that randomly assigned patients with higher-risk MDS and chronic myelomonocytic leukemia (CMML) 1:1:1 to azacitidine (75 mg/m^2/day on days 1 to 7 of a 28-day cycle); azacitidine plus lenalidomide (10 mg/day on days 1 to 21); or azacitidine plus vorinostat (300 mg twice daily on days 3 to 9). The primary phase II end point was improved ORR.

Results

Of 277 patients from 90 centers, 92 received azacitidine, 93 received azacitidine plus lenalidomide, and 92 received azacitidine plus vorinostat. Median age was 70 years (range, 28 to 93 years), 85 patients (31%) were female, and 53 patients (19%) had CMML. Serious adverse events were similar across arms, although combination-arm patients were more likely to undergo nonprotocol-defined dose modifications ($P < .001$).With a median follow-up of 23 months (range, 1 to 43 months), the ORR was 38% for patients receiving azacitidine, 49% for azacitidine plus lenalidomide ($P = .14$ *v* azacitidine), and 27% for azacitidine plus vorinostat ($P = .16$ *v* azacitidine). For patients with CMML, ORR was higher for azacitidine plus lenalidomide versus azacitidine (68% *v* 28%, $P = .02$) but similar for all arms across cytogenetic subgroups, as was remission duration and overall survival. ORR was higher with mutations in *DNMT3A* and lower for *SRSF2*, whereas ORR duration improved with fewer mutations. Lenalidomide dose reduction was associated with worse overall survival (hazard ratio, 1.30; $P = .05$).

Conclusion

Patients with higher-risk MDS treated with azacitidine-based combinations had similar ORR to azacitidine monotherapy, although patients with CMML benefitted from azacitidine plus lenalidomide. The efficacy of combination regimens may have been affected by dose modifications.

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ASSOCIATED CONTENT

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Appendix

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Data Supplement

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INTRODUCTION

The myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemias (CMML; an MDS/myeloproliferative neoplasm [MPN] overlap) comprise a spectrum of distinct bone marrow disorders associated with cytopenias, a consequent increased risk of bleeding and infection, and, in higher-risk subtypes, a high likelihood of transformation to acute myeloid leukemia.[1-3] They are the most common myeloid malignancies, with approximately 15,000 to 20,000 new diagnoses in the United States yearly, 25% to 30% of which constitute higher-risk disease.[4]

Three drugs, azacitidine, decitabine, and lenalidomide, were approved by the US Food and Drug Administration for the treatment of MDS or one of its subtypes.[5-7] Lenalidomide purportedly works through inhibition of phosphatase activity in the common deleted region, which plays a key role in cell cycle regulation; through a defect in ribosomal protein function via ubiquitination and degradation of CK1 alpha in patients with the deletion 5q cytogenetic abnormality; and through bone marrow microenvironment effects in patients without the deletion 5q cytogenetic abnormality.[8-10] Lenalidomide has demonstrated transfusion independence response rates of 67% in patients with lower-risk MDS with the deletion 5q abnormality and of 26% in lower-risk patients without the abnormality.[6,11] It has also shown activity in patients with MPN.[12,13] Azacitidine and decitabine exert their effects via DNA methyltransferase inhibition and direct cytotoxicity.[14] Each drug can affect peripheral cytopenias, transfusion needs, and quality of life.[5,7,15] In addition, azacitidine improves overall survival (OS) in patients with higher-risk MDS or CMML.[15]

Histone acetylation facilitates active gene transcription and is highly regulated by histone deacetylases (HDACs).[16] HDAC inhibition can restore normal acetylation of histone proteins and transcription factors. Vorinostat, a small molecule inhibitor of class I and II HDAC enzymes, has been combined with azacitidine in phase I and II studies in higher-risk MDS,[17] with an overall response rate (ORR) among 33 patients of 70%.[18] Lenalidomide has also been combined with azacitidine in the phase I and II setting,[19] with an ORR among 36 patients of 72%.[20]

We conducted a multicenter, randomized, three-arm, phase II/III study of azacitidine combined with lenalidomide or with vorinostat versus azacitidine monotherapy in patients with higher-risk MDS and CMML. The phase II analysis was to determine whether combination therapy could improve the response rate compared with azacitidine to justify phase III evaluation for OS. Secondary objectives included assessing outcomes in predefined subgroups, such as CMML and common MDS cytogenetic and molecular abnormalities.

PATIENTS AND METHODS

Study Design and Treatment

In the North American Intergroup Study S1117 (ClinicalTrials.gov identifier NCT01522976), patients were randomly assigned 1:1:1 using a dynamic allocation scheme stratified on MDS versus CMML to one of three study arms: azacitidine monotherapy (75 mg/m^2/day intravenously or subcutaneously on days 1 to 7 of a 28-day cycle for both monotherapy and combination arms), with allowance for azacitidine to be administered on either a 7-day continuous or a 7-day interrupted schedule (eg, 5-2-2: azacitidine administered on days 1 to 5, followed by 2 days of no treatment, followed by 2 days of azacitidine)[21,22]; azacitidine plus lenalidomide (10 mg/day orally on days 1 to 21); or azacitidine plus vorinostat (300 mg twice daily orally on days 3 to 9; Fig 1). Dose modification or interruption guidelines are listed in Appendix Table A1 (online only). Antibiotic prophylaxis could be used per individual institutional practices.

Patients

Eligibility criteria included higher-risk MDS (International Prognostic Scoring System [IPSS] Intermediate-2 or High and/or bone marrow blasts $\geq$ 5%) or CMML with $<$ 20% blasts, reviewed centrally; and age $\geq$ 18 years (Appendix, online only).

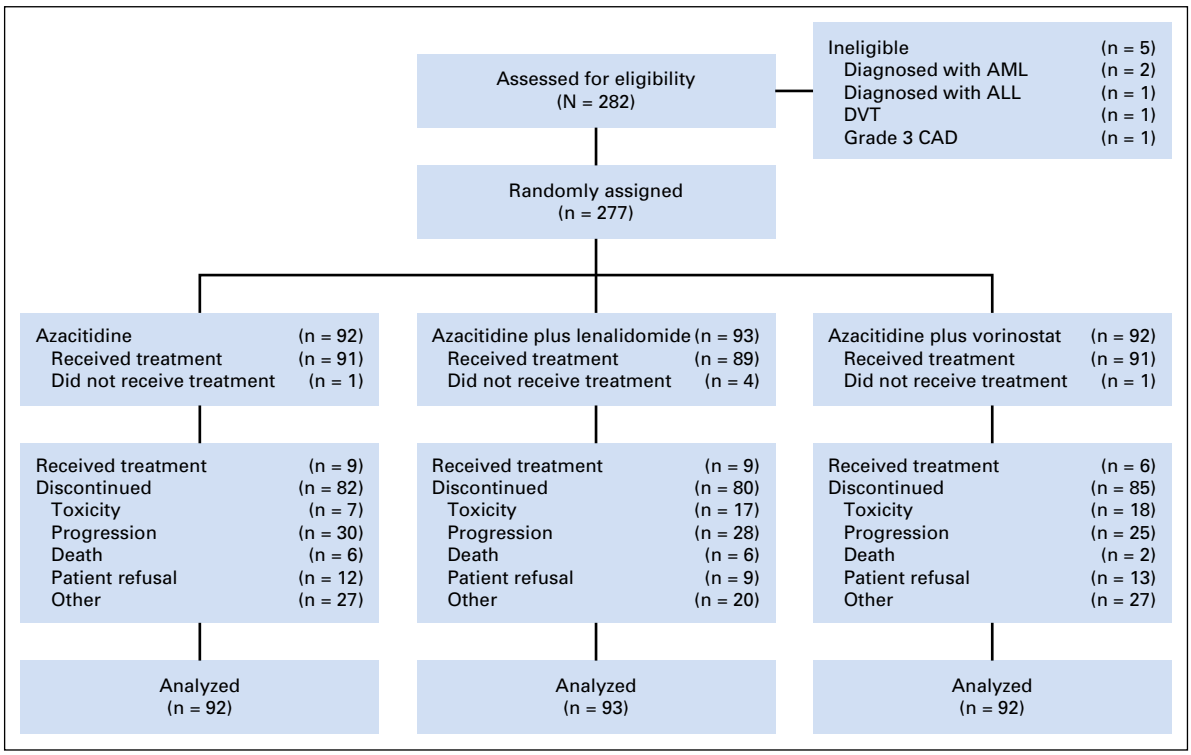

Fig 1. CONSORT diagram. ALL, acute lymphatic leukemia; AML, acute myelogenous leukemia; CAD, coronary artery disease; DVT, deep vein thrombosis.

Outcomes

The primary phase II end point was ORR reviewed centrally on a patient-by-patient basis per 2006 International Working Group MDS response criteria (complete response [CR] plus partial response [PR] plus hematologic improvement [HI]).[23] OS was measured from study entry, response duration from the time response was first documented to loss of response, progression, or death. MDS Centers of Excellence were defined per the MDS Foundation[24]; center volume was defined as low (one to four patients enrolled) or high (five to 17 patients enrolled) on the basis of the median volume in this study (n = 4; Appendix).

Mutation Analysis

Genomic DNA was isolated from cryopreserved peripheral blood or bone marrow mononuclear cells and subjected to targeted, amplicon-based, next-generation sequencing of coding regions of MDS-associated genes, using Ion Torrent (National Cancer Institute of Canada/Canadian Cancer Trials Group Alliance and Nationwide samples; n = 97) or Illumina (San Diego, CA) platforms (Cleveland Clinic and Albert Einstein University; n = 16). Somatic mutation calls were made (present or absent) with a minimum average depth of coverage of 500× magnification. The lower limit of detection was estimated between variant allele fractions of 0.02 to 0.05 (Supplemental Methods in the Appendix).

Statistics

The phase II objective of this phase II/III study was to select, on the basis of ORR, one combination arm for phase III evaluation of OS compared with azacitidine. A total of 240 eligible patients (80 per arm) were required for the phase II analysis. If the true ORR of azacitidine was 35% (null hypothesis) and the true ORR of a combination arm was 55% (alternative hypothesis), each comparison of a combination arm versus azacitidine had a power of 81% with a one-sided alpha of 5%. Additional statistics are described in the Supplemental Methods section in the Appendix.

RESULTS

Baseline Characteristics

From June 2012 through June 2014, 282 patients were enrolled from 90 centers in the United States and Canada. Five patients were ineligible because of a diagnosis other than MDS or CMML or pre-existing toxicity, leaving 277 eligible patients: 92 received azacitidine, 93 received azacitidine plus lenalidomide, and 92 received azacitidine plus vorinostat. Baseline characteristics were similar across arms (Table 1). The median age was 70 years (range, 28 to 93 years), 85 patients (31%) were female, 53 patients (19%)

Table 1. Patient Characteristics

Factor	AZA (n = 92)	AZA + LEN (n = 93)	AZA + VOR (n = 92)	AZA *v* AZA + LEN *P*	AZA *v* AZA + VOR *P*
Age, years	69 (42, 88)	70 (51, 86)	70 (28, 93)	.31	.72
Female	31 (34)	32 (34)	22 (24)	1	.19
Male	61 (66)	61 (66)	70 (76)		
PS 0	28 (31)	38 (41)	36 (39)	.20	.31
PS 1	54 (59)	43 (46)	44 (48)		
PS 2	9 (10)	12 (13)	12 (13)		
CMML-1	11 (13)	14 (17)	11 (13)	.57	.44
CMML-2	7 (9)	5 (6)	5 (6)		
RAEB-1	29 (35)	22 (27)	23 (27)		
RAEB-2	35 (43)	40 (49)	47 (55)		
IPSS Low	3 (3)	2 (2)	2 (2)	.78	.63
IPSS Int-1	25 (28)	21 (23)	30 (34)		
IPSS Int-2	40 (45)	46 (50)	42 (48)		
IPSS High	20 (23)	23 (25)	14 (16)		
No prior chemo	86 (93)	87 (94)	87 (95)	1	1
Prior chemo	6 (7)	6 (6)	5 (5)		
No prior radiation	88 (96)	91 (98)	90 (98)	.44	.68
Prior radiation	4 (4)	2 (2)	2 (2)		
No prior transplant	90 (98)	91 (98)	92 (100)	1	.50
Prior transplant	2 (2)	2 (2)	0 (0)		
HgB (g/dL)	9 (3, 99)	9 (6, 14)	9 (6, 15)	.76	.71
Platelets ($\times 10^3$)	70 (8, 4000)	75 (3, 452)	62 (3, 1462)	.89	.40
WBC ($\times 10^3$)	3 (1, 205)	3 (0, 61)	3 (0, 65)	.12	.63
ANC ($\times 10^3$)	2 (0, 110)	1 (0, 336)	2 (0, 36)	.14	.88
Blood blasts (%)	0 (0, 18)	0 (0, 23)	0 (0, 19)	.40	.036
Marrow blasts (%)	8 (0, 22)	10 (0, 20)	9 (1, 18)	.72	.89
Not transfusion dependent at prestudy	40 (43)	43 (46)	34 (37)	.77	.45
Transfusion dependent at prestudy	52 (57)	50 (54)	58 (63)		
IPSS-R good/very good	29 (32)	35 (38)	34 (37)	.89	.90
IPSS-R Intermediate	16 (17)	13 (14)	18 (20)		
IPSS-R poor	10 (11)	8 (9)	8 (9)		
IPSS-R very poor	23 (25)	23 (25)	19 (21)		
Missing	14 (15)	14 (15)	13 (14)		

NOTE. Median (range) or N (%) reported, unless otherwise indicated.
Abbreviations: ANC, absolute neutrophil count; AZA, azacitidine; chemo, chemotherapy; CMML, chronic myelomonocytic leukemia; HgB, hemoglobin; Int, intermediate; IPSS, International Prognostic Scoring System; IPSS-R, Revised IPSS; LEN, lenalidomide; PS, performance status; RAEB, refractory anemia with excess blasts; VOR, vorinostat.

had CMML, and 18 patients (6%) had treatment-related MDS. IPSS risk group distribution was similar across arms, with all patients with Low/Intermediate-1 IPSS classifications having excess blasts or nonproliferative CMML-1. The revised IPSS-defined cytogenetic risk group distribution and distinct cytogenetic abnormalities of interest occurred at similar rates across treatment arms, and baseline characteristics of patients were similar whether they were treated at an MDS Center of Excellence or a high volume center (Appendix Table A2). A majority of patients (58%) were dependent on packed RBC transfusions at baseline.

Adverse Events

Serious adverse events attributed to therapy were similar across arms (Table 2), with two exceptions: patients in the azacitidine plus vorinostat arm had more grade 3 or higher gastrointestinal toxicities (14 patients [15%] *v* four patients [4%] in the azacitidine arm; $P = .02$), whereas patients in the azacitidine plus lenalidomide arm had more grade 3 or higher rash (14 patients [16%] *v* three patients [3%] in the azacitidine arm; $P = .005$). Rates of grade 3 or higher febrile neutropenia were similar across the three arms, as were rates of infection and infestations for all three cohorts, across grades: 89% for azacitidine monotherapy, 91% for azacitidine plus lenalidomide, and 91% for azacitidine plus vorinostat. Patients in combination arms were significantly more likely to have therapy stopped because of toxicities or complications than were patients receiving azacitidine monotherapy (8% for azacitidine, 20% for azacitidine plus lenalidomide [$P = .05$ *v* azacitidine], and 21% for azacitidine plus vorinostat [$P = .03$ *v* azacitidine, with $P = .02$ for both combination arms *v* azacitidine]) and to undergo nonprotocol-defined dose modifications (24% for azacitidine, 43% for azacitidine plus lenalidomide [$P = .002$], and 42% for azacitidine plus vorinostat [$P = .01$, with $P < .001$ for combinations *v* azacitidine]).

ORR and Duration

Patients received a median of 22 weeks of therapy: 23 weeks for patients receiving azacitidine, 25 weeks for those receiving azacitidine plus lenalidomide ($P = .61$ *v* azacitidine), and 20 weeks for azacitidine plus vorinostat ($P = .33$ *v* azacitidine, $P = .9$ for combinations *v* azacitidine). With a median follow-up among patients still alive of 23 months (range, 1 to 43 months), the ORR for the entire cohort was 38%: 38% for patients receiving azacitidine; 49% for patients receiving azacitidine plus lenalidomide ($P = .14$ *v* azacitidine); and 27% for patients receiving azacitidine plus vorinostat ($P = .16$ *v* azacitidine; Table 3). Rates of CR/PR/HI and marrow CR were also not significantly different across groups, although within HI, patients receiving azacitidine plus lenalidomide had higher rates of HI-neutrophil than did azacitidine monotherapy patients (19% *v* 5%; $P = .007$). Among previously untreated patients, there was a trend toward improved ORR for those treated with azacitidine plus lenalidomide (n = 81) versus azacitidine (n = 79; 49% *v* 35%; $P = .08$). Time to best response did not differ across treatment arms. On the basis of the phase II analysis, neither combination arm was selected for phase III testing of OS. The median response duration for the cohort was 15 months: 10 months for azacitidine, 14 months for azacitidine plus lenalidomide ($P = .85$ *v* azacitidine), and 18 months for azacitidine plus vorinostat ($P = .37$). For patients remaining on therapy for ≥ 6 months (n = 119), patients receiving azacitidine plus lenalidomide had a higher ORR (87%) versus patients receiving azacitidine (62%; $P = .01$), although no difference in response duration ($P = .98$).

Response Rates and Duration Within Predefined Subgroups

For patients with CMML, the ORR (38% for the entire cohort) was significantly higher for those receiving azacitidine plus lenalidomide than for those receiving azacitidine monotherapy (68% *v* 28%; $P = .02$). Median response duration for patients with CMML was 19 months and similar across arms. No differences

Table 2. Toxicity

	Azacitidine Plus Lenalidomide (n = 89) Grade No.				Azacitidine (n = 91) Grade No.				Azacitidine Plus Vorinostat (n = 91) Grade No.			
Adverse Event	≤ 2	3	4	5	≤ 2	3	4	5	≤ 2	3	4	5
Blood and lymphatic system	73	13	3	0	80	10	1	0	78	12	1	0
Cardiac disorders	86	1	2	0	91	0	0	0	90	1	0	0
Gastrointestinal disorders	77	12	0	0	87	4	0	0	77	14	0	0
General disorders and administration	78	11	0	0	84	6	0	1	75	16	0	0
Hepatobiliary disorders	89	0	0	0	91	0	0	0	90	1	0	0
Infections and infestations	73	11	3	2	83	5	2	1	80	7	4	0
Injury, poisoning and procedure	89	0	0	0	91	0	0	0	90	1	0	0
Investigations	85	4	0	0	91	0	0	0	84	7	0	0
Metabolism and nutrition disorders	68	20	1	0	88	2	1	0	76	14	1	0
Musculoskeletal and connective tissue disorders	85	4	0	0	90	1	0	0	88	3	0	0
Nervous system disorders	86	2	0	1	91	0	0	0	85	6	0	0
Psychiatric disorders	89	0	0	0	91	0	0	0	89	2	0	0
Renal and urinary disorders	88	1	0	0	91	0	0	0	88	3	0	0
Respiratory, thoracic and mediastinum	84	3	1	1	89	2	0	0	83	6	2	0
Skin and subcutaneous tissue	75	14	0	0	88	3	0	0	90	1	0	0
Vascular disorders	83	6	0	0	91	0	0	0	84	7	0	0
Maximum grade any adverse event	30	49	6	4	61	24	4	2	39	46	6	0

Table 3. Responses

Variable	AZA n = 92 (33%)	AZA + LEN n = 93 (34%); *P* *v* AZA	AZA + VOR n = 92 (33%); *P* *v* AZA	Total n = 277 (100%)
ORR (%)	35 (38)	46 (49); *P* = .14	25 (27); *P* = .16	106 (38)
CR/PR/HI, %	24/0/14	24/1/25; *P* = 1 for CR, *P* = .007 for HI-n	17/1/9; *P* = .36 for CR, *P* = 1 for HI-n	22/1/16
Marrow CR, No. %	11 (12)	8 (9)	13 (14)	32 (12)
CMML ORR (n = 53 [18/19/16]; %)	5 (28)	13 (68); *P* = .02	2(12); *P* = .41	20 (38)
tMDS ORR (n = 18; %)	4 (57)	4 (67)	1 (20)	9 (50)
IPSS Int-1 ORR (n = 76; %)	9 (36)	9 (43)	11 (37)	29 (38)
IPSS Int-2 ORR (n = 128; %)	16 (40)	26 (57)	11 (26)	53 (41)
IPSS High ORR (n = 57; %)	7 (35)	8 (35)	3 (21)	18 (32)
Transfusion-dependent ORR (n = 160; %)	20 (38)	19 (38)	14 (24)	53 (33)
Allogeneic transplantation, No. (%)	15 (16)	10 (11)	15 (16)	40 (14)
Median ORR duration, months	10	14; *P* = .41	15; *P* = .31	14
Median CMML ORR duration, months	19	17; *P* = .82	28 ; *P* = .69	19
ORR: pts receiving therapy > 6 months (n = 119; %)	24 (62)	39 (87); *P* = .01	20 (57); *P* = .81	83 (70)
Median ORR duration, pts receiving therapy > 6 months, months	14	14; *P* = .98	18; *P* = .36	16

Abbreviations AZA, azacitidine; CMML, chronic myelomonocytic leukemia; CR, complete response; HI, hematologic improvement; HI-n, hematologic improvement-neutrophils; Int, intermediate; IPSS, International Prognostic Scoring System; LEN, lenalidomide; MDS, myelodysplastic syndrome; ORR, overall response rate; PR, partial response; pts, patients; tMDS, treatment-related myelodysplastic syndrome; VOR, vorinostat.

in ORR were seen for therapy-related MDS, IPSS subgroups, transfusion-dependent patients, or allogeneic transplantation rates.

Within the Revised IPSS–defined cytogenetic risk groups and for distinct cytogenetic abnormalities of interest, ORR across treatment arms were, in general, similar (Table 4). ORR across arms was better for patients with chromosome 5 abnormality versus those without (odds ratio, 2.17; $P = .008$). Patients with chromosome 5 abnormalities and +8 receiving azacitidine plus lenalidomide had a nonsignificant higher ORR than did patients receiving azacitidine (62% *v* 50%, $P = .56$, and 60% *v* 36%, $P = .41$, respectively), with no significant difference in response duration.

Early predictors of ORR, which did not differ across arms, included doubling of platelet count and increase in platelet count > 30,000 after the first cycle (n = 45 and n = 74, $P < .001$ and $P < .001$, respectively). Early increase in neutrophil count or hemoglobin were not associated with ORR.

Of 113 patients with available mutational data, 103 (91%) had at least one mutation, the most common being *ASXL1* (n = 31), *TET2* (n = 26), *SRSF2* (n = 23), *TP53* (n = 22), *RUNX1* (n = 21), and *U2AF1* (n = 19; Table 5). The median number of mutations was two (range, 0 to 7). This mutation profile was consistent with other studies enriched for higher-risk MDS and CMML.[25,26]

Compared with patients without mutations, ORR was significantly higher for those with mutations in *DNMT3A* (67% *v* 34%; $P = .025$) and numerically higher for those with *BCOR* (57% *v* 34%; $P = .23$) and *NRAS* (60% *v* 36%; $P = .28$), but lower for *SRSF2* (17% *v* 41%; $P = .037$) and *ASXL1* (23% *v* 43%; $P = .049$; Appendix Table A3). Response duration was worse for those with mutations in *TET2* ($P = .046$) and *TP53* ($P = .003$), with a trend for *ASXL1* ($P = .069$). Response duration improved significantly with fewer mutations (hazard ratio [HR], 6.86 for two or more mutations *v* 0; $P = .01$; Appendix Fig A1, online only).

Table 4. Cytogenetic Response and Outcomes

Cytogenetic Variable, No. or Median (% or range)	AZA n = 92	AZA + LEN n = 93 (*P* *v* AZA)	AZA + VOR n = 92; (*P* *v* AZA)	ORR: Odds Ratio (*P* *v* patients without abnormality)	OS: Hazard Ratio (*P* *v* patients without abnormality)
Normal ORR (n = 26/27/31)	42%	59% (*P* = .28)	16% (*P* = .04)	.93 (*P* = .82)	
Normal OS, months	20	26 R (*P* = .56)	NR (*P* = .85)		.44 (*P* < .001)
+8 ORR (n = 14/10/9)	36%	60% (*P* = .41)	44%(*P* = 1.0)	1.19 (*P* = .65)	
+8 OS (months)	14	25 (*P* = .44)	13 (*P* = .57)		1.08 (*P* = .74)
Chr 5 abn ORR (n = 22/26/18)	50%	62% (*P* = .56)	44%(*P* = .76)	2.17 (*P* = .008)	
Chr 5 abn OS, months	11	11 (*P* = .36)	11 (*P* = .12)		2.86 (*P* < .001)
−7 ORR (n = 16/12/9)	38%	42% (*P* = 1)	33%(*P* = 1)	.92 (*P* = .83)	
−7 OS, months	11	11 (*P* = .55)	13(*P* = .34)		2.17 (*P* < .001)
17p ORR (n = 7/10/12)	43%	30% (*P* = .64)	42%(*P* = 1.0)	.94 (*P* = .88)	
17p OS, months	12	8 (*P* = .10)	11 (*P* = .91)		2.78 (*P* < .001)
IPSS-R good/very good (%)	29 (32%)	34 (38%)	34 (37%)	(Reference)	(Reference)
IPSS-R intermediate (%)	16 (17%)	13 (14%)	18 (20%)	.81 (*P* = .58)	1.30 (*P* = .29)
IPSS-R poor (%)	10 (11%)	8 (9%)	8 (9%)	1.16 (*P* = .74)	2.01 (*P* = .01)
IPSS-R very poor (%)	23 (25%)	23 (25%)	19 (21%)	1.12 (*P* = .72)	3.98 (*P* < .001)

Abbreviations: abn, abnormality; AZA, azacitidine; chr, chromosome; IPSS-R, Revised International Prognostic Scoring System; LEN, lenalidomide; NR = median not reached; ORR, overall response rate; OS, overall survival; VOR, vorinostat.

Table 5. Mutation Incidence and Response Among 113 Tested Patients

Mutation	Incidence (%)	No. With Data	No. Mutated A,A+L,A+V	Response Among Mutated, No. (%) A,A+L,A+V	$P_{interaction}$ Between Arm and Mutation for Response
ASXL1	31 (27)	113	10,10,11	2(20),5(50),0(0)	.84
BCOR	7 (6)	109	2,3,2	2(100),1(33),1(50)	.99
BCORL1	1 (1)	109	1,0,0	0(0),0(0),0(0)	—
BOD1L	0 (0)	97	0,0,0	0(0),0(0),0(0)	—
CBL	8 (7)	113	6,2,0	2(33),1(50),0(0)	.85
CEBPA	9 (8)	109	4,3,2	0(0),2(67),0(0)	.99
CUX1	4 (4)	109	3,0,1	1(33),0(0),0(0)	.99
DNMT3A	12 (11)	113	3,6,3	2(67),5(83),1(33)	.81
ETV6	7 (6)	113	2,1,4	0(0),1(100),2(50)	.99
EZH2	7 (6)	113	3,1,3	1(33),0(0),0(0)	.99
FLT3	1 (1)	109	0,0,1	0(0),0(0),0(0)	—
GATA1	1 (1)	97	0,1,0	0(0),0(0),0(0)	—
GATA2	3 (3)	109	1,1,1	0(0),0(0),0(0)	1
GNAS	8 (8)	97	3,3,2	0(0),0(0),0(0)	1
IDH1	1 (1)	113	0,0,1	0(0),0(0),0(0)	—
IDH2	5 (4)	113	2,0,3	0(0),0(0),0(0)	1
JAK2	2 (2)	113	1,0,1	0(0),0(0),0(0)	1
KDM6A	3 (3)	109	1,2,0	0(0),0(0),0(0)	1
KIT	1 (1)	113	1,0,0	1(100),0(0),0(0)	—
KRAS	4 (4)	109	1,1,2	0(0),1(100),0(0)	.99
MPL	1 (1)	97	1,0,0	0(0),0(0),0(0)	—
NF1	6 (6)	109	0,2,4	0(0),0(0),1(25)	—
NRAS	5 (4)	113	2,1,2	2(100),1(100),0(0)	.99
PHF6	5 (4)	113	2,1,2	0(0),0(0),0(0)	1
PTPN11	3 (3)	109	1,2,0	0(0),0(0),0(0)	1
RAD21	2 (2)	109	0,1,1	0(0),1(100),0(0)	—
RIT1	2 (2)	97	2,0,0	0(0),0(0),0(0)	—
RUNX1	21 (19)	113	7,9,5	0(0),4(44),2(40)	.99
SETBP1	7 (6)	113	2,3,2	0(0),1(33),0(0)	.99
SF3B1	10 (9)	113	3,4,3	1(33),1(25),1(33)	.68
SH2B3	2 (2)	97	1,1,0	1(100),1(100),0(0)	1
SMC1A	1 (1)	97	0,1,0	0(0),1(100),0(0)	—
SMC3	3 (3)	109	2,0,1	0(0),0(0),0(0)	1
SRSF2	23 (22)	106	9,4,10	1(11),2(50),1(10)	.74
STAG2	14 (13)	109	7,4,3	2(29),3(75),0(0)	.79
TET2	26 (23)	113	12,8,6	2(17),3(38),3(50)	.23
TP53	22 (19)	113	9,8,5	4(44),5(62),1(20)	.66
U2AF1	19 (17)	113	7,6,6	4(57),2(33),2(33)	.12
WT1	1 (1)	109	0,0,1	0(0),0(0),1(100)	—
ZRSR2	3 (3)	97	1,0,2	0(0),0(0),0(0)	1
SUS	51 (45)	113	18,14,19	6(33),5(36),4(21)	.19
SRE	34 (30)	113	10,13,11	0(0),6(46),4(36)	.99
SR	16 (15)	109	7,5,4	2(29),4(80),0(0)	.73
NK	8 (7)	113	3,2,3	2(67),2(100),0(0)	.31
CNP	16 (14)	113	7,5,4	2(29),1(20),1(25)	.53

Abbreviations: A, azacitidine; CNP, the combination variable CBL/NF1/PTPN11AZA; L, lenalidomide; NK, the combination variable NRAS/KRAS; NR = median not reached; SR, the combination variable STAG2/RAD21; SRE, the combination variable SETBP1/RUNX1/ETV6; SUS, the combination variable SF3B1/U2AF1/SRSF2; V, vorinostat.

Overall Survival

The median OS for the entire cohort was 17 months: 15 months for azacitidine patients; 19 months for azacitidine plus lenalidomide patients (P = .68 *v* azacitidine); and 17 months for those receiving azacitidine plus vorinostat (P = .22 *v* azacitidine; Fig 2).The median OS after treatment failure for the entire cohort was 9 months: 7 months for azacitidine patients; 9 months for azacitidine plus lenalidomide patients (P = .74 *v* azacitidine); and 10 months for azacitidine plus vorinostat patients (P = .07 *v* azacitidine; P = .21 for combination arms after failure *v* azacitidine; Fig 3). For patients receiving therapy for > 6 months, the median OS for the cohort was 25 months: 20 months for those receiving azacitidine; 26 months for those receiving azacitidine plus lenalidomide (P = .74 *v* azacitidine); and 27 months for those receiving azacitidine plus vorinostat (P = .40 *v* azacitidine). There was a significant association between mean dose reduction of lenalidomide during the first four cycles of therapy and worse OS in multivariable analyses adjusting for IPSS and age (HR, 1.30; P = .05), but not for vorinostat (HR, 1.21; P = .13).

The OS for patients with CMML was similar across treatment arms: median not reached for those receiving azacitidine, those receiving azacitidine plus lenalidomide (P = .87 *v* azacitidine), and those receiving azacitidine plus vorinostat (P = .78 *v* azacitidine). Within cytogenetic risk categories, the OS (compared with Very Good/Good) was worse for Poor (HR, 2.01; P = .01) and Very Poor (HR, 3.98; P < .001), without significant modification by treatment arm (Table 4). Compared with patients without a given

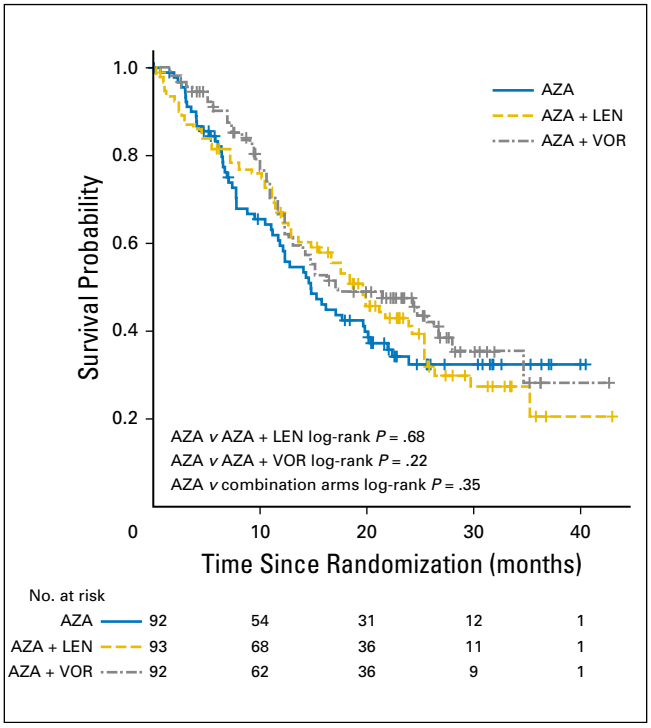

Fig 2. Overall survival. Comparisons are between combination arms and azacitidine (AZA) monotherapy. LEN, lenalidomide; VOR, vorinostat.

cytogenetic abnormality, OS was better for normal (HR, 0.45; $P < .001$) and worse for chromosome 5 abnormalities (HR, 2.86; $P < .001$), −7 (HR, 2.18; $P < .001$), and 17p (HR, 2.81; $P < .001$). Although small numbers prevented definitive conclusions, combinations hinted at better OS in patients with chromosome 5

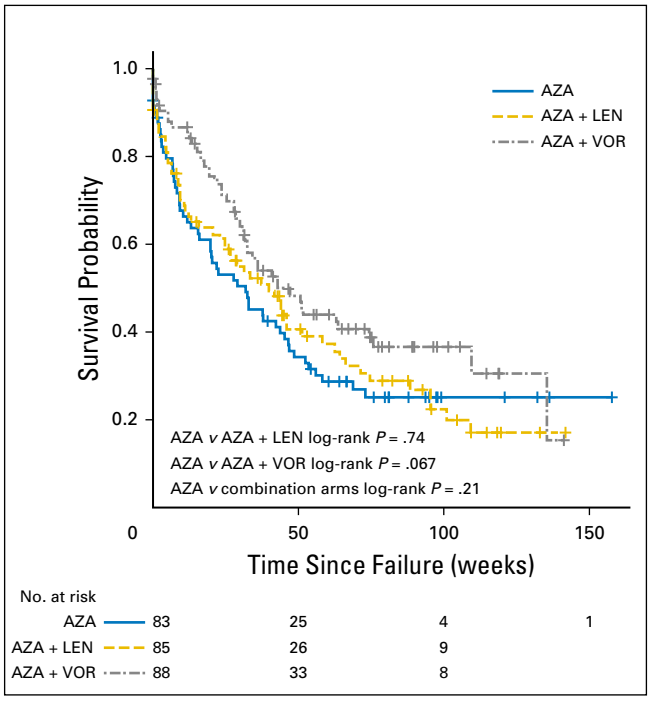

Fig 3. Overall survival after failure. AZA, azacitidine; LEN, lenalidomide; VOR, vorinostat.

($P = .15$) and for patients without 17p ($P = .22$) abnormalities. Patients with fewer mutations had better OS (HR, 4.55; $P = .04$); those with *SETBP1* ($P = .03$) and *TP53* ($P < .001$) had worse OS, with trends for worse OS in those with mutations in *CUX1* (log-rank $P = .08$), and *TET2* ($P = .07$).

Treatment Center Effect

Controlling for treatment arm, the baseline characteristics and outcome of all patients and patients on discrete study arms treated at MDS Centers of Excellence (n = 75) or high-volume (n = 137) sites were similar to other centers (Appendix Table A2).

DISCUSSION

Patients with higher-risk MDS and CMML have limited treatment options. Although hematopoietic cell transplantation is potentially curative, it is implemented in < 5% of patients.[27] Because these diseases are biologically and prognostically similar to acute myeloid leukemia in older adults, it is appealing to consider more aggressive therapeutic approaches, such as combinations of drugs that work in theoretically complementary or synergistic ways.

We randomly assigned patients with higher-risk MDS or CMML to receive azacitidine, azacitidine plus lenalidomide, or azacitidine plus vorinostat on the basis of single-arm phase II trials in which the ORR for each of the combinations was approximately double what had been seen previously for azacitidine monotherapy.[15,18,20] Unfortunately, those outcomes were not realized in the current study, with one exception: patients with CMML treated with azacitidine plus lenalidomide had twice the ORR as with azacitidine. This makes some sense in the context of previous trials, which have demonstrated activity of azacitidine in MDS and of lenalidomide in MPNs for this overlap disorder.

The single-arm studies on which this trial was based were small phase II trials with larger variances, which may explain higher ORR. It is also possible that results were affected by variations in patient selection and treatment practices. Patients on combination arms may have been undertreated. They were significantly more likely to undergo nonprotocol-defined treatment modifications and to be withdrawn from therapy because of toxicities, despite the overall similarity in adverse events across arms. This is not entirely surprising, because one study found that the physicians were twice as likely as patients to attribute treatment toxicities to poor drug tolerability, leading to treatment discontinuation.[28] There was a significant association between lenalidomide dose reductions and worse OS similar to what was seen in patients with deletion 5q lower-risk MDS treated with lenalidomide.[29] It is thus unresolved whether combination therapies can be realistically implemented on a broad scale. Finally, the response criteria have limitations in higher-risk patients treated with a hypomethylating agent, including the response assessment being confounded by temporary treatment-related cytopenias, and best response does not always reflect the most common response.

This study was not powered to assess OS differences among treatment groups, the ultimate measure of a clinically meaningful end point and one for which it is not entirely clear that ORR is an adequate interim marker. It is intriguing that median OS was 3 to

4 months longer for combination arms compared with azacitidine and that a trend for OS improvement emerged for patients treated with azacitidine plus vorinostat compared with azacitidine after treatment failure, possibly indicating a deeper response for combination therapies. Still, any effects on OS can only be validated in an adequately powered prospective study.

For cytogenetic subgroups, ORR was better for patients with chromosome 5 abnormalities compared with those without these abnormalities, but essentially did not differ across treatment arms. For the entire cohort, OS was worse for patients with abnormalities of chromosome 5, −7, and 17p and may be improved by combinations in patients with chromosome 5 or absence of 17p abnormalities. The distribution of molecular mutations was similar to previous reports, with *DNMT3A* lesions and fewer mutations associated with significantly higher ORR and *TET2*, and *TP53* ($P = .001$) associated with compromised response durations.[30-32] Patients with fewer mutations had better OS, whereas those with *SETBP1* and *TP53* had worse OS. Although a direct comparison with the recent study by Welch et al[33] is challenging given different patient populations (22% MDS), treatment (decitabine for 10 days), and definitions of response, the ORR for patients with *TP53* mutations in this study (45% *v* 35% for wild-type) seemed to be similar, as did the negative impact on OS.

In conclusion, patients with higher-risk MDS treated with azacitidine plus lenalidomide or azacitidine plus vorinostat had a similar ORR to patients treated with azacitidine monotherapy. Although specific patient subgroups, (CMML, normal cytogenetics, or chromosome 5 abnormalities), may derive benefit from azacitidine-based combinations, this should be confirmed in studies focused on these subgroups. Because underdosing may have been associated with compromised response and survival in combination arms, in most circumstances, patients with higher-risk MDS should be treated without dose adjustment for an induction phase of the first 4 months of therapy. Future studies in higher-risk MDS and CMML should be adequately powered to demonstrate an improvement in OS as a primary end point, or at minimum, response duration for molecular subtypes between study arms, in which innovative designs, such as Bayesian randomization, could optimize therapies for uncommon molecular lesions.

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Disclosures provided by the authors are available with this article at jco.org.

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Randomized Phase II Study of Azacitidine Alone or in Combination With Lenalidomide or With Vorinostat in Higher-Risk Myelodysplastic Syndromes and Chronic Myelomonocytic Leukemia: North American Intergroup Study SWOG S1117

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Appendix

Supplemental Methods

Patients. Eastern Cooperative Oncology Group performance status of 0 to 2; no previous treatment with study drugs or allogeneic transplantation, although prior autologous transplantation was allowed; no radiation therapy or chemotherapy within the previous 12 months, although therapy-related myelodysplastic syndrome (MDS) was allowed; and no specific level of organ dysfunction or abnormality of blood counts was excluded. Cytogenetic risk groups were defined per the Revised International Prognostic Scoring System and reviewed centrally.

Statistics. Fisher's exact tests and Wilcoxon rank-sum tests were used to compare categorical and quantitative factors between combination arms and azacitidine. Survival end points were estimated using the Kaplan-Meier method and analyzed with the log-rank test and Cox proportional hazard regression models. A two-sided alpha value of .05 denoted significance, *P* values were not adjusted for multiple comparisons. The following results were based on data available as of February 12, 2016, with survival updated as of April 13, 2015.

Outcomes. Baseline transfusion requirements were recorded during the 8 weeks preceding study registration to determine whether transfusion reduction or independence occurred. In instances in which therapy-related cytopenias were suspected, peripheral blood counts before and after bone marrow assessments or 8-week duration time points were also reviewed. Blood counts were assessed at least every 4 weeks, with bone marrow biopsies after cycles 4 and 7 and at suspicion of progression. Among 66 patients without a bone marrow biopsy or with an inadequate biopsy after cycle 4, there were no significant differences across study arms. Disease progression was defined as $>$ 50% increase in myeloblasts from baseline; non–treatment-related $\geq$ 50% decrement in neutrophil or platelet count; reduction of hemoglobin of $\geq$ 2g/dL from baseline; or becoming transfusion dependent. Treatment failure with azacitidine failure was defined as a lack or loss of response or disease progression.

Response Definitions

a. Accurate counts of RBC units and platelet transfusions received before and after starting protocol treatment are necessary to determine patients' responses to therapy. The numbers of RBC units and platelet transfusions during the 8 weeks before registration in the study will be recorded for use as baselines. Only RBC transfusions given for hemoglobin $<$ 9 g/dL or platelet transfusions for platelets $<$ 50,000/mm^3 before registration will be considered in the RBC transfusion response evaluation.

   1. Transfusion dependence: Patients who receive one or more RBC or platelet transfusions will be considered RBC or platelet transfusion dependent, respectively, in the absence of another explanation, such as gastrointestinal bleeding, hemolysis, etc.
   2. Relevant reduction in RBC transfusion requirement: This is defined for patients who were RBC transfusion dependent and received $\geq$ 4 units during the 8 weeks before registration on study. If, during an 8-week period after entering the study, the total number of RBC units transfused has decreased by at least 4 units compared with the number transfused during the 8 weeks before registration, then the patient will have a relevant reduction in RBC transfusion requirement.
   3. Transfusion independence: For assessment of response, RBC or platelet transfusion independence requires that the patient receive no RBC or platelet transfusions, respectively, for a period of at least 8 weeks.

b. Accurate measurements of hemoglobin and platelet counts before and after starting protocol treatment are also necessary to determine patients' responses to therapy. Whenever patients are transfusion dependent, hemoglobin and platelet counts should be measured immediately before transfusions to ensure that they reflect the patient's true hematologic status.

Complete Remission. The patient must satisfy all of the following bone marrow and peripheral blood criteria, and the patient must not receive RBC or platelet transfusions, erythropoietin, myeloid growth factor, or thrombopoietic agent within 28 days before this disease assessment:

Bone marrow evaluation must meet the following criteria:

Myeloblasts must be $\leq$ 50%. (NOTE: Persistence of dysplasia will be noted but does not preclude achievement of complete response [CR].)

b. Peripheral blood evaluation: The patient must satisfy all of the following for blood examinations performed during at least a 4-week period:

1. Hemoglobin $\geq$ 11.0 g/dl.
2. Neutrophils $\geq$ 1,000/mm^3.
3. Platelets $\geq$ 100,000/mm^3.
4. Blasts = 0%.
5. No evidence of dysplasia. (NOTE: The presence of mild megaloblastoid changes may be permitted if they are thought to be consistent with treatment effect. However, persistence of pretreatment abnormalities, for example, pseudo–Pelger-Hüet cells, ringed sideroblasts, or dysplastic megakaryocytes, is not consistent with CR.) Note: Transient cytopenias during repeated chemotherapy courses should not be considered as interrupting durability of response, as long as they recover to the improved counts of the previous course.

Marrow CR. The patient must satisfy the definition of CR for the bone marrow examination, and the marrow myeloblasts must have decreased by $>$ 50% from pretreatment. Marrow CR may be achieved with or without improved blood counts, and any hematologic improvement will be noted.

Partial Remission. The patient must satisfy all of the following criteria, and the patient must not receive RBC or platelet transfusions, erythropoietin, myeloid growth factor, or thrombopoietic agent within 28 days before this disease assessment:

a. Bone marrow evaluation: blasts $>$ 5% but decreased by $>$ 50% from pretreatment or a WHO subtype of MDS that is less advanced than pretreatment; cellularity and morphology are not relevant.

b. Peripheral blood evaluation: all of the peripheral blood results required for CR during at least a 4-week period.

Stable Disease. Failure to achieve at least a partial remission (PR), but with no evidence of progression for at least 8 weeks.

Failure. Death during treatment or disease progression or progression to acute myeloid leukemia or a WHO subtype of MDS or chronic myelomonocytic leukemia that is more advanced than pretreatment.

Relapse (after a CR, marrow CR, or PR). One or more of the following criteria (a. to e.) in the absence of another explanation, such as acute infection, gastrointestinal bleeding, hemolysis, etc. Note that the detection of circulating blasts is not by itself a sufficient criterion for relapse, but should trigger a bone marrow examination to determine whether relapse has occurred.

a. Return to pretreatment bone marrow blast percentage.
b. 50% or greater decrement from the maximum absolute granulocyte count during CR or PR. Granulocyte counts during periods of active infection will not be considered in determining the maximum.
c. 50% or greater decrement from the maximum platelet count during CR or PR.
d. A reduction in hemoglobin concentration by at least 1.5 g/dL from the maximum level during CR or PR.
e. Becoming transfusion dependent.

DNA Sequencing

DNA sequencing methods for each participating institution are summarized below.

Canadian Sites, Alliance, and Nationwide. Genomic DNA (gDNA) was extracted after thawing viably frozen bone marrow mononuclear cells, using the DNeasy Blood and Tissue Kit (69504; Qiagen, Hilden, Germany) per the manufacturer's protocol, but with the substitution of proteinase K from Sigma (P5568, Sigma-Aldrich, St. Louis, MO) and with the substitution of 50 μL low Tris-EDTA buffer (602-1297-01; Life Technologies, Carlsbad, CA) as the final suspension solution. gDNA was quantified using the TaqManRNase P qPCR Detection Kit (4316831; Life Technologies). Barcoded libraries were prepared from 15 ng of extracted gDNA, profiling 589 coding regions in 48 recurrently mutated genes, using a custom, pan-myeloid, two-tube, 1,552-amplicon, Ion Torrent AmpliSeq polymerase chain reaction (PCR) panel (ThermoFisher Scientific, Carlsbad, CA) and Ion AmpliSeq Library Kit 2.0 (4475435; ThermoFisher Scientific). Targets included all coding exons or hotspots for *ASXL1, BCOR, BCORL1, BOD1L, BRAF, BRCC3, CALR, CBL, CEBPA, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FLT3, GATA1, GATA2, GNAS, GNB1, IDH1, IDH2, JAK2, KDM6A, KIT, KRAS, MPL, NF1, NF-E2, NPM1, NRAS, PHF6, PTPN11, RAD21, RIT1, RUNX1, SETBP1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG2, TET2, TLR2, TP53, U2AF1, WT1,* and *ZRSR2* (information provided on request). Libraries were then quantified using the Ion Library TaqMan Quantitation Kit (4468802; ThermoFisher Scientific), diluted to 100 pM, pooled and sent to the Queen's Genomics Laboratory at Ongwanada in Kingston, Canada. Libraries were templated using the Ion OneTouch 2 system and Ion PI Template OT2 200 Kit v3 (4488318; Life Technologies), then sequenced using the Ion Proton System and Ion PI Sequencing 200 Kit v3 (4488315; Life Technologies). Barcoded libraries, in a batch of 12 to 30 libraries, were run together on a single Ion PI v3 chip (4488315; Life Technologies). Sequences were aligned to the human genome hg19, and variants were called in Ion Torrent Suite (Version 3.2.0). Files were uploaded into Ion Reporter (Version 5.2), and each sample was independently filtered

through a workflow of optimized variant calling geared to our custom AmpliSeq panel. Variants were further filtered through the Strict filter to exclude UCSC Common SNPs (University of California, Santa Cruz, common single nucleotide polymorphisms) and nonexonic and synonymous variants, and to initially include only variants with an allele ratio of variant allele fractions 0.2 to 1.0 and a depth coverage of $>$ 25. Next, we searched for lower-frequency variants (variant allele fractions 0.02 to 0.19) reported in the Catalogue of Somatic Mutations in Cancer (COSMIC; http://cancer.sanger.ac.uk/cosmic) database and/or with Ion Reporter $P \leq$ .001. Next, candidate variants were visually inspected using the Integrative Genomics Viewer (Broad Institute, Cambridge, MA). Variants were excluded if they appeared only in the ends of short sequence reads or consistently exhibited forward or reverse strand bias. Finally, all known false discoveries (as confirmed through independent PCR and Sanger sequencing, not shown), and suspected mis-priming events were removed from the final variant calls.

Cleveland Clinic. Genomic DNA was extracted from peripheral blood or bone marrow mononuclear cells in blood samples that were stored at the Stem Cell Tissue Bank at the Cleveland Clinic. Direct sequencing was performed on coding exons of 62 genes: *APC, ASXL1, BCOR, BCORL1, BTRC, C7orf55, LUC7L2, CBL, CCDC42B, CDH23, CEBPA, CFTR, CSF1R, CUX1, DDX41, DDX54, DHX29, DNMT3A, EED, ERBB4, ETV6, EZH2, FLT3, GATA2, GLI1, GLI2, GNB1, GPR98, IDH1, IDH2, IRF4, JAK2, JAK3, KDM6A, KIT, KRAS, MECOM, MED12, MLL, NF1, NPM1, NRAS, OGT, PHF6, PRPF8, PTCH1, PTPN11, RAD21, RNF25, RUNX1, SETBP1, SF3B1, SMC3, SRSF2, STAG2, STAT3, SUZ12, TET2, TP53, U2AF1, WT1, ZRSR2,* and *SRSF2* using IlluminaTrueSeq Custom Amplicon kit per manufacturer protocol. For germline confirmation, mutations were analyzed in nonclonal CD3þ cells whenever DNA was available. Bidirectional sequencing was performed by standard techniques using an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA). All mutations were scored as pathogenic on the basis of the observation that they were not detected in normal samples, in germline source, or in published SNP databases (dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP), and/or they were not reported as SNPs in previous publications.

Albert Einstein University. Genomic DNA was isolated from bone marrow aspiration or peripheral blood and then coding regions of 21 genes were assessed as follows: *ASXL1, EZH2, ETV6, RUNX1, TP53, CBL, DNMT3A, IDH1, IDH2, JAK2, KIT, MPL, NPM1, NRAS, PHF6, SETBP1, SF3B1, SRSF2, TET2, U2AF1,* and *ZRSR2*. These 21 genes amplified by PCR. The DNA sequences of these regions were then determined using next-generation sequencing technology. Somatic mutations consistent with MDS or other myeloid neoplasms within these samples were identified after cross-referencing all identified sequence variants with selected databases, including but not limited to COSMIC, dbSNP, and the listing of MDS mutations as identified in the numerous publications. The limit of detection of this assay is 5% (ie, if 5% of the gene copies in a specimen contain the mutant allele, then the mutated base should be consistently detected). Other assay limitations may include potential mis-calls due to limited depth of coverage (target of 500$\times$ minimum for this assay), amplicon design limitations (read quality can decrease toward the middle of amplicons), and platform basis. Poor-quality DNA resulting from poor specimen quality may cause assay failures.

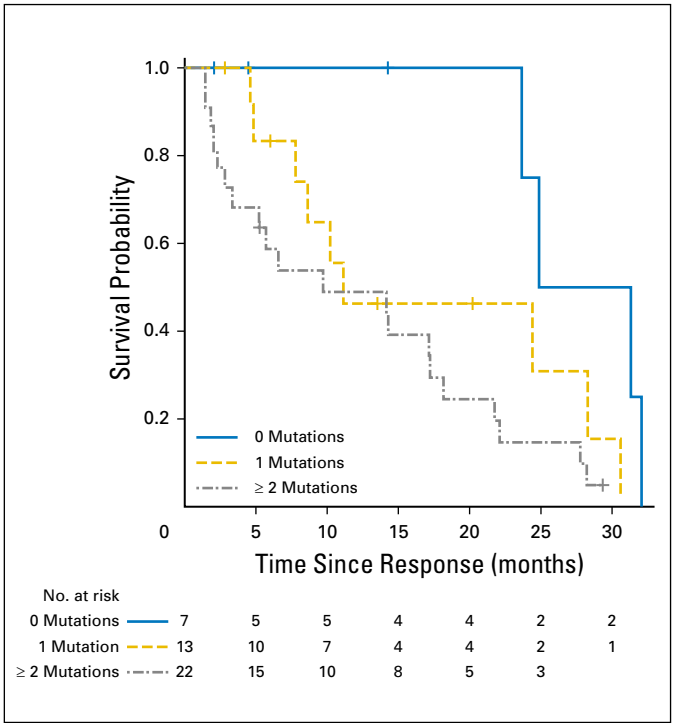

Fig A1. Response duration by number of mutations.

Table A1. Dose Modifications for Adverse Events

Drug	Starting Dose	Dose Level -1	Dose Level -2	Dose Level -3
Azacitidine	75 mg/m^2 daily, days 1-7	50 mg/m^2 days 1-7	50 mg/m^2 days 1-5	Discontinue
Lenalidomide	10 mg/day × 21 days	5 mg/day × 21 days	5 mg/day × 14 days	5 mg every other day × 21 days
Vorinostat	300 mg 2 times/day	200 mg 2 times/day	100 mg 2 times/day	100 mg 1 time/day

NOTE. Dose interruptions or modifications were implemented for prolonged cytopenias (defined as failure to recover to ≥ 50% of baseline from the start of the previous treatment cycle) or for National Cancer Institute Common Terminology Criteria for Adverse Events ≥ Grade 3 nonhematologic adverse events. In all cases, if an adverse event could reasonably be attributed to a single study drug during combination therapy, the dose of that drug was reduced or held. If the adverse event could not reasonably be attributed to a single drug, both drugs were reduced or held. Patients continued treatment on protocol as long as they derived clinical benefit in the opinion of the treating physician or until treatment failure (defined as disease progression, relapse, or significant or unresolved toxicity), patient preference, or treatment delay for any reason > 42 days.

Table A2. Patient Characteristics for Those Treated at MDS CEs or Not and Treated and at Higher and Lower Volume Centers

Factor	Not Treated at MDS CE (n = 202) No. (%)	Treated at MDS CE (n = 75) No. (%)	*P*
AZA	65 (32)	27 (36)	.86
AZA + LEN	69 (34)	24 (32)	
AZA + VOR	68 (34)	24 (32)	
Age	71 (29, 93)	68 (39, 89)	.086
Female	57 (28)	28 (37)	.15
Male	145 (72)	47 (63)	
PS 0	76 (38)	26 (35)	.39
PS 1	99 (49)	42 (57)	
PS 2	27 (13)	6 (8)	
CMML-1	28 (15)	8 (12)	.27
CMML-2	12 (7)	5 (7)	
RAEB-1	48 (26)	26 (39)	
RAEB-2	94 (52)	28 (42)	
IPSS Low	6 (3)	1 (1)	.20
IPSS Int-1	49 (25)	27 (38)	
IPSS Int-2	98 (50)	30 (42)	
IPSS High	44 (22)	13 (18)	
No prior chemo	189 (94)	71 (95)	1
Prior chemo	13 (6)	4 (5)	
No prior radiation	195 (97)	74 (99)	.69
Prior radiation	7 (3)	1 (1)	
No prior transplant	200 (99)	73 (97)	.30
Prior transplant	2 (1)	2 (3)	
HgB (g/dL)	9 (3, 99)	9 (7, 15)	.30
Platelets ($\times 10^3$)	68 (3, 4000)	69 (3, 388)	.65
WBC ($\times 10^3$)	3 (0, 205)	3 (1, 113)	.55
ANC ($\times 10^3$)	1 (0, 336)	2 (0, 110)	.78
Blood blasts (%)	0 (0, 23)	0 (0, 20)	.73
Marrow blasts (%)	10 (0, 20)	7 (1, 22)	.16
Not transfusion dependent at prestudy	86 (43)	31 (41)	.89
Transfusion dependent at prestudy	116 (57)	44 (59)	
IPSS-R good/very good	70 (35)	28 (37)	.95
IPSS-R intermediate	36 (18)	11 (15)	
IPSS-R poor	19 (9)	7 (9)	
IPSS-R very poor	46 (23)	19 (25)	
IPSS-R missing	31 (15)	10 (13)	
	Low Volume, 1-4 patients (n = 140)	High Volume, 5-17 patients (n = 137)	*P*
Age	71 (29, 93)	68 (39, 89)	.23
Female	40 (29)	45 (33)	.51
Male	100 (71)	92 (67)	
PS 0	50 (36)	52 (38)	.47
PS 1	69 (50)	72 (53)	
PS 2	20 (14)	13 (9)	
CMML-1	15 (12)	21 (17)	.089
CMML-2	6 (5)	11 (9)	
RAEB-1	34 (27)	40 (33)	
RAEB-2	71 (56)	51 (41)	
IPSS Low	1 (1)	6 (5)	.038
IPSS Int-1	38 (28)	38 (29)	
IPSS Int-2	61 (45)	67 (51)	
IPSS High	37 (27)	20 (15)	
No prior chemo	132 (94)	128 (93)	.81
Prior chemo	8 (6)	9 (7)	
No prior radiation	138 (99)	131 (96)	.17
Prior radiation	2 (1)	6 (4)	
No prior transplant	139 (99)	134 (98)	.37
Prior transplant	1 (1)	3 (2)	
HgB (g/dL)	9 (3, 14)	9 (6, 99)	.20
Platelets ($\times 10^3$)	73 (3, 4000)	62 (3, 481)	.21
WBC ($\times 10^3$)	3 (0, 90)	3 (1, 205)	.8
ANC ($\times 10^3$)	1 (0, 336)	1 (0, 110)	.86
Blood blasts (%)	0 (0, 23)	0 (0, 20)	.79
Marrow blasts (%)	10 (0, 22)	8 (0, 18)	.0035
Not transfusion dependent at prestudy	55 (39)	62 (45)	.33

(continued on following page)

Table A2. Patient Characteristics for Those Treated at MDS CEs or Not and Treated and at Higher and Lower Volume Centers (continued)

Factor	Not Treated at MDS CE (n = 202) No. (%)	Treated at MDS CE (n = 75) No. (%)	*P*
Transfusion dependent at prestudy	85 (61)	75 (55)	
IPSS-R good/very good	45 (32)	53 (39)	.13
IPSS-R intermediate	22 (16)	25 (18)	
IPSS-R poor	11 (8)	15 (11)	
IPSS-R very poor	34 (24)	31 (23)	
IPSS-R missing	28 (20)	13 (9)	

NOTE. The baseline characteristics and outcome of all patients and patients on discrete study arms treated at myelodysplastic syndromes (MDS) Centers of Excellence (CEs; n = 75) or high-volume (n = 137) sites were similar to other centers not identified as MDS Centers of Excellence or low-volume sites for overall response rate (odds ratio [OR] = 0.95, *P* = .45, and OR = 0.98, *P* = .75), nonprotocol defined dose modifications (OR = 0.93, *P* = 0.29, and OR = 0.99, *P* = .91), dose adjustments of azacitidine in first 4 cycles (OR = 0.97, *P* = .69, and OR=0.99, *P* = .86), or time to off-protocol (HR = 1.25, *P* = .11, and HR = 0.94, *P* = .61). Median (range) and No. (%) reported.
Abbreviations: ANC, absolute neutrophil count; AZA, azacitidine; chemo, chemotherapy; CMML, chronic myelomonocytic leukemia; HgB, hemoglobin; Int, intermediate; IPSS, International Prognostic Scoring System; IPSS-R, Revised IPSS; LEN, lenalidomide; PS, performance status; RAEB, refractory anemia with excess blasts; VOR, vorinostat.

Table A3. Association Between Mutation and Outcomes

Mutation	No. With Data	No. Mutated	WT Response, No. (%)	Mutated Response, No. (%)	Response OR (95% CI); *P*	OS HR (95% CI); *P*	Response Duration HR (95% CI); *P*
ASXL1	113	31	35 (43)	7 (23)	0.82 (0.67 to 1); .049	1.16 (0.65 to 2.09); .61	2.41 (0.93 to 6.23; .069
BCOR	109	7	35 (34)	4 (57)	1.26 (0.87 to 1.82); .23	0.64 (0.16 to 2.63); .54	1.09 (0.37 to 3.18; .87
CBL	113	8	39 (37)	3 (38)	1 (0.71 to 1.42); .98	0.79 (0.29 to 2.19); .65	0.77 (0.18 to 3.24; .72
CEBPA	109	9	37 (37)	2 (22)	0.86 (0.62 to 1.2); .38	0.73 (0.23 to 2.34); .59	1.16 (0.27 to 5.03; .84
DNMT3A	113	12	34 (34)	8 (67)	1.39 (1.05 to 1.85) ;.025	0.69 (0.3 to 1.62); .4	1.15 (0.51 to 2.6; .73
ETV6	113	7	39 (37)	3 (43)	1.06 (0.73 to 1.54); .75	1.17 (0.42 to 3.24); .76	1.23 (0.37 to 4.07; .74
NF1	109	6	38 (37)	1 (17)	0.82 (0.55 to 1.21); .32	1.54 (0.56 to 4.29); .4	0.52 (0.07 to 3.87; .52
NRAS	113	5	39 (36)	3 (60)	1.27 (0.82 to 1.96); .28	0.71 (0.17 to 2.93); .64	0.57 (0.14 to 2.41; .45
RUNX1	113	21	36 (39)	6 (29)	0.9 (0.71 to 1.13); .37	0.57 (0.26 to 1.26); .16	1.03 (0.36 to 2.97; .96
SETBP1	113	7	41 (39)	1 (14)	0.78 (0.54 to 1.13); .2	2.49 (1.06 to 5.84); .036	1.49 (0.2 to 11.21; .7
SF3B1	113	10	39 (38)	3 (30)	0.92 (0.67 to 1.27); .63	1.43 (0.61 to 3.36); .41	2.42 (0.72 to 8.19; .15
SRSF2	106	23	34 (41)	4 (17)	0.79 (0.63 to 0.98); .037	0.73 (0.36 to 1.5); .39	1.07 (0.32 to 3.55; .92
STAG2	109	14	34 (36)	5 (36)	1.00 (0.76 to 1.31); 1	0.52 (0.19 to 1.45); .21	1.09 (0.37 to 3.23; .88
TET2	113	26	34 (39)	8 (31)	0.92 (0.74 to 1.14); .45	1.79 (0.96 to 3.35); .068	2.4 (1.02 to 5.66; .046
TP53	113	22	32 (35)	10 (45)	1.11 (0.88 to 1.39); .37	3.14 (1.74 to 5.68); < .001	4 (1.61 to 9.93; .0028
U2AF1	113	19	34 (36)	8 (42)	1.06 (0.83 to 1.35); .63	0.88 (0.43 to 1.81); .73	1.09 (0.46 to 2.54; .85
SUS	113	51	27 (44)	15 (29)	0.87 (0.73 to 1.04); .12	0.87 (0.51 to 1.49); .61	1.42 (0.68 to 2.93; .35
SRE	113	34	32 (41)	10 (29)	0.89 (0.74 to 1.09); .27	0.92 (0.52 to 1.65); .79	1.18 (0.52 to 2.64; .7
SR	109	16	33 (35)	6 (38)	1.02 (0.79 to 1.32); .88	0.67 (0.29 to 1.57); .36	1.29 (0.47 to 3.52; .62
NK	113	8	38 (36)	4 (50)	1.15 (0.81 to 1.63; .44	0.71 (0.22 to 2.26); .56	0.52 (0.12 to 2.2; .38
CNP	113	16	38 (39)	4 (25)	0.87 (0.67 to 1.12); .28	1.06 (0.52 to 2.16); .88	0.66 (0.2 to 2.22; .51

NOTE. Odds ratios (ORs) > 1 indicate that the mutation is associated with increased odds of response compared with wild type (WT). Hazard ratios (HRs) > 1 indicate that the mutation is associated with decreased response duration or survival compared with WT.
Abbreviations: CNP, the combination variable CBL/NF1/PTPN11; NK, the combination variable NRAS/KRAS; OS, overall survival; SR, the combination variable STAG2/RAD21; SRE, the combination variable SETBP1/RUNX1/ETV6; SUS, the combination variable SF3B1/U2AF1/SRSF2.