Figure 1.
CONSORT diagram. Schematic representation of patient disposition in the trial.

diagnosis of MDS. Of the 217 patients with AML, 111 were newly diagnosed, 73 had relapsed disease, and 33 were refractory to at least one prior line of therapy.

Treatment administration and toxicities
Patients received a median of six cycles (IQR: 2, 8) of treatment in both arms of the trial. Average compliance to AZA across all

cycles of treatment was 73% in the AZA arm and 71% in the combination arm. There was no difference in dose intensity across treatment arms with a median intensity of 100% of the dose delivered in the first six cycles of treatment. A total of 106 patients in the AZA arm experienced one or more toxicity compared to 110 patients in the combination arm and there was no difference between treatment arms ($P = 0.87$). Adverse events (grades 3

Table 1. Demographics of study population

	Whole population (*n* = 259) No. (%)	Azacitidine alone (*n* = 129) No. (%)	Azacitidine + Vorinostat (*n* = 130) No. (%)
Age, years			
<70 years old	96 (37)	48 (37)	48 (37)
≥70 years old	163 (63)	81 (63)	82 (63)
Gender			
Male	156 (60)	75 (58)	81 (62)
Female	103 (40)	54 (42)	49 (38)
AML disease stage			
Newly diagnosed	111 (43)	57 (44)	54 (42)
Relapsed	73 (28)	34 (26)	39 (30)
Refractory	33 (13)	17 (13)	16 (12)
MDS disease stage			
Newly diagnosed	36 (14)	16 (12)	20 (15)
Relapsed	5 (2)	4 (3)	1 (1)
Refractory	1 (0)	1 (1)	0 (0)
ECOG performance status			
0	84 (32)	52 (40)	32 (25)
1	133 (51)	63 (49)	70 (54)
2	26 (10)	9 (7)	17 (13)
Missing	16 (6)	5 (4)	11 (8)
Cytogenetic group			
Favorable risk	13 (5)	2 (2)	11 (8)
Intermediate risk	109 (42)	58 (45)	51 (39)
Poor risk	54 (21)	26 (20)	28 (22)
Risk not known/not done	73 (28)	38 (29)	35 (27)
Missing	10 (4)	5 (4)	5 (4)
BM morphology, % blasts			
Mean	46.2	48	44.4
SD	28.4	27.7	29.1
Hemoglobin, g/L			
Mean	131.1	120.9	141.1
SD	184.9	167.5	200.9
Platelets, 10^9/L			
Mean	85.4	78.1	92.7
SD	131.2	79.2	167.7
WCC, 10^9/L			
Mean	14.1	15.6	12.6
SD	24.6	29	19.4
Neutrophils, 10^9/L			
Mean	3.1	3	3.2
SD	9.2	8.4	9.9

and 4) experienced by 5% or more of patients are listed in Supplementary Table S6.

Response and survival

There was no difference in either ORR [41% vs. 42%; OR, 1.05 (95% CI, 0.64–1.72); *P* = 0.84] or CR/CRi/mCR rate [22% and 26%; OR, 0.82 (95% CI, 0.46–1.45); *P* = 0.49] between the control and combination therapy arms. Time to first response and duration of response at 1 year was similar in the AZA and combination arm (6.2 months vs. 5.7 months and 67% vs. 58%, respectively; Supplementary Fig. S2). In predetermined subgroup analysis, patients with relapsed/refractory disease demonstrated an increased CR in the AZA/VOR arm (*P* = 0.02), although this did not translate to an improvement in OS.

No difference was observed in OS between patients treated with AZA monotherapy (median OS = 9.6 months; 95% CI, 7.9–12.7) and patients in the AZA/VOR arm (median OS = 11.0 months; 95% CI, 8.5–12.0; HR, 1.15; 95% CI, 0.87–1.51; *P* = 0.32). Specifically, there was no difference in OS between treatment arms in patients with newly diagnosed or relapsed/refractory AML (Fig. 2 and Supplementary Fig. S3).

Clinical and molecular factors predicting outcome after AZA-based therapy

We next wished to identify clinical factors predictive of response to AZA-based therapy in the trial cohort. Multivariable logistic regression demonstrated higher ORR rates in newly diagnosed disease versus refractory/relapsed disease (*P* = 0.038). Neither diagnosis (AML vs. MDS, *P* = 0.22) nor presentation karyotype (favorable vs. intermediate vs. poor, *P* = 0.76) predicted ORR in the same model. Cox regression analysis demonstrated increased OS in patients with MDS as opposed to AML (*P* = 0.012), a low ECOG score (*P* = 0.09), and a presentation WBC <10 × 10^9/l (*P* = 0.019). Presentation karyotype did not correlate with OS.

The impact of diagnostic mutational status on clinical response and OS was then studied using the results of NGS performed on 250 patients at trial entry (Fig. 3A). The mean mutation number per patient was 3.4 (Fig. 3B). Mutations in *RUNX1* were most frequent (73 patients, 29%). Mutations in *DNMT3A* (59 patients 23%), *IDH2* (57 patients, 23%), and *TET2* (56 patients, 22%) were also common (Fig. 3A). The observed mutational frequency was broadly consistent with that previously reported in older, but
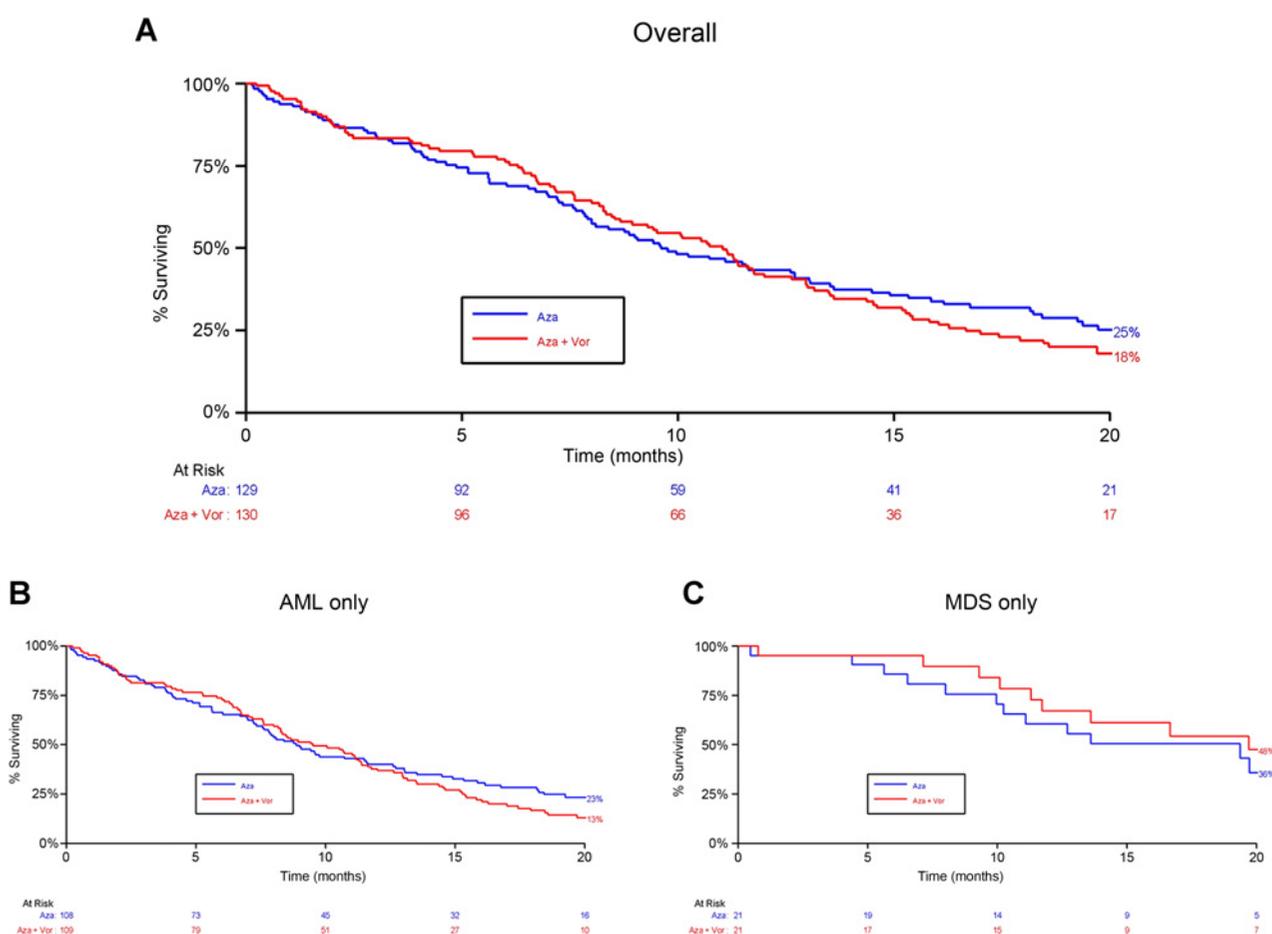
Figure 2.
Overall survival of trial patients. **A,** Survival in all study patients. **B,** Survival in patients with AML. **C,** Survival in patients with MDS.

not younger, AML and MDS patients (19–21) (Fig. 3C). In univariate analysis there was a lower complete response (CR, CRi, mCR) rate in patients with an *IDH2* mutation ($P = 0.029$) and *STAG2* mutation ($P = 0.002$) but an increased CR rate in patients with an *NPM1* mutation ($P = 0.038$; Table 2). When considered in a multivariable analysis and adjusted for all clinical variables, the presence of *STAG2* and *IDH2* mutations was not shown to have a significant association with acquisition of CR (Table 2). However, *NPM1* mutation remained of prognostic significance ($P = 0.012$).

Mutations in *CDKN2A* ($P = 0.0001$), *IDH1* ($P = 0.004$), *TP53* ($P = 0.003$), *NPM1* ($P = 0.037$), and *FLT3-ITD* ($P = 0.04$) were associated with reduced OS in univariate analysis. In multivariate analysis adjusted for all clinical variables, mutations in *CDKN2A*, *IDH1*, and *TP53* were associated with decreased OS (Table 2). No mutations were associated with improved OS. Mutations in *ASXL1* ($P = 0.035$) and *ETV6* ($P = 0.033$) were associated with a reduced duration of response. No mutations were associated with improved duration of response.

Among other frequently co-occurring mutations, we observed significant co-occurrence of *NPM1* mutations with *DNMT3A*, *FLT3-ITD*, *FLT3-other*, and *IDH1* as well as *DNMT3A* with *FLT3-other*, *IDH1*, and *IDH2* ($P < 0.05$ for all compar-isons). Patients with mutations in both *DNMT3A* and *IDH1* had reduced OS (median OS = 9.8 months; 95% CI, 1.5–11.6 months) compared to patients without both mutations (median OS = 10.7; 95% CI, 8.9–12). Patients with both *NPM1* and *IDH1* mutations had reduced OS (median OS = 3.8 months; 95% CI, 1.6–NE) compared to patients without both mutations (median OS = 10.7; 95% CI, 9.0–11.8). No significantly co-occurring mutations were found to be predictors of acquisition of CR (Fig. 3D).

Impact of AZA-based therapy on the LSC population

An expanded CD34$^+$ progenitor population was observed in 42/45 studied patients at diagnosis, whereas a CD34$^-$ expanded precursor population was observed in 3/45 (Fig. 4A). The majority of expanded populations were lymphoid-primed multipotential progenitors (LMPP: Lin-CD34$^+$CD38$^-$CD90$^-$CD45RA$^+$), which have been previously characterized as an LSC population with functional leukemia-propagating activity in serial xeno-transplant assays (14), and as a novel biomarker of AML disease response and relapse (4). Quantitatively, the immunophenotypic LMPP population is usually very small in normal BM (<2 in 10^5 cells; Vyas *et al.*, data under review). Therefore, expansion of the LMPP
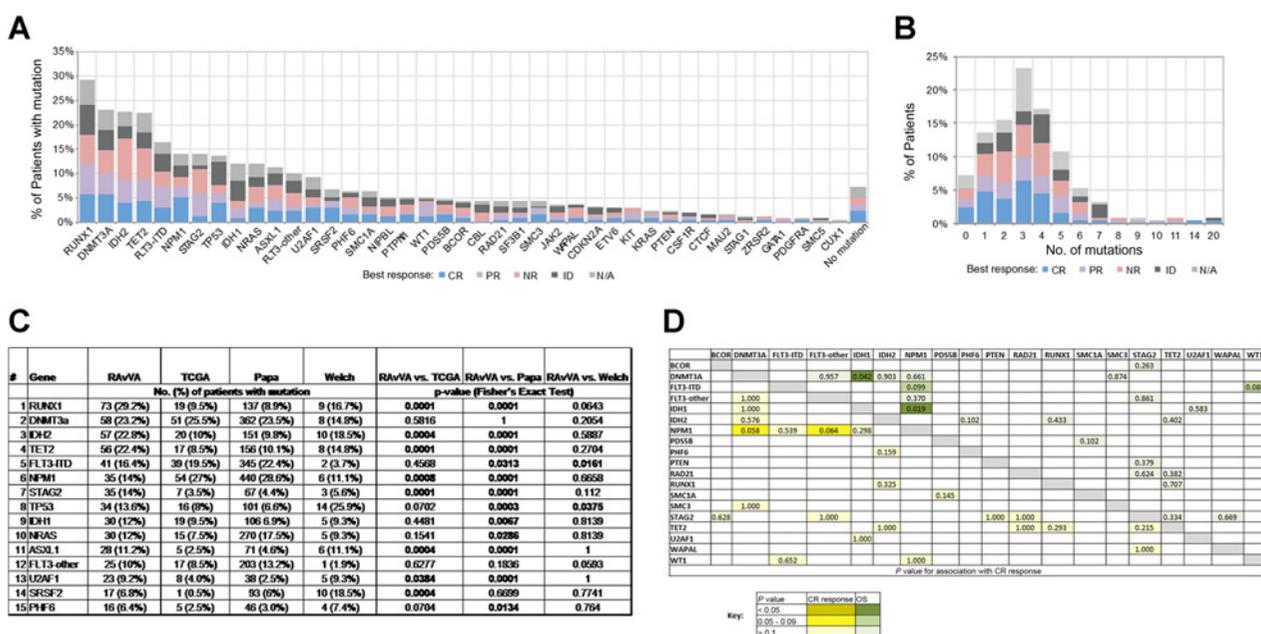
Figure 3.
Mutation profile of study population and correlation with clinical response. **A,** Frequency of mutations (as % of patients) in patients at trial entry. Patients are further divided according to best response achieved. **B,** Frequency of number of mutations detected per patient pretreatment. **C,** Comparison of mutations detected in RAvVA cohort compared with recently published AML cohorts [Papa: Papaemmanuil et al. (19); TCGA(20): Welch et al. (21)]. **D,** Correlation of combinations of detected mutations with CR and OS: only genes where there were at least five patients with two mutations are included in this analysis. Unadjusted P values from a Fisher exact test are shown. The top right half of the table (values in shades of green) show mutation combinations significantly associated with decreased OS. The bottom left half of the table (with values in shades of yellow) shows absence of significant mutation combinations predictive of CR. Key: CR (includes CR, CRi), PR, NR (no response including stable disease and progressive disease), ID (induction death), NA (response data not available).

population can be a sensitive measure of residual disease at CR in patients with AML. For these reasons, we focused on quantitation LMPP by immunophenotyping to measure the impact of therapy on putative LSC populations at best response and relapse.

In seven patients with resistant disease, there was no reduction in LMPP numbers measured as a fold change (Fig. 4B). Of interest, there was no significant reduction of LMPP numbers in eight patients achieving a PR, where the average BM blast percentage was reduced by 50%. In contrast, in 22 patients with CR/CRi/mCR, there was a significant reduction in LMPP numbers with AZA-based therapy. However, even here, LMPP numbers failed to normalize in 16/22 (Fig. 4B). In seven patients with expanded LMPP numbers, who achieved a CR, sequential monitoring demonstrated expansion progenitor populations prior to disease relapse (Fig. 4C).

Discussion

Co-administration of the HDAC inhibitor VOR did not improve response or survival in patients with AML or MDS treated with AZA. This observation is consistent with previous randomized studies in high-risk MDS but is the first demonstration that HDAC inhibitors have no impact on clinical outcomes in patients with newly diagnosed or relapsed AML-treated with AZA (10–12). Why might our study have failed to replicate earlier single arm studies of strikingly increased clinical activity of combined AZA and HDAC inhibitor treatment

(8, 9, 22)? Clinical and molecular characterization demonstrates comparability between study arms and confirms that the trial population was broadly representative of older patients with high-risk AML and MDS. Alternatively, the clinical activity of the experimental study arm might have been blunted because VOR associated drug toxicity resulted in under-dosing of AZA. Detailed pharmacovigilance studies excluded this possibility and indeed AZA dose intensity was similar in both treatment arms. Consideration should however be given to the possibility that co-administration of HDAC inhibitors might inhibit cellular uptake of aza-nucleosides and exploration of alternative dosing schedules may be worth exploring.

The search for novel drug partners with the potential to improve the clinical activity of AZA has been hampered by the fact that its mechanism of clinical activity remains unknown. Cell line and animal data have identified upregulation of epigenetically silenced genes and consequent restoration of cell-cycle checkpoints as an important potential mechanism of action and indeed previous in vitro studies have correlated the antitumor activity of both AZA and DEC with their ability to effect changes in cell-cycle gene expression and induce G_2 phase arrest (7, 23, 24). Consequently, the observation that heterozygous predicted loss of function mutations in CDKN2A, a cell-cycle checkpoint activator, are correlated with decreased survival in AZA-treated patients is supportive of the hypothesis that induction of cell-cycle arrest is a potentially important mechanism of action of this agent. In our study the CDKN2A mutations were nonsense in two patients and in the other seven were either nonsynonymous SNVs that had

Table 2. Univariate and multivariate analysis of predictors of CR and OS in the study population

		Overall response			
		Univariate analysis		Multivariable analysis[a]	
Covariate		OR (95% CI)	P[b]	OR (95% CI)	P
Clinical variables					
Disease status			(<0.001)		
	Refractory (vs. relapsed)	0.2 (0.1, 0.9)	0.03	Not estimable	
	Newly diagnosed (vs. relapsed)	2.1 (1.0, 4.6)	0.051	3.6 (1.1, 11.7)	0.037
Baseline WBC	≥10 (vs. <10)	0.7 (0.3, 1.5)	0.39	0.5 (0.2, 1.8)	0.292
Cytogenetic risk			(0.416)		
	Intermediate (vs. poor)	0.6 (0.3, 1.3)	0.204	0.6 (0.2, 2.0)	0.424
	Favorable (vs. poor)	1.0 (0.2, 4.9)	0.951	0.8 (0.1, 5.6)	0.843
Age	≥70 (vs. <70)	1.3 (0.7, 2.5)	0.447	1.3 (0.4, 3.8)	0.674
ECOG P.S.			(0.98)		
	1 (vs. 0)	1.0 (0.5, 1.9)	0.92	1.6 (0.6, 4.4)	0.395
	2 (vs. 0)	1.1 (0.4, 3.2)	0.902	1.0 (0.2, 5.8)	0.981
Mutations					
STAG2 mutation	Present (vs. absent)	0.2 (0.1, 0.6)	0.002	0.3 (0.1, 1.4)	0.117
IDH2 mutation	Present (vs. absent)	0.4 (0.2, 0.9)	0.029	0.4 (0.1, 1.3)	0.139
NPM1 mutation	Present (vs. absent)	2.5 (1.0, 6.2)	0.038	8.6 (1.6, 45.8)	0.012

		Overall survival				
		Median OS	Univariate analysis		Multivariable analysis[c]	
Covariate		(95% CI), months	HR (95% CI)	P[d]	HR (95% CI)	P
Clinical variables						
Diagnosis	MDS	19.4 (11.3, 22.7)	1		1	
	AML	9.1 (8.0, 11.1)	2.0 (1.3, 3.0)	0.0008	2.3 (1.3, 4.3)	0.007
Baseline WBC	<10	11.5 (9.8, 13.6)	1		1	
	≥10	8.8 (6.7, 10.5)	1.5 (1.1, 2.0)	0.0116	2.2 (1.4, 3.5)	0.001
Disease Status				(0.0132)		
	Relapsed	7.6 (6.4, 10.5)	1		1	
	Refractory	9.8 (8.3, 13.2)	0.8 (0.5, 1.2)	0.218	1.0 (0.5, 1.8)	0.976
	Newly diagnosed	11.7 (10.1, 14.9)	0.6 (0.5, 0.9)	0.005	0.5 (0.3, 0.8)	0.003
ECOG P.S.				(0.0235)		
	0	12.7 (9.6, 19.4)	1		1	
	1	10.1 (8.0, 11.5)	1.6 (1.1, 2.1)	0.009	1.6 (1.0, 2.6)	0.035
	2	9.5 (7.8, 15.4)	1.5 (0.9, 2.4)	0.0968	1.6 (0.9, 2.9)	0.131
Age	<70	9.3 (7.6, 11.6)	1		1	
	≥70	11.1 (9.0, 13.5)	0.8 (0.6, 1.1)	0.1706	1.6 (0.9, 1.8)	0.448
Cytogenetic risk				(0.8589)		
	Poor	9.5 (7.1, 11.1)	1		1	
	Intermediate	11.4 (8.1, 15.3)	0.9 (0.6, 1.3)	0.6367	1.2 (0.7, 1.9)	0.549
	Favorable	12.0 (1.7, N/E)	0.8 (0.4, 1.8)	0.6392	1.1 (0.5, 2.8)	0.802
Mutations						
CDKN2A mutation	Absent	11.0 (9.3, 12.6)	1		1	
	Present	4.5 (0.2, 7.8)	3.9 (1.9, 8.0)	0.0001	10.0 (3.3, 30.3)	<0.001
TP53 mutation	Absent	11.3 (9.4, 13.0)	1		1	
	Present	7.6 (2.4, 9.6)	1.8 (1.2, 2.6)	0.003	4.7 (2.5, 9.0)	<0.001
IDH1 mutation	Absent	11.1 (9.4, 12.7)	1		1	
	Present	5.6 (2.8, 9.8)	1.9 (1.2, 2.9)	0.004	3.6 (1.7, 7.6)	0.001
NPM1 mutation	Absent	11.1 (9.1, 12.6)	1		1	
	Present	8.1 (5.6, 10.7)	1.5 (1.0, 2.2)	0.037	0.6 (0.4, 1.1)	0.122
FLT3ITD mutation	Absent	11.1 (9.0, 12.7)	1		1	
	Present	8.8 (6.1, 11.6)	1.5 (1.0, 2.1)	0.04	1.0 (0.6, 1.8)	1

Abbreviations: ECOG P.S., Eastern Cooperative Oncology Group Performance Status; N/E, not estimable values in brackets refer to P value of the overall test for the specified variable; WBC, white blood cell.
[a]Logistic regression model adjusted for all variables in the table.
[b]Given by the Chi-square or Fisher exact test, corresponding to pairwise comparisons or the overall comparison as indicated between parentheses.
[c]Cox proportional hazards model adjusted for all variables in the table.
[d]Given by the log-rank test, corresponding to pairwise comparisons, or the overall comparison as indicated between parentheses.

previously been reported (six patients) or within two codons of a previously reported mutation (one patient). *CDKN2A* encodes P14, P16, and ARF. P14 and P16 inhibit the cyclin-dependent kinase CDK4, which regulates the G_1 cell-cycle checkpoint. ARF sequesters the E3 ubiquitin-protein ligase MDM2, a protein responsible for the degradation of p53. Thus, if loss of *CDKN2A* abrogates the clinical activity of AZA it raises the possibility that

AZA induces G_1 cell-cycle arrest and requires at least some p53 function for its antileukemic activity. We acknowledge that the findings of this study are based on a small sample size and that it is important to replicate this clinical association of *CDKN2A* mutations with poor clinical response to AZA in larger studies. If confirmed, our data highlight further study of P14, P16, and ARF function as a potentially fruitful line of investigation in
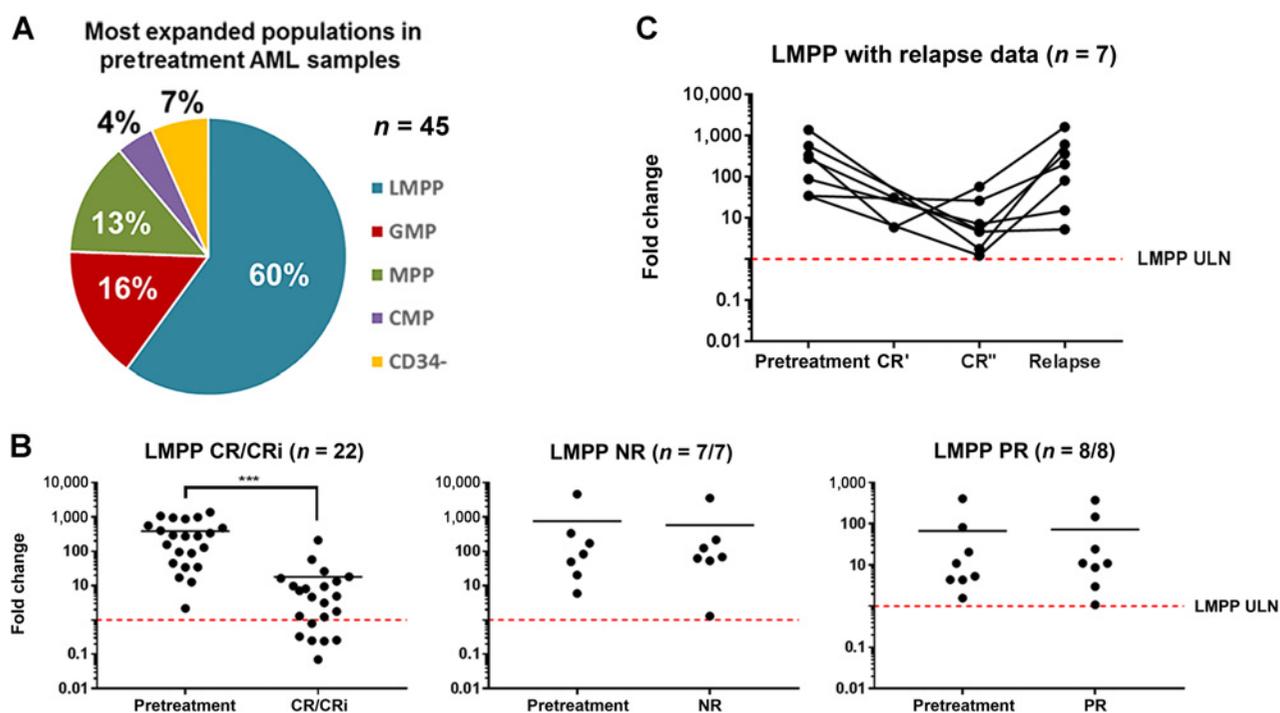
Figure 4.
Flow cytometric measurement of LSC populations. **A,** Quantitation of expanded CD34$^+$ progenitor or CD34$^-$ precursor LSC populations in AML patients pretreatment. **B,** Quantitation of LMPP-like LSC pretreatment and at CR expressed as fold change of upper limit of LMPP frequency in normal BM (upper limit of normal, ULN, dotted line, assessed in 12 normal donors). **C,** Longitudinal quantitation of LMPP-like LSC in patients pretreatment, at CR (multiple time points in two patients: CR′ and CR″) and at relapse.

understanding and potentially improving the outcomes of AZA-based therapy.

The identification of both clinical and molecular predictors of outcome with AZA therapy is important if this agent is to be optimally deployed. Improved survival was observed in patients with newly diagnosed disease, a low presentation white count and ECOG score. Importantly, and in contrast to patients treated with myelosupressive chemotherapy, we observed no impact on survival of an adverse risk karyotype after AZA-based therapy (25). Our data also demonstrate that NGS improves risk stratification because mutations in CDKN2A, IDH1, and TP53 were independently associated with decreased survival in AZA-treated patients. We did not identify any impact of mutations in TET2 or DNMT3A on outcome, in contrast to previous smaller retrospective studies (26–29). Although TP53 mutations have previously been shown to be associated with decreased survival in patients treated with intensive chemotherapy (30), it has recently been reported that the presence of a TP53 mutation was associated with a higher response rate in patients treated with DEC (21). In contrast, our data demonstrating no impact of TP53 on response rate to AZA but decreased OS in mutated patients implies that these two DNMT inhibitors may have distinct mechanism of action.

The development of strategies to overcome the inevitability of disease relapse in patients with AML treated with AZA is essential if outcomes are to improve. It is postulated that disease recurrence in patients with AML treated with either myelosuppressive chemotherapy or DNMT inhibitors occurs as a result of expansion of chemo-resistant LSC. However, correlative data in large cohorts of patients treated with either modality of therapy has been lacking.

Thus, the demonstration in this study of LSC persistence in AZA-treated patients who achieve a CR is consistent with the hypothesis that this recently identified cellular population may serve as a reservoir of resistant disease in AZA-treated patients. These data contrast with observations in patients treated with conventional chemotherapy where durable clearance of LSC appears to correlate with long-term remission and highlight the potential importance of quantitation of this cellular population as a biomarker of response in future studies of novel AZA-based combinations (4, 31, 32).

Disclosure of Potential Conflicts of Interest

C. Craddock reports receiving other commercial research support and speakers bureau honoraria from Celgene. L.S. Quek reports receiving commercial research grants and speakers bureau honoraria from Celgene. M. Raghavan reports receiving speakers bureau honoraria from Celgene. M. McMullin reports receiving speakers bureau honoraria from Celgene and Novartis. P. Vyas reports receiving commercial research grants from Celgene, and speakers bureau honoraria from Celgene and Jazz. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: C.F. Craddock, E. Gbandi, M. Raghavan, K. Wheatley, S. Siddique, J.D. Cavenagh, P. Vyas
Development of methodology: L.S. Quek, M. Dennis, P. Vyas
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.F. Craddock, L.S. Quek, P. Ferguson, M. Metzner, N. Garcia-Martin, A. Kennedy, A. Hamblin, M. Raghavan, S. Nagra, M.F. McMullin, R.J. Kelly, M. Dennis, J.D. Cavenagh, P. Vyas
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.E. Houlton, L.S. Quek, E. Gbandi, C. Roberts, M. Metzner, N. Garcia-Martin, A. Kennedy, K. Wheatley, P. Vyas

References

1. Ossenkoppele G, Lowenberg B. How I treat the older patient with acute myeloid leukemia. Blood 2015;125:767–74.
2. Dombret H, Seymour JF, Butrym A, Wierzbowska A, Selleslag D, Jang JH, et al. International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. Blood 2015;126:291–9.
3. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Gattermann N, Germing U, et al. Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. J Clin Oncol 2010;28:562–9.
4. Craddock C, Quek L, Goardon N, Freeman S, Siddique S, Raghavan M, et al. Azacitidine fails to eradicate leukemic stem/progenitor cell populations in patients with acute myeloid leukemia and myelodysplasia. Leukemia 2013;27:1028–36.
5. Itzykson R, Thepot S, Berthon C, Delaunay J, Bouscary D, Cluzeau T, et al. Azacitidine for the treatment of relapsed and refractory AML in older patients. Leuk Res 2015;39:124–30.
6. Ram R, Gatt M, Merkel D, Helman I, Inbar T, Nagler A, et al. Second line azacitidine for elderly or infirmed patients with acute myeloid leukemia (AML) not eligible for allogeneic hematopoietic cell transplantation-a retrospective national multicenter study. Ann Hematol 2017; 96:575–9.
7. Min C, Moore N, Shearstone JR, Quayle SN, Huang P, van Duzer JH, et al. Selective inhibitors of histone deacetylases 1 and 2 synergize with azacitidine in acute myeloid leukemia. PLoS One 2017;12:e0169128.
8. Garcia-Manero G, Yang H, Bueso-Ramos C, Ferrajoli A, Cortes J, Wierda WG, et al. Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [SAHA]) in patients with advanced leukemias and myelodysplastic syndromes. Blood 2008;111:1060–6.
9. Silverman LVA, Odchimar-Reissig R, LeBlanc A, Najfeld V, Gabrilove J, Isola L, Espinoza-Delgado I, Zwiebel J. A phase I trial of the epigenetic modulators vorinostat, in combination with azacitidine (azaC) in patients with the myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML): a study of the New York Cancer Consortium. Blood 2008;112:3656.
10. Prebet T, Sun Z, Figueroa ME, Ketterling R, Melnick A, Greenberg PL, et al. Prolonged administration of azacitidine with or without entinostat for myelodysplastic syndrome and acute myeloid leukemia with myelodysplasia-related changes: results of the US Leukemia Intergroup trial E1905. J Clin Oncol 2014;32:1242–8.
11. Garcia-Manero G, Montalban-Bravo G, Berdeja JG, Abaza Y, Jabbour E, Essell J, et al. Phase 2, randomized, double-blind study of pracinostat in combination with azacitidine in patients with untreated, higher risk myelodysplastic syndromes. Cancer 2017;15;123(6):994-1002.
12. Sekeres MA, Othus M, List AF, Odenike O, Stone RM, Gore SD, et al. 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. J Clin Oncol 2017:JCO2015662510.
13. Pleyer L, Burgstaller S, Girschikofsky M, Linkesch W, Stauder R, Pfeilstocker M, et al. Azacitidine in 302 patients with WHO-defined acute myeloid leukemia: results from the Austrian Azacitidine Registry of the AGMT-Study Group. Ann Hematol 2014;93: 1825–38.
14. Goardon N, Marchi E, Atzberger A, Quek L, Schuh A, Soneji S, et al. Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer Cell 2011;19:138–52.
15. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009;114:937–51.
16. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, et al. Revised recommendations of the international working group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol 2003;21:4642–9.
17. Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 2006;108:419–25.
18. Quek L, Ferguson P, Metzner M, Ahmed I, Kennedy A, Garnett C, et al. Mutational analysis of disease relapse in patients allografted for acute myeloid leukemia. Blood Adv 2017;1:193–204.
19. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med 2016;374:2209–21.
20. Cancer Genome Atlas Research N. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 2013;368: 2059–74.
21. Welch JS, Petti AA, Miller CA, Fronick CC, O'Laughlin M, Fulton RS, et al. TP53 and decitabine in acute myeloid leukemia and myelodysplastic syndromes. N Engl J Med 2016;375:2023–36.
22. Silverman LRAV, Odchimar-Reissig R, Cozza A, Najfeld V, Licht JD, Zwiebel JA. A phase I/II study of vorinostat, an oral histone deacetylase inhibitor, in combination with azacitidine in patients with the myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Initial results of the phase I trial: A New York Cancer Consortium. J Clin Oncol 2008;26:suppl); abstr 7000.
23. Liu J, Xie YS, Wang FL, Zhang LJ, Zhang Y, Luo HS. Cytotoxicity of 5-Aza-2'-deoxycytidine against gastric cancer involves DNA damage in an ATM-P53 dependent signaling pathway and demethylation of P16(INK4A). Biomed Pharmacother 2013;67:78–87.
24. Shang D, Han T, Xu X, Liu Y. Decitabine induces G2/M cell cycle arrest by suppressing p38/NF-kappaB signaling in human renal clear cell carcinoma. Int J Clin Exp Pathol 2015;8:11140–8.
25. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a

Craddock et al.

Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood 2000;96:4075–83.

26. Bejar R, Lord A, Stevenson K, Bar-Natan M, Perez-Ladaga A, Zaneveld J, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood 2014;124:2705–12.

27. Itzykson R, Kosmider O, Cluzeau T, Mansat-De Mas V, Dreyfus F, Beyne-Rauzy O, et al. Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. Leukemia 2011;25:1147–52.

28. Traina F, Visconte V, Elson P, Tabarroki A, Jankowska AM, Hasrouni E, et al. Impact of molecular mutations on treatment response to DNMT inhibitors in myelodysplasia and related neoplasms. Leukemia 2014;28:78–87.

29. Jung SH, Kim YJ, Yim SH, Kim HJ, Kwon YR, Hur EH, et al. Somatic mutations predict outcomes of hypomethylating therapy in patients with myelodysplastic syndrome. Oncotarget 2016;7:55264–75.

30. Takahashi K, Patel K, Bueso-Ramos C, Zhang J, Gumbs C, Jabbour E, et al. Clinical implications of TP53 mutations in myelodysplastic syndromes treated with hypomethylating agents. Oncotarget 2016;7:14172–87.

31. Gerber JM, Smith BD, Ngwang B, Zhang H, Vala MS, Morsberger L, et al. A clinically relevant population of leukemic CD34(+)CD38(−) cells in acute myeloid leukemia. Blood 2012;119:3571–7.

32. Terwijn M, Zeijlemaker W, Kelder A, Rutten AP, Snel AN, Scholten WJ, et al. Leukemic stem cell frequency: a strong biomarker for clinical outcome in acute myeloid leukemia. PLoS One 2014;9:e107587.

Clinical Cancer Research

Outcome of Azacitidine Therapy in Acute Myeloid Leukemia Is not Improved by Concurrent Vorinostat Therapy but Is Predicted by a Diagnostic Molecular Signature

Charles F. Craddock, Aimee E. Houlton, Lynn Swun Quek, et al.

Updated version	Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-17-1423
Supplementary Material	Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2017/08/01/1078-0432.CCR-17-1423.DC1

Cited articles	This article cites 30 articles, 11 of which you can access for free at: http://clincancerres.aacrjournals.org/content/23/21/6430.full#ref-list-1
Citing articles	This article has been cited by 2 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/23/21/6430.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/23/21/6430. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.