HLA-DR expression at diagnosis is not predictive of relapse risk post allogeneic hematopoietic stem cell transplantation for pediatric acute myeloid leukemia: a report from the Children's Oncology Group

The therapeutic role of allogeneic hematopoietic stem cell transplantation (allo-HSCT) for patients with relapsed, refractory, or high-risk acute myeloid leukemia (AML) in first remission is well-recognized. It remains the best curative option for these groups of patients. However, relapse after allo-HSCT remains a significant challenge, seen in up to 40-50% of transplant recipients.[1]

Human leukocyte antigen (HLA) class I and II are essential for presenting antigens to $CD8^+$ and $CD4^+$ T cells, respectively. In the context of allo-HSCT, reductions or loss of HLA can enable leukemic cells to evade detection by the donor's immune system, leading to disease recurrence. While natural killer cells can recognize some forms of HLA class I loss, the reduction of class II may be more consequential.[2] Studies have shown that both genetic and non-genetic mechanisms can result in decreased HLA expression, contributing to immune escape and subsequent relapse.[3] For instance, downregulation of HLA class II molecules, such as HLA-DR, is associated with relapse in adult AML patients after allo-HSCT.[4,5]

HLA-DR is expressed in 80-90% of newly diagnosed AML, although expression levels are variable. Based on these prior findings indicating an association of HLA-DR expression in AML with relapse risk, we hypothesized that the magnitude of HLA-DR expression at initial diagnosis of AML would be inversely associated with the risk of relapse in pediatric patients receiving an allo-HSCT for AML in first remission. To test this hypothesis, we analyzed combined data for pediatric patients receiving HSCT in first complete remission on two consecutive frontline pediatric AML trials (AAML0531 [ClinicalTrials.gov identifier: NCT00372593] and AAML1031 [ClinicalTrials.gov identifier: NCT01371981]) conducted by the Children's Oncology Group (COG).[6-8] Each trial protocol was approved by the National Cancer Institute's central institutional review board and the local institutional review board for each participating institution. Informed consent was provided by patients or families.

Data on clinical outcomes for eligible patients who received a per protocol therapy transplant on these two trials, AAML0531 and AAML1031, were analyzed as of March 31, 2020, or March 31, 2023, respectively. We identified 235 patients who received an allo-HSCT in first complete remission as part of their per protocol therapy for AML and had available mean fluorescent intensity (MFI) data. Relevant information on demographics, diagnostic cytogenetics and molecular abnormalities, risk group (as per AAML1031), minimal residual disease (MRD) status at end-of-induction therapy, and HSCT data, including outcomes (relapse-free survival and overall survival) was available for all patients. Data on HLA-DR expression density on leukemia blasts, as quantitated by MFI, was extracted from testing performed at the time of diagnosis. Subsequently, log-MFI-based quantitative HLA-DR expression was divided into quartiles, with quartile 1 (Q1) representing the lowest MFI and quartile 4 (Q4) the highest.

Based on our hypothesis of the potential adverse impact of low HLA-DR expression on post-HSCT outcomes, we compared data for patients demonstrating the lowest MFI at diagnosis (Q1) with those demonstrating higher (Q2-Q4) HLA-DR expression. The median (range) follow-up time for patients alive at last contact from transplant was 7.3 (0.5-12.7) years. The statistical significance of the observed difference in proportions was tested by the Pearson χ^2 or Fisher exact test when data were sparse. The Mann-Whitney test was used to compare medians. The Kaplan-Meier method was used to estimate 5-year overall survival and relapse-free survival.[9] Methods that account for competing events were used to estimate relapse risk and transplant-related mortality. Data were analyzed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Sixty patients were classified as Q1 and a total of 175 were included in the combined Q2-Q4 group based on log-MFI HLA-DR expression. The baseline characteristics for the two groups are compared in Table 1 with statistical analyses excluding patients for whom the relevant data were unknown or not available. As expected in this cohort of patients receiving an allo-HSCT in first complete remission, known high-risk cytogenetic or molecular variants, as previously defined, were identified in a significant number (96 with *FLT3*-ITD, 12 with monosomy 7, 7 with monosomy 5).[6,7] The rate of *FLT3*-ITD positivity was higher in patients in Q2-Q4 than in those in Q1 (44.8% *vs*. 30%; P=0.044). Q1 had a higher proportion of young children (<2 years) as compared to Q2-Q4 (26.7% *vs*. 9.1%; P<0.001). Congruently, the median age (in years) at diagnosis of AML was significantly lower (P=0.003) for those in Q1 (7.1; range, 0.06-18.8) than for those in Q2-Q4 (11.4; range, 0.2-23.6). Response to therapy assessed by end-of-induction course

1 (EOI1) MRD showed a higher rate of MRD positivity in Q1 than in Q2-Q4 (70.2% *vs*. 51.3%; *P*=0.014). Pre-HSCT MRD was available for 137 patients across the four quartiles and demonstrated no statistically significant difference between the two groups (Q1 *vs*. Q2-Q4). These results are summarized in Table 1.

There were no statistically significant differences in HSCT outcomes between patients in Q1 (N=60) and those in Q2-Q4 (N=175) in terms of 5-year relapse risk (38.6%±12.8% *vs*. 33.2%±7.3%; *P*=0.340), 5-year overall survival (54.1%±13.1% *vs*. 64.9%±7.3%; *P*=0.175), 5-year relapse-free survival (51.4%±13% *vs*. 55.9%±7.6%; *P*=0.554), and 5-year transplant-related mortality (10%±7.8% *vs*. 10.9%±4.7%; *P*=0.704) (Table 2). Multivariable analysis using a model that adjusted for treatment arm, *FLT3*-ITD positivity, MRD at EOI1, and age at diagnosis did not show any significant differences in overall survival, relapse-free survival, relapse risk, and transplant-related mortality from HSCT for Q1 *versus* Q2-Q4 (Table 3).

This analysis found no prognostic impact of HLA-DR expression at diagnosis on outcomes of allo-HSCT in first complete remission in patients from the recent COG AML trials AAML0531 and AAML1031. Unlike the potentially adverse impact of low HLA-DR expression on AML blasts at relapse after allo-HSCT described in populations of adult patients, there was not an analogous association with the level of HLA-DR expression at diagnosis on post-HSCT outcomes in this pediatric cohort.[4,5] While an approximately 10% difference was noted in 5-year overall survival between the two groups, it was not statistically significant in our analyses. Pertinently, the current analysis was not appropriately powered to detect differences between these groups. The intensity of HSCT conditioning regimens for pediatric AML is predominantly myeloablative, in contrast to the significant use of reduced-intensity conditioning in adult patients. The preferential use of myeloablative conditioning and its potential to better eradicate residual AML may explain the absence of an adverse impact of low HLA-DR expression in our cohort of pediatric patients.

However, we did note an unanticipated association of HLA-DR expression at diagnosis on the probability of achieving MRD negativity at EOI1 in the patients included in this analysis. A higher proportion of patients with the lowest

Table 1. Hematologic, clinical trial enrollment and demographic data correlates for HLA-DR quartiles.

	HLA-DR FITC log-MFI quartiles			
	Q1	Q2	Q3	Q4
Log MFI – median (range)	0.89 (0.46-1.46)	1.75 (1.47-1.95)	2.12 (1.95-2.25)	2.46 (2.26-3.68)

Characteristic	Q1		Q2-Q4		Q1 *vs.* Q2-Q4
	N	%	N	%	*P*
Total N of patients	60	-	175	-	-
Study/arm					
AAML0531: arm A	10	16.7	42	24.0	0.238
AAML0531: arm B	11	18.3	51	29.1	0.101
AAML1031: arm A	10	16.7	26	14.9	0.737
AAML1031: arm B	21	35.0	25	14.3	<0.001
AAML1031: arm C	8	13.3	31	17.7	0.431
Cytogenetic/molecular features*					
Inv(16) positive	0	0	1	0.6	1.000
t(8;21) positive	0	0	0	0	-
Monosomy 7 positive	2	3.4	10	5.8	0.735
Monosomy 5/del(5q) positive	2	3.4	5	2.9	1.000
MLL (9:11)/11q23 positive	9	15.3	37	21.6	0.291
CEBPA positive	0	0	10	5.7	0.069
NPM positive	12	20	22	12.6	0.158
FLT3-ITD positive	18	30	78	44.8	0.044
MRD at EoI**, positive	40	70.2	80	51.3	0.014
Pre-HSCT MRD#, positive	2	6.7	15	14	0.362
Age at diagnosis, years	7.1 (median)	0.06-18.8 (range)	11.4 (median)	0.2-23.6 (range)	0.003

*Data not available/unknown: inv(16), t(8;21), monosomy 7, monosomy 5/del(5q), *MLL* (9;11)/11q23 – one patient in Q1 and four patients in Q2-Q4; *FLT3*-ITD – one patient in Q2-Q4. **Data on minimal residual disease at end of induction not available/unknown for three patients in Q1 and 19 patients in Q2-Q4. #Data on pre-transplant minimal residual disease not available/unknown for 30 patients in Q1 and 68 patients in Q2-Q4. HLA: human leukocyte antigen; FITC: fluorescein isothiocyanate; MFI: mean fluorescent intensity; Q: quartile; N: number; MRD: minimal residual disease; EoI: end of induction; HSCT: hematopoietic stem cell transplant.

Table 2. Outcome measures of hematopoietic stem cell transplant based on HLA-DR expression quartiles.

Outcome measures	HLA-DR FITC log-MFI quartiles				
	Q1		Q2-Q4		Q1 *vs.* Q2-Q4
	N	%±2SE%	N	%±2SE%	*P*
5-year OS from HSCT	60	54.1±13.1	175	58.9±12.7	0.175
5-year RFS from HSCT	60	51.4±13.0	175	49.1±12.9	0.554
5-year RR from HSCT	60	38.6±12.8	175	39.5±12.8	0.340
5-year TRM from HSCT	60	10.0±7.8	175	11.4±8.2	0.704

HLA: human leukocyte antigen; FITC: fluorescein isothiocyanate; MFI: mean fluorescent intensity; Q: quartile; 2SE: two standard errors; OS: overall survival; HSCT: hematopoietic stem cell transplant; RFS: relapse-free survival; RR: relapse risk; TRM: transplant-related mortality.

Table 3. Multivariable analysis for impact of HLA-DR expression quartiles on hematopoietic stem cell transplant outcomes.

HLA-DR FITC log MFI quartiles	OS from HSCT		RFS from HSCT		RR from HSCT		TRM	
	HR	*P*	HR	*P*	HR	*P*	HR	*P*
Q1 N=57	1	-	1	-	1	-	1	-
Q2-Q4 N=156	0.92	0.730	1.18	0.477	1.1	0.694	1.29	0.58

HLA: human leukocyte antigen; FITC: fluorescein isothiocyanate; MFI: mean fluorescent intensity; OS: overall survival; HSCT: hematopoietic stem cell transplant; RFS: relapse-free survival; RR: relapse risk; TRM: transplant-related mortality; Q: quartile.

MFI of HLA-DR at diagnosis (i.e., those in Q1) remained MRD-positive on bone marrow by flow cytometry at EOI1 (Q1: 70.2% *vs*. Q2-Q4: 51.3%). The adverse impact of MRD positivity at EOI on curative outcomes with chemotherapy for pediatric AML is now well described.[10]

While this analysis did not support our initial hypothesis on the association of HLA-DR expression on allo-HSCT outcomes for pediatric AML, the unexpected finding of a statistically significant association of HLA-DR expression with EOI1 MRD status was notable. This should be interpreted cautiously as a proportion of pediatric patients with low HLA-DR expression at diagnosis may have molecular alterations that are now recognized as high-risk, such as the *CBFA2T3-GLIS* and *FUS-ERG* fusions.[11,12] Interestingly, a recent analysis of HLA-DR expression levels at diagnosis in adult AML failed to show any impact on antigenicity or outcomes with chemotherapy.[13] This study also reported no impact of HLA-DR expression on 5-year overall survival and relapse rates after allo-HSCT in their cohort of patients. There are early data supporting further exploration of a therapeutic role for interferon-γ in the setting of low HLA-DR expression, as it induces MHC class II proteins on myeloid cells, including leukemic blasts.[4,5]

Our analysis has inherent limitations due to its retrospective nature, and potentially skewed data due to the inclusion of only those patients from AAML0531 and AAML1031 who proceeded to receive an allo-HSCT, rather than the whole cohort enrolled on both of these studies. An analysis of all diagnostic samples as mentioned above may be considered to further explore our current findings.

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Contributions

JHC, JTH, MRV, KRS, and VC conceived the study. ASG, RA, CAH, TAA, and RBG contributed to provision of patients or study materials, and data collection. TAA and RBG performed the statistical analysis. All the authors contributed to interpreting the analyzed data, and writing, reviewing, and approving the final version of this manuscript.

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Data-sharing statement

The Children's Oncology Group (COG) data-sharing policy describes the release and use of COG individual subject data for use in research projects in accordance with National Clinical Trials Network (NCTN) Program and NCI Community Oncology Research Program (NCORP) guidelines. Only data expressly released from the oversight of the relevant COG Data and Safety Monitoring Committee (DSMC) are available to be shared. Data sharing will ordinarily be considered only after the primary study manuscript is accepted for publication. For phase III studies, individual-level de-identified datasets that would be sufficient to reproduce results provided in a publication containing the primary study analysis can be requested from the NCTN/NCORP Data Archive at *https://nctn-data-archive.nci.nih.gov/*. Data are available to researchers who wish to analyze the data in secondary studies to enhance the public health benefit of the original work and who agree to the terms and conditions of use. For non-phase III studies, data are available following the primary publication. An individual-level de-identified dataset containing the variables analyzed in the primary results paper can be expected to be available upon request. Requests for access to COG protocol research data should be sent to: *datarequest@childrensoncologygroup.org*. Data are available to researchers whose proposed analysis is found by COG to be feasible and of scientific merit and who agree to the terms and conditions of use. For all requests, no other study documents, including the protocol, will be made available and no end date exists for requests. In addition to the above, release of data collected in a clinical trial conducted under a binding collaborative agreement between COG or the NCI Cancer Therapy Evaluation Program (CTEP) and a pharmaceutical/biotechnology company must comply with the data-sharing terms of the binding collaborative/contractual agreement and must receive the proper approvals.

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