Comprehensive Ara-C SNP score predicts leukemic cell intracellular ara-CTP levels in pediatric acute myeloid leukemia patients

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Aim: Cytarabine (Ara-C), a mainstay of acute myeloid leukemia (AML) treatment, is a prodrug requiring activation to ara-CTP for its antileukemic activity. Aim of this study was to evaluate impact of genetic variants in the key genes involved in ara-C metabolism on the leukemic cell intracellular levels of ara-CTP.

Method: We investigated SNPs in 14 ara-C metabolic-pathway genes, for association with intracellular ara-CTP levels, in leukemic cells obtained post-initiation of cytarabine infusion in pediatric AML patients (n = 68). **Results:** Nine SNPs were significantly associated with leukemic cell intracellular concentration of ara-CTP. A comprehensive ara-CTP-SNP-score (ACSS) was further developed from top four SNPs identified in regression model. Patients were classified into three groups based on ACSS: high-ACSS (score >0), intermediate-ACSS (score = 0) and low-ACSS (score <0). ACSS designation was significant predictor of intracellular ara-CTP levels (p = 0.00012), suggesting a cumulative or synergistic effect of the significant SNPs. **Conclusion:** ACSS score designation holds promise in defining ara-C dose. Validation of the clinical utility of ACSS score in other independent cohorts will help identification of patients with potentially lower or higher levels of the ara-CTP in leukemic cells, thereby opening up opportunities for dose management to reduce toxicity and enhance efficacy.

First draft submitted: 25 May 2018; Accepted for publication: 3 July 2018; Published online: 8 August 2018

Keywords: acute myeloid leukemia • cytarabine • pharmacogenomics • SNPs

Acute myeloid leukemia (AML) is a cancer of the myeloid precursor stem cells with excess production and accumulation of abnormal undifferentiated blast cells in the bone marrow [1]. Despite the major advances in AML drug combination strategies and treatment options, resistance and disease relapse represents a major obstacle to reach the desired outcomes [2,3]. Although significant proportion of patients achieve complete remission after initial therapy, subset of patients experience relapse with significant impact in long-term survival rates [4]. For more than four decades, cytarabine, a nucleoside analog has been used as the backbone of the AML induction therapy [5,6]. However, there is wide interpatient variation in clinical response to cytarabine-based chemotherapeutic regimens and our understanding of these factors and mechanisms is still limited.

Cytarabine is a prodrug that requires activation to ara-CTP through series of phosphorylation steps and thus intracellular levels of ara-CTP are critical for achieving significant leukemic cell death. Previous *in vitro* studies have shown that the intracellular concentrations of ara-CTP are higher in ara-C sensitive cells than in resistant cells [7]. Further, leukemic cells from patients with chronic myelogenous leukemia (which is not responsive to ara-C), have only half the ara-CTP levels as compared with leukemic cells from patients with AML (which is responsive to ara-C) [2,8]. Thus, one of the mechanisms underlying ara-C resistance is insufficient intracellular levels of ara-CTP, which may be due to inefficient cellular uptake due to low levels of the transporters (*SLC29A1*,

SLC28A3 and *SLC28A1*); reduced activation due to alterations in enzymes as *DCK*, *CMPK1*, *NME3* which is a member of the nucleoside diphosphate kinase family which includes nine kinases, catalyzing the synthesis of nucleosides triphosphates other than ATP; increased inactivation rates due to *NT5C2*, *CDA* or *DCTD*; and/or increased cellular dCTP pools, that can compete with DNA incorporation of ara-CTP and also inhibit *DCK* activity which, in turn, are regulated by the enzyme ribonucleotide reductase (consisting of *RRM1* and *RRM2* subunits). Moreover, *CTPS1* or cytidine-5'-triphosphate synthetase can play an important role in regulation of ara-C activation, it catalyzes the conversion of UTP to CTP and inhibition of *CTPS* has been reported previously to be associated with depletion of CTP/dCTP pools and increase in sensitivity to ara-C treatment [9].

Reports in literature have shown association of SNPs within key candidate cytarabine metabolism genes and clinical response in AML patients [10–14] or with cytarabine *in vitro* chemosensitivity [15,16]. Our group has previously reported association of SNPs in *DCK*, *NT5C2* and RRM with intracellular levels of ara-CTP in leukemic cells [10–12]. However, to the best of our knowledge, other than previous reports from our group on selected ara-C pathway genes, no other study has comprehensively evaluated genetic variation in cytarabine metabolism genes with leukemic cell intracellular levels of active drug ara-CTP. Though very critical, it is very challenging to perform these studies due to unavailability of bone marrow samples at an early time point after initiation of induction treatment. Collection of bone marrow samples 24 or 48 hr postinitiation of cytarabine is not a part of standard practice and this is one of the major reasons for lack of investigations focusing on cytarabine pharmacogenetics on active drug levels in leukemic cells. Our study is unique in that aspect and is probably the biggest cohort with 68 pediatric AML patients where leukemic cell intracellular levels of ara-CTP were obtained postinitiation of cytarabine therapy in patients enrolled in St Jude AML 97 study. Given that patients had received only cytarabine on the first day of remission induction therapy, the intracellular ara-CTP levels determined at this time point are free of potentially confounding effects of other agents and are well suited for comprehensive pharmacogenetic evaluation ara-C metabolic pathway genes. We further report a cytarabine combinatorial SNP-score developed using the most promising SNPs predictive of leukemic cell ara-CTP levels and once validated in other cohorts this score holds promise for personalizing cytarabine chemotherapy.

Materials & methods

Patient cohort

Patients included in this study were enrolled in the St Jude AML-97 clinical trial. The details of study design, patient eligibility and clinical outcome are published elsewhere [17,18]. Briefly, between March 1997 and June 2002, 96 patients younger than 22 years with previously untreated AML or myelodysplastic syndrome, were randomized to receive cytarabine as a 2 h 500 mg/m² intravenous (iv.) bolus infusion (arm A, n = 50) or as a 500 mg/m²/day continuous infusion (arm B, n = 46) for 5 days. The patients in both arms received cladribine 9 mg/m²/day as a 30-min iv. infusion for 5 days that was initiated 24 hr after the start of cytarabine infusion, thus patients in both arms received cytarabine alone either as short daily or continuous infusion on day 1 (overall study schema is shown in Figure 1).

Intracellular ara-CTP levels

Bone marrow aspirates were obtained on day 1 after initiation of cytarabine, as previously described [17,18]. The patients with ≥70% leukemic blast percentage in the bone marrow were assessed for intracellular ara-CTP levels. Briefly, mononuclear cells were isolated using Ficoll-Hypaque density-gradient centrifugation and intracellular ara-CTP was quantified. Intracellular levels were calculated as nmol nucleotide per 2.0×10^7 cells as described previously [17,18]. Bone marrow samples were also obtained on day 2 (details have been published elsewhere [17,18]).

Genotyping for SNPs

We genotyped 108 SNPs in 14 genes known to be involved in ara-C metabolism and transport including *DCK*, *CMPK1*, *CDA*, *DCTD*, *RRM1*, *RRM2*, *NT5C2*, *NT5C3*, *CTPS1*, *NME4*, *NME3*, in addition to drug transporters *SLC29A1*, *SLC28A1* and *SLC28A3*). SNPs were genotyped using Sequenom (CA, USA) based genotyping that uses MALDI-TOF-based chemistry at University of Minnesota, Biomedical Genomics Center. SNPs with call rate of <95%, minor allele frequency of <0.05 were excluded. Additionally, we tested for SNPs on a gene locus for linkage disequilibrium and for SNPs co-occurring with high LD $r^2 > 0.8$, we included one SNP from the group for further analysis. Overall, we evaluated 88 SNPs in 14 genes for association with intracellular levels of ara-CTP (details provided in Supplementary Table 1).

Figure 1. Overall schema of the study.

AML: Acute myeloid leukemia; LD: Linkage disequilibrium.

Statistical analysis

Kruskal–Wallis or Wilcoxon rank test was used to compare ara-CTP levels between genotype groups or groups defined by combinatorial risk scores. Ara-CTP levels were log transformed before analysis. We evaluated all 88 SNPs using linear regression model to test impact for possible combinations of two SNPs per model. This method can extend the linear modeling framework to include variables that are not normally distributed. Bayesian Information Criterion (BIC) has been used to compare and order the models by the weight of evidence in favor of each model. Information measures have penalties if the added variable did not significantly improve the model fit. So that, the models with lowest BIC is preferred and they are more likely to have generated the observed intracellular ara-CTP levels. Thereafter, 1000 permutation tests have been done for each model to evaluate if by chance we can get better models to fit the data compared with the observed model. Permutation p-value <0.05 was considered significant. Univariate multiple linear regression models were built by stepwise addition of SNP and other major demographic and diagnostic factors such as race, cytogenetic risk groups and treatment arms. We have been able to obtain percentage of variability in ara-CTP, which can be explained by the independent variables individually.

Development of comprehensive ara-C SNP score results

Comprehensive ara-C SNP score (ACSS) was constructed using four SNPs representing top three models demonstrating significant association with ara-CTP levels. As a step 1, we designated directional genotype score for each SNP in each patient by taking into account direction of association of variant allele with ara-CTP levels as well as the potential mode of inheritance. The genotype score was then utilized for generation of a composite score by addition of individual genotype scores. Thus, instead of multiple genotypes each patient now had one composite score. Addition of genotype scores for four unique SNPs from top three models resulted in composite ACSS ranging from -2 to 3 with negative score indicating more number of low-ara-CTP associated alleles and positive score indicating more number of high ara-CTP associated alleles.

Results

Interpatient variation in ara-CTP levels

Intracellular ara-CTP levels at day 1 and the genotype data were successfully obtained for 68 patients (arm A, n = 35 and arm B, n = 33) making this to date the largest cohort of AML patients with intracellular ara-CTP levels following treatment with ara-C. The patient characteristics are listed in [Table 1](#). Wide interpatient variation in intracellular levels of ara-CTP was observed: 40.5-fold in arm A (range: 0.06–2.43 nmol ara-CTP per 2×10^7

Table 1. Patient demographics and characteristic features in all patients and by treatment arm.			
Characteristics	Overall (n = 68)	Arm A (n = 35)	Arm B (n = 33)
Age (years)			
Median	10	9	12
Range	0.5–21	0.5–21	1–20
Race			
White	43	26	17
Black	13	4	9
Other	12	5	7
Gender			
Male	31	18	13
Female	37	17	20
Risk group			
Favorable	26	18	8
Intermediate	38	16	22
Adverse	2	0	2
NA	2	1	1

leukemic cells) and 100-fold in arm B (range: 0.012–1.222 nmol ara-CTP per 2×10^7 leukemic cells). Intracellular ara-CTP levels did not differ by treatment arm, age, gender, initial cytogenetic risk group or race (all $p > 0.05$). Given that cytarabine was the only agent patients received before day 1 sample collection we focused primarily on the ara-CTP levels determined at this time point.

Association of ara-C metabolic pathway SNPs with intracellular levels of ara-CTP at day 1 post-treatment

Nine SNPs demonstrated association with day 1 intracellular ara-CTP levels at $p < 0.05$ (Table 2). Among these, SNPs with variant allele associated with lower intracellular levels included: rs4643786 T > C in 3'UTR of *DCK* (TT vs CT + CC; $p = 0.023$; consistent with our previous results in smaller subset of patients); *SLC28A1* intronic SNP rs11853372 G > T (TT vs GT + GG; $p = 0.018$); *DCTD* synonymous coding SNP rs4742 T > C (CC vs CT + TT; $p = 0.016$), which occurs in strong LD with at least 17 different intronic variants within *DCTD* locus and *CTPS1* intronic SNP rs4364871 C > T which occurs in LD with 15 additional SNPs on *CTPS1* locus (CC vs CT + TT; $p = 0.038$).

SNPs with significant association of variant allele with higher ara-CTP level included: *SLC28A3* SNP rs17343066 G > A, which occurs in LD with 7 other *SLC28A3* SNPs (AA vs AG + GG; $p = 0.028$); rs12067645 G > A (GG vs AG + AA; $p = 0.047$) which occurs in linkage with multiple promoter and intronic SNP in *CTPS1* and another 3'UTR *CTPS1* SNP rs11577910 (GG vs AG $p = 0.045$); *CDA* SNP rs12404655 A > G (AA vs AG + GG $p = 0.017$), which occurs in LD with six intronic and four 3'UTR SNPs within *CDA* and previously reported SNP in *RRM1* rs11030918 (occurs in LD with at least 22 other SNPs within *RRM1*¹⁰). Box plots of association of these SNPs with intracellular levels of ara-CTP are shown in Supplementary Figure 1.

Cytarabine score determination using generalized linear models

We evaluated all 88 SNPs using linear models for any combination of two SNPs with the most significant prediction of leukemic cell cytarabine levels at day 1. As shown in Table 3, our results identified three models for two SNP combinations (that included four unique SNPs) with significant association with ara-CTP levels. These models included combinations of *DCK*-rs4643786 and *RRM1*-rs11030918 in model 1; *DCK*-rs4643786 and *CTPS1*-rs12067645 in model 2 and *RRM1*-rs11030918 and *SLC28A3*-rs17343066 in model 3. Figure 2 shows distribution of the genotypes for these four SNPs across patient's sorted by low to high levels of ara-CTP. A comprehensive score was created using genotype information for four SNPs that passed the models. Directional genotype score was provided for each SNP in each patient after consideration for direction of association of variant allele with ara-CTP levels as well as the potential mode of inheritance. Addition of directional genotype scores resulted in composite ACSS ranging from -2 to 3 with negative score indicating more number of low-ara-CTP associated alleles and positive score indicating more number of high ara-CTP associated alleles. The

Table 2. SNPs demonstrating significant association with day 1 intracellular ara-CTP levels post-infusion of cytarabine in pediatric acute myeloid leukemia patients treated under St Jude AML97 clinical trial (n = 68).

Gene name	SNP rsID	SNP location	MAF	Ara-CTP levels (nmol/2 × 10 ⁷ cells)			Direction association with ara-CTP levels	Genotype score (inheritance mode)
				Genotype	Mean ± SD	p-value		
<i>DCK</i>	rs4643786 [†] ; T >C	Intron (occurs in LD with several intronic SNPs [11])	0.125	TT (n = 51) CT (n = 11) CC (n = 3)	0.488 ± 0.391 0.267 ± 0.221 0.145 ± 0.091	TT vs CT + CC; p = 0.023	Low	Additive (TT = 0, CT = -1, CC = -2)
<i>SLC28A1</i>	rs11853372; G >T	Intron	0.328	GG (n = 32) GT (n = 30) TT (n = 6)	0.510 ± 0.508 0.461 ± 0.384 0.146 ± 0.095	TT vs GT + GG, p = 0.018	Low	Recessive (GG/GT = 0, TT = -1)
<i>DCTD</i>	rs4742; T >C	Synonymous (occurs in LD with 17 intronic SNPs in <i>DCTD</i>)	0.364	TT (n = 25) CT (n = 32) CC (n = 10)	0.450 ± 0.352 0.533 ± 0.528 0.239 ± 0.274	CC vs CT + TT; p = 0.016	Low	Recessive (TT/CT = 0, CC = -1)
<i>CTPS1</i>	rs4364871; C >T	Intron (occurs in LD with 15 SNPs 2 in <i>CTPS1</i>)	0.123	CC (n = 47) CT (n = 19) TT (n = 1)	0.539 ± 0.490 0.271 ± 0.218 0.36	CC vs CT + TT, p = 0.038	Low	Dominant (CC = 0, CT/TT = -1)
<i>CTPS1</i>	rs12067645; G >A	Intergenic (occurs in LD with 35 SNPs in 5' region of <i>CTPS1</i>)	0.222	GG (n = 45) AG (n = 20) AA (n = 2)	0.379 ± 0.353 0.602 ± 0.557 0.873 ± 0.704	GG vs AG + AA, p = 0.047	High	Additive (GG = 0, AG = 1, AA = 2)
<i>CTPS1</i>	rs11577910; G >A	3'UTR (occurs in LD with 12 SNPs in <i>CTPS1</i>)	0.093	GG (n = 61) AG (n = 7)	0.425 ± 0.433 0.732 ± 0.439	GG vs AG, p = 0.045	High	(GG = 0, AG = 1)
<i>CDA</i>	rs12404655; A >G	Occurs in LD with 4 SNPs in 3' region and 6 in <i>CDA</i> introns	0.21	AA (n = 44) AG (n = 21) GG (n = 3)	0.372 ± 0.362 0.642 ± 0.555 0.385 ± 0.269	AA vs AG + GG; p = 0.017	High	Dominant (AA = 0, AG/GG = 1)
<i>RRM1</i>	rs11030918 [†] ; T >C	5' UTR (occurs in LD with 2-5' UTR one 3'UTR and 19 intronic SNPs in <i>RRM1</i>)	0.317	TT (n = 25) CT (n = 32) CC (n = 9)	0.396 ± 0.326 0.354 ± 0.348 0.785 ± 0.411	CC vs CT + TT; p = 0.0025	High	Recessive (TT/CT = 0, CC = 1)
<i>SLC28A3</i>	rs17343066; G >A	Occurs in LD with 7 intronic SNPs in <i>SLC28A3</i>	0.457	GG (n = 20) AG (n = 34) AA (n = 12)	0.558 ± 0.649 0.361 ± 0.311 0.591 ± 0.293	AA vs AG + GG, p = 0.028	High	Recessive (GG/AG = 0, AA = 1)

[†]SNPs previously reported [10,11]; LD = Linkage disequilibrium: data from Haploreg database (Version4.1) variants with $r^2 \geq 0.8$ are indicated in third column (<http://archive.broadinstitute.org/mammals/haploreg/haploreg.php>)

Table 3. Top three models for two SNP combinations that were significantly associated with ara-CTP levels at day 1.

Model	Coefficient	p-value	BIC-wt	Pperm
rs4643786 (<i>DCK</i>) + rs11030918 (<i>RRM1</i>)	-0.339 0.383	0.006 0.009	0.038097	0.004
rs4643786 (<i>DCK</i>) + rs12067645 (<i>CTPS1</i>)	-0.42 0.228	0.0009 0.016	0.030693	0.001
rs11030918 (<i>RRM1</i>) + rs17343066 (<i>SLC28A3</i>)	0.521 0.406	0.0005 0.0022	0.030467	0

BIC: Bayesian information criterion.

Table 4. Association of ara-C SNP score group with intracellular ara-CTP levels.

ACSS groups	Ara-CTP level by ACSS group at day 1 (nmol/2 × 10 ⁷ cells)		
	Score	Mean ± SD	p-value
L-ACSS	<0 (n = 7)	0.133 ± 0.150	1.20E-04
I-ACSS	0 (n = 32)	0.342 ± 0.324	
H-ACSS	>0 (n = 29)	0.660 ± 0.508	

ACSS: Ara-C SNP score.

score was further compressed to classify patients into three groups low-ACSS (L-ACSS) with scores <0 (ara-CTP levels 0.133 ± 0.150 nmol/2 × 10⁷ cells); intermediate-ACSS (I-ACSS) group with score = 0 (ara-CTP levels 0.342 ± 0.324 nmol/2 × 10⁷ cells) and high-ACSS (H-ACSS) group with scores >0 (ara-CTP levels 0.660 ± 0.508 nmol/2 × 10⁷ cells). ACSS score demonstrated significant association with intracellular levels of ara-CTP (p = 0.0001) (Table 4 & Figure 3). Further, the ACSS could explain approximately 30% of observed

Figure 2. Genotype HeatMap showing distribution of genotypes for four SNPs across patients. The patients ($n = 68$) are arranged from low to high levels of intracellular ara-CTP (the levels are depicted as $\text{pmol}/2 \times 10^7$ cells). Genotypes with significant association of variant allele with low levels of ara-CTP are depicted in red and those with significant associated with higher ara-CTP are depicted in blue, grey boxes reflect wild type genotype.

Figure 3. Association of comprehensive ara-C SNP score with intracellular levels of active ara-CTP (Log transformed ara-CTP $\text{pmol}/2 \times 10^7$ cells). Box plot showing association of low-ACSS (score <0), intermediate-ACSS (score $=0$) and high-ACSS (score >0) score groups with intracellular ara-CTP levels obtained at day 1 post-initiation of ara-C infusion. ACSS: Ara-C SNP score.

variation in ara-CTP levels which was significantly higher than contribution from any other covariates such as race, treatment arm, cytogenetic-risk group and gender, which together explained only approximately 8% of the observed variation (Table 5). Although day 1, ara-CTP levels was the primary focus of this study, we observed similar association between ACSS score and intracellular ara-CTP levels in samples obtained on day 2 (Supplementary Figure 2).

Table 5. Ara-C SNP score group classification explains majority of interpatient variation in ara-CTP as compared with other factors.

Independent variable	Variability in day 1 intracellular ara-CTP levels explained	R-squared
ACSS score groups	0.302	0.302
Race	0.027	0.329
Arm	0.032	0.361
Cytogenetic-risk group	0.007	0.368
Gender	0.007	0.375

CSS score groups are given in bold to emphasize its importance in explaining 30 out of 37% of total variability in ara-CTP levels.
ACSS: Ara-C SNP score.

Discussion

Cytarabine has been used for AML treatment for more than 40 years. It is a prodrug that requires uptake to cells and activation by series of phosphorylation steps to form the active form of the drug, ara-CTP, which competes with dCTP for incorporation into DNA and inhibits DNA synthesis, and triggers apoptosis. Thus, intracellular abundance of ara-CTP is critical for its therapeutic efficacy [19] and at the same time higher levels might be contributing to observed toxicity and adverse events. Genetic variation in genes involved in ara-C uptake, activation of ara-CTP or inactivation as well as genes involved in regulating pools of dCTP can impact its intracellular levels of ara-CTP and thus its therapeutic efficacy. Several studies on association of SNPs within ara-C metabolic pathway genes with clinical end points have been reported [10–14,16,20–30]; however, there is lack of information on direct association of ara-C pathway SNPs with abundance of ara-CTP within leukemic cells of AML patients. One of biggest challenges associated with designing such a study is obtaining bone marrow aspirates post cytarabine infusion, which is not a standard clinical procedure in the treatment of AML. We have previously reported SNPs in select genes *DCK*, *NT5C2* and *RRM1/RRM2* to be associated with intracellular ara-CTP levels in patients treated on St Jude AML97 clinical trial [10–12]. In the current study, we performed a comprehensive evaluation of 14 genes and 88 SNPs involved in ara-C pathway.

We observed SNPs in two influx transporters: *SLC28A1* (rs11853372 G >T) with presence of variant allele predictive of low-intracellular ara-CTP levels and *SLC28A3* rs17343066 G >A with variant allele associated with higher ara-CTP levels. The 3'UTR SNP in *DCK* rs4643786 T >C has been previously been associated with lower *DCK* mRNA levels and intracellular ara-CTP levels in a smaller cohort of patients [11]; however in contrast to this, it has been associated with higher remission rate in adult Chinese AML patients [20]. *CDA* and *DCTD* both are involved in deamination and convert ara-C >ara-U and ara-CMP >ara-UMP, respectively. A synonymous SNP rs4742 C >T in *DCTD* occurs in high LD with at least 17 other SNPs on *DCTD* locus and predicted lower ara-CTP levels where as *CDA* SNP was associated with high ara-CTP levels. Among the genes involved in regulating cellular pools of CTP, one SNP in *RRM1* and three SNPs in *CTPS1* showed significant association with intracellular levels of ara-CTP. *RRM1* codes for ribonucleotide reductase and *CTPS1* encodes for cytidine-triphosphate synthase and both play critical role in pyrimidine synthesis and maintaining cellular pools of dCTP. Intracellular dCTP can impact ara-CTP production and its efficacy by feedback inhibition of *DCK*, by allosteric activation of the inactivating enzyme *CDA* and by competing with the ara-CTP for incorporation into DNA. Although, the functional relevance of these SNPs still needs to be investigated, majority of the significant SNPs occurred in LD with multiple SNPs on the respective gene locus, warranting in-depth functional characterization of these and potential linked SNPs. As an exploratory evaluation for the potential functional impact of the nine SNPs significantly associated with we utilized the Genotype-Tissue Expression (GTEx) project (www.gtexportal.org). Of the nine SNPs, seven were represented in the GTEx database. The single tissue (whole blood) expression quantitative trait loci (eQTL) analysis from GTEx database showed association of *CDA* SNP rs12404655 A >G with lower *CDA* mRNA expression which is consistent with G allele predictive of high ara-CTP concentration. Interestingly for *CTPS1* SNPs, variant alleles for rs12067645 G >A, and rs11577910 C >T with higher and for rs4364871 C >T with lower mRNA levels, which was not consistent with the direction of association of these SNPs with ara-CTP levels. Although this requires further investigation, *CTPS1* gene has 12 alternatively spliced transcripts, which might interfere with accurate mRNA quantification of the wild-type reference transcript. Similarly, *RRM1* SNP rs11030918 T >C demonstrated association with the higher mRNA levels in whole blood, and was associated with higher ara-CTP levels. Although molecular mechanisms underlying this observation require further investigation,

previous report has shown that higher *RRM1* expression is associated with higher ara-C sensitivity as well as better DFS [2]. *SLC28A3* and *DCTD* SNPs were not significantly associated with the respective genes mRNA levels.

As multiple SNPs can co-exist in a patient, we evaluated influence of SNP combinations on intracellular ara-CTP levels, three top two SNP models with significant impact on ara-CTP levels consisted of four unique SNPs. Thus, we developed a comprehensive ara-C SNP risk score (ACSS) for each patient by taking into account the directional genotype and inheritance mode for each of the four SNPs. Our results show that the comprehensive ACSS has improved power to significantly predict leukemic cell intracellular levels of ara-CTP and explained approximately 30% of variation observed in intracellular levels of ara-CTP. Given the ACSS score subclassifies patients into three groups: low-ACSS, intermediate-ACSS and high-ACSS independent of patient demographics and risk groups, once validated this score has a potential to identify patients with potentially lower levels ara-CTP (L-ACSS group) in the leukemic cells with high risk of treatment failure. Conversely, patients with H-ACSS might be at higher risk of ara-C related side effects.

The uniqueness of AML97 study is the availability of bone marrow aspirates from day 1 and day 2 post ara-C infusion allowing for intracellular ara-CTP level quantification; however, one of the limitations in extending our analysis to outcome was differences duration between initiation of course 2 in treatment arms, in timing of chemotherapy and two major amendments during the course of the study that influenced clinical outcome (described in detail in Rubnitz *et al.* [17]). Due to these reasons for the purpose of this study, we primarily focused our evaluation on intracellular ara-CTP levels obtained at day 1 post ara-C infusion. Since patients received only ara-C as a single agent on the first day of remission induction therapy, so ara-CTP measurements at this time-point were free of potentially confounding effects of other agents.

Conclusion & future perspectives

ACSS developed using potentially relevant SNPs in ara-C metabolic pathway can predict intracellular levels of ara-CTP in leukemic cells. Future studies are needed to validate the clinical utility of ACSS in predicting response and toxicity after induction treatment. Although, current AML chemotherapeutic regimens use low- and high-dose of ara-C, these are not guided by pharmacogenomics. Once validated the ACSS holds potential for designing individualized ara-C dosing for maximizing benefit for the patient. Our results open up opportunities to pair L-ACSS score with high-dose ara-C, H-ACSS with low-dose and I-ACSS with intermediate dose of ara-C to treat AML patients.

Summary points

Purpose of the study

- Cytarabine is the mainstay of acute myeloid leukemia (AML) chemotherapy since 1973; however, the comprehensive understanding of genetic factors contributing to interpatient variation in levels of the active form of drug ara-CTP within leukemic cell is lacking.
- This study was designed to evaluate the role of SNPs within 14 genes in ara-C activation pathway for association with intracellular levels of ara-CTP in pediatric AML patients.

Results

- Nine SNPs in seven genes demonstrated significant association with ara-CTP levels determined day 1 after initiation of ara-C infusion.
- Multi-SNP modeling approach identified four SNPs which were utilized to create an ACSS score for each patient.
- The patients were classified into three groups: high-ACSS (score >0), intermediate-ACSS (score = 0) and low-ACSS (score <0) with significant association with intracellular ara-CTP levels, low levels in patients with low ACSS score.

Conclusion

- Classification of patients with respect of ACSS score might help in personalizing cytarabine therapy to achieve maximum therapeutic efficacy and avoid unwanted toxicity.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at:

<https://www.futuremedicine.com/doi/suppl/10.2217/pgs-2018-0086>

Acknowledgements

The authors would like to acknowledge the support provided by Biomedical Genomics Center (BMGC) and Minnesota Supercomputing Institute at the University of Minnesota.

Financial & competing interests disclosure

This study was supported by NIH grant R01CA132946 (JK Lamba), American Society of Hematology Bridge funding and by the American Lebanese Syrian Associated Charities (ALSAC). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

St Jude Children's Research Hospital Institutional Review board approved the AML 97 study. Specimens were obtained from patients after obtaining informed consent from parents/guardians, with assent from patients as appropriate.

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