Transfusion independence and survival in patients with acute myeloid leukemia treated with 5-azacytidine

Reanalysis of the CALGB[1] and AZA001[2] studies in advanced myelodysplastic syndrome (MDS) suggests that 5-azacytidine (AZA) is effective for acute myeloblastic leukemia (AML) with less than 30% bone marrow blasts. Most AML patients are elderly (>65 years old) and unfit for intensive chemotherapy and allogeneic transplantation;[3] prognosis is extremely poor with median survival of a few months.[4] For these patients, appropriate treatments are best supportive care or low-dose cytarabine.[3] Compared with best supportive care, low-dose cytarabine more frequently induces complete remissions (18% *vs*. 1%) and prolongs survival (3.8 *vs*. 2.5 months).[3] AZA targets DNA methyltransferase and reactivates quiescent genes,[5] decreases leukemic cell proliferation and induces cell differentiation. In higher risk MDS or in AML with less than 30% bone marrow blasts,[6] AZA outperformed conventional care (best supportive care, low-dose cytarabine, and intensive chemotherapy)[7] with longer hematologic improvement and transfusion independence (TI).[5] Here, we postulated that patients unfit for standard AML chemotherapy might also benefit from AZA, irrespectively of bone marrow blast count.

We systematically reviewed charts of all patients treated with AZA at our institution between 1st January 2007 and 31st December 2011, and identified 52 consecutive AML patients. These patients were not eligible for intensive chemotherapy due to advanced age (n=33) or comorbidities (n=9), or were refractory to initial intensive chemotherapy (n=10). AZA was administered at 100 mg/m^2 sc Days 1-5, over 28-day cycles. This schedule is equivalent to standard Days 1-7 MDS treatment[8] and allows weekday outpatient treatment.[2] Therapy was continued until disease progression, as survival benefit is extended beyond achievement of best response.[9] Indication for transfusion was based on symptoms, comorbidities, and laboratory values (generally Hb<80 g/L and PLT<10^9/L). Transfusion independence was defined as eight weeks or over without red blood cell (RBC-TI) and/or platelet (PLT-TI) transfusion.[1,7] The indication to treat patients with baseline RBC-TI was based on appearance of agranulocytosis (n=3), high peripheral blood or marrow blast counts (n=5), and/or AML relapse (n=1). Overall survival (OS) was measured from the first administration of AZA to death. Five surviving patients were censored on 31st December 2011. The median follow up of censored patients was 12.2 months. Statistical analysis was performed using Stata software (Statacorp LP, College Station, Texas, USA).

At baseline, 15% (n=9) of our patients had RBC-TI and 25% (n=13) PLT-TI. Median overall survival of the 52 enrolled patients was 8.6 months and 12-month survival rate was 28% (95 CI: 15-43%). We excluded 14 patients from transfusion and survival analyses since they did not reach the minimal 8-week observation period. Among them, 11 patients received only one cycle of AZA and 3 received 2 cycles. Treatment was interrupted due to premature death or disease progression (n=12), or availability of a donor for allogeneic stem cell transplantation (n=2). Among these 14 patients, at the start of therapy, 3 had RBC-TI and 3 had PLT-TI. Median OS was 1.3 months (range 0.5-23.7).

Table 1. Baseline characteristics of study groups according to RBC transfusion independence.

		Total	Baseline or acquired RBC-TI	Never reached RBC-TI	*P***
N.		38	22	16	
Age	Years	68 [25-86]	72 [62-83]	66 [39-80]	0.04
	≥70	21 (55%)	14 (67%)	7 (41%)	NS
Gender	Female	24 (63%)	14 (67%)	10 (59%)	NS
Values at baseline					
Hb	g/L	85 [72-125]	87 [73-125]	82 [72-108]	NS
PLT	x10^9/L	38 [7-517]	40 [7-128]	37 [14-517]	NS
WBC	x10^9/L	3.1 [0.6-36.6]	3.9 [1.4-21.4]	2.9 [0.6-36.6]	NS
ANC	x10^9/L	0.5 [0.0-6.0]	0.6 [0.0-3.1]	0.3 [0.1-6.0]	NS
PB blasts	%	14 [0-91]	10 [0-91]	39 [0-90]	NS
BM blasts	%	70 [20-90]	73 [20-90]	70 [20-90]	NS
	≥30%	29 (83%)	15 (80%)	14 (87%)	NS
	≥50%	21 (60%)	11 (55%)	10 (67%)	NS
RBC units over 8 weeks prior to AZA start	2 [0-16]	1 [0-16]	2 [0-16]	NS	
Cytogenetic risk group*	Favorable	3 (8%)	2 (10%)	1 (6%)	
	Intermediate	21 (64%)	12 (57%)	11 (65%)	NS
	Adverse	11 (29%)	7 (33%)	4 (24%)	
Diagnosis	Primary AML	16 (42%)	8 (28%)	8 (62%)	
	Transformed MDS	17 (45%)	14 (63%)	3 (19%)	NS
	Refractory/relapsed	5 (13%)	2 (9%)	3 (19%)	
Time from diagnosis to AZA	Months	0.4 [0.0-12.9]	0.3 [0.1-12.6]	0.6 [0.0-13.0]	NS

*For continuous data median is shown, [ ] range. RBC-TI: red blood cell transfusion independence. *Definition of cytogenetic groups was according to HOVON classification,[10] NA for one patient in never TI group. ** Mann-Whitney test: NS, P>0.05.*

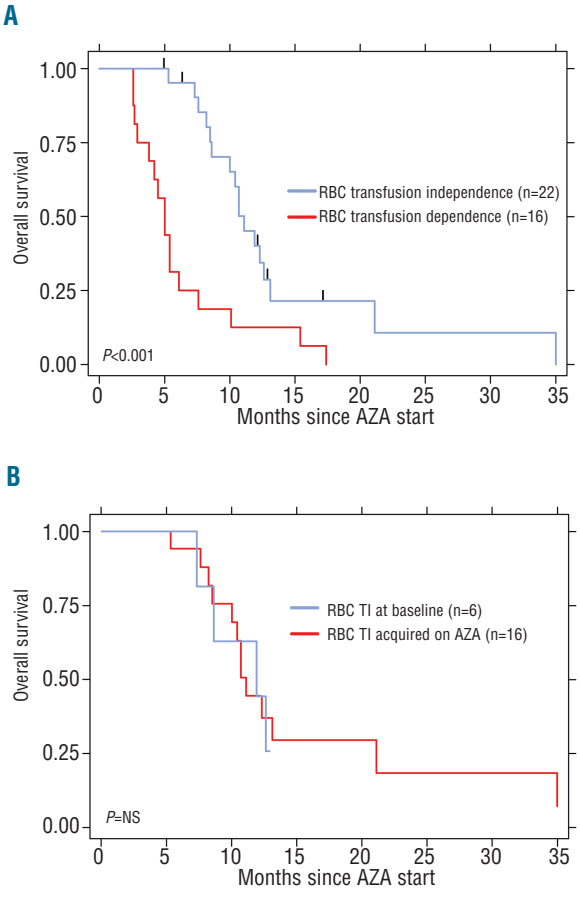

Figure 1. Survival according to RBC-transfusion independency. (A) Overall survival of patients according to transfusion needs. We analyzed 38 patients: 22 RBC-transfusion independent (blue) and 16 RBC-transfusion dependent (red). OS 11.1 *versus* 5.0 months, *P*=0.0006. Twelve-month survival rates were 40% (95% CI: 19-60%) and 13% (95% CI: 2-32%), respectively. (B) Overall survival of patients with transfusion independence (TI) according to baseline transfusion needs (n=22). Median OS was 10.7 months for baseline TI (n=6, blue) *versus* 11.9 months for TI acquired under AZA (n=16, red), 12-month OS 40 (95% CI: 17-63%) *versus* 45% (95% CI: 5-75%), *P*=NS.

Characteristics of the 38 remaining patients are presented in Table 1. Patients with baseline or acquired RBC-TI (n=22) were grouped and compared to patients who never reached RBC-TI (n=16). Among patients with transfusion independence, 6 had RBC-TI at baseline while 16 achieved RBC-TI under therapy. Except for age, which was older for patients with RBC-TI, we found no differences in baseline characteristics. Our 38 patients received a median of 6 (range 3-20) cycles of AZA. Overall response rate was 23% (n=9), distributed in 7 complete response with incomplete blood count recovery (CRi) and 2 partial remission (PR).[11] The remaining 29 patients (76%) exhibited resistant disease (RD). RD status was equally distributed among transfusion independent and transfusion dependent groups (13 *vs*. 16).

RBC-TI at baseline or under AZA therapy (n=22) was associated with prolonged survival. The median OS of transfusion independent patients was 11.1 months *versus* 5.0 months for transfusion dependent patients: 12-month OS 40% (95% CI: 19-60%) *versus* 13% (95% CI: 2-32%) (*P*=0.0006; Figure 1A). Presence of transfusion independence at baseline (n=6) or its achievement under treatment (n=16) had similar survival outcome: median OS 10.7 *versus* 11.9; 12-month OS 40% (95% CI: 17-63%) *versus* 45% (95% CI: 5-75%) (*P*=NS; Figure 1B). Median OS of transfusion dependent patients reported here (5.0 months) is similar to that observed for patients enrolled in the AML14 trial who received best supportive care (2.5 months) or low-dose cytarabine (3.8 months).[3] The impact of PLT-TI on survival was similar although not significant: OS 10.7 *versus* 5.4 months; 12-month OS 32% (95% CI: 14-52%) *versus* 21% (95% CI: 5-45%) (*P*=0.053). The median delay to RBC-TI or PLT-TI after therapy initiation was 85 days (range 60-143) and 73 days (35-154), respectively, (approx. 3 AZA cycles). As observed in MDS patients,[1] transfusion independence was achieved in all responding patients by the end of the 6th cycle. In patients with baseline RBC-TI, the median duration of transfusion independence was 22 weeks (range 8-42). When obtained in response to AZA, the median duration of transfusion independence reached 30 weeks (range 10-88). In our country, blood product expenses in transfusion dependent patients (median 5 RBC and 6 PLT transfusions per month for our cohort) were comparable to those of continued AZA therapy in transfusion independent patients.

Univariate analysis of survival showed significant differences in peripheral blood blast count of 20% and over, relapsed/refractory disease, baseline PLT-TI and baseline or acquired RBC-TI. Interestingly, BM blast level was not predictive of survival or response to treatment. In multivariate analysis, baseline or acquired RBC-TI remained the single parameter favorably affecting survival (HR 0.36, 95% CI: 0.16-0.77) (*P*=0.009).

In conclusion, we report here for the first time that AZA can induce transfusion independence in half of previously transfusion dependent patients and that transfusion independence is a strong prognostic factor in unfit AML patients. Observations made in MDS[12] may now be extended to AML. In addition, AZA may improve quality of life by reducing requirements for transfusions and anemia symptoms. Prospective randomized trials are required to validate these observations in larger cohorts.

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Clonal patterns of X-chromosome inactivation in peripheral blood cells of female patients with chronic idiopathic neutropenia

Chronic idiopathic neutropenia (CIN) is an acquired disorder of granulopoiesis characterized by prolonged, unexplained reduction in peripheral blood (PB) neutrophils due to accelerated apoptosis of the bone marrow (BM) granulocytic progenitors.[1] There has been growing evidence to suggest that CIN shares common pathogenetic features with immune-mediated BM failure syndromes, such as aplastic anemia (AA) and myelodysplastic syndromes (MDS).[2]

Clonal hematopoiesis has been associated with the pathophysiology of the immune-mediated BM failure syndromes.[3,4] The possible existence of clonal stem/progenitor cell populations in CIN using methods other than classical karyotype has not yet been investigated. The X-chromosome inactivation pattern (XCIP) method is based on the Lyon–Beutler hypothesis of the random inactivation of one of the two X-chromosomes early in embryogenesis.[5,6] Normal female tissues display a mosaic expression of genes from maternal and paternal X-chromosomes whereas monoclonal populations express either maternally or paternally-derived X-linked genes.[5,6] The XCIP assay has been widely used to identify clonality in MDS and AA.[7-10] In the current study, we have probed the existence of clonal hematopoiesis in CIN by investigating the XCIP of the glucose-6-phosphate dehydrogenase (G6PD) and iduronate-2-sulfatase (IDS) gene polymorphisms in PB granulocytes and lymphocytes using reverse transcription polymerase chain reaction (RT-PCR).[10]

Genomic DNA extracted from PB samples of 134 female patients fulfilling the diagnostic criteria for CIN[1] and 124 age-matched female healthy subjects, was initially screened for C/T heterozygocity at nucleotides 1311 of G6PD and 438 of IDS using a PCR-based restriction fragment length polymorphism assay.[10] Total RNA isolated from PB lymphocytes and granulocytes of subjects showing heterozygocity in at least one of the two genes was transcribed into cDNA and amplified by a nested (G6PD) or conventional (IDS) PCR using previously described primers.[10] Samples showing expression of only one allele at a ratio equal to or more than 9:1 compared to the other allele despite the existence of both at the genomic level, were classified as clonal. The χ^2 test was used for comparisons between patients and controls.

Seventy-six CIN patients (56.7%) and 76 healthy individuals (61.3%) showing heterozygocity for at least one polymorphism were assessed for clonality in PB lymphocytes and granulocytes (Figure 1A and B). Patients' characteristics are shown in *Online Supplementary Table S1*. No statistically significant difference was observed in the age distribution between CIN patients and controls that were assessed for clonality. Twenty of 41 (48.78%) CIN patients heterozygous for the G6PD polymorphism showed a monoclonal pattern in at least one cell population; 16 of 41 patients (39.02%) displayed monoclonal pattern in both lymphocytes and granulocytes indicating the involvement of an early hematopoietic stem cell whereas 4 of 41 patients (9.76%) displayed monoclonal pattern in granulocytes and polyclonal in lymphocytes implying clonality within the myeloid lineage (Figure 1C). Of 28 healthy subjects showing heterozygocity for G6PD, 6 individuals (21.43%) displayed a monoclonal pattern that concerned both cell populations in all cases. This frequency is significantly lower than the respective 48.78% observed in the patients (P=0.0213).

Similarly, 23 of 50 (46%) CIN patients heterozygous for the IDS polymorphism displayed a monoclonal pattern in at least one PB cell population (Figure 1D); 20 of 50 (40%) CIN patients displayed monoclonal pattern in both cell populations and 3 of 50 patients (6%) displayed monoclonal pattern in granulocytes and polyclonal in lymphocytes suggesting the involvement of a primitive and committed stem cell, respectively. Of the 57 healthy subjects showing heterozygocity for IDS, 12 individuals (21.05%) displayed monoclonal pattern that concerned both cell populations in all cases (Figure 1D). This proportion is significantly lower than the respective 46% observed in CIN patients (P=0.0061).

Of the 76 CIN patients displaying heterozygosity in the expression of at least one gene polymorphism, 32