Dexamethasone in hyperleukocytic acute myeloid leukemia

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ABSTRACT

Patients with acute myeloid leukemia and a high white blood cell count are at increased risk of early death and relapse. Because mediators of inflammation contribute to leukostasis and chemoresistance, dexamethasone added to chemotherapy could improve outcomes. This retrospective study evaluated the impact of adding or not adding dexamethasone to chemotherapy in a cohort of 160 patients with at least 50×10^9 white blood cells. *In silico* studies, primary samples, leukemic cell lines, and xenograft mouse models were used to explore the antileukemic activity of dexamethasone. There was no difference with respect to induction death rate, response, and infections between the 60 patients in the dexamethasone group and the 100 patients in the no dexamethasone group. Multivariate analysis showed that dexamethasone was significantly associated with improved relapse incidence (adjusted sub-HR: 0.30; 95% CI: 0.14-0.62; P=0.001), disease-free survival (adjusted HR: 0.50; 95% CI: 0.29-0.84; P=0.010), event-free survival (adjusted HR: 0.35; 95% CI: 0.21-0.58; P<0.001), and overall survival (adjusted HR: 0.41; 95% CI: 0.22-0.79; P=0.007). In a co-culture system, dexamethasone reduced the frequency of leukemic long-term culture initiating cells by 38% and enhanced the cytotoxicity of doxorubicin and cytarabine. In a patient-derived xenograft model treated with cytarabine, chemoresistant cells were enriched in genes of the inflammatory response modulated by dexamethasone. Dexamethasone also demonstrated antileukemic activity in *NPM1*-mutated samples. Dexamethasone may improve the outcome of acute myeloid leukemia patients receiving intensive chemotherapy. This effect could be due to the modulation of inflammatory chemoresistance pathways and to a specific activity in acute myeloid leukemia with *NPM1* mutation.

Introduction

Acute myeloid leukemias (AML) are myeloid malignancies induced by the oncogenic transformation of hematopoietic progenitors in the bone marrow leading to the destruction of blood tissue and, therefore, to profound pancytopenia, severe bleeding, and infection.[1] Approximately 20% of patients present at diagnosis with high white blood cell (WBC) counts (i.e. >50x10^9/L).[2] In this high-risk situation, the probability of severe complications is increased because of leukemic organ infiltration, severe hemorrhage, or metabolic disorders, including tumor lysis syndrome, renal failure, and disseminated intravascular coagulopathy, which is further worsened by the induction of antileukemic treatment. Hyperleukocytosis is also associated with leukostasis syndrome within the lung or brain, which can potentially lead to acute respiratory distress syndrome or stroke. Thus, patients with a high WBC count share an increased risk of death during the initial phase of the disease. Hyperleukocytosis is also independently associated with shorter relapse-free survival in patients treated by intensive chemotherapy, indicating a potential link with chemoresistance.[2]

Dexamethasone is an anti-inflammatory drug widely used in acute lymphoblastic leukemia and other lymphoid malignancies.[3] Much less frequently used in myeloid disorders, this drug is often offered to prevent or treat a severe inflammatory status, so-called differentiation syndrome in patients with acute promyelocytic leukemia treated with all trans-retinoic acid and/or arsenic trioxide.[4,5] Mediators of inflammation induced by leukemic blasts and endothelial cells contribute to the pathogenesis of leukostasis.[6] Studies on the molecular mechanisms of leukostasis and leukemic cell invasion have shown that leukemic blasts use integrins and selectins to attach to cytokine-activated endothelium and directly activate endothelial cells by secreting inflammatory cytokines, such as tumor necrosis factor-α, interleukin-1β, and interleukin-6, which induce the conditions necessary for their adhesion to vascular endothelium, migration to tissues, proliferation, and chemoresistance.[6,7] The central role of the inflammatory response prompted us to assess the impact of dexamethasone in this setting because this drug exerts a potent inhibitory effect on cytokine production.[8] We hypothesized that introducing a short course of dexamethasone into routine practice during the early phase of induction chemotherapy would improve the outcome of hyperleukocytic AML patients.

Methods

Patients

Between January 2004 and December 2015, 802 patients aged between 18 and 75 years with cytologically confirmed AML were consecutively treated with intensive chemotherapy at Toulouse University Hospital. Patients with acute promyelocytic leukemia were not considered. Patients were classified into three prognostic categories based on cytogenetics.[9] *FLT3*-ITD and *NPM1* mutations were assessed in patients with intermediate-risk cytogenetics. Data were collected from the patients' files and certified by the Data Management Committee of the AML database of Toulouse University Hospital registered at the Commission Nationale de l'Informatique et des Libertés (CNIL, #1778920).[10] In accordance with the Declaration of Helsinki, the study was reviewed and approved by the research ethics committee at Toulouse University Hospital.

Treatment

Study patients received induction chemotherapy that included daunorubicin at a daily dose of 60–90 mg/m^2 of body surface area daily for 3 days, or idarubicin at a daily dose of 8-9 mg/m^2 daily for 5 days, together with a continuous intravenous infusion of cytarabine at a daily dose of 100–200 mg/m^2 daily for 7 days.[10] No patient received an FLT3 inhibitor in combination with chemotherapy during first-line induction. Lomustine was added in patients aged over 60 years.[11] Hydroxyurea could be started promptly at diagnosis for leukocytic reduction. Leukapheresis was not performed. Starting in January 2010, dexamethasone (10 mg b.i.d. given for 3 days) was systematically added to induction chemotherapy in all patients who had a WBC count of at least 100 x 10^9/L or in patients with a WBC count over 50 x 10^9/L and clinical symptoms of leukostasis. This dexamethasone schema was used based on our previous experience in patients with acute promyelocytic leukemia.[4] Supportive care, which included prevention of invasive fungal infections with voriconazole from 2004 to 2008 then posaconazole, treatment of febrile neutropenia and disseminated intravascular coagulopathy, and blood-product transfusions were given according to standard guidelines that did not change over the study period.[12,13] Patients who achieved complete remission proceeded to subsequent treatment steps. Post-remission therapy was based on relapse risk and whether an HLA-identical donor had been identified or not. Patients at low risk of relapse (i.e. patients with a core-binding factor AML, *NPM1*, or *CEBPA* mutation without *FLT3*-ITD) received only chemotherapy as post-remission therapy. All other patients with an HLA-matched donor underwent allogeneic stem-cell transplantation, whereas those without such a donor received chemotherapy.

Response criteria and end points

Complete response was defined according to standard criteria.[14] Relapse was defined as leukemia recurrence after a first complete remission. Disease-free survival was measured from the time of complete remission evaluation to the date of relapse or death, whatever the cause. Event-free survival was measured from the date of diagnosis to the date of failure to enter complete remission, relapse, or death, whichever came first. Overall survival was defined as the time interval from diagnosis until death, whatever the cause.

The statistical analyses and exploratory analyses are described in the *Online Supplementary Appendix*.

Results

Study population

The flowchart of the 160 patients with a WBC count of at least 50 x 10^9/L included in this retrospective study is shown in Figure 1 and the patients' characteristics are summarized in Table 1. The median follow-up period of patients still alive at the date of last contact was 52.2 months [inter-quartile range (IQR); 23.7-72.9 months]; the median periods in the dexamethasone and the no dexamethasone groups were 44.1 months (IQR; 19.6-55.8) and 65.7 months (IQR 52.0-79.7), respectively. The median age of the patients was 60.1 years (IQR: 49.2-67.3); 50% of patients were aged ≥60 years. Compared to the 100 patients in the no dexamethasone group, the 60 patients in the dexamethasone group were more likely to have a poor

performance status, features of leukostasis syndrome, and higher WBC count. Hydroxyurea treatment was given to 49 patients in the dexamethasone group and to 59 patients in the no dexamethasone group. Allogeneic stem cell transplantation was given to 19 patients in the dexamethasone group and to 25 patients in the no dexamethasone group. Of note, overall survival of patients (aged 18–75 years) with a WBC count <50 x 10^9/L who were treated with intensive chemotherapy between 2004 and 2009 (336 patients with 232 deaths; median overall survival, 21.5 months; IQR: 7.6–158.8) did not differ significantly from that of patients treated between 2010 and 2015 (295 patients with 164 deaths, median overall survival, 25.8 months; IQR: 9.1–not achieved) (hazard ratio for 2010–2015 *vs.* 2004–2009=0.95; 95% CI: 0.77–1.16; *P*=0.595).

Impact of dexamethasone during the induction phase

Fifty patients (83.3%) from the dexamethasone group and 74 (74%) from the no dexamethasone group achieved a complete response (*P*=0.171) (Table 2). At day 60 of induction chemotherapy, 7 patients (11.7%) in the dexamethasone group had died compared to 20 patients (20%) in the no dexamethasone group (*P*=0.173). There were no significant differences between the two groups in terms of fungal (*P*=0.710) or bacterial (*P*=0.192) infections. However, grade 3-4 bleeding events were more frequent in the dexamethasone group compared to the no dexamethasone group [13 (21.7%) *vs.* 6 (6.2%); *P*=0.038] as were admissions to the intensive-care unit by day 90 [29 (48.3%) *vs.* 17 (17.0%); *P*<0.0001].

Impact of dexamethasone on relapse and survival

In the univariate analyses, the use of dexamethasone was associated with an improved outcome, with the improvement reaching statistical significance for relapse incidence (sub-HR: 0.43; 95% CI: 0.25-0.74; *P*=0.003), disease-free survival (HR: 0.48; 95% CI: 0.29-0.80; *P*=0.005), event-free survival (HR: 0.52; 95% CI: 0.34-0.79; *P*=0.002), and overall survival (HR: 0.55; 95% CI: 0.35-0.85; *P*=0.005) (Figure 2). In a Fine and Gray competing risks model, the use of dexamethasone was associated with a significantly lower risk of relapse (adjusted sub-HR: 0.30; 95% CI: 0.14-0.62; *P*=0.001) (*Online Supplementary Table S1*). In multivariate analyses, the use of dexamethasone was associated with significantly better outcomes when considering disease-free survival (adjusted HR: 0.50; 95% CI: 0.29-0.84; *P*=0.010) (*Online Supplementary Table S2*), event-free survival (adjusted HR: 0.35; 95% CI: 0.21-0.58;

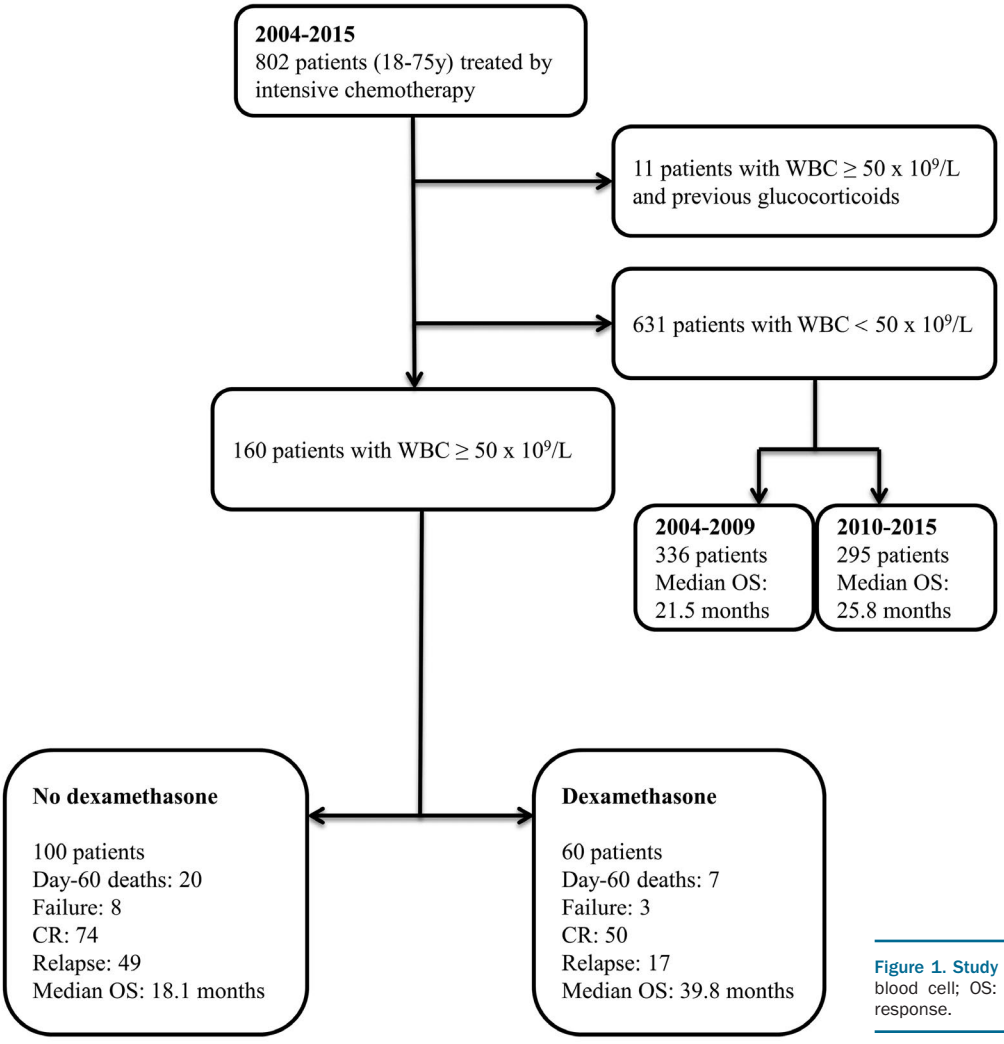

Figure 1. Study flowchart. y: years; WBC: white blood cell; OS: overall survival; CR: complete response.

Table 1. Characteristics of the 160 acute myeloid leukemia patients with hyperleukocytosis.

Characteristics	No dexamethasone n=100 (62.5%)	Dexamethasone n=60 (37.5%)	*P*	All patients n=160 (100%)
Sex – n. (%)				
Male	56 (56.0)	27 (45.0)	0.178	83 (51.9)
Female	44 (44.0)	33 (55.0)		77 (48.1)
Age – years				
Median (IQR)	60.7 (48.3-68.5)	58.8 (50.5-66.5)	0.244	60.1 (49.2-67.3)
<60 years – n. (%)	47 (47.0)	33 (55.0)		80 (50.0)
≥60 years – n. (%)	53 (53.0)	27 (45.0)	0.327	80 (50.0)
ECOG performance status – n. (%)				
0-1	60 (77.9)	31 (56.4)	0.008	91 (68.9)
2-4	17 (22.1)	24 (43.6)		41 (31.1)
Extramedullary involvement – n. (%)				
No	38 (46.3)	25(42.4)	0.640	63 (44.7)
Yes	44 (53.7)	34 (57.6)		78 (55.3)
AML status – n. (%)				
De novo	81 (81.0)	55 (91.8)	0.067	136 (85.0)
Secondary	19 (19.0)	5 (8.3)		24 (15.0)
Leukostasis – n. (%)				
No	84 (84.0)	34 (56.7)	<0.001	118 (73.8)
Central nervous system	2 (2.0)	9 (15.0)		11 (6.9)
Lung	10 (10.0)	12 (20.0)		22 (13.8)
Central nervous system and lung	4 (4.0)	5 (8.3)		9 (5.6)
Infection at diagnosis – n. (%)				
No	78 (80.4)	46 (76.7)	0.576	124 (70.9)
Yes	19 (19.6)	14 (23.3)		33 (21.0)
White blood cell count – x10^9/L				
Median (IQR)	86.3 (66.1-115.5)	119 (90.7-181.4)	<0.001	97.6 (71.0-142.6)
<100 – n. (%)	62 (62.0)	21 (35.0)		83 (51.9)
≥100 – n. (%)	38 (38.0)	39 (65.0)	<0.001	77 (48.1)
Platelet count – x10^9/L				
Median (IQR)	54.5 (35.0-85.5)	49.5 (24.5-72.0)	0.062	52.0 (31.0-77.0)
<50 – n. (%)	44 (44.0)	30 (50.0)		74 (46.3)
≥50 – n. (%)	56 (56.0)	30 (50.0)	0.461	86 (53.8)
CD56 – n. (%)				
≤20%	71 (78.9)	44 (75.9)	0.666	115 (77.7)
>20%	19 (21.1)	14 (24.1)		33 (22.3)
Cytogenetic risk – n. (%)				
Favorable	8 (8.0)	7 (11.7)	0.530	15 (9.4)
Intermediate	79 (79.0)	48 (80.0)		127 (79.4)
Adverse	13 (13.0)	5 (8.3)		18 (11.3)
ELN classification – n. (%)				
Favorable	23 (23.0)	17 (28.3)	0.155	40 (25.0)
Intermediate-1	32 (32.0)	25 (41.7)		57 (35.6)
Intermediate-2	21 (21.0)	12 (20.0)		33 (20.6)
Adverse	13 (13.0)	5 (8.3)		18 (11.3)
Unknown[§]	11 (11.0)	1 (1.7)		12 (7.5)
NPM1 mutations[¶]				
No	27 (39.7)	17 (35.4)	0.639	44 (37.9)
Yes	41 (60.3)	31 (64.6)		72 (62.1)
FLT3-ITD mutations[¶]				
No	33 (48.5)	20 (41.7)	0.465	53 (45.7)
Yes	35 (51.5)	28 (58.3)		63 (54.3)
Creatinine - mg/dL				
Median (IQR)	1.05 (0.89-1.25)	0.91 (0.71-1.24)	0.029	1.01 (0.83-1.24)
<1.36 – n. (%)	82 (82.0)	50 (83.3)		132 (82.5)
>1.36– n. (%)	18 (18.0)	10 (16.7)	0.830	28 (17.5)
Bilirubin - mg/dL				
Median (IQR)	0.54 (0.38-0.79)	0.53 (0.35-0.79)	0.649	0.53 (0.36-0.79)
≤1.47 – n. (%)	95 (95.0)	55 (94.8)		150 (94.9)
>1.47 – n. (%)	5 (5.0)	3 (5.2)	1.000	8 (5.1)

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Characteristics	No dexamethasone n=100 (62.5%)	Dexamethasone n=60 (37.5%)	P	All patients n=160 (100%)
Albumin - g/dL				
Median (IQR)	3.50 (3.30-3.90)	3.50 (3.20-4.00)	0.500	3.50 (3.20-4.00)
<3.5 – n. (%)	41 (41.4)	29 (49.2)		70 (44.3)
≥3.50 – n. (%)	58 (58.6)	30 (50.8)	0.344	88 (55.7)
Ferritin - ng/mL				
Median (IQR)	928.5 (570.0-1342.0)	1103.0 (606.0-2249.0)	0.287	1064.0 (599.5-1904.0)
≤1000 – n. (%)	24 (52.2)	25 (46.3)		49 (49.0)
>1000 – n. (%)	22 (47.8)	29 (53.7)	0.558	51 (51.0)
Lactate dehydrogenase – IU/L				
Median (IQR)	1618.0 (913.0-2506.0)	1498.5 (790.0-2369.0)	0.455	1551.5 (840.5-2476.0)
≤1550 – n. (%)	48 (48.0)	32 (53.3)		80 (50.0)
>1550 – n. (%)	52 (52.0)	28 (46.7)	0.514	80 (50.0)
Fibrinogen – g/L				
Median (IQR)	4.0 (2.8-4.8)	3.7 (2.6-4.7)	0.197	3.9 (2.7-4.8)
≤1.5 – n. (%)	8 (8.0)	7 (11.7)		15 (9.4)
>1.5 – n. (%)	92 (92.0)	53 (88.3)	0.441	145 (90.6)
Study period – n. (%)				
2004-2009	67 (67.0)	0 (0.0)	<0.001	67 (41.9)
2010-2015	33 (33.0)*	60 (100.0)	93 (58.1)	
Hydroxyurea – n. (%)				
No	41 (41.0)	11 (18.3)	0.003	52 (32.5)
Yes	59 (59.0)	49 (81.7)		108 (67.5)
Chemotherapy – n. (%)				
Daunorubicin-cytarabine	32 (32.0)	16 (26.7)	0.281	48 (30.0)
Idarubicin-cytarabine	15 (15.0)	13 (21.7)		28 (17.5)
Idarubicin-cytarabine-lomustine	45 (45.0)	21 (35.0)		66 (41.3)
Time sequential induction	5 (5.0)	5 (8.3)		10 (6.3)
Other	3 (3.0)	5 (8.3)		8 (5.0)
Allo-SCT – n. (%)				
No	75 (75.0)	41 (68.3)	0.361	116 (72.5)
Yes	25 (25.0)	19 (31.7)		44 (27.5)

IQR: interquartile range; ECOG: Eastern Cooperative Oncology Group; ELN: European LeukemiaNet; Allo-SCT: allogeneic stem cell transplantation (16 from sibling and 28 from HLA 9/10 or 10/10 matched unrelated donors). §ELN is unknown if *FLT3*-ITD or *NPM1* mutation was missing for normal karyotypes or if karyotype is missing. §*NPM1* and *FLT3*-ITD mutations in patients with intermediate-risk cytogenetics. * Among the 33 patients who did not receive dexamethasone, only 3 patients had symptoms of pulmonary leukostasis and more than 100 x10^9/L white blood cell count (WBC), whereas 3 additional patients had more than 100 x10^9/L WBC without leukostasis.

P<0.001) (*Online Supplementary Table S3*), and overall survival (adjusted HR: 0.41; 95% CI: 0.22-0.79; *P*=0.007) (Table 3). Of note, when put in the multivariate model, FLT3-ITD mutations had no significant impact. Among patients who had undergone allogeneic stem cell transplantation in first complete response, the outcome of the dexamethasone group was still better than that of the no dexamethasone group (*Online Supplementary Figure S1*).

Impact of dexamethasone on leukemia-initiating cells in a co-culture system

The use of dexamethasone was unexpectedly associated with a lower relapse rate suggesting that this drug could display potent antileukemic activity against AML cells at the origin of relapse and/or by enhancing the cytotoxicity of chemotherapy. Leukemic long-term culture initiating cells have been shown to be a reliable functional readout for monitoring the activity of leukemia-initiating cells, an AML subpopulation of cells thought to be at the origin of relapse.[15,16] Using an optimized niche-like co-culture system capable of maintaining leukemia-initiating cells *ex vivo*, dexamethasone reduced the frequency of leukemic long-term culture initiating cells by 38±14% as compared to untreated primary AML cells (Figure 3A). Interestingly, primary AML cells treated with dexamethasone presented a higher expression profile of the CD38 marker after one week, and a higher percentage of myeloid and lymphoid lineage positive cells as well as monocytic CD11b/CD14-positive cells within the long-term culture weeks suggesting that dexamethasone may have a differentiation effect on AML (Figure 3B and C).

Impact of dexamethasone on chemoresistance

We next sought to determine whether dexamethasone could improve the cytotoxicity of genotoxic drugs used in AML. In liquid culture, short-term dexamethasone treatment with or without cytarabine or doxorubicin did not show synergy or an additive effect in a panel of genetically diverse AML cell lines (*Online Supplementary Figure S2* and *Online Supplementary Table S4*). However, in a co-culture system, one week of exposure to dexamethasone significantly enhanced cytarabine activity in most AML cell lines (Figure 3D). Recently, it has been shown that cytarabine resistance of AML cells is associated with increased sensitivity to glucocorticoids.[17,18] We thus wondered whether AML cells resistant to cytarabine display specific tran-

Table 2. Patients' outcomes during induction chemotherapy according to study group.

	No dexamethasone n=100 (62.5%)	Dexamethasone n=60 (37.5%)	*P*	All patients n=160
Admission in intensive care unit*– n (%)				
No	83 (83.0)	31 (51.7)	<0.0001	114 (71.3)
Yes	17 (17.0)	29 (48.3)		46 (28.8)
Bacterial infections - n (%)				
No	71 (73.2)	38 (63.3)	0.192	109 (69.4)
Yes	26 (26.8)	22 (36.7)		48 (30.6)
Fungal infections - n (%)				
No	86 (88.7)	52 (86.7)	0.710	138 (87.9)
Yes	11 (11.3)	8 (13.3)		19 (12.1)
Bleeding events (grade 3-4) - n (%)				
No	91 (93.8)	47 (78.3)	0.004	138 (87.9)
Yes	6 (6.2)	13 (21.7)		19 (12.1)
Day-60 deaths - n (%)				
No	80 (80.0)	53 (88.3)	0.173	133 (83.1)
Yes	20 (20.0)	7 (11.7)		27 (16.9)
Induction failure - n (%)				
No	92 (92.0)	57 (95.0)	0.538	149 (93.1)
Yes	8 (8.0)	3 (5.0)		11 (6.9)
Complete response - n (%)				
No	26 (26.0)	10 (16.7)	0.171	36 (22.5)
Yes	74 (74.0)	50 (83.3)		124 (77.5)

*During the first three months following chemotherapy.

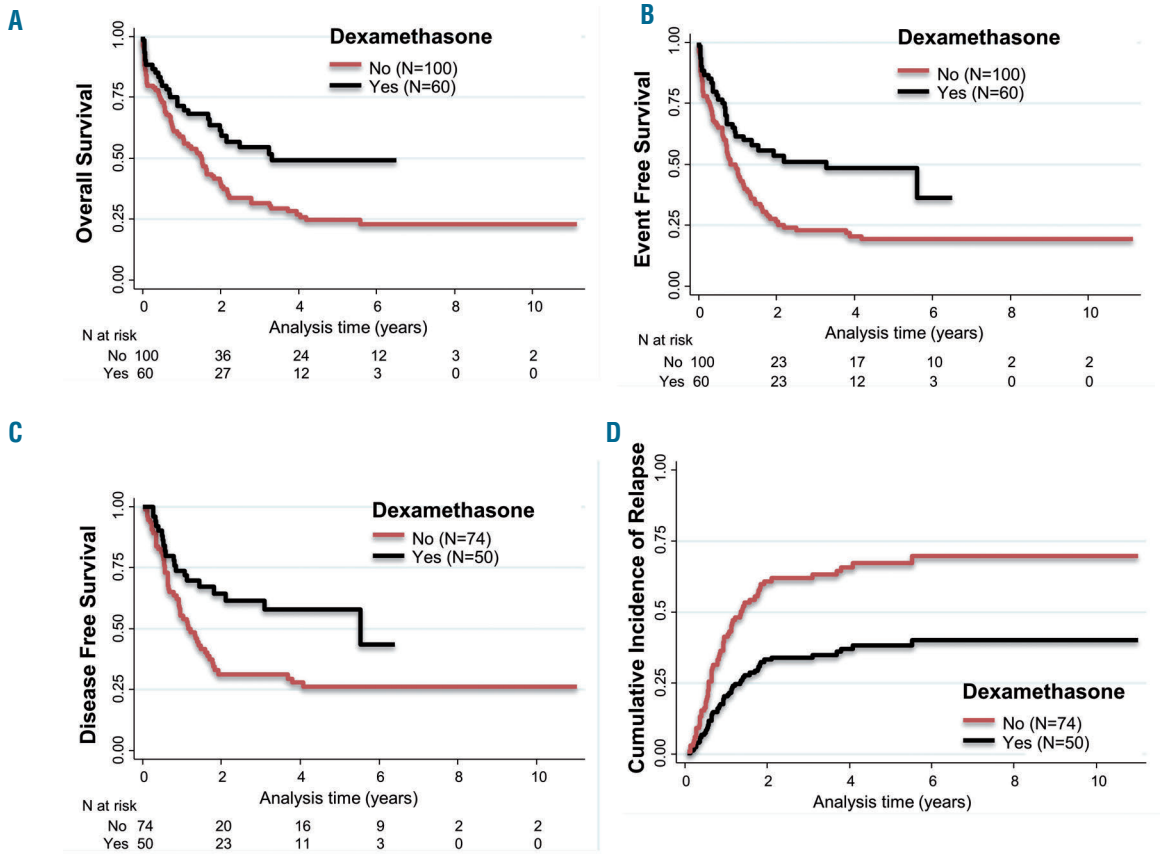

Figure 2. Estimates of survival end points and incidence of relapse. (A) Kaplan–Meier curves for overall survival in patients treated with dexamethasone (black line) or not (solid line in red). (B) Show event-free survival, (C) disease-free survival and (D) cumulative incidence of relapse. It is worth nothing that the follow up concerning relapse seems to be equal in the dexamethasone and no dexamethasone groups. This is due to the competing risk analyses. Indeed, in the competing risk analyses, a subject having a non-relapse death was not censored at the date of death and was still virtually considered to be at risk of having a relapse (and was still in follow up).

scriptomic characteristics *in vivo*. To test this, we used a patient-derived xenograft model of chemoresistance (*Online Supplementary Figure S3A*).[19] Eight to 18 weeks after transplantation of the AML sample, the mice were given daily intraperitoneal injections of 60 mg/kg cytarabine or vehicle for 5 days. Three days after the last dose of cytarabine or vehicle, viable human AML blasts were collected from the bone marrow, then purified and processed for transcriptomic analysis. The transcriptome of residual human AML cells exhibiting *in vivo* resistance to cytarabine treatment displayed a strong upregulation of the genes involved in immune and inflammatory responses, including the nuclear factor-κB network (Figure 3E). Furthermore, this gene signature of chemoresistance displayed a highly significant interaction with the dexamethasone gene signature (Figure 3F and *Online Supplementary Table S5*). Similarly, interrogation of a publicly available transcriptomic data set established from AML patients in first relapse and a data mining algorithm (Genomatix) revealed that the dexamethasone signature was also enriched within AML cells collected at relapse (Figure 3G and *Online Supplementary Table S6*).[20] Moreover, in two patient-derived xenograft models, treatment of NSG mice with the dexamethasone-cytarabine combination induced a deeper therapeutic response compared to that achieved with cytarabine alone (Figure 3H). All together, these data strongly suggest that the impact of dexamethasone with intensive chemotherapy observed in the clinic could result from the targeting of chemoresistant AML cells.

Pre-clinical antileukemic activity of dexamethasone in acute myeloid leukemia with *NPM1* mutations

A recent pre-clinical study demonstrated that AML cells with *RUNX1* mutations were sensitive to glucocorticoids while earlier *in vitro* studies suggested an antileukemic activity in AML with the t(8;21)/*RUNX1-RUNX1T1* translocation.[21,22] To find out whether other molecular subgroups could benefit from glucocorticoids, we first tested the *in vitro* activity of dexamethasone against AML cell lines with various genetic backgrounds. As expected, dexamethasone had no significant activity as a single agent in most AML cell lines cultured in suspension (Figure 4A). Only two out of seven AML cell lines were moderately sensitive to the growth inhibition effect of dexamethasone, including OCI-AML3, an *NPM1*-mutated cell line.

Table 3. Multivariate analysis for overall survival.

	Numbers	Events	Adjusted HR	95% CI	*P*
Dexamethasone					
No	100	74	1	0.22-0.79	0.007
Yes	60	27	0.41		
AML status					
De novo	136	80	1	1.45-4.11	0.001
Secondary	24	21	2.44		
Infection at diagnosis					
No	124	74	1	1.06-2.93	0.029
Yes	33	24	1.76		
Albumin- g/dL					
> 3.5	70	47	1	0.39-0.94	0.027
≥ 3.5	88	52	0.61		
Lactate dehydrogenase – UI/L					
≤1550	80	41	1	1.14-2.70	0.010
>1550	80	60	1.76		
Fibrinogen – g/L					
≤ 1.5	15	12	1	0.17-0.62	0.001
> 1.5	145	89	0.32		
Cytogenetic risk					
Favorable	15	3	1		
Intermediate	127	87	4.18	1.28-13.62	0.018
Unfavorable	18	11	7.07	1.83-27.29	0.005
Hydroxyurea					
No	52	37	1	0.39-0.97	0.037
Yes	108	64	0.61		
Admission to intensive care unit*					
No	114	70	1	2.21-6.38	<0.001
Yes	46	31	3.76		
Study period					
2004-2009	67	54	1	0.36-1.07	0.087
2010-2015	93	47	0.62		
Allogeneic stem cell transplantation					
No	116	79	1	0.29-0.92	0.026
Yes	44	22	0.51		

*During the first three months following chemotherapy. HR: Hazard Ratio; CI: Confidence Interval.

We thus explored the impact of dexamethasone treatment in the OCI-AML3 xenotransplantation model (*Online Supplementary Figure S3B*). Dexamethasone treatment resulted in a significant survival advantage compared to vehicle. Moreover, the combination of dexamethasone plus cytarabine significantly improved mouse survival compared to that following cytarabine treatment alone (Figure 4B). We then tested the *in vitro* activity of dexamethasone against primary samples from patients with or without an *NPM1* mutation. Primary AML samples with

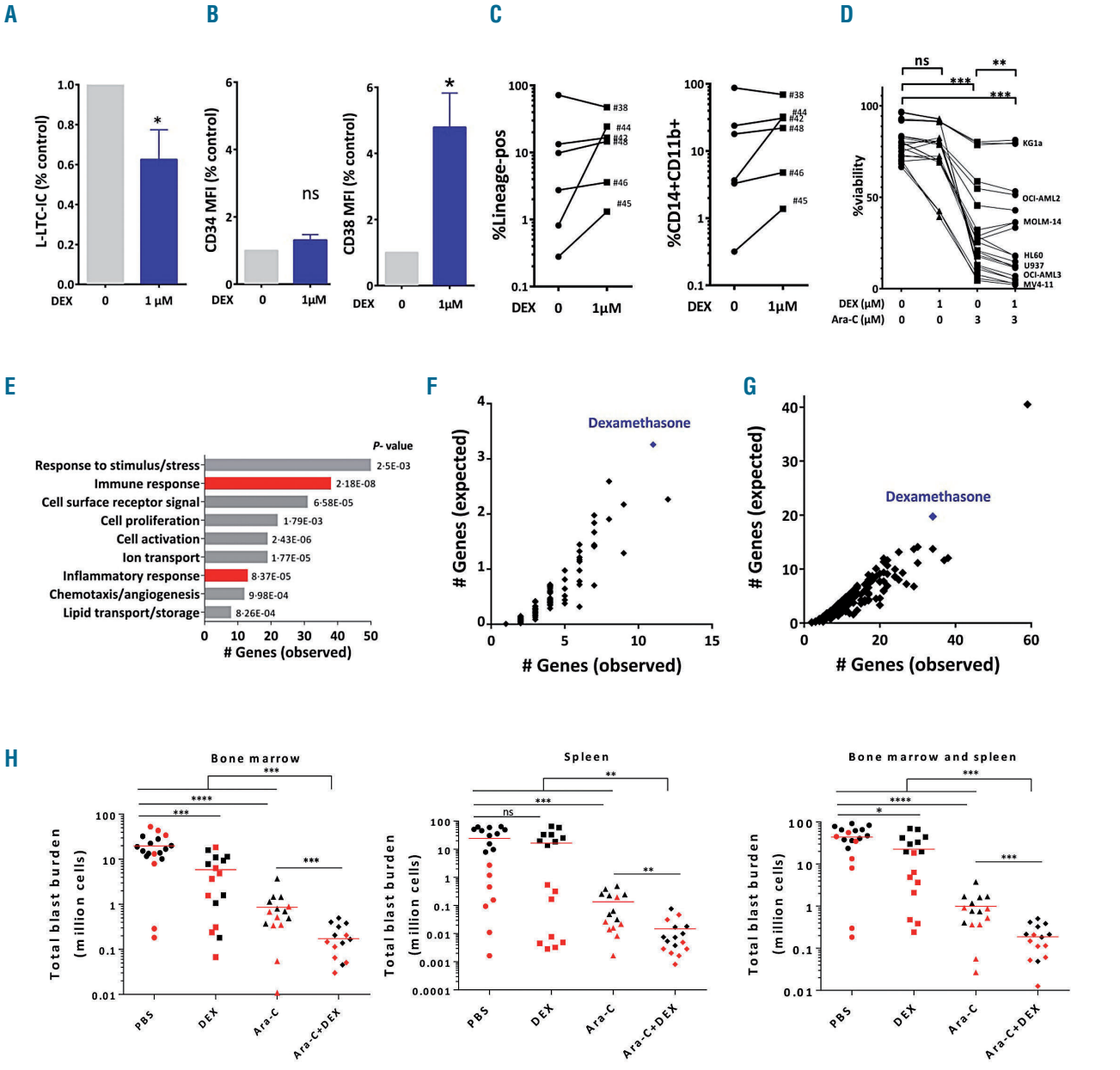

Figure 3. Impact of dexamethasone on chemoresistance. (A) Leukemia long-term culture initiating cell (L-LTC-IC) frequency in acute myeloid leukemia (AML) samples upon dexamethasone treatment (#38 to 49, *Online Supplementary Table S8*). (B) Expression of CD34 and CD38 upon dexamethasone treatment in co-culture with AML samples and MS-5 stromal cells. (C) Expression of lineage and CD14/11b markers upon dexamethasone treatment in co-culture with AML samples and MS-5 stromal cells. (D) Seven AML cell lines incubated in a co-culture system with MS-5 stromal cells were treated for 1 week with vehicle, dexamethasone, cytarabine, or dexamethasone plus cytarabine. (E) Gene ontology enrichment analysis of down-regulated and up-regulated genes from RNA expression profiles of viable AML cells following cytarabine *versus* vehicle-treated AML-patient-derived xenograft (PDX) mice, by 1.5-fold or more. (F and G) Gene-to-small molecule associations that are significantly enriched within residual post-cytarabine AML cells (Figure 3F and *Online Supplementary Table S5*) or in relapse (compared to pairwise diagnosis, Figure 3G and *Online Supplementary Table S6*) using a data-mining algorithm (Genomatix) from GSE97631[19] or GSE66525[20] publicly accessible transcriptomic databases, respectively. These two graphs shown a gene signature ranking assessed by the number of observed *versus* expected genes significantly modulated in transcriptomes after treatment with diverse small molecules and significantly enriched in AML transcriptomes of residual post-cytarabine AML cells or of relapse. (H) Treatment of PDX models from 2 AML samples collected at diagnosis (black dots: normal karyotype, *NPM1* mutation, wild-type for *FLT3*-ITD, *DNMT3A*-exon23, *CEBPA*, *IDH1*, and *IDH2*, red dots: normal karyotype, *NPM1* mutation, *DNMT3A*-exon23 mutation, *FLT3* wild type) with vehicle, dexamethasone (10 mg/kg/day, 5 days), cytarabine (30 mg/kg/day, 5 days), or dexamethasone plus cytarabine. At day 8, the reduction of the total AML cell burden was assessed by the absolute quantification of the hCD45$^+$hCD33$^+$mCD45.1$^-$ cell population in bone marrow and spleen. Mann Whitney test: ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05; ns: not significant.

NPM1 mutations were more sensitive to dexamethasone-induced apoptosis than samples without *NPM1* mutations (Figure 4C).

Overlap between mutant *NPM1* up-regulated target genes and a dexamethasone-associated gene expression signature

In line with these results, interrogation of a transcriptomic data set from a series of AML patients with *NPM1* mutations and a data mining algorithm revealed that the *NPM1* mutation gene signature was highly enriched in genes responsive to several small molecules, including dexamethasone as well as all-trans retinoic acid and dactinomycin, which have previously demonstrated therapeutic activity in this subgroup of AML (Figure 4D and E, and *Online Supplementary Table S7*).[23] To address the genetic heterogeneity of AML, further transcriptomic analyses from two independent data sets revealed more complex molecular interactions between dexamethasone and some AML subgroups, such as those with RUNX1 mutations or the *CBF-MYH11* rearrangement (*Online Supplementary Figure S4A*).[23,24] Overall, enrichment in the dexamethasone

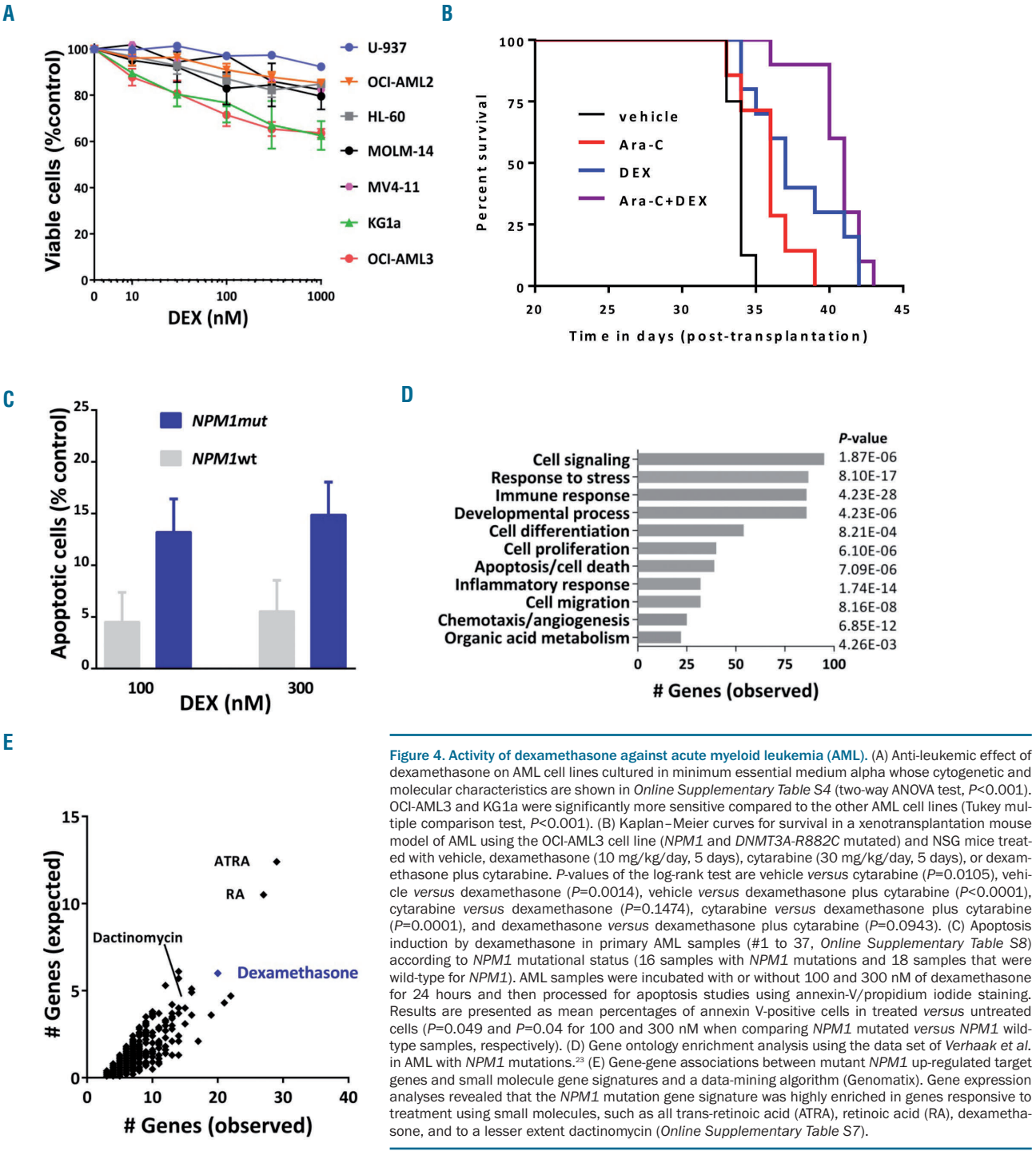

Figure 4. Activity of dexamethasone against acute myeloid leukemia (AML). (A) Anti-leukemic effect of dexamethasone on AML cell lines cultured in minimum essential medium alpha whose cytogenetic and molecular characteristics are shown in *Online Supplementary Table S4* (two-way ANOVA test, $P<0.001$). OCI-AML3 and KG1a were significantly more sensitive compared to the other AML cell lines (Tukey multiple comparison test, $P<0.001$). (B) Kaplan–Meier curves for survival in a xenotransplantation mouse model of AML using the OCI-AML3 cell line (*NPM1* and *DNMT3A-R882C* mutated) and NSG mice treated with vehicle, dexamethasone (10 mg/kg/day, 5 days), cytarabine (30 mg/kg/day, 5 days), or dexamethasone plus cytarabine. *P*-values of the log-rank test are vehicle *versus* cytarabine ($P=0.0105$), vehicle *versus* dexamethasone ($P=0.0014$), vehicle *versus* dexamethasone plus cytarabine ($P<0.0001$), cytarabine *versus* dexamethasone ($P=0.1474$), cytarabine *versus* dexamethasone plus cytarabine ($P=0.0001$), and dexamethasone *versus* dexamethasone plus cytarabine ($P=0.0943$). (C) Apoptosis induction by dexamethasone in primary AML samples (#1 to 37, *Online Supplementary Table S8*) according to *NPM1* mutational status (16 samples with *NPM1* mutations and 18 samples that were wild-type for *NPM1*). AML samples were incubated with or without 100 and 300 nM of dexamethasone for 24 hours and then processed for apoptosis studies using annexin-V/propidium iodide staining. Results are presented as mean percentages of annexin V-positive cells in treated *versus* untreated cells ($P=0.049$ and $P=0.04$ for 100 and 300 nM when comparing *NPM1* mutated *versus* *NPM1* wild-type samples, respectively). (D) Gene ontology enrichment analysis using the data set of *Verhaak et al.* in AML with *NPM1* mutations.[23] (E) Gene-gene associations between mutant *NPM1* up-regulated target genes and small molecule gene signatures and a data-mining algorithm (Genomatix). Gene expression analyses revealed that the *NPM1* mutation gene signature was highly enriched in genes responsive to treatment using small molecules, such as all trans-retinoic acid (ATRA), retinoic acid (RA), dexamethasone, and to a lesser extent dactinomycin (*Online Supplementary Table S7*).

gene signature was observed in 45-60% of AML patients (*Online Supplementary Figure S4B*).

All together, these results demonstrate that dexamethasone has significant activity against *NPM1*-mutated AML cells, corresponding to ~65% of the patients treated by dexamethasone in the clinical study.

Discussion

Three very recent pre-clinical studies have demonstrated that glucocorticoids could be of real interest in AML. Malani *et al.* and Kurata *et al.* have shown that the development of cytarabine resistance in AML cells is associated with increased sensitivity to glucocorticoids.[17,18] Using a chemogenomic approach, Simon *et al.* demonstrated that AML samples bearing inactivating *RUNX1* mutations are particularly sensitive to glucocorticoids.[21] Our study is the first to detect a significant clinical correlation between dexamethasone treatment and outcome in adult patients with AML. Indeed, we showed that the addition of dexamethasone to intensive chemotherapy was associated with significantly better disease-free and overall survival in hyperleukocytic AML patients. We must, however, acknowledge that Turkish investigators previously reported their long-lasting experience on the potential impact of high-dose methylprednisolone in pediatric AML.[25]

The strong and unexpected impact of dexamethasone in preventing relapses prompted us to undertake *in silico*, *in vitro*, and *in vivo* exploratory analyses. The gene signatures of some molecular subgroups of AML were highly enriched in genes responsive to dexamethasone, including AML with *NPM1* mutations, which were particularly sensitive to the antileukemic activity of dexamethasone both *in vitro* and *in vivo*. Moreover, using a xenotransplantation model of chemoresistance, we demonstrated that the transcriptome of viable AML cells in xenograft NSG mice following cytarabine exposure is highly enriched in inflammatory response genes as well as in genes responsive to dexamethasone.[19] Although inflammation is a hallmark of cancer, its role has been neglected in AML in which other oncogenic pathways, including transcriptional dysregulation, sustained proliferative signaling, epigenetic or metabolic alterations, as well as deregulated splicing have been more deeply assessed. Yet, several aspects of inflammation could be explored to increase our knowledge of AML pathophysiology and to expand therapeutic opportunities or prognostic markers.[26] The results of our study led us to speculate that dexamethasone, by affecting specific transcriptomic programs and/or by modulating the early inflammatory response which is associated with chemoresistance, might sensitize AML cells to chemotherapy-induced cell death and thereby limit the risk of leukemic regrowth and relapse. Thus, although there was only a trend for a higher complete response rate in the dexamethasone group, this effect was translated in our study into a reduction of cumulative incidence of relapse, which is a better end point than the complete response rate for assessing the quality of response and impact on chemoresistant disease. Our findings also suggest that dexamethasone, used as a chemosensitizer in combination with intensive chemotherapy, should be assessed in prospective trials regardless of the WBC count.

Dexamethasone has both cytoplasmic and nuclear activities that interfere with signal transducers or transcription factors such as PI3-kinase, activating protein-1, and nuclear factor-κB, which are both involved in leukemic stem-cell biology.[8,27] Inflammatory cytokines can induce both nuclear factor-κB and activating protein-1 to support leukemic stem-cell survival in a synergistic manner.[28] Thus, by suppressing cytokine release and targeting specific intracellular pathways, dexamethasone could make leukemic stem cells more susceptible to chemotherapy-induced cell death. The mechanisms of action underpinning dexamethasone activity in AML are likely to be multiple as leukemic stem cells are subject to different levels of regulation which are either cell autonomous or driven by interactions with the microenvironment.[29,30]

In most studies that have focused on hyperleukocytosis in AML, the early mortality rate is about 20–30%, which is similar to that in the no dexamethasone group in our study.[31] Early mortality has remained very high compared to that of patients without hyperleukocytosis even in recent series, and therapeutic strategies, aimed at reducing leukocytosis through the use of leukapheresis or low-dose chemotherapy, failed to demonstrate any benefit.[2,31] Our results show that dexamethasone treatment was associated with a lower rate of early mortality following induction chemotherapy despite a higher rate of admission to the intensive care unit. There was, however, no significant difference compared to patients in the no dexamethasone group, which may reflect the low number of events. The criteria for intensive care unit admission in our center changed in 2015 when AML patients with a WBC count >100x10^9/L or leukostasis were admitted directly into the unit. However, we analyzed the 2004-2013 period and found the same difference with more intensive care unit admissions in the dexamethasone group (31% *vs.* 15%; P=0.028). Thus, it is likely that this difference is related to the selection criteria for giving dexamethasone, including higher WBC count and leukostasis syndrome, which are the main risk factors for transfer into the intensive care unit. Furthermore, although many physicians may be reluctant to use steroids in AML because of the potential risk of invasive fungal infections, we did not identify such adverse effects in dexamethasone-treated patients who also received antifungal prophylaxis.

Because this study is retrospective and included only a relatively low number of patients, there are some limitations. However, it also reflects some real-life aspects of the care of AML patients with a high WBC count, a difficult-to-treat population requiring immediate medical treatment, which means that these patients are often excluded from prospective trials. In our study, the impact of dexamethasone was adjusted for several clinical and biological factors to limit the potential biases inherent to non-randomized studies, and a biological rationale was further provided to strengthen the clinical findings. Although prospective randomized clinical trials are needed to confirm the results of this study, our findings argue for a repositioning of dexamethasone use within the backbone of intensive chemotherapy in AML patients.

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