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Pharmacogenomic Score Effectively Personalizes Treatment of Acute Myeloid Leukemia

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Abstract

Background: Cytarabine (also known as ara-C) has been the backbone of acute myeloid leukemia (AML) chemotherapy for over five decades. Recent pharmacogenomics-based 10-SNP ara-C score (ACS10) showed low ACS10 (≤0) to be associated with poor outcome in AML patients treated with standard chemotherapy. Here, we evaluated ACS10 score in the context of three different induction 1 regimens in pediatric AML patients.

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Contributors:

JKL, SP conceptualized and designed the study, JKL, PKP, SP generated data, RJM, AE, XC, HW, SP and JKL performed data analysis and interpretation. C-HP, RCR, HI, JER, BAD, DJK, NL provided clinical expertise, data and interpretation. All authors contributed to manuscript editing and approved the final version.

Declaration of Interests: We declare no competing interests.

Materials and Methods: ACS10 score groups (low,≤0 or high,>0) were evaluated for association with event-free survival (EFS) and overall survival (OS) by three randomized treatment arms in patients treated on the AML02 (NCT00136084) and AML08 (NCT00703820) clinical trials: AML02 low-dose cytarabine (LDAC arm, n=91), AML02+AML08 high-dose cytarabine (HDAC arm, n=194) and AML08 clofarabine+ cytarabine (Clo/Ara-C arm, n=105) induction 1 regimens.

Results: Within the low-ACS10 score (≤0) group, significantly improved EFS and OS was observed among patients treated with Clo/Ara-C as compared to LDAC (EFS, HR=0.45, 95% CI, 0.23–0.88, p=0.020; OS, HR=0.44, 95% CI, 0.19–0.99, p=0.048). In contrast, within the high-ACS10 score group (score >0) augmentation with Clo/Ara-C was not favorable as compared to LDAC (Clo/Ara-C vs. LDAC, EFS, HR=1.95, 95% CI: 1.05–3.63, p=0.035; OS HR=2.17, 95%CI: 1.05–4.49; p=0.037). Personalization models predicted 9% improvement in outcome in ACS10 score-based tailored induction (Clo/Ara-C for low and LDAC for high-ACS10 groups) as compared to non-personalized approaches (p<0.002).

Conclusion: Our findings suggest that tailoring induction regimens using ACS10 scores can significantly improve outcome in patients with AML. Given the SNPs are germline, preemptive genotyping can accelerate matching the most effective remission induction regimen.

Introduction

Acute myeloid leukemia (AML) is a heterogenous disease with suboptimal outcome. Although induction regimens typically consisting of cytarabine (also known as ara-C) in combination with an anthracycline and etoposide induce remission in most pediatric AML patients, approximately 30% of patients who initially achieve remission relapse and subsequently have very dismal outcomes[1–3]. Cytarabine (Ara-C) is a prodrug that requires activation to the active form: cytarabine-triphosphate (ara-CTP), which induces leukemic cell death[4,5]. Thus, intracellular ara-CTP abundance is critical for its antileukemic activity. Pharmacogenomic studies in AML have previously reported SNPs associated with genes in metabolic pathways of drugs such as ara-C, however most of this work was done on single genes[6–18]. We recently performed a comprehensive pharmacogenomics evaluation of single nucleotide polymorphisms (SNPs) in cytarabine pathway genes and established a clinically relevant polygenic 10-SNP based score (ACS10) consisting of 10 SNPs in nine genes[19]. These genes included those involved in cellular uptake of ara-C (SLC28A3 and SLC29A1), its inactivation (CDA, CTPS1) and activation (DCK, CMPK1), or those regulating cellular pools of CTP which in turn impact ara-C sensitivity and impact cytidine mediation feedback inhibition of DCK (Supplementary Table 1). Low ACS10 score (score≤0) implies inefficiency in cytarabine uptake and activation or efficient inactivation, as reflected by its association with lower intracellular levels of ara-CTP[20]. In this study, patients within low ACS10 score group had poor outcomes after treatment with standard induction regimen consisting of low-dose cytarabine (100 mg/m^2 every 12 hours on days 1–10; LDAC arm) in combination with daunorubicin and etoposide. However, when given an augmented induction regimen with a higher dose of cytarabine (3g/m^2, every 12 hours on days 1, 3, and 5; HDAC arm) in combination with daunorubicin and etoposide, there was significant improvement in outcome in the low-ACS10 score group[19]. In contrast, within

the high ACS10 score group (score >0; reflective of higher intracellular ara-CTP levels) no significant difference in outcome was observed between patients treated with low-dose- or high-dose cytarabine based regimens. In fact, a trend toward poor outcome was observed in patients with high ASC10 group treated with high dose cytarabine. These results suggest that pharmacogenomics-based inefficiency of cytarabine activation within the low ACS10 score group can be overcome by intensifying the dose of cytarabine.

In the present study, we investigated whether augmentation of therapy by combining two nucleoside analogs can serve as a therapeutic option in patients with low ACS10 scores. Clofarabine is another nucleoside analog that inhibits both DNA polymerase as well as ribonucleotide reductase and has been shown to enhance the therapeutic efficacy of cytarabine[21–23]. Previous work has shown that clofarabine can result in biochemical modulation of ara-CTP and synergistic cell death[24]. A recently completed clinical trial AML08[25] (NCT00703820) investigated the combination of clofarabine and cytarabine versus conventional induction therapy in newly diagnosed pediatric AML patients, thus providing us a unique opportunity to evaluate ACS10 score groups by the three treatment arms across two clinical trials (AML02: NCT00136084 and AML08: NCT00703820).

Materials and Methods

Patient Cohorts

Multi-site AML02 Cohort (NCT00136084): Children, adolescents, and young adults with newly diagnosed AML treated on the multicenter AML02 trial with both clinical and genotype data available (N=166) were included in this study. Details of study design and clinical outcome have been reported elsewhere[26]. In brief, patients were randomly assigned to receive either high-dose cytarabine (HDAC) (3 g/m^2, every 12 hours on days 1, 3, and 5; HDAC arm, n=75) or low-dose cytarabine (LDAC) (100 mg/m^2 every 12 hours on days 1–10; LDAC arm, n=91) combined with daunorubicin (50 mg/m^2 over 6 hours on days 2, 4, and 6) and etoposide (100 mg/m^2 over 4 hours on days 2–6) as induction I. Please note that low-dose cytarabine LDAC, referred to in this study is same as the standard dose of ara-C used approved for treating AML. Subsequent therapy was assigned as per risk characteristics and minimal residual disease (MRD) status after induction I; details of trial outcome are reported elsewhere[26].

Multisite AML08 Cohort (NCT00703820): Newly diagnosed AML patients less than 22 years old treated on the multicenter AML08 trial with both clinical and genotype data available (N=224) were included in the current evaluation. Details of AML08 study design and clinical outcome have been reported elsewhere[25]. Overall, patients were randomized to receive clofarabine and cytarabine (Clo/Ara-C arm: clofarabine 52 mg/m^2 per day on days 1 to 5 and cytarabine 1 g/m^2 per day on days 1 to 5), or high-dose cytarabine, daunorubicin, and etoposide (HDAC arm similar to AML02 trial above- cytarabine 3 g/m^2 every 12 hours on days 1, 3, and 5, daunorubicin 50 mg/m^2 on days 2, 4, and 6, and etoposide 100 mg/m^2 per day on days 2 to 6) as induction I. Induction II regimens consisted of low-dose cytarabine with daunorubicin, and etoposide given with or without sorafenib or

vorinostat. Consolidation included additional courses of chemotherapy or hematopoietic cell transplantation (HSCT).

Clinical Endpoints Definitions

For both cohorts, event-free survival (EFS) was defined as the time from study enrollment to induction failure, relapse, secondary malignancy, death, or study withdrawal for any reason, with event-free patients censored on the date of last follow-up. Overall survival (OS) was defined as the time from study enrolment to death, with living patients censored on the date of last follow-up. MRD1 positivity was defined as presence of ≥0.1% leukemic cells after induction 1. The study was conducted in accordance with declaration of Helsinki and was approved by the Institutional Review Boards. Informed consent was obtained from parents/guardians or patients and assents from the patients, as appropriate, for the approved clinical trial protocols.

Genotyping

For the AML02 cohort, genomic DNA was genotyped as previously reported using sequenom iPlex platform at the University of Minnesota, Biomedical Genomics Center[19]. ACS10 scores were calculated for each patient as per previous publication[19]. For the AML08 cohort, genomic DNA was genotyped using the Illumina Omni 2.5M Exome Beadchip (Illumina, San Diego, CA, USA) at Hussman Institute for Human Genetics, University of Miami, Miami, FL, USA. Genotype calling was performed using Illumina's Genome Studio software V2011.1 (Illumina, San Diego, CA, USA). Six of the ten ACS10 SNPs were typed on the illumina 2.5 Omni array and two were genotyped using Taqman allelic discrimination assay using QuantStudio 5 (Applied Biosystems, Foster City, CA). Two SNPs were not represented on the array, therefore genotype data for SNPs that occurred in high linkage disequilibrium was used to derive the score (details of the SNPs included to build ACS10 score in AML08 cohort is provided in Supplementary Table 2).

Statistical Analysis: MRD1, EFS, and OS were evaluated for association with ACS10 score across the three treatment regimens in the AML02 and AML08 trials. EFS and OS probabilities for ACS10 groups within treatment arms or for treatment arms within a ACS10 groups were estimated using the Kaplan-Meier method. Cox proportional hazard regression models were used to evaluate the associations of ACS10 with EFS and OS and logistic regression models were used for associations with MRD1. The Wilcoxon rank-sum test and Kruskal-Wallis test were used to compare medians of numeric variables across groups and Chi-square tests and Fisher's exact test were used to evaluate the association among pairs of categorical variables. Significance levels for associations of ACS10 score with clinical outcome were set at $P<0.05$. Multivariable Cox proportional hazard models were utilized for association with EFS and OS that included ACS10 score groups, initial risk group assignment, MRD1, ancestry, WBC count at diagnosis and age as other study covariates. The 95% confidence interval (CI) of hazard ratios (HR) was calculated to quantitatively measure the effect on clinical outcome. All statistical analyses were performed using the statistical computing environment R (www.r-project.org). Survival analyses were performed using survival and survminer packages in R4.1.0. Resampling methods were used to evaluate the statistical reproducibility and significance of the best ASC10-score group-induction I

regimen pair. The statistical reproducibility was evaluated by repeating calculations for each of a collection of 10,000 bootstrap data sets obtained by resampling patients with replacement from each treatment group separately. The statistical significance of these results was evaluated by repeating these calculations for each of a collection of 10,000 permuted data sets obtained by shuffling the assignment of outcome (EFS, OS) to ACS10 score group within each treatment group separately.

Results

Figure 1. shows the study schema with information on the three induction I regimens between the two clinical trials. Consistent with the overall clinical trials results[25,26], no difference in EFS and OS was observed by treatment arm or by clinical trial for patients included in the present evaluation (survival curves embedded in Figure 1 and Supplementary Figure 1). Thus, subsequent analyses by ACS10 scores were performed within the 3 treatment arms within the two trials among 390 evaluable patients: i) AML02-LDAC arm (n=91); ii) AML02+AML08-HDAC arm (n=194) and iii) AML08-Clo/Ara-C arm (n=105). ACS10 score distribution across AML02 and AML08 trials was similar (frequency distribution histogram Figure 1). Except for ancestry, no significant differences in the patient characteristics between the two ACS10 score groups were observed (Table 1).

Among the low ACS10 group, Clo/Ara-C based augmented induction I regimen demonstrated significant survival advantage:

Within the low-ACS10 score group (score ≤0), treatment with Clo/Ara-C was associated with significant improvement as compared to standard LDAC induction in EFS (HR=0.45, 95% CI, 0.23–0.88, p=0.020; Figure 2A) and OS (HR=0.44, 95% CI, 0.19–0.99, p=0.048; Figure 2B). Consistent with our previous report, within the expanded HDAC treatment group improvement in EFS was observed as compared to the reference LDAC arm in patients with low ACS10 score though it did not reach statistical significance (EFS, HDAC vs. LDAC; HR=0.58, 95% CI, 0.34–1.01, p=0.055; OS, HR=0.62, 95% CI, 0.32–1.18, p=0.15). Incidence of relapse or resistance disease at 5 year was also higher in patients on standard LDAC arm (45%) as compared to HDAC (25%) or Clo/Ara-C (30%) induction arm (HDAC vs LDAC: p=0.03 Clo/Ara-C vs. LDAC p=0.14, Supplementary Figure 2). Overall, among the ACS10 low score group, Clo/Ara-C demonstrated the most favorable outcome, while LDAC was associated with least favorable outcome (Figure 2).

Among high ACS10 group, augmented Clo/Ara-C or HDAC was not an optimal induction I regimens:

Within the high ACS10 score group, significantly worse EFS and OS were observed in the Clo/Ara-C arm as compared to the LDAC arm (EFS, HR=1.95, 95% CI: 1.05–3.63, p=0.035; OS HR=2.17, 95%CI: 1.05–4.49; p=0.037, Figure 2C and 2D). Similarly, outcome of patients treated within the HDAC arm were inferior as compared to those treated with LDAC (EFS: HR=1.72, 95% CI, 0.97–3.07, p=0.064; OS: HR=2.10, 95% CI, 0.96–4.59, p=0.063, Figure 2C and 2D, respectively). It is important to note that these results are unlikely to suffer from a historical comparison bias as the outcomes of the patients between randomly assigned arms of the AML02 and AML08 or the trials were similar (Figure 1 and

Supplementary Figure 1). Though limited by numbers, non-leukemia associated deaths were observed to be higher in augmented arm as compared to LDAC (at 5yrs HDAC: 11.1%, Clo/Ara-C: 7.7% and LDAC: 1.9%, Supplementary Figure 3).

Multivariable analysis of outcomes in low or high ACS10 score groups by treatment arms:

In multivariable Cox-proportional hazard models including age, WBC count, diagnostic risk group, ancestry and MRD1 as predictor variables, within the low ACS10 score group, Clo/Ara-C arm remained a significantly better treatment option, followed by HDAC as compared to the reference LDAC as an induction I regimen, EFS: Clo/Ara-C vs. LDAC; HR = 0.38; 95% CI, 0.19 to 0.75; p=0.006; HDAC vs. LDAC, HR = 0.52; 95% CI, 0.29 to 0.92; p=0.026 (Figure 3A) and OS: Clo/Ara-C vs. LDAC, HR = 0.37 95% CI, 0.15 to 0.87; p=0.023; HDAC vs. LDAC, HR = 0.51; 95% CI, 0.25 to 1.03; p=0.061 (Figure 3B).

Within the high ACS10 score group, adjusting for age, WBC count, diagnostic risk group, ancestry and MRD1, Clo/Ara-C and HDAC arms remained poor treatment options as compared to standard LDAC induction I regimen (EFS: Clo/Ara-C vs. LDAC, HR=1.94; 95% CI, 1.02 to 3.72; p=0.045; HDAC vs. LDAC, HR=2.44; 95% CI, 1.32 to 4.50; p=0.004, Figure 3C) and (OS: Clo/Ara-C vs. LDAC, HR=1.88; 95% CI, 0.84 to 4.22; p=0.124; HDAC vs. LDAC, HR = 2.75; 95% CI, 1.30 to 5.82; p=0.008, Figure 3D). Overall, within the high ACS10 score group, LDAC remained the best treatment option followed by Clo/Ara-C or HDAC after adjusting for other known prognostic/confounding variables.

ACS10 as a single predictor of EFS and OS for each treatment arm:

Using ACS10 score as a continuous variable, we observed that five-year EFS and OS improved with increasing ACS10 score for patients treated on the LDAC arm but not for those treated with HDAC and Clo/Ara-C (Figure 4). In single-predictor Cox models of EFS, each unit decrease of ACS10 score significantly impacted the event rate by a factor of HR=1.41 (95%CI: 1.16 to 1.72; p=0.0007) and in models of OS each unit decrease of ACS10 impacted the death rate by a factor of HR=1.33 (95% CI: 1.07 to 1.64; p = 0.008) among 91 patients given LDAC. Similar analysis within HDAC and Clo/Ara-C arms demonstrated that each unit decrease in ACS10 score did not impact EFS and OS (EFS, HR=1.03; 95% CI: 0.90 to 1.17; p=0.68; OS, HR=1.04; 95% CI: 0.89 to 1.21; p=0.58 among 194 patients given HDAC, and EFS, HR=0.91, 95% CI: 0.74 to 1.11; p = 0.35; HR=0.95, 95% CI: 0.74 to 1.21; p=0.67 among 105 patients given Clo/Ara-C, Figure 4). These predictions show potential benefit to patients by using ACS10 score to personalize therapy assignment. Patients with lower scores have the best outcomes with Clo/Ara-C while patients with higher scores have the best outcomes with LDAC.

ACS10-Level Based Benefit of Personalization—Among 154 patients in low-ACS10 score group, we observed more than 10-percentage point improvement in 5-year EFS and OS with augmentation with Clo/Ara-C (EFS 64.14% and OS 78.9%) or HDAC (EFS, 60.22% and OS, 71.67%) as compared to standard LDAC based induction I (EFS, 42.1% and OS, 57.9 %). Among 236 patients with high-ACS10 score group, 5-year EFS and OS was improved with LDAC (EFS, 71.24% and OS, 82.9 %) as compared to Clo/Ara-C (EFS, 53.4% and OS, 66.7%) or HDAC treatment (EFS, 56.9 % and OS, 66.7%) Supplementary

Table 3. These results suggest that 5-year EFS and OS for pediatric AML can be improved with matching the best induction regimen after consideration of patients' ACS10 score group.

We further used resampling methods as described in the statistical methods to evaluate the statistical reproducibility and significance of these results. Overall, with two ACS10 score groups and three nucleoside-based induction I treatment options (LDAC, HDAC and Clo-Ara-C), there are nine possible treatment assignment strategies: each of the three treatments may be given to each of the two ACS10 score groups. We used the existing data to estimate the cohort-level 5-year EFS and OS for each strategy, followed by bootstrap to estimate the probability that each is best (Supplementary Table 4). For the whole cohort, assignment of LDAC to high ACS10 score patients and Clo/Ara-C to low ACS10 score patients gave the best 5-year EFS in 6,235 of the 10,000-bootstrap data-sets and best 5-year OS in 7,645 of the 10,000-bootstrap data-sets. Across the 10,000 bootstrap data sets, the mean improvement in 5-year EFS was 0.066 (95% bootstrap CI: 0–0.14) and 5-year OS was 0.065 (95% bootstrap CI: 0–0.13). This data strongly recommends that LDAC is best for high-ACS10 score patients (top two options both give LDAC to high-score patients with total probability of ~80–90% of being best, Supplementary Table 4).) and that Clo/Ara-C is the best option for patients within low-ACS10 score group (selected as the best option with highest probability of ~76%). Population estimates for 5-year EFS for pediatric AML patients improves from 59.07% when given LDAC irrespective of ACS10 score to 68.43% when personalizing LDAC for patients with high ACS10 score and Clo/Ara-C to low ACS10 score patients. Similarly, 5-year OS for pediatric AML patients may be improved from 73% when given LDAC irrespective of ACS10 score to 81.3% when personalizing LDAC for patients with high ACS10 score and Clo/Ara-C to low ACS10 score patients. Figure 5. shows the personalization model for selecting the most effective induction I regimen based on ACS10 score in pediatric AML patients that can result in overall population level benefit of 9.46% (as compared to 1.4% benefit by chance determined by permutation analysis, p=0.0013, Figure 5A) for 5-year EFS and 8.9% for 5- year OS (as compared to 1.5% benefit by chance, p=0.0017, Figure 5B).

Discussion

Cytarabine has been the backbone of AML treatment for more than five decades and has been given to patients at different dosages/regimens without consideration of patient's pharmacogenomics. We recently reported a comprehensive cytarabine pharmacogenomic score composed of 10 SNPs (ACS10) that is reflective of each patient's ability to activate cytarabine to its active metabolite ara-CTP[19]. Low ACS10 scores (≤0) implies reduced ability to activate to ara-CTP and high ACS10 (>0) efficient cytarabine activation. In the AML02 cohort, patients with low ACS10 scores had poor outcomes when given LDAC-based induction, however an improvement was observed when HDAC is given during induction I. It should be noted that all patients received HDAC in consolidation. These findings suggest that therapy augmentation by increasing cytarabine dose during remission induction I, can potentially overcome reduced efficacy due to pharmacogenomics driven inefficiency in drug activation despite subsequent therapies involving HDAC. Other nucleoside analogs such as clofarabine have been used in AML and have shown improved

outcome when given in combination with cytarabine in adult AML[27,28]. In pediatric AML, clofarabine given with ara-C during remission induction was well-tolerated in the AML08 trial[25]. Although statistically significant differences in outcome were not observed between treatment arms, the study demonstrated that reducing the cumulative anthracycline dose in the Clo/Ara-C arm was feasible without compromising efficacy and has the potential to reduce the risk of cardiotoxicity in pediatric AML[25]. As clofarabine can potentiate cytarabine activation by inhibiting ribonucleotide reductases, it seems to be a reasonable option to overcome pharmacogenomics-based impact on the efficacy of cytarabine. In the present study, we show that ACS10 has a significant impact on treatment outcome based on the three different nucleoside analog-based induction I regimens (LDAC, HDAC and Clo/Ara-C) given across two clinical trials AML02 and AML08. Patients with low ACS10 scores had significantly worse EFS and OS when treated with LDAC. However, their outcome was improved significantly when treated with Clo/Ara-C, while those who received intensification to HDAC achieved intermediate outcomes. It should be noted clofarabine inhibits ribonucleotide reductase and that 2 SNPs within ACS10 are in ribonucleotide reductases (one each in RRM1 and RRM2). Though the functional characterization of clofarabine chemosensitivity with respect to these SNPs requires further investigation, rs11030918 has been associated higher ara-CTP levels previously[20]. The results presented here suggest that the best induction I regimen for low-ACS10 group is Clo/Ara-C and that these patients should not be treated with LDAC. With respect to HDAC, though improved response as compared to LDAC arm was observed in low-ACS10 group, implying it as an alternative option but not as optimal as Clo/Ara-C, leaving it upto clinician to make the decision by considering other factors. It should be however noted that cost of treatment with ara-C is more economical than use of clofarabine and may impact the choice of induction regimen. In contrast, for patients with high ACS10 score the best induction regimen is LDAC, with Clo/Ara-C and HDAC showing inferior outcome. This evidence suggests that patients' efficient in activating nucleoside analogs within intensified regimens might be contributing to poor outcome, which may be attributed to high toxicity, although this remains to be investigated.

We further show the population level benefit using resampling methods. The most optimal model pairing of high ACS10 score with LDAC and low ACS10 score with Clo/Ara-C resulted in approximately 9% improvement in outcome as compared to a non-personalized approach. This improvement is higher than those historically observed in any randomized clinical trial for AML, raising a question if the clinical trials were to be designed taking into account the current discovery rather than being randomized, we might have observed significant improvements in outcome based on the induction 1 regimens. Based on these results we propose a pharmacogenomics-guided personalized induction I regimen for improving AML treatment.

It should be noted that we observed racial differences with a significantly greater abundance of low ACS10 score group in black patients (70%), as compared to white patients (30%). This observation holds significant clinical value as black patients have historically shown poor outcome as compared to white patients[29–31]. A comprehensive evaluation of racial differences in pediatric AML within AML02 and AML08 trials along with its interaction with ACS10 score distribution by race and its impact on outcome will be reported separately

(Lamba et al JAMA network in Press). However as anticipated given the higher abundance of low-ACS10 in Black significantly better outcome was observed with Clo/Ara-C induction arm as compared to LDAC arm suggesting racial differences in outcome could in part be mitigated by use of Clo/Ara-C based induction regimen (meeting abstract[32]).

In conclusion, this study highlights the potential for pharmacogenomics based-ACS10 score for personalizing remission induction regimen to enhanced efficacy in AML patients. The results of the current study also warrant the need to expand pharmacogenomics studies to incorporate SNPs associated with anthracycline and etoposide, this expansion may further improve the PGx based predictive power by developing a more comprehensive scoring system. The data presented strongly suggest that low-dose cytarabine-based induction is the most beneficial regimen for patients with high ACS10 scores but is not an optimal option for the low ACS10-score group. For patients with low ACS10 scores, we recommend that Clo/Ara-C is the most-effective induction regimen. Additionally, given that these are germline polymorphisms the impact of the pharmacogenomics score should be applicable to adult AML and our results highlights the need to evaluate these in adult AML.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability statement:

Data is available upon request from the corresponding author: jatinderklamba@ufl.edu.

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Translational Statement

Cytarabine based regimens have been the mainstay of AML therapy for more than five decades. Clinical trials that randomly assign patients to receive standard combinations of cytarabine and anthracyclines with or without an additional agent have generally failed to show improvements in survival. Recently reported pharmacogenetics based 10-SNP score (ACS10) predicts poor prognosis in patients treated with standard low-dose cytarabine. Here, we show that augmentation with clofarabine is an effective option to improve the outcomes patients with low ACS10. At population level, ACS10 based personalization can improve long-term event-free survival by 9%, which is significantly greater than those observed in traditional randomized trials. Our findings suggest that tailoring induction regimens using ACS10 scores can significantly improve outcome in patients with AML and has a potential to be incorporated in upcoming trials.

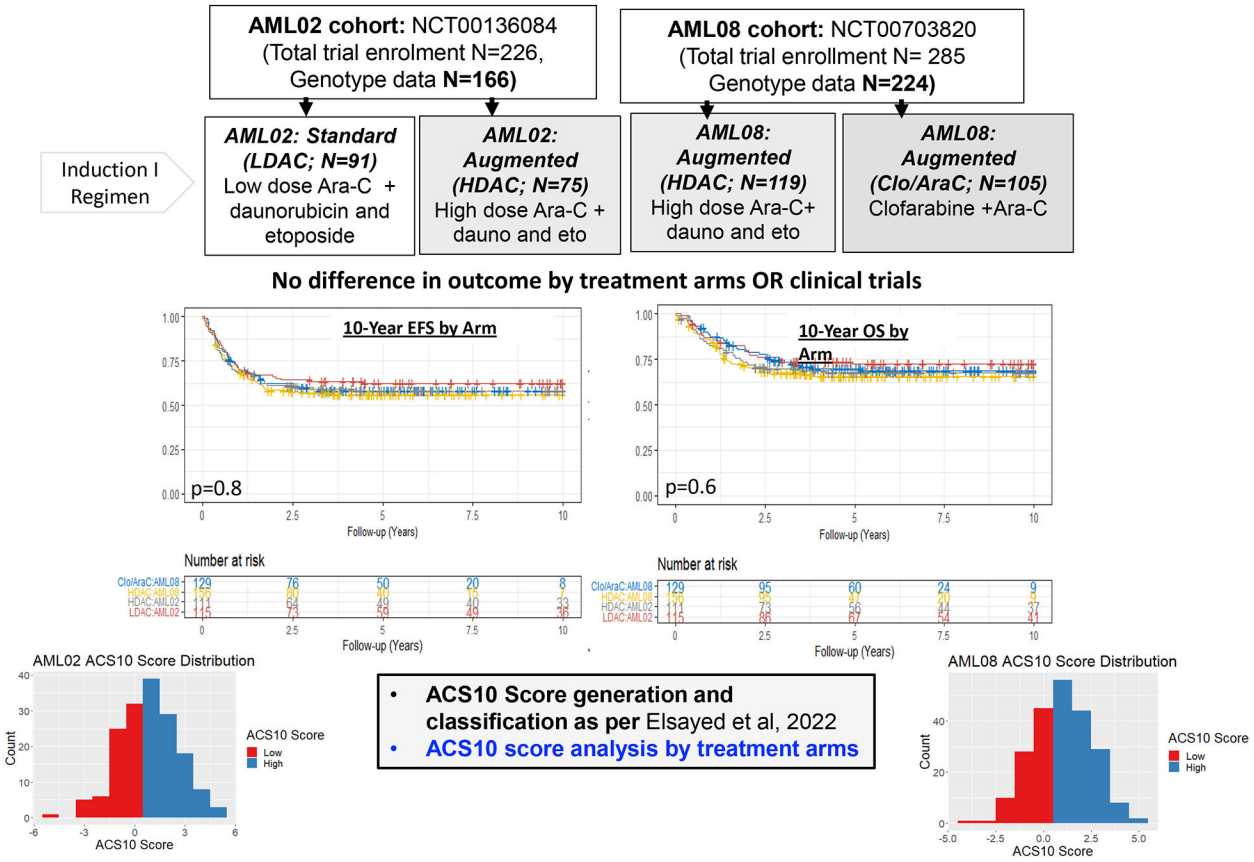

FIG 1. Overall study design.

Abbreviations: EFS, event-free survival; LDAC: Low dose ara-C HDAC, high-dose ara-C; EFS: event free Survival; OS, overall survival; ACS10, ara-C pharmacogenomic 10-SNP score.

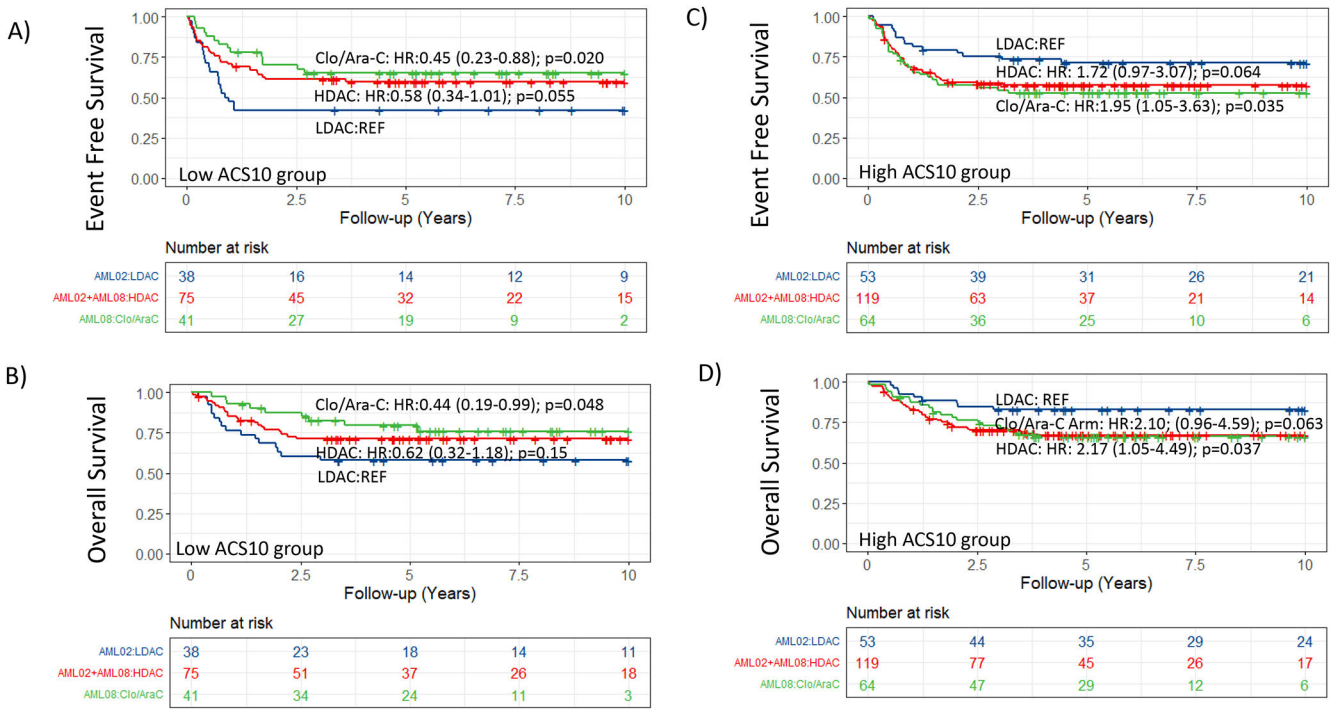

FIG 2. Patient outcomes by composite ACS10 score groups within three treatment arms in patients enrolled on AML02 and AML08 cohorts:

(A) EFS and (B) OS by 3 treatment arms within low ACS10 score group; (C) EFS and (D) OS by treatment arms within High ACS10 score group; EFS, event-free survival; OS, overall survival; HR, hazard ratio; LDAC: Low dose ara-C regimen; HDAC: High dose ara-C regimen; Clo/Ara-C: clofarabine and Ara-C combination regimen.

A) EFS for **Low ACS10 group**: LDAC vs. HDAC vs. Clo/Ara-C

Variable		N	Hazard ratio	p
ARM_COMB	LDAC	36	Reference	
	HDAC	69	0.52 (0.29, 0.92)	0.026
	Clo/AraC	37	0.38 (0.19, 0.75)	0.006
Age	<10 (Years)	78	Reference	
	>= 10 (Years)	64	1.07 (0.64, 1.81)	0.787
Risk_Group	Low	42	Reference	
	Standard	63	3.20 (1.35, 7.59)	0.008
	High	37	3.68 (1.44, 9.42)	0.007
WBC.bnry	<30 (G/L)	81	Reference	
	>= 30 (G/L)	61	1.19 (0.71, 1.98)	0.511
Race	White	79	Reference	
	Black	46	0.94 (0.53, 1.66)	0.822
	Other	17	0.70 (0.30, 1.61)	0.403
MRD1	Negative	78	Reference	
	Positive	64	2.22 (1.25, 3.96)	0.007

C) EFS for **High ACS10 group**: LDAC vs. HDAC vs. Clo/Ara-C

Variable		N	Hazard ratio	p
ARM_COMB	LDAC	51	Reference	
	HDAC	111	2.44 (1.32, 4.50)	0.004
	Clo/AraC	60	1.94 (1.02, 3.72)	0.045
Age	<10 (Years)	106	Reference	
	>= 10 (Years)	116	1.23 (0.79, 1.90)	0.358
Risk_Group	Low	62	Reference	
	Standard	116	2.45 (1.29, 4.66)	0.006
	High	44	3.20 (1.53, 6.69)	0.002
WBC.bnry	<30 (G/L)	116	Reference	
	>= 30 (G/L)	106	1.34 (0.86, 2.06)	0.192
Race	White	178	Reference	
	Black	18	0.96 (0.41, 2.23)	0.929
	Other	26	0.51 (0.24, 1.06)	0.072
MRD1	Negative	138	Reference	
	Positive	84	2.81 (1.76, 4.47)	<0.001

B) OS for **Low ACS10 group**: LDAC vs. HDAC vs. Clo/Ara-C

Variable		N	Hazard ratio	p
ARM_COMB	LDAC	36	Reference	
	HDAC	69	0.51 (0.25, 1.03)	0.061
	Clo/AraC	37	0.37 (0.15, 0.87)	0.023
Age	<10 (Years)	78	Reference	
	>= 10 (Years)	64	1.40 (0.73, 2.68)	0.311
Risk_Group	Low	42	Reference	
	Standard	63	4.99 (1.39, 17.96)	0.014
	High	37	7.30 (1.92, 27.74)	0.004
WBC.bnry	<30 (G/L)	81	Reference	
	>= 30 (G/L)	61	1.01 (0.55, 1.87)	0.967
Race	White	79	Reference	
	Black	46	1.39 (0.72, 2.68)	0.327
	Other	17	0.45 (0.13, 1.57)	0.208
MRD1	Negative	78	Reference	
	Positive	64	1.51 (0.74, 3.06)	0.253

D) OS for **High ACS10 group**: LDAC vs. HDAC vs. Clo/Ara-C

Variable		N	Hazard ratio	p
ARM_COMB	LDAC	51	Reference	
	HDAC	111	2.75 (1.30, 5.82)	0.008
	Clo/AraC	60	1.88 (0.84, 4.22)	0.124
Age	<10 (Years)	106	Reference	
	>= 10 (Years)	116	1.45 (0.87, 2.42)	0.154
Risk_Group	Low	62	Reference	
	Standard	116	4.97 (1.75, 14.17)	0.003
	High	44	6.11 (1.98, 18.91)	0.002
WBC.bnry	<30 (G/L)	116	Reference	
	>= 30 (G/L)	106	1.39 (0.83, 2.34)	0.210
Race	White	178	Reference	
	Black	18	1.41 (0.59, 3.35)	0.434
	Other	26	0.61 (0.26, 1.43)	0.256
MRD1	Negative	138	Reference	
	Positive	84	2.89 (1.65, 5.06)	<0.001

FIG 3. Forest plots of multivariable Cox proportional hazard models within low and high ACS10 groups by the three treatment arms with inclusion of age, risk-group assignment, WBC, ancestry and MRD1.

(A) EFS and (B) OS by LDAC, HDAC and Clo/Ara-C arms within low ACS10 group patients; (C) EFS and (D) OS by LDAC, HDAC and Clo/Ara-C arms within low ACS10 group patients. ACS10, ara-C pharmacogenomic 10-SNP score; EFS, event-free survival; OS, overall survival; HR, hazard ratio; LDAC: Low dose ara-C regimen; HDAC: High dose Ara-C regimen; Clo/Ara-C: clofarabine and Ara-C combination regimen.

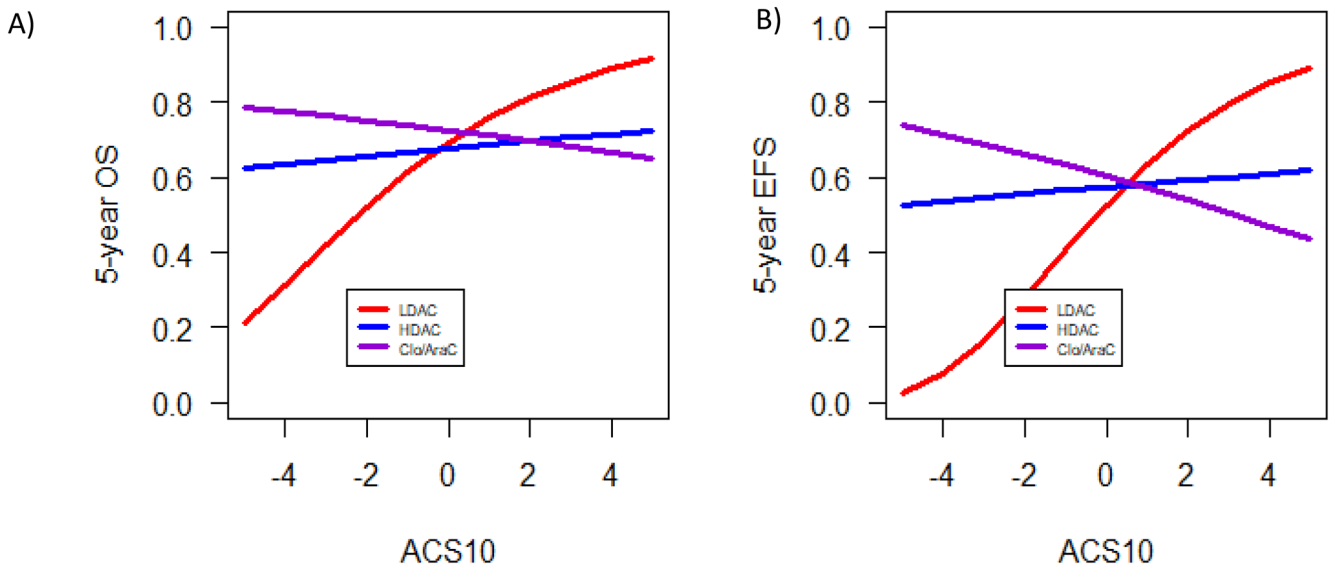

FIG 4. Impact of interaction between numerical ACS10 scores by treatment arms on 5-year OS and EFS.

(A) Five-year OS and (B) 5-year EFS in AML02 LDAC, HDAC and Clo-Ara-C treatment arms by ACS10 scores. ACS10, ara-C pharmacogenomic 10-SNP; EFS, event-free survival; OS, overall survival; HR, hazard ratio; LDAC: Low dose ara-C regimen; HDAC: High dose Ara-C regimen; Clo/Ara-C: clofarabine and Ara-C combination regimen.

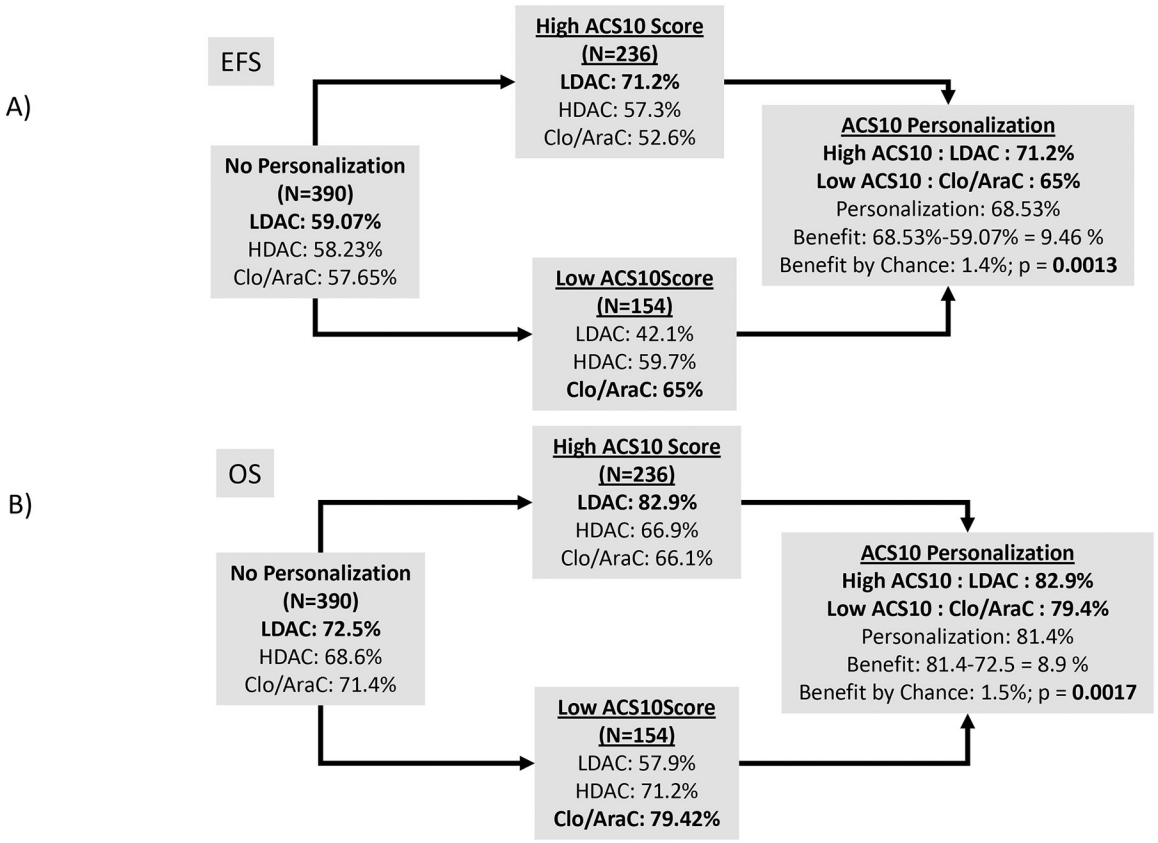

FIG 5.

Personalization model for 5-year EFS (A) and OS (B) based on ACS10 score groups in pediatric AML.

Table 1.

Patient Characteristics by ACS10 Score Groups

Characteristic	High ACS10 (N=236)	Low ACS10 (N=154)	P
Sex			
Female	109	69	0.87
Male	127	85	
Age group			
< 10 yrs	112	86	0.1296
≥ 10 yrs	124	68	
Provisional Risk			
Low	62	43	0.257
Standard	127	71	
High	47	40	
WBC Group, G/L			
< 30	123	89	0.291
≥ 30	112	65	
Unknown	1	0	
Ancestry			
Black	19	51	**1E-05**
Other	25	20	
Unknown	1	1	
White	191	82	
Cohorts			
AML02 (n=166)	97	69	
LDAC Arm	53	38	0.956
HDAC Arm	44	31	
AML08 (n=224)	139	85	
Clo/Ara-C Arm	65	40	0.967
HDAC Arm	74	45	

Note: *P* values < 0.05 indicated in bold

Abbreviations: ACS10, ara-C pharmacogenomic 10-SNP score; Ara-C, cytarabine; Clo, clofarabine; HDAC, high-dose cytarabine and daunorubicin and etoposide; LDAC, low-dose cytarabine and daunorubicin and etoposide; WBC, white blood cell