REGULAR ARTICLE

blood advances

CDA as a predictive marker for life-threatening toxicities in patients with AML treated with cytarabine

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Key Points

- Ara-C is the mainstay of treatment for patients with AML, and life-threatening toxicities are common.
- We demonstrated that cytidine deaminase downregulation predicts severe/lethal toxicities with cytarabine.

Cytarabine (Ara-C) is the backbone of acute myeloid leukemia (AML) chemotherapy. Little is known about possible risk factors predictive for the frequent (ie, up to 16%) life-threatening or lethal toxicities caused by Ara-C. Ara-C is detoxified in the liver by a single enzyme, cytidine deaminase (CDA), coded by a gene known to be highly polymorphic. In this proof-of-concept study, we particularly investigated the role of the CDA poor metabolizer (PM) phenotype in Ara-C toxicities. CDA phenotyping (measurement of CDA residual activity in serum) and genotyping (search for the *CDA*2* allelic variant) were performed in 58 adult patients with AML treated with the standard 7+3 (Ara-C + anthracyclines) protocol. Statistically significantly lower CDA activity was observed in patients experiencing severe/lethal toxicities as compared with patients who did not (1.5 ± 0.7 U/mg vs 3.95 ± 3.1 U/mg; Student *t* test $P < .001$). Subsequent receiver operating characteristic analysis identified a threshold in CDA activity (ie, 2 U/mg) associated with PM syndrome and increased risk of developing severe toxicities. Five percent of patients experienced lethal toxicities, all displaying CDA PM status (1.3 ± 0.5 U/mg). In terms of efficacy, a trend toward higher response rates and longer progression-free survival and overall survival were observed in patients with low CDA activity. Taken together, the results of this study strongly suggest that CDA is a predictive marker of life-threatening toxicities in patients with AML receiving induction therapy with standard Ara-C.

Introduction

Standard induction chemotherapy for patients with acute myeloid leukemia (AML) consists of cytarabine (Ara-C) administered as a continuous infusion for 7 days combined with anthracyclines for 3 days (7+3 regimen).[1,2] Attempts to challenge this standard of care by adding a third drug, increasing Ara-C dosing, or augmenting dose-intensity have been performed.[3,4] However, these strategies have not convincingly improved clinical outcome, and the sole significant modification of the original 7+3 protocol was the recent increase in the dose of daunorubicin to achieve higher efficacy.[5]

Beyond improvements in response rates, better progression-free survival (PFS) and overall survival (OS) have been reported using intensified induction chemotherapy, but with increased treatment-related toxicities. Indeed, high-dose Ara-C triggers severe gastrointestinal toxicities such as grade 3 and 4 oral mucositis, intestinal ulceration, and ileus, thus increasing the risk of severe neutropenia, sepsis, and death resulting from toxicity.[6] Usually, 23% to 61% of severe nonhematological toxicities are regularly

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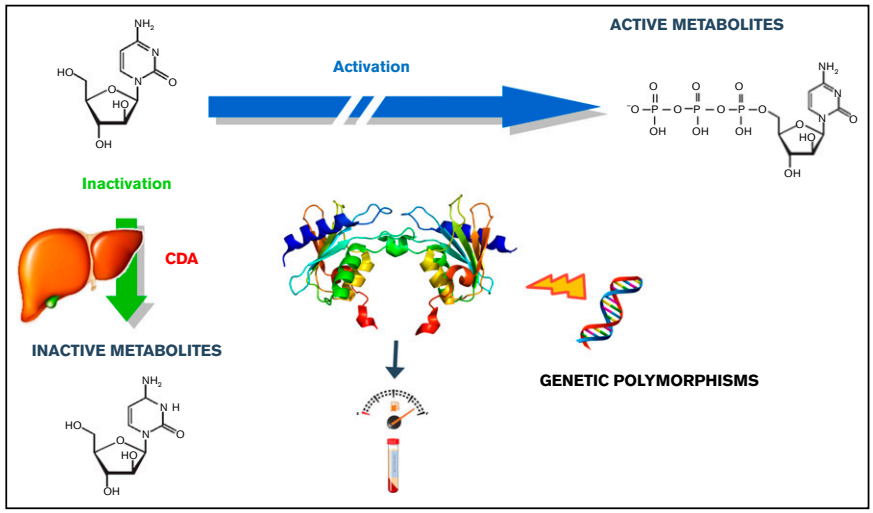
Figure 1. Cytarabine metabolic pathway.



reported with Ara-C, depending on the dose (ie, 100 or 200 mg/m^2), with 6% to 16% experiencing lethal toxicities.[7-9] Monitoring drug-induced hematological toxicities remains difficult in patients with hematological disorders; however, febrile neutropenia has already been reported in up to 43% of patients with AML treated with high-dose Ara-C.[10] The pathogenesis, prophylaxis, and therapeutic management of such life-threatening toxicities remain challenging issues. These toxicities can be age related, and therefore, the use of high-dose Ara-C is precluded in the elderly, because such toxicities can rapidly become life threatening in frail patients. However, because patients with cancer typically have several comorbidities, establishing a clear relationship between drug intake and clinical outcome is often challenging.

In this context, the only predictive factors regarding Ara-C are based on parameters that are patient specific and disease related. [11] Of these, cytogenetic classification remains the strongest criterion for predicting complete response, PFS, and OS, although mutations in the *FLT3*, *NPM1*, and *CEBPA* genes improve the prognostic classification of patients with a normal karyotype.[12] Of note, no markers for tolerance have been identified thus far for Ara-C. However, drug overexposure in patients with impaired liver detoxification could be a critical condition triggering severe toxicities, as observed with major cytotoxics such as fluorouracil, capecitabine, gemcitabine, mercaptopurine, and irinotecan.[13-15] With these drugs, drug elimination is also dependent on a single liver enzyme (ie, DPD, TPMT, and UGT1A1, respectively) coded by a polymorphic gene affecting enzymatic activity and liver clearance.

As a nucleoside analog, Ara-C undergoes liver detoxification by cytidine deaminase (CDA), an enzyme implicated in the metabolism of other pyrimidine derivatives such as gemcitabine, capecitabine, and azacytidine.[16] CDA is the pivotal enzyme involved in the liver degradation of Ara-C by deaminating the parent drug to inactive uracil arabinoside, leading to an extremely short plasma half-life (Figure 1).[17,18] CDA is a ubiquitous enzyme coded by a gene known to be highly polymorphic, with subsequent changes in resulting activity and either poor metabolizer (PM), extensive metabolizer (EM), or ultrarapid metabolizer (UM) phenotype.[16,19,20] Low CDA activity (ie, PM phenotype) is associated with severe toxicities with nucleoside analogs, whereas high activity leads to better tolerance and but lower efficacy.[19,21] PM status has already been identified as the culprit in reports of cases of death resulting from toxicity with azacytidine.[22] Regarding Ara-C, so far the issue of CDA activity has been mainly addressed at the tumor level as a possible resistance factor[23] resulting from drug deactivation after cellular uptake. Case reports in children suggest PM CDA status is predictive of Ara-C toxicity, but to date, no such relationship has been investigated in adults.[24]

Here we establish for the first time the link between CDA phenotype and incidence of severe toxicities in adult patients with AML treated with Ara-C.

Patients and methods

Patients

This was a noninterventional, proof-of-concept study conducted at the Hematology and Cellular Therapy Department of the Conception University Hospital of Marseille, Marseille, France. Designed as a real-life observational study, the only inclusion criterion was being a patient with AML scheduled for Ara-C–based induction therapy, with no exclusion criteria. AML was diagnosed based on World Health Organization criteria and required the presence of at least 20% myeloblasts in bone marrow. Beyond patient evaluation, physicians were free to exercise their best judgment about the suitability of patients for intensive induction chemotherapy following routine clinical practice. Patient care and sampling were performed following standard procedures in our institute, including sampling to determine CDA status routinely performed at the Assistance Publique–Hôpitaux de Marseille in any patient with cancer scheduled for nucleoside analogs.[16] As such, per French legislation, neither clinical registration number nor ethical committee approval was required to start collecting and analyzing the data. Written informed consent was only required before performing germinal *CDA* genotyping, to comply with the 2013 French law regarding constitutional genetics.

Treatments

Induction consisted of 90 mg/m^2 of daunorubicin and 200 mg/m^2 of Ara-C in patients age $<$65 years and 12 mg/m^2 of idarubicin and 200 mg/m^2 of Ara-C for those age $>$65 years, following the standard 7+3 protocol (ie, 7 days of Ara-C including 3 days of the associated drug). Bone marrow examination was scheduled on day

30; presence of persistent leukemia, defined as at least 5% blasts in the biopsy with at least 20% cellularity, called for a second course of induction therapy.

Pharmacodynamic end points

Toxicity was graded according to the Common Terminology Criteria for Adverse Events (version 4.0) during or after the induction phase. Because some patients with long-lasting aplasia received granulocyte colony-stimulating factor as supportive care, we focused primarily on nonhematological toxicities. Efficacy was evaluated 1 month after starting the induction. Complete remission (CR) was defined as bone marrow blasts <5%, absence of blasts with Auer rods, absence of extramedullary disease, absolute neutrophil count $>1 \times 10^9$/L, platelets $>100 \times 10^9$/L, and no requirement for red blood cell transfusions. Additionally, CR with incomplete recovery (CRi) was defined as CR with incomplete marrow recovery. Progressive disease (PD) was defined as not meeting any of the previous requirements. PFS was defined as the period of time during and after treatment that a patient lived with no worsening of the disease. OS was defined as the period of time after treatment during which a patient was still alive.

CDA status determination

CDA status was established following 2 complementary strategies. First, residual CDA activity in serum was considered a surrogate for liver activity, as extensively described previously and routinely implemented in our institute.[19] Briefly, CDA activity secreted in serum from hepatocytes and erythrocytes was spectrophotometrically measured ex vivo using cytidine as substrate and measurement of ammonium release after overnight incubation. CDA phenotype was finally expressed as units per milligram of total protein, with $1\ U = 4.10^{-3}$ μmol of ammonium released per minute and per milliliter of serum. On the basis of previous studies in patients with solid tumors, mean CDA activity of 3.6 ± 2.7 U/mg is considered a normal phenotype (ie, EM) in adults.[17] Additionally, genotypic investigations focused on the search for the canonical *CDA*2* allelic variant (ie, *CDA79A>C*)[25] by high-resolution melting analysis as described previously.[19] Genotyping *CDA* was performed only in patients for whom written informed consent to undergo germinal pharmacogenetic investigation was obtained, following the French Agence de la Biomédecine–Haute Autorité de Santé guidelines.

Statistical analysis

Because of the small number of patients in this observational study, CR and CRi were grouped as a single subset of responding patients. Comparisons between patient subgroups were performed by Mann-Whitney test for continuous variables. Influence of CDA status on toxicities was tested using χ^2 test (SigmaStat 2.0.; San Jose, CA) and receiver operating characteristic analysis (R Project, Vienna, Austria). Influence of CDA status on efficacy and toxicity was evaluated using 1-way analysis of variance (ANOVA) testing for response (SigmaStat 2.0). PFS and OS were analyzed by Kaplan-Meier curves, and influence of CDA status was tested by calculating hazard ratios (HRs) and by log-rank test (MedCalc 17.7.2; MedCalc Software, Ostend, Belgium).

Results

Patient characteristics

A total of 58 adult patients (25 women, 33 men; age 63 ± 15 years; range, 21-85 years) with AML routinely treated in La Conception University Hospital of Marseille were monitored in our study. Patient characteristics are summarized in Table 1. Mean total Ara-C dose at induction was 2208 ± 638 mg over 7 days. For younger patients (ie, age <65 years), mean Ara-C dose was 2402 ± 592 mg over 7 consecutive days, with daunorubicin. For older patients (ie, age >65 years), Ara-C dose was 2296 ± 579 mg over 7 consecutive days, with idarubicin. Median initial white blood cell count was 22 ± 44 $\times 10^9$/L. AML risk status was categorized as favorable, intermediate, or unfavorable based on cytogenetic and molecular abnormalities as defined by the European LeukemiaNet guidelines.[11] Karyotype was established as follows: 7 patients (12%) were categorized as favorable, 31 patients (53%) as intermediate, 17 patients (29%) as unfavorable, and 3 patients (5%) as unknown.

Table 1. Patient characteristics

Patients	N (%)
Total	58
Median age (range), y	61 (21-85)
Sex	
Male	33 (57)
Female	25 (43)
Cytogenetic risk	
Favorable	7
Intermediate	31
Unfavorable	17
Unknown	3
CDA status	
PM (≤2 U/mg)	28 (48)
EM (2-7 U/mg)	23 (40)
UM (>7 U/mg)	7 (12)
ECOG PS	
BM blasts, %	54
WBC, $\times 10^9$/L	22 ± 44
FAB subtype	
M0	7
M1	15
M2	14
M4	11
M5	9
M6	1
M7	—
Unclassified AML	1

ECOG PS, Eastern Cooperative Oncology Group performance status; FAB, French American British; WBC, white blood cell count.

CDA status

Mean CDA activity in the studied population was 3.14 ± 3 U/mg (range, 0.60-16 U/mg; median, 2.1 U/mg; first quartile, 1.3 U/mg; third quartile, 3.2 U/mg; Figure 2). Distribution in CDA values was not described by a normal law (Kolmorov Smirnov test, 0.231; $P < .001$), thus suggesting a multimodal distribution among patients. Only 16 patients (ie, 27%) correctly filled in the

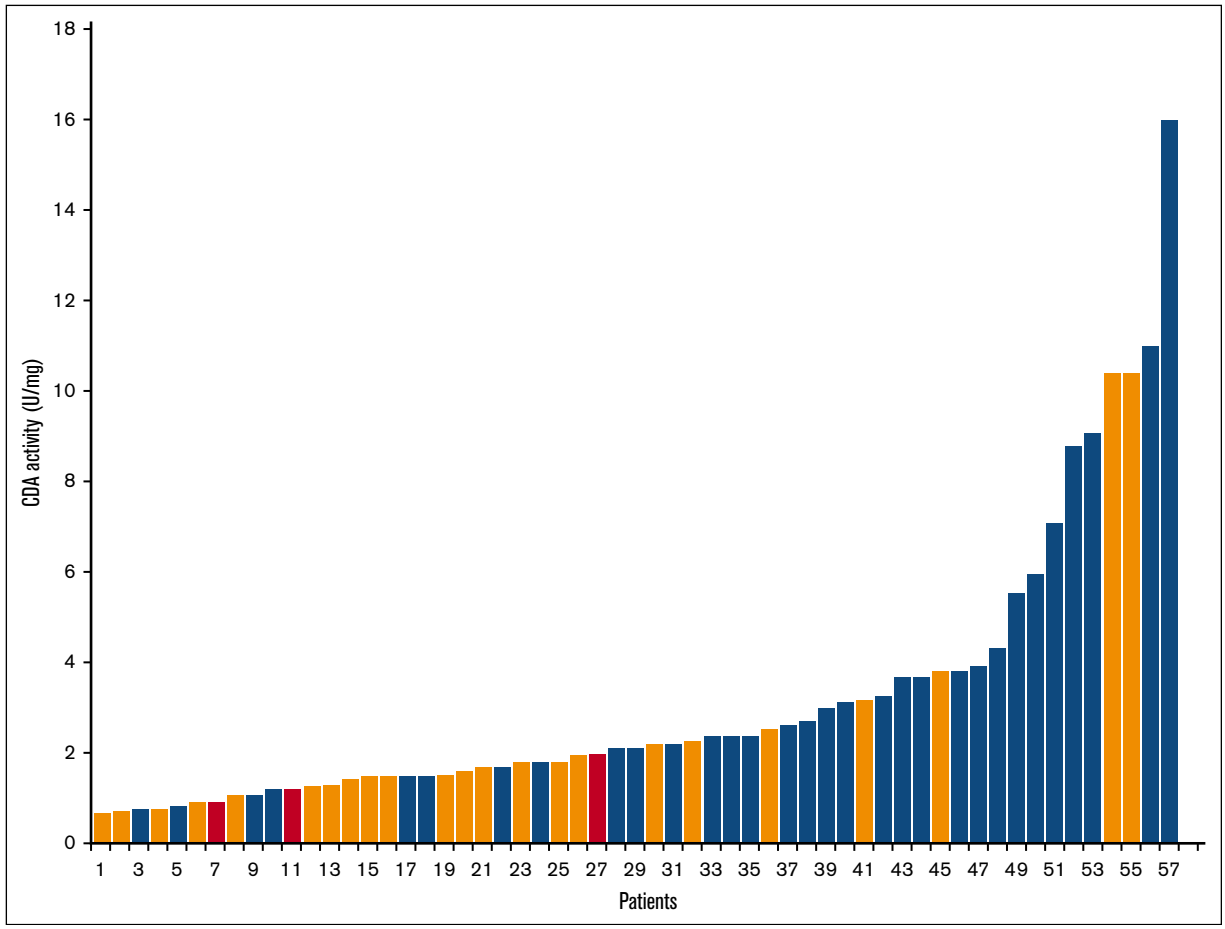


Figure 2. Distribution of CDA activities at diagnosis in patients with AML. Blue bars, no severe toxicities; orange bars, severe toxicities; red bars, deaths resulting from toxicity.

form related to germinal genetic investigation and were genotyped. High-resolution melting screening for the canonical *79A>C* polymorphism showed that incidence of this polymorphism (ie, 40% of heterozygous patients) was comparable to that seen in previous studies,[26] and no genotype-to-phenotype relationship was evidenced (data not shown), as previously reported.[19] No *CDA*2* homozygous patient was found.

Treatment-related nonhematological toxicity

Table 2 summarizes the nonhematological toxicities observed among the patients. A total of 25 patients (43%) experienced severe toxicities (ie, grade ≥3 per Common Terminology Criteria for Adverse Events) upon induction phase, including 3 unrecoverable grade 5 toxicities (5%). Age was not associated with severe toxicities (data not shown). Digestive toxicities were the most frequent (12 [21%] of 58 patients), as regularly reported with Ara-C.[27] The 3 deaths resulting from toxicity (5% of the patients) were due to gastrointestinal bacterial translocation (2 patients) and postneutropenia sepsis with skin toxicity and multiorgan failure (1 patient). Relationship between CDA activity and severe toxicity was first investigated using receiver operating characteristic analysis (Figure 3). We identified a cutoff in CDA value of <2 U/mg associated with increased risk of experiencing severe toxicity, regardless of the toxicity type, with a specificity of 65% and sensitivity of 74%. Figure 4 displays the comparative CDA values between patients with and without severe toxicities. Student *t* testing showed that patients without severe toxicities had CDA values higher than those of patients with severe or lethal toxicities (3.95 ± 3.1 vs 1.5 ± 0.7 U/mg; Student *t* test $P < .001$). More precisely, mean CDA activity was 3.95 ± 3.1, 1.6 ± 0.7, and 1.3 ± 0.5 U/mg in patients without severe toxicities, patients with severe toxicities, and patients with lethal toxicities (1-way ANOVA with Newman-Keuls multiple comparison testing $P < .01$). No significant difference was found in CDA value between patients with severe toxicities and those with lethal toxicities. Among the 25 patients with severe toxicities, 18 (72%) were categorized as PM with CDA <2 U/mg (PM patients), 5 had CDA values between 2.2 and 7 U/mg (EM patients), and 2 had CDA >7 U/mg (UM patients). Overall, 64% of PM patients displayed severe toxicities, and 11% had lethal toxicities. In comparison, only 23% of non-PM patients experienced severe toxicities during Ara-C induction, and no deaths resulting from toxicity were observed in this subset. A significant difference in toxicity was observed between the groups (Student *t* test $P < .005$). Figure 5 shows the repartition between PM, EM, and UM patients depending on the severity of the toxicities. Search for the *CDA 79A>C* polymorphism was not associated with severe toxicities, but because of the small number of genotypes collected, the power of the statistical test was below the

Table 2. Relationship between toxicity and CDA status

Toxicity, n (%)	CDA activity, U/mg	
	<2 (PM) (n = 28)	≥2 (EM + UM) (n = 30)
Severe	18 (64)	7 (23)
Digestive	10 (36)	2 (7)
Cutaneous	3 (11)	1 (3)
Other*	5 (16)	4 (13)
Death resulting from toxicity	3 (11)	0 (0)

Distribution in severe toxicities (grade ≥3 per Common Terminology Criteria for Adverse Events) in patients with CDA PM status (<2 U/mg) or non-PM status (CDA ≥2 U/mg).
*Others toxicities were: 4 cases of sepsis and 1 hyperthermia (CDA <2 U/mg) and 2 cases of sepsis, 1 renal impairment, and 1 hyperthermia (CDA ≥2 U/mg).

desired power, thus making this observation inconclusive (data not shown).

Efficacy

Cytologic response. After a single course of induction therapy, CR was achieved in 35 patients (60%) and CRi achieved in 4 patients (7%), whereas 16 patients (27%) did not respond (PD). With regard to karyotype, complete response was achieved in 5 (71%) of 7 patients with favorable karyotype, in 20 (64%) of 31 patients in the intermediate group, and in 8 (47%) of 17 patients in the unfavorable cytogenetic group. Regardless of karyotype, mean CDA activity was 2.9 ± 2.4 U/mg in patients with CR/CRi and 3.9 ± 4.3 U/mg in patients with PD (Figure 6). However, this difference was not statistically significant (1-way ANOVA on ranks testing $P = .922$). Because of the limited number of patients, it was not possible to test the combined effects of karyotype and CDA status on response. No relationship between CDA genotype and efficacy was found (data not shown).

Survival. Because of the limited number of patients, it was not possible to test the combined effects of karyotype and CDA status on survival. Regardless of karyotype, we observed that patients with CDA value <2 U/mg tended to have longer PFS (278 vs 517 days; Figure 7A) and OS (570 days vs not reached; Figure 7B) as compared with patients with CDA ≥2 U/mg. However, both HR (HR, 0.59; 95% confidence interval [CI], 0.29-1.19 and HR, 0.73; 95% CI, 0.30-1.77) and log-rank testing ($\chi^2 = 2.036$; $P = .153$ and $\chi^2 = 2.004$; $P = .156$) showed that the observed differences in PFS and OS were not statistically significant.

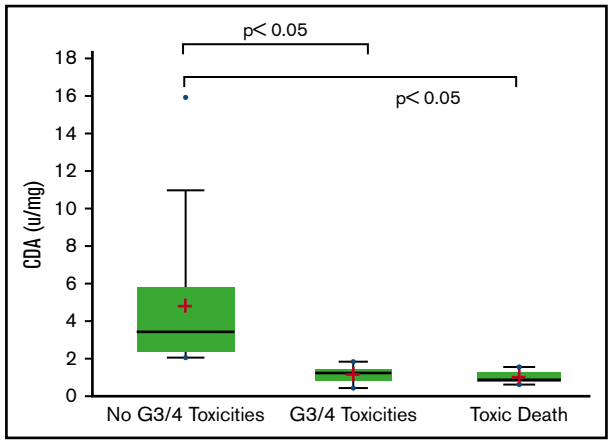


Figure 4. Distribution of CDA activity in patients with no severe toxicities, in patients with severe toxicities, and in patients with lethal toxicities. One-way ANOVA with Newman-Keuls multiple comparison testing showed that CDA values in patients with no severe toxicities were significantly different than those in patients with severe/lethal toxicities. No difference was found in CDA values between patients with severe toxicities and those with lethal toxicities. G, grade.

Discussion

In our institute, we have developed an expertise in screening for CDA deficiency as a possible predictive factor for toxicity with several nucleoside analogs.[28] CDA deregulation is a risk factor for the occurrence of severe and life-threatening toxicities with gemcitabine, capecitabine, and azacytidine.[25,29] Case reports in children have already suggested a possible link between decreased CDA activity and severe toxicities upon Ara-C administration.[30]

In this proof-of-concept study, for the first time we present additional evidence about the possible relationship between CDA PM status and severe toxicities in adult patients treated with Ara-C. In this study, the coadministration of either idarubicin or daunorubicin was not considered a confounding factor, because cardiotoxicity and liver toxicity associated with those 2 drugs were not

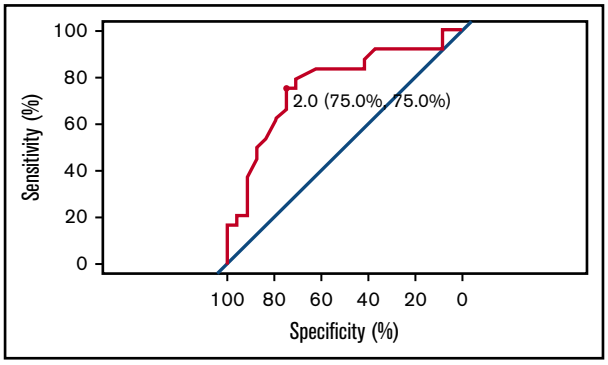


Figure 3. Receiver operating characteristic (ROC) analysis for determining optimal CDA value associated with severe toxicities. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold.

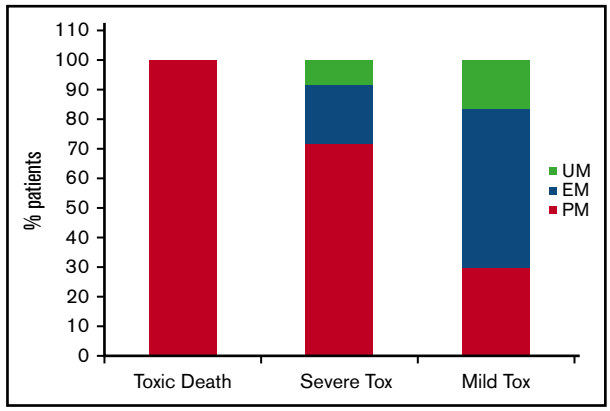


Figure 5. Distribution of the CDA phenotypes in patients with AML. Proportions of patients with PM, EM, and UM status with no severe toxicities (Mild Tox), severe toxicities (Severe Tox), and lethal toxicities (Toxic Death).

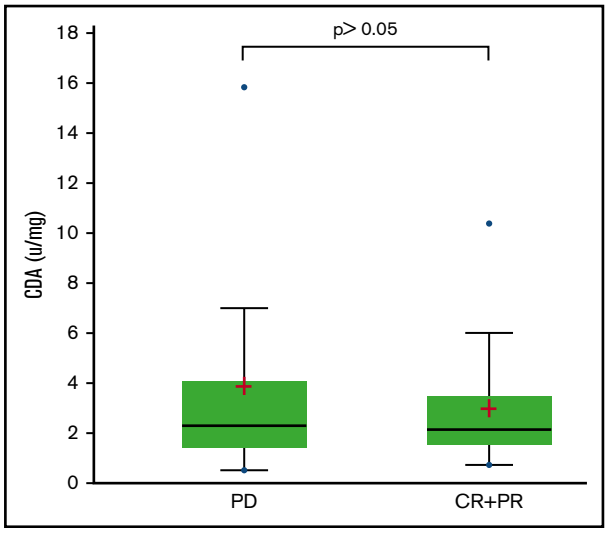


Figure 6. Distribution of CDA activity in patients with PD and with therapeutic response (CR + CRi). No statistical difference was found (Student *t* test $P > .05$).

observed in our patients. Conversely, digestive toxicities, including lethal bacterial translocation, were frequently observed and were in line with Ara-C–induced intestinal lesions reported previously.[27,31] Screening for the *CDA*2* allelic variant was inconclusive, because only a small subset of our patients (ie, <30%) had their *CDA* gene screened; however, the exact role *79A>C* polymorphism plays in nucleoside drug-related toxicities remains a controversial issue.[32,33] In this respect, the poor accrual for collecting CDA genotype was not considered a limitation in this observational study, because CDA status evaluation was based primarily on CDA phenotype, not genotype. Interestingly, functional testing for CDA status, routinely checked in our institute since the mid 2010s in patients scheduled for nucleosidic analogs,[34] showed that all patients with AML with lethal toxicities after standard Ara-C induction were PM with markedly decreased CDA activity (ie, enzymatic activity 73% lower than in the population without toxicities). In addition, overall rate of severe toxicities in PM patients was up to 2.7 times the rate in non-PM patients (ie, 64% vs 23%). Despite the fact that in this noninterventional study it was not possible to monitor drug levels nor to perform pharmacokinetics, CDA deficiency strongly suggests that plasma overexposure to Ara-C could explain the severe or lethal toxicities we observed, a relationship already established with the nucleoside analog gemcitabine, both in animals and in patients.[19]

Here we identified a threshold in CDA activity (ie, 2 U/mg) statistically associated with an increased risk for severe or lethal toxicities upon Ara-C treatment. Once confirmed in larger studies, this threshold could be used as a predictive marker for Ara-C toxicities to be used at bedside as part of routine bioguided medicine to avoid life-threatening toxicities. Interestingly, we also observed a trend toward lower response rates in patients with elevated CDA activity. Although not statistically significant, this relationship is in line with our previous work demonstrating the link between CDA UM status and treatment failure with gemcitabine in pancreatic cancer[35] and with azacytidine in patients with chronic myelomonocytic leukemia.[22] Similarly, we also observed a trend toward longer PFS and OS in patients with CDA below the 2 U/mg

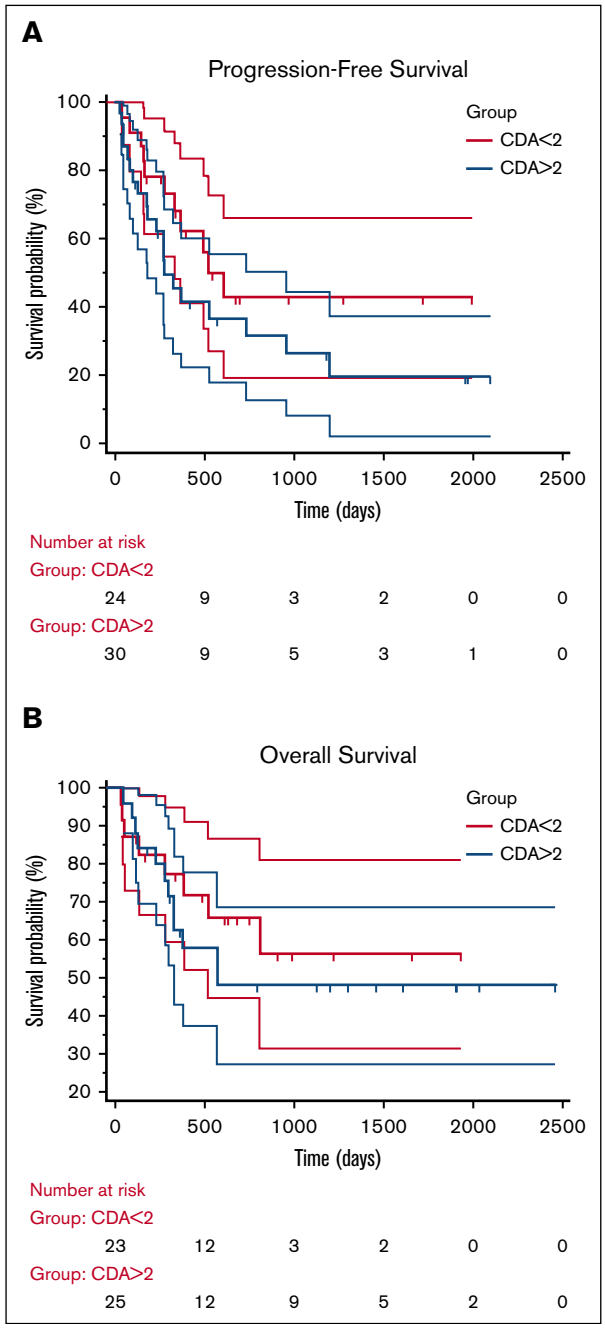


Figure 7. Kaplan-Meier representation for survival. (A) PFS and (B) OS. Both HR and log-rank testing failed to show a statistically significant difference between the curves.

threshold, thus illustrating how toxicity and efficacy are intricately linked with Ara-C in this setting. Of note, in the first part of the Kaplan-Meier curve, patients with higher CDA activity seemed to perform better, probably because as previously underlined, early deaths resulting from toxicity occurred only in PM patients, thus leading to a drop in the curve. The curves then separated in opposite directions in favor of extended survival in CDA-deficient patients, possibly because low CDA activity is associated with higher Ara-C exposure and subsequently higher antiproliferative efficacy.

Developing biomarker-based medicine is an increasingly used strategy in oncology to minimize unnecessary use of drugs while reducing toxicity risks and maintaining optimal efficacy. Integrating pharmacogenetic covariates in decision algorithms is already part of the routine clinical use of irinotecan (*UGT1A1*) and mercaptopurin (*TPMT*). Other polymorphisms such as *Cyp2D6* (tamoxifen) and *DPYD* (fluorouracil, capecitabine) have yet to be officially recommended by regulatory and health agencies and clinical societies.[36] We are in the early stages of understanding to what extent and how often CDA deficiency could be associated with poor clinical outcome with Ara-C. On the basis of our previous studies with gemcitabine, CDA PM status is usually found in 7% to 10% of patients with solid tumors.[19] Here, the incidence of CDA deficiency (28 [48%] of 58 patients) was surprisingly markedly higher than previously observed in patients with solid tumors such as sarcoma or pancreatic cancer monitored for CDA in our institute. To date, we have little explanation for the discrepancy observed in distribution of CDA PM status in patients with hematological disorders as compared with solid tumors. In particular, whether hematological disease in adults could affect CDA function or conversely CDA PM status could be a factor for increased risk of developing acute myeloid leukemia remains to be fully elucidated in future studies. CDA is implicated in a variety of metabolic reactions and homeostasis of several physiological compounds, and whether such metabolic disorder could be a risk factor for hematological disorder remains unknown. Of note, CDA deficiency has been associated with cytidine and deoxycytidine accumulation in tumor cells, a condition that could lead to genomic instability through PARP deregulation.[37] However, this hypothesis does not explain the marked discrepancy in CDA deficiency between patients with solid tumors and patients with AML. In addition, 7 patients (12%) displayed hyperleucocytosis (mean white blood cell count, 132×10^9/L; range, 93-282 $\times 10^9$/L); all had UM phenotype (mean CDA activity, 6.7 U/mg; range, 3.7-16 U/mg). Because CDA is ubiquitous and is expressed in many cell types, including white blood cells, one can hypothesize that hyperleucocytosis leads to increased residual activity in serum as measured by our phenotyping test.

Overall, and although preliminary and performed in a limited number of adult patients from a single institute, this proof-of-concept study strongly suggests that developing upfront functional testing for CDA could be a convenient and rapid strategy to identify outlier PM patients likely to experience life-threatening toxicities with Ara-C. It has to be underlined that in our study, other cofactors such as age were not associated with higher risk for toxicity and therefore could not be used to this end. Prospective studies including full pharmacokinetic support and pharmacokinetic/pharmacodynamic modeling are now warranted to confirm the possible role deregulated CDA could play on the poor clinical outcome of adults patients treated with Ara-C–containing regimens. Should this role be confirmed, CDA could be used as a biomarker to customize dosing in patients (ie, by cutting dosing in frail PM patients and increasing dosing in UM patients so as to ensure an optimal efficacy/toxicity balance). Ultimately, CDA phenotype measured before starting Ara-C–based therapy could be used as a covariate to individualize treatment as part of more personalized medicine. Our present data suggest that precision medicine is not reserved for trendy new molecules and that older but widely prescribed cytotoxics such as Ara-C deserve special attention to optimize clinical outcome in patients with AML.

Authorship

Contribution: R.F., L.F., J.C., B.L., and R.C. designed the observational study; R.F., L.F., Y.B.-H., M.D., V.I., J.C., and L.O. collected the data; M.D., C.R., Y.B.-H., and L.O. analyzed the samples; R.F., D.-C.I., and J.C. performed statistical analyses; R.F., L.F., J.C., B.L., G.V., and R.C. wrote the manuscript; R.F. and J.C. produced the figures; and all authors read and approved the manuscript.

Conflict-of-interest disclosure: J.C. declares research funding and honoraria from Roche France and owns a patent on CDA testing (WO2009021551 A1). The remaining authors declare no competing financial interests.

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