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Azacitidine with or without lenalidomide in higher risk myelodysplastic syndrome & low blast acute myeloid leukemia

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ABSTRACT

Standard treatment for higher risk myelodysplastic syndromes, chronic myelomonocytic leukemia and low blast acute myeloid leukemia is azacitidine. In single arm studies, adding lenalidomide had been suggested to improve outcomes. The ALLG MDS4 phase II trial randomized such patients to standard azacitidine or combination azacitidine (75mg/m^2/d days 1 to 5) with lenalidomide (10mg days 1-21 of 28-day cycle from cycle 3) to assess clinical benefit (alive without progressive disease) at 12 months. A total of 160 patients were enrolled; median age 70.7 years (range 42.5-87.2), 31.3% female with 14% chronic myelomonocytic leukemia, 12% acute myeloid leukemia and 74% myelodysplastic syndromes. Adverse events were similar in both arms. There was excellent delivery of protocol therapy (median azacitidine cycles 11 both arms) with few dose reductions, delays or early cessations. At median follow up 33.1 months (range 0.7-59.5), the rate of clinical benefit at 12 months was 65% azacitidine arm and 54% lenalidomide+azacitidine arm (P=0.2). There was no difference in clinical benefit between each arm according to WHO diagnostic subgroup or IPSS-R. Overall response rate was 57% in azacitidine arm and 69% in lenalidomide+azacitidine (P=0.14). There was no difference in progression- free or overall survival between the arms (each P>0.12). Although the combination of lenalidomide and azacitidine was tolerable, there was no improvement in clinical benefit, response rates or overall survival in higher risk myelodysplastic syndrome, chronic myelomonocytic leukemia or low blast acute myeloid leukemia patients compared to treatment with azacitidine alone. This trial was registered at *www.anzctr.org.au* as *ACTRN12610000271000*.

Introduction

The myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML), an MDS/myeloproliferative neoplasm overlap syndrome, are a group of clonal bone marrow disorders characterized by active but ineffective and clonal hematopoiesis accompanied by morphological dysplasia and variable cytopenias. Cytogenetic abnormalities and/or recurrent somatic mutations are present in the majority of cases.[1,2] The prognosis is variable with 30% of patients transforming to acute myeloid leukemia (AML).[2,3] AML with a "low blast" count of 20-29% has a similar prognosis to MDS with blasts of 10-19%.[4] The most widely used tool for stratifying clinical risk in MDS is the IPSS score.[4,5]

Azacitidine is approved and available for use in subsets of intermediate- to high-risk MDS. It is a nucleoside analogue that has direct cytotoxicity and gives rise to DNA hypomethylation through interference with DNA methyltransferase.[6] Clinical responses are manifest by an improvement in hematologic parameters and quality of life in a broad population of MDS patients including those with lower-risk disease but significant cytopenias.[7,8] Overall survival is prolonged in those with higher-risk disease.[9] There is also an established role for azacitidine in low-blast count AML and elderly AML with >30% BM blasts.[10,11] Azacitidine is an established standard of care in these patients, but even so the disease does not respond in many patients and survival remains suboptimal. Ball *et al.* reviewed a number of studies that combined hypomethylating agents (azacitidine and decitabine) with a number of different medication classes including small molecules, immunomodulators and monoclonal antibodies, but found a lack of survival advantage in these combinations compared to HMA monotherapy.[12] Emerging data suggests molecular profiles may influence response to azacitidine.[13]

Lenalidomide is a thalidomide analogue and is both more potent and tolerable relative to thalidomide.[14,15] Its efficacy in MDS is most pronounced in patients with 5q- MDS (low risk MDS).[16] Targeted degradation of CK1a (encoded by the retained allele of *CSNK1A1* at 5q32 in cells with 5q-) achieves cytogenetic remission and transfusion independence in the majority of patients.[15] Clinically relevant responses are also seen in lower-risk disease without 5q-.[17,18,19] In MDS without 5q-, the primary mechanism of disease control with lenalidomide appears to be immunomodulation.[14] Defective or reduced immune interaction between host and tumor contributes to the pathogenesis of MDS. Lenalidomide overcomes this by reducing pro-inflammatory cytokines, upregulation of T- and NK-cell activity and inhibition of angiogenic activity. These effects prevent apoptosis of healthy stem cells, improve erythropoiesis and direct immune responses against abnormal hematopoietic clones.[14]

The combination of a demethylating agent and an immunomodulatory drug has been explored in phase-I and -II studies in MDS, CMML and low blast AML in an attempt to improve outcomes. The ALLG MDS3 trial of azacitidine and thalidomide[20] showed promising response rates, and a phase-II study by Sekeres *et al.*[21] including the combination of azacitidine and lenalidomide in higher-risk MDS (blasts ≥ 5% or IPSS ≥1.5) or CMML resulted in an overall response rate of 49% compared to 38% azacitidine alone (P=0.14), with the subgroup of CMML patients on combination therapy achieving an improved ORR compared to aza alone (68% *vs.* 28%, P=0.02). Other groups have gone on to review the safety and efficacy of this combination in similar disease groups; elderly AML patients and high risk MDS and AML with ≤30% blasts.[22,23] Narayan *et al.* demonstrated a modest 25% response rate in elderly patients with previously treated AML and high-risk MDS. In these responders, the overall survival was 9.6 months compared to 4 months for non responders.[22]

We conducted an open-label, multicentre randomized phase-II study across 30 sites in Australia to assess the efficacy of azacitidine in combination with lenalidomide compared to standard azacitidine alone in the treatment of higher-risk MDS, CMML and low blast AML.

Methods

Study design and treatment

ALLG MDS4 was an open-label, multi-centre study conducted across 30 Australian sites. The study was registered at *anzctr.org.au ACTRN12610000271000*, was reviewed and approved by the Human Research Ethics Committees of each centre and conducted according to the Declaration of Helsinki. All patients provided written informed consent prior to participation.

The primary objective was to demonstrate improved efficacy with the combination compared to azacitidine alone. Secondary objectives were to describe response rates, response duration, overall survival, tolerability and changes in quality of life, and to explore biomarkers of response and mechanism of action of azacitidine and lenalidomide.

Patients were stratified according to IPSS (low-Int1 or Int2-high),[5] by centre and by disease category (MDS, AML or CMML),[24] and randomized 1:1 to either azacitidine alone at standard dosing of 75mg/m^2/d x 7 days (on a 5-2-2 interrupted schedule[25]) each 28 day cycle subcutaneously, or to the combination azacitidine plus lenalidomide. Patients on the combination arm received azacitidine alone at the above dose and schedule for the first 2 cycles, then commenced lenalidomide 10mg/d from day 1 of cycle 3 with a reduction in azacitidine dose with the combination to 75mg/m^2/d for 5 consecutive days per cycle as per phase 1 data available at the time.[26] The rationale for this was to limit the expected myelotoxicity typically seen in the first 2 cycles of treatment with azacitidine and so to improve the deliverability of combination treatment. Lenalidomide was continued only until completion of C12 due to limited data on longer-term combination toxicity. Azacitidine as a single agent was continued after the primary endpoint assessment at 12 months, until disease progression or unacceptable toxicity. Patients were followed for transformation to AML and survival until the last registered patient had been followed for a minimum 2 years after completion of the first 12 months of treatment.

Patient population

Patients were eligible with a diagnosis of non-proliferative CMML, AML with blasts <30% or MDS by WHO criteria;[24] those with refractory cytopenia with unilineage dysplasia (RCUD) and refractory anemia with ringed sideroblasts (RARS) had to have at least one clinically significant cytopenia as defined in the protocol (refer *Online Supplementary Appendix*), consistent with early studies of azacitidine in a broader group of patients with MDS.[7] Patients were 18 years or older and could have either *de novo* or secondary disease. They must have received no prior chemotherapy for MDS or AML except low dose cytarabine or hydroxyurea,

and no prior demethylating agent or immunomodulatory drug. They were to have a performance status of ECOG 0-2 and adequate organ function (*Online Supplementary Appendix*). GCSF was only used for short term management of severe neutropenic infections with no response assessment performed within 21 days of use. Patients on a stable dose of EPO prior to study entry were allowed to continue unchanged while on study.

Statistical plan

Analyses were carried out using the SAS (Statistical Analysis System, Version 9.3, SAS Institute, North Carolina, USA) software and graphs were produced in R version 3.2.3 software (R Foundation for Statistical Computing, Vienna, Austria, *http://www.R-project.org*). All comparisons were by intention to treat. The close-out date for this analysis was 12th March 2016.

A sample size of 160 patients (80 per arm) would provide 90% power, assuming a two-sided type I error of 5%, to detect an improvement of at least 25% in clinical benefit at 12 months, where the expected rate of clinical benefit at 12 months in the control arm of 50%, given a median time to progressive disease, relapse or death in the AZA001 study of 14.1 months,[9] and an expected rate of clinical benefit in the combination arm being 75%.

Toxicity

Adverse event rates are based on the worst grade reported during study treatment for those patients who commenced treatment. Fisher's exact test was used to compare adverse event rates between the randomized arms. All events and grades are based on the CTCAE v4.0 unless otherwise specified. Emerging grade 3+ haematologic toxicity applied to patients who did not have a haematologic toxicity at baseline but developed whilst on treatment. Results are based on absolute value from the screening averaged haematology counts. A grade 3+ toxicity for neutrophil and platelet data was defined as a reduction of more than 50% from baseline but for haemoglobin Grade 3+ was defined as Hb <80g/L.

Efficacy

All patients who were randomized (intention to treat group) were considered in efficacy analysis. 2006 IWG criteria were used for all responses.[27] The primary endpoint was "clinical benefit at 12 months", defined as the patient being alive and progression/relapse free at 12 months (+/- 1 month) post commencement of treatment, and so included those patients with stable disease at 12 months as achieving clinical benefit. Best response was determined using all assessments performed at the commencement of each cycle from C3 until treatment discontinuation, with bone marrow biopsies performed after C2, C4, C8 and C12. The overall response rate (ORR) included all patients achieving improvement (HI), marrow CR, PR and CR as best response.

Univariable logistic regression models were used to assess the impact of the following pre-defined variables on response (marrow CR or better): treatment arm, IPSS-R, IPSS, cytogenetic risk group, WHO diagnosis (MDS *vs.* AML *vs.* CMML).

Progression-free survival (PFS) was measured from the commencement of treatment to disease progression or death from any cause. Overall survival (OS) was measured from the commencement of treatment to death from any cause. OS and PFS duration was censored at the study close-out date for patients who were still being followed up without having experienced the relevant event by the close-out date, or at the date of last contact for patients who were lost to follow up before the study close-out date. The Kaplan-Meier (product-limit) method was used to estimate PFS and OS and median follow-up time (using the censoring distribution). The logrank test was used to compare survival between the treatment arms and IPSS-R subgroups.

Quality of life (QoL)

The EORTC QLQ C30 was utilized to describe differences in QoL parameters. These analyses were performed on five functional scales (physical, role, emotional, social and cognitive), three symptom scales (fatigue, nausea & vomiting and pain) and a global health status/QoL scale and six single items. Regression methods accounting for repeated measures (i.e., generalized estimating equations (GEE) with an exchangeable correlation structure) were used to estimate the difference between the treatment arms adjusted for baseline QoL score and weeks on trial. Differences between arms are expressed such that positive differences favour the LEN+AZA arm and negative differences favour the AZA arm.

Molecular and Biomarkers

Next generation sequencing (NGS). Sequence analysis of targeted regions within 26 genes involved in myeloid malignancy (Peter MacCallum Cancer Centre Myeloid Amplicon Panel (v5.4)) was performed in duplicate using Access Array methodology (Fluidigm, South San Francisco, CA, USA) to prepare amplicon-based, indexed libraries that were sequenced to a depth of ~1000 reads per amplicon on a MiSeq instrument using v2 chemistry (Illumina, San Diego, CA, USA). Alignment, variant calling and annotation were performed using a custom pipeline. Variants were evaluated using multiple functional and quality filters to identify likely pathogenic variants.

SNP-Array testing. DNA (200 ng) was hybridized to CytoSNP-12 BeadChip arrays (Illumina, San Diego, CA) according to the manufacturer's instructions. Analysis was performed using Karyostudio v1.4 software (Illumina). Karyotypes were reported according to the International System for Cytogenetic Nomenclature (ISCN 2013).

Exploratory analyses include changes in promoter DNA methylation during cycle 1 and at additional time points on treatment, immunophenotyping of MDS population, T-cell subsets, NK-cell function and cytokine profile as predictors of response to treatment and will be reported separately.

Results

Baseline demographics and disease features (Table 1)

One hundred and sixty patients with a median age of 70.7 years (range 42.5-87.2) were enrolled on study between August 2010 and August 2012; 159 received study drug. The median time from diagnosis to treatment was 1.0 year (0.0-13.2). Twenty-two patients (14%) had CMML, 19 (12%) AML and the remaining 74% MDS were mostly RCMD or RAEB 1/2 subtypes. Overall IPSS was Low-Int 1 in 61%; by IPSS-R Very Low/Low/Intermed in 63% with no difference in prognostic or cytogenetic subgroups between arms. Fifty-seven percent of patients were transfusion dependent at study entry. The 5q- cytogenetic abnormality was present as an isolated abnormality in only 3 patients (IPSS Int-1 in 2 patients, Int-2 in 1).

Molecular characteristics at baseline

Targeted amplicon sequencing and SNP-A testing was successfully performed in 66 cases. Targeted amplicon sequencing detected pathogenic mutations in one or more genes in 94% (62/66) of patients and SNP-array detected

abnormalities in 50% (33/66) of patients consistent with published literature.[28,29] Of the 4 patients without abnormalities detectable by NGS, 1 patient had monosomy 7 detectable on SNP-A resulting in 95.4% (63/66) of cases having a detectable molecular aberration. The average number of SNP-A abnormalities per case was 7 (range 0-37). Identification of additional SNP-A abnormalities upstaged cytogenetic risk in 24% (16/65) cases (G-banded karyotype not available in one case). SNP-A and mutational data are summarized in Table 2.

Table 1. Clinical and hematologic characteristics of 160 patients by assigned treatment cohort.

Baseline characteristics	AZA (n=80)	LEN+AZA (n=80)	Total (n=160)
Age years, median (range)	69.1 (42.5-85.9)	71.4 (44.1-87.2)	70.7 (42.5-87.2)
Male	52 (65)	58 (73)	110 (68.8)
Female	28 (35)	22 (28)	50 (31.3)
ECOG 0	42 (53)	41 (51)	83 (51.9)
1	36 (45)	33 (41)	69 (43.1)
2	2 (3)	6 (8)	8 (5.0)
Diagnosis WHO			
RCUD (RA, RN, RT)	1 (1)	0 (0)	1 (0.6)
RARS	6 (8)	3 (4)	9 (5.6)
RCMD	24 (30)	28 (35)	52 (32.5)
RAEB-1	11 (14)	11 (14)	22 (13.8)
RAEB-2	16 (20)	15 (19)	31 (19.4)
MDS-U	0 (0)	1 (1)	1 (0.6)
MDS isolated del5q-	2 (3)	1 (1)	3 (1.9)
CMML	12 (15)	10 (13)	22 (13.8)
AML	8 (10)	11 (14)	19 (11.9)
IPSS risk group			
Low	12 (15)	10 (13)	22 (13.8)
Int-1	37 (46)	38 (48)	75 (46.9)
Int-2	22 (28)	17 (21)	39 (24.4)
High	9 (11)	15 (19)	24 (15.0)
IPSS-R risk group			
Very Low	2 (3)	3 (4)	5 (3.3)
Low	24 (32)	18 (24)	42 (28.0)
Intermediate	23 (31)	25 (33)	48 (32.0)
High	16 (21)	12 (16)	28 (18.7)
Very High	10 (13)	17 (23)	27 (18.0
missing	5	5	10
Cytogenetics (IPSS-R)			
Very Good	1 (1)	2 (3)	3 (2.0)
Good	55 (73)	51 (68)	106 (70.7)
Intermed	11 (15)	11 (15)	22 (14.7)
Poor	3 (4)	2 (3)	5 (3.3)
Very poor	5 (7)	9 (12)	14 (9.3)
Carrying 5q-	10 (13)	13 (17)	23 (15.3)
missing	5	5	10
Past therapy			
EPO	3 (4)	0 (0)	3 (1.9)
GCSF	2 (3)	2 (3)	4 (2.5)
Low dose cytarabine	0 (0)	1 (1)	1 (0.6)
Baseline cytopenias			
Hb (<100g/L)	57 (71)	53 (66)	110 (68.8)
Neutrophils (<1.5x10^9/L)	47 (59)	37 (46)	84 (52.5)
Platelet (<100x10^9/L)	42 (53)	48 (60)	90 (56.3)

Hb: hemoglobin; ECOG: Eastern Cooperative Oncology Group; Int: intermediate; MDS-(U): myelodysplastic syndrome-(unclassifiable); RA: refractory anemia, RAEB: refractory anemia with excess blasts; RCMD: refractory cytopenia with multilineage dysplasia; RARS: refractory anemia with ringed sideroblasts; RCUD: refractory cytopenia with unilineage dysplasia; RN: refractory neutropenia; RT: refractory thrombocytopenia; WHO: World Health Organisation. Median (range) reported and N (%) unless otherwise specified.

Most CMML cases (5/7, 71%) had a normal SNP-A karyotype. Rates of SNP-A karyotypic complexity and del(5q) were highest in RAEB-2 (7/16, 43%) and AML (2/4, 50%). Both AML cases with an abnormal karyotype also had deletion of 17p. There were no significant differences between the AZA and AZA+LEN groups in terms of high-risk molecular profile, SNP-A complexity or cytogenetic risk group. Clinical benefit was most frequent in the normal SNP-A (29/39, 82%) and IPPS-Rsnp good cytogenetic risk group (28/39, 82%) cases.

Treatment

With a close out date of 12th March 2016, median follow up was 33.1 months (range 0.7-59.5). There was excellent drug delivery, with the median number of azacitidine cycles per patient administered of 11 in both arms with

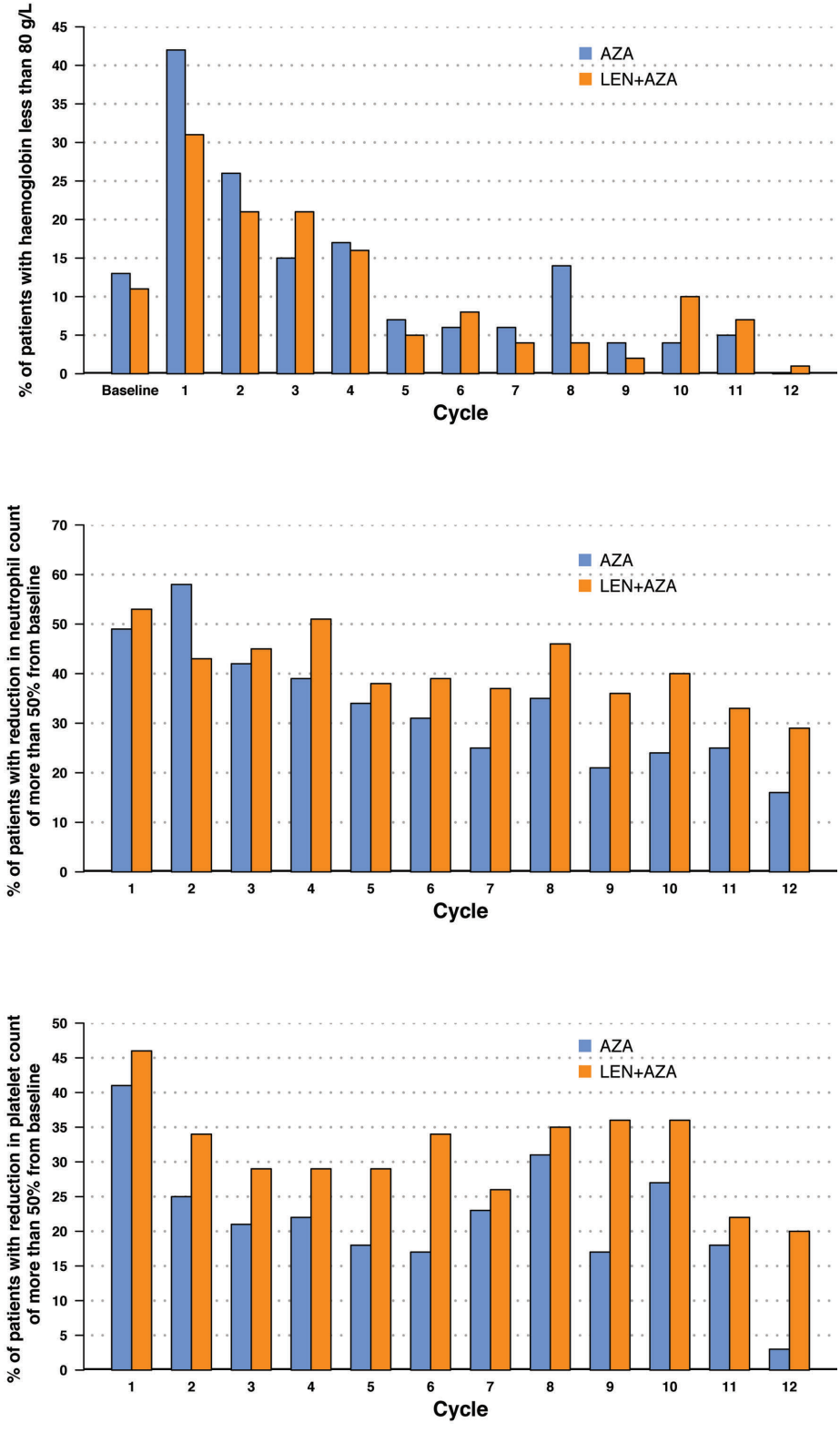

Figure 1. Rates of Grade 3+ Anemia (Hb less than 80g/L) baseline and on treatment, rates of Grade 3+ neutropenia (reduction neutrophils to less than 50% baseline) and rates of Grade 3+ thrombocytopenia (reduction in platelets to less than 50% baseline).

only 2.6% cycles dose- reduced. For those on combination treatment the median duration of lenalidomide treatment was 9 cycles (range 1-12) with only 2.8% lenalidomide cycles dose- reduced. Early discontinuation of azacitidine was mainly due to investigator/patient decision, relapse/progressive disease, or death. Early discontinuation of lenalidomide was mainly due to toxicity (11 patients) or investigator/patient decision (9 patients). Six patients were treated with lenalidomide after completing the protocol-specified 12 months of study therapy; 2 in AZA arm and 4 in LEN+AZA. This was initiated by individual investigators, and continued for up to 2 years post study therapy. The extended lenalidomide treatment was associated with grade 3 diarrhea in one patient and resulted in no improvement in response in these patients.

Safety

Non hematologic toxicity. Rates of all adverse events grade 3 or higher according to system and treatment arm are summarised in *Online Supplementary Table S1* with no differences observed. The most common non-hematologic toxicity was infection; the overall number of infectious episodes grade 3 or worse was 132 in 42.8% patients.

There was no difference between the arms for overall rates of infection with sepsis being the most common infection type. The difference between the severity of sepsis between the two treatment arms was significant with greater severity in the combination arm; sepsis Grade 4+ was seen in 11 patients in the combination arm compared to 2 patients in azacitidine alone arm (Table 3). There were 17 deaths due to

Table 2. Molecular characteristics of cohort with baseline samples.

	AZA (n=35)	LEN-AZA (n=31)	TOTAL (n=66)
Targeted Amplicon Sequencing			
$TP53^{mut}$	3	5	8
$TET2^{mut}$	14	10	24
High-risk molecular profile ($TP53^{mut}$ and/or $ASXL1^{mut}$ and/or $RUNX1^{mut}$ and/or $EZH2^{mut}$)	21	21	42
low-risk molecular profile ($SF3B1^{mut}$ only)	4	1	5
SNP-Array			
5q-	6	6	12
Monosomy 7/7q-	6	3	9
20q-	4	4	8
Trisomy 8	0	6*	6
17p-	2	4	6
7q CN LOH	4	2	4
Combined molecular profile			
TP53 abnormality ($TP53^{mut}$ and/or 17p-)	3	5	8
High-risk molecular profile ($TP53^{mut}$, $ASXL1^{mut}$, $RUNX1^{mut}$, $EZH2^{mut}$, IPSS-RsnpP, IPSS-RsnpVP)	22	21	43

mut: mutated; LOH: loss of heterozygosity; IPSS-RsnpP or VP: IPSS-R SNP-A poor cytogenetic or very poor cytogenetic risk.

Table 3. Non-hematologic toxicity; infections, grade 3 and above.

Infection type	AZA		LEN+AZA	
	N. patients	No. episodes	N patients	No. episodes
Any Infection	34 (43%)	62	34 (43%)	71
GI/Abdo	6 (8%)	7	7 (9%)	10
Renal/Urologic	0 (0%)	0	2 (3%)	2
Respiratory	9 (11%)	10	14 (18%)	18
Sepsis overall	18 (23%)	29	19 (24%)	29
Sepsis grade 3	16		8	
Sepsis grade 4	2		10	P=0.02
Sepsis grade 5	0		1	
Skin/Mucosal/Eye	9 (11%)	11	9 (11%)	9
All other infections	5 (6%)	5	6 (8%)	6

infection (7 in AZA, 10 in LEN+AZA). For full listing of cause of death according to treatment arm see *Online Supplementary Table S2.*

The only other non hematologic toxicity seen at rates >5% was raised GGT in 15 patients with no significant difference between the arms (AZA n=4 LEN+AZA n=11; *P*=0.1).

Hematologic toxicity. Comparing data from cycle 3 to cycle 12 (as LEN was introduced from cycle 3 onwards), there was no association between treatment arm and cycle for any of the hematologic grade 3+ toxicity rates was observed. For Hb <80g/L and for neutrophils and platelets >50% reduction from baseline count, there was no difference between treatment arms but a statistical significant difference across the cycles for both arms. See Figure 1.

Emerging Grade 3+ hematologic toxicity. For those patients who did not have a grade 3+ toxicity at baseline as defined by CT CAE V4.0 (N), there was a non significant trend to greater treatment emergent neutropenia (78% *vs.* 68%) and thrombocytopenia (63% vs. 50%) in the combination arm (*Online Supplementary Table S3*).

Efficacy

Summary of efficacy endpoints is provided in Table 4.

The primary endpoint of rate of clinical benefit at 12 months (alive with stable disease or better) in the AZA arm was 65%, and 54% in the LEN+AZA arm (Fishers Exact test, *P*=0.2). There was no difference in rate of clinical benefit between each treatment arm according to WHO diagnostic subgroup (MDS, AML or CMML) or according to IPSS-R.

There was no difference in clinical benefit across disease subtype within either the LEN+AZA or the AZA treated groups.

The overall response rate (best response of HI, PR, marrow CR or CR) with AZA was 57% and 69% in LEN+AZA (*P*=0.14).

CR was achieved in 17 patients (22%) on AZA and 20 patients (25%) on LEN+AZA.

There was no difference in type of HI across the 2 arms.

The median time to best response for those achieving a HI or better was not different between treatment arms; 5.5 months (range 1.8-11.7) AZA and 4.8 months (range 1.8-12.4) LEN+AZA. Median time to first response (of HI or better) was 2.8 months (range 1.6-9.2), with no difference between the arms (*P*=0.13).

There were no significant associations found for these variables with respect to response – either clinical benefit at 12 months as defined by primary endpoint, or for overall response rate of best response HI or better.

Using univariable subgroup logistic regression models of treatment effect on primary endpoint response (clinical benefit stable disease or better at 12 months), there were no significant associations for age, sex, WHO diagnosis, IPSS, IPSS-R or cytogenetic risk group (*Online Supplementary Figure S1*).

Cytogenetic response

Fifty-nine patients had a karyotypic abnormality detected at baseline, 28 in the AZA arm and 31 in the LEN+AZA arm. A total of 29% (8) of patients on the AZA arm had a cytogenetic response. Half (4) of those achieved a complete cytogenetic response while the other half (4) achieved a partial response with a ≥50% reduction in the chromosomal abnormality. A total of 39% (12) patients in the LEN+AZA arm had a cytogenetic response, 11 of them

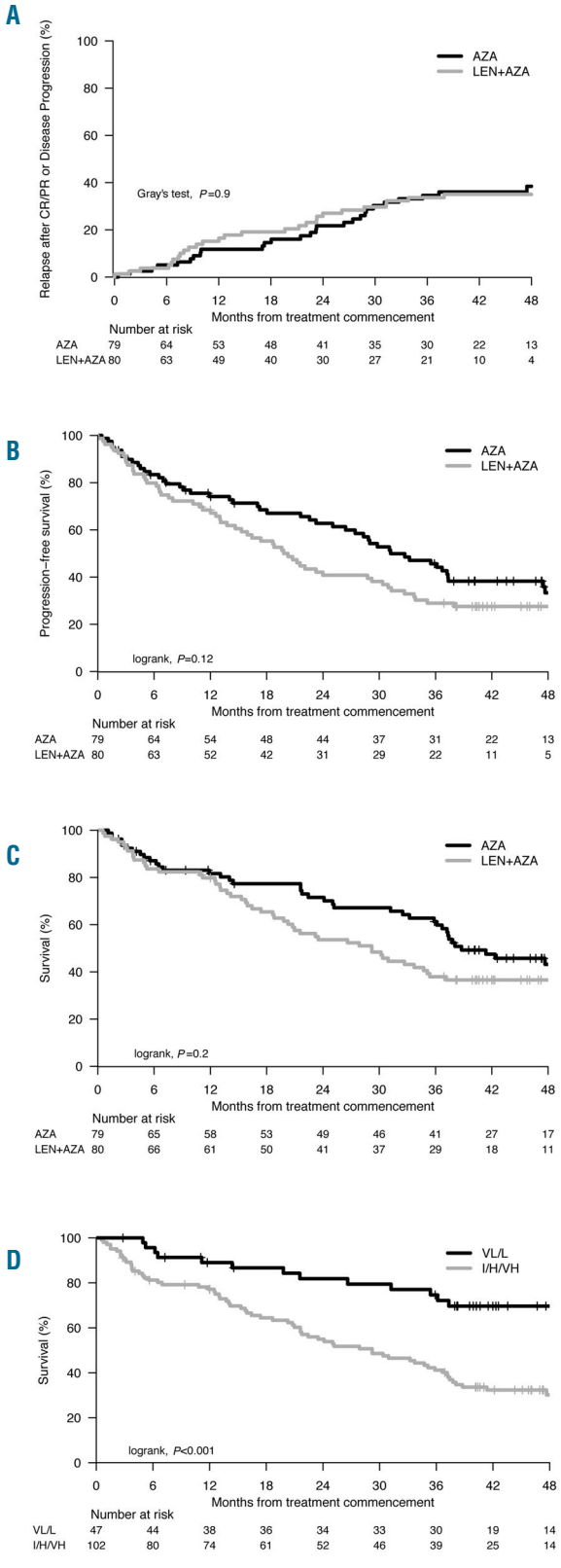

Figure 2. Time to relapse, progression-free and overall survival between treatment cohorts, and overall survival according to risk. A. Kaplan-Meier curves of time to relapse after achieving CR/PR, or disease progression between both treatment cohorts. B. Kaplan-Meier curves of progression-free survival (PFS). C. Overall survival according to assigned treatment cohort. D. Overall survival according to IPSS-R; very low/low *versus* intermed/high/very high risk.

achieving a complete cytogenetic response whilst 1 achieved a partial response..

Time to disease relapse or progression (Figure 2A)

No association with treatment arm was found for time to relapse after CR/PR or PD.

Median time to progression to AML (MDS and CMML patients by WHO criteria) or death (all patients) from any cause was 37.2 months in the AZA arm and 28.8 months in the LEN+AZA arm.

Progression free-survival (PFS) (Figure 2B)

PFS was measured from first day of treatment to date of first confirmed disease progression or death from any cause. Median PFS time on AZA was 31.2 months (95% CI 25.0-37.4) and on LEN+AZA was 19.8 months (95% CI 14.7-29.2) with no difference between the arms observed (Figure 2B, logrank P=0.12).

Overall survival (Figures 2C and 2D)

The median follow-up time (estimated with the inverse Kaplan-Meier method) was 47.2 months (range 0.7-59.5); median survival time on AZA was 38.8 months (95%CI 35.8-52.6) compared to 29.2 months on LEN+AZA (95%CI 19.8-35.1) (logrank P=0.2). Forty-one patients on AZA had died compared to 49 on LEN+AZA (Table 8). Cause of death was mostly due to disease progression and infections with 5 overall due to hemorrhage and 9 other/unknown. There was a significant difference in median overall survival in IPSS-R Very Low/Low-risk and Intermediate/High/Very High-risk groups (Figure 2D, logrank P<0.001).

Quality of Life (Figure 3)

Completion rates for the EORTC QLQ-C30 at baseline/screening was 96%, at C4D22 83%, C8D22 82% and C12D22 or at primary endpoint visit was 84%. The only effect of treatment on QoL scores during study was a higher rate of diarrhea in LEN+AZA arm.

Discussion

This randomized phase II study aimed to find out whether outcomes were improved for patients with higher risk MDS, CMML and low blast AML by adding lenalidomide treatment to the established regimen of azacitidine. These two agents have shown synergistic activity in vitro, with promising early results of the combination treatment from smaller single arm clinical trials. In this study, there was excellent duration and delivery of treatment in both arms due to strong recognition of the value of prolonged therapy, particularly with a clinical benefit endpoint at 12 months. Despite this and the good tolerability of the combination, there was no improvement in response rates, clinical benefit or survival. As in the study by Sekeres *et al.*,[21] we showed a trend towards improved responses without translation to improved clinical benefit or survival, though this study was not adequately powered to show a difference in overall survival. The lack of clinical benefit was not due to an excess of toxicity in the combination arm.

No subgroup in this study, including those with CMML, and in contrast to the recent report by Sekeres *et al.*,[21] had improved responses with the addition of lenalidomide to

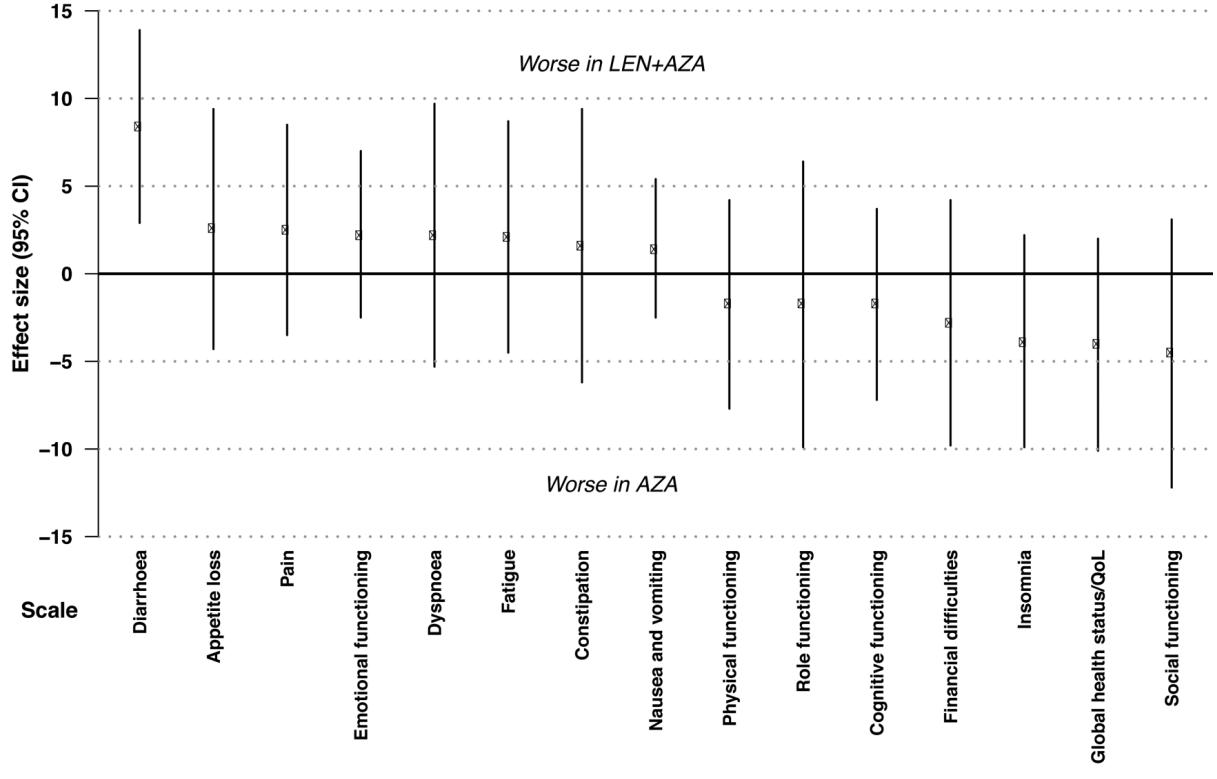

Figure 3. Quality of life differences on EORTC QLQ C30 questionnaire between treatment cohorts using GEE regression model

Table 4. Efficacy: clinical benefit at 12 months, overall response rate (ORR) & best response achieved by assigned treatment cohort; those who received treatment.

	AZA n=79 n (%[Exact 95% CI])	LEN+AZA n=80 n (%[Exact 95% CI])	P
Clinical benefit at 12 months (SD or better)	52 (65% [54-75])	43 (54% [42-65])	0.2
MDS	38 (63% [50-75])	34 (58% [44-70])	0.6
AML	5 (62% [24-91])	4 (36% [11-69])	0.4
CMML	9 (75% [43-95])	5 (50% [19-81])	0.4
	P=0.7	P=0.4	
IPSS-R Very Low/Low	22 (85% [65-96])	12 (57% [34-78])	0.052
IPSS-R Intermed/High/Very High	28 (57% [42-71])	28 (52% [38-66])	0.7
Overall response rate (Best response)	45 (57% [45-68])	55 (69% [57-79])	0.14
MDS	36 (60% [47 - 72])	41 (69% [56 - 81])	0.3
AML	3 (43% [10-82])	6 (55% [23-83])	>0.99
CMML	6 (50% [21-79])	8 (80% [44-97])	0.2
IPSS-R Very Low/Low	15 (58% [37-77])	14 (67% [43-85])	0.6
IPSS-R Intermed/High/Very High	28 (58% [43-72])	37 (69% [54-80])	0.3
Best response achieved			
CR	17 (22%)	20 (25%)	
PR	0	2 (2%)	
Marrow CR	2 (2%)	5 (6%)	
Marrow CR+HI	8 (10%)	5 (6%)	
HI only	18 (23%)	23 (29%)	
SD	22 (28%)	15 (19%)	
PD	3 (4%)	4 (5%)	
Death prior to C3 first response assessment	6 (8%)	4 (5%)	
Missing data/not evaluable	3 (4%)	2 (2%)	

CR: complete response; HI: hematologic improvement; PD: progressive disease; PR: partial response; SD: stable disease.

azacitidine. Overall, there was very good durability of responses and good survival. Unsurprisingly, those with lower-risk disease according to established prognostic scores lived longer.

Our eligibility included patients with potentially lower-risk disease subtypes in contrast to other recent clinical trials such as AZA001[9] and SWOG S1117[21] which defined eligibility based on prognostic score. This inclusion was based on earlier data showing similar response rates across all IPSS groups[7] and an acknowledgement that a proportion of those with apparent lower-risk disease have outcomes more in keeping with those with higher prognostic scores. Despite this, and though it is an indirect comparison of populations, our cohort risk compares similarly to the SWOG S1117 cohort with respect to proportion of patients with Very Low/Low IPSS-R; 31.3% Sekeres cohort compared to our ALLG MDS4 32% (AZA) and 38% (AZA + LEN).

It is possible that the dose and scheduling of treatment in this protocol may have impacted on responses. Phase 1 data by Sekeres[26] supported the decision to reduce the number of days of azacitidine dosing to five when combining with lenalidomide, in order to reduce the risk of treatment limiting toxicity in the first two cycles. Given the lack of excessive toxicity in our combination arm and the high median number of azacitidine cycles (11 cycles in our cohort compared to SWOG 1117 median 23-25 weeks treatment) we did achieve the implementation of this treatment combination on a broad multi-centre setting. However, we have not shown that full azacitidine dosing of seven days per cycle in combination with lenalidomide is feasible. In addition, the concurrent as opposed to consecutive administration of the two agents on this protocol may have reduced overall efficacy. The dose of lenalidomide selected for this study was based on a Phase II study in MDS,[21] however, subsequent studies have utilised higher doses of lenalidomide in combination – mostly sequential - in AML[30] and high-risk MDS[31] which may improve efficacy. The option of using lenalidomide prior to the introduction of azacitidine could be considered as an extrapolation of the findings by Zeidan *et al.* who demonstrated enhanced erythroid improvement in low -risk (non-5q deletion) MDS.[31] Finally, consideration could be given to commencing both agents from C1 rather than delaying the introduction of lenalidomide until C3 in an attempt to improve efficacy, although early progressions or deaths in our study were uncommon with 10 deaths or disease progression within the first 2 cycles of treatment (5 in each arm).

There was no central review of pathology in this study. Responses were provided by the site investigators and only reviewed centrally if there were discrepancies or questions. IWG criteria for response was adopted, though its application in patients experiencing both disease and treatment related cytopenias is complex, and the application and consistency across many sites was

difficult to ensure. A more robust, refined IWG criteria is awaited and would make this more consistent in future studies.

We have shown the feasibility on a broad scale of the combination of lenalidomide and azacitidine in patients with higher risk MDS, CMML and low blast count AML, but the lack of improvement in responses and clinical benefit do not support the utilization of this combination in higher-risk MDS in clinical practice. Other combinations and novel agents are needed to improve the outcomes for this large and vulnerable group of patients, who at this stage have limited therapeutic options. Other groups are currently exploring novel combination studies with azacitidine such as enasidinib and venetoclax.

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