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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

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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 availability statement:

The Children's Oncology Group 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 3 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 agree to the terms and conditions of use. For non-phase 3 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 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.

Manuscript:

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 post 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 (CR1) 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 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 CR1 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. Significance of the observed difference in proportions was tested by the Pearson's Chi-squared test or Fisher's 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 was unknown or not available. As expected in this cohort of patients receiving an allo-HSCT in CR1, known high risk (HR) cytogenetic or molecular variants, as previously defined, were identified in a significant number [n = 96 (FLT3/ITD) + 12 (monosomy 7) + 7 (monosomy 5)].[6, 7] The rate of FLT3/ITD positivity was higher in patients in Q2-Q4 in comparison to 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) versus the median 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 as compared to Q2-Q4 (70.2% vs 51.3%; p=0.014). Pre-HSCT MRD was available for 137 patients across the 4 quartiles and demonstrated no statistically significant difference between the two groups (Q1 versus Q2-Q4). These results are summarized in **Table 1**.

There were no statistically significant differences in HSCT outcomes between patients in Q1 (n=60) versus 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 CR1 allo-HSCT outcomes 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 post allo-HSCT described in adult patient populations, 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] Whilst 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 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]

Whilst 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 on 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 allogeneic HSCT in their patient cohort. There is 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 allogeneic HSCT, rather than the whole patient cohort enrolled on both these studies. An analysis of all diagnostic samples as mentioned above may be considered to further explore our current findings.

References:

1. Bejanyan N, Weisdorf DJ, Logan BR, et al. Survival of patients with acute myeloid leukemia relapsing after allogeneic hematopoietic cell transplantation: a center for international blood and marrow transplant research study. Biol Blood Marrow Transplant. 2015;21(3):454-459.
2. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295(5562):2097-2100.
3. Sauerer T, Velázquez GF, Schmid C. Relapse of acute myeloid leukemia after allogeneic stem cell transplantation: immune escape mechanisms and current implications for therapy. Mol Cancer. 2023;22(1):180.
4. Christopher MJ, Petti AA, Rettig MP, et al. Immune Escape of Relapsed AML Cells after Allogeneic Transplantation. N Engl J Med. 2018;379(24):2330-2341.
5. Toffalori C, Zito L, Gambacorta V, et al. Immune signature drives leukemia escape and relapse after hematopoietic cell transplantation. Nat Med. 2019;25(4):603-611.
6. Gamis AS, Alonzo TA, Meshinchi S, et al. Gemtuzumab ozogamicin in children and adolescents with de novo acute myeloid leukemia improves event-free survival by reducing relapse risk: results from the randomized phase III Children’s Oncology Group trial AAML0531. J Clin Oncol. 2014;32(27):3021-3032.
7. Aplenc R, Meshinchi S, Sung L, et al. Bortezomib with standard chemotherapy for children with acute myeloid leukemia does not improve treatment outcomes: a report from the Children's Oncology Group. Haematologica. 2020;105(7):1879-1886.
8. Pollard JA, Alonzo TA, Gerbing R, et al. Sorafenib in combination with standard chemotherapy for children with high allelic ratio FLT3/ITD+ acute myeloid leukemia: A

report from the Children's Oncology Group protocol AAML1031. J Clin Oncol. 2022;40(18):2023-2035.

9. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53(282):457-481.
10. Loken MR, Alonzo TA, Pardo L, et al. Residual disease detected by multidimensional flow cytometry signifies high relapse risk in patients with de novo acute myeloid leukemia: a report from Children's Oncology Group. Blood. 2012;120(8):1581-1588.
11. Eidenschink Brodersen L, Alonzo TA, et al. A recurrent immunophenotype at diagnosis independently identifies high-risk pediatric acute myeloid leukemia: a report from Children's Oncology Group. Leukemia. 2016;30(10):2077-2080.
12. Buteyn NJ, Burke CJ, Smith JL, et al. EZH2-Mediated MHC Class II Silencing Drives Immune Evasion in AML with t(16;21) (*FUS-ERG)*. Blood. 2021;138 (Supplement 1):374.
13. Roerden M, Märklin M, Salih HR, et al. Expression levels of HLA-DR in acute myeloid leukemia: implications for antigenicity and clinical outcome. Leuk Lymphoma. 2021;62(8):1907-1919.

Tables for manuscript

Table 1: Hematologic, clinical trial enrolment 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

		Q1		**Q2 – Q4**		**Q1 vs Q2 – Q4**
Characteristic		**N**	**%**	**N**	**%**	**P-value**
Total number 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) pos**	0	0%	1	0.6%	1.000
	t(8;21) pos	0	0%	0	0%	-
	Monosomy 7 pos	2	3.4%	10	5.8%	0.735
	Monosomy 5 / del(5q) pos	2	3.4%	5	2.9%	1.000
	MLL (9:11) / 11q23 pos	9	15.3%	37	21.6%	0.291
	CEBPA pos	0	0%	10	5.7%	0.069
	NPM pos	12	20%	22	12.6%	0.158
	FLT3/ITD pos	18	30%	78	44.8%	**0.044**
MRD at EoI**	**pos**	40	70.2%	80	51.3%	**0.014**
Pre-HSCT MRD#	**Pos**	2	6.7%	15	14%	0.362
Age at diagnosis in 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 – 1 patient in Q1 and 4 patients in Q2 -Q4; FLT3/ITD – 1 patient in Q2 – Q4.*

***: Data on MRD at EOI not available / unknown for 3 patients in Q1 and 19 patients in Q2 – Q4.*

#: Data on pre-HSCT MRD not available / unknown for 30 patients in Q1 and 68 patients in Q2 – Q4.

FITC – Fluorescein isothiocyanate; HLA – Human leukocyte antigen; MFI – Mean fluorescent intensity; MRD – Minimal residual disease; Q – Quartile

Table 2: HSCT outcome measures based on HLA-DR expression quartiles

	HLA-DR FITC log-MFI quartiles				
	Q1		**Q2 – Q4**		**Q1 vs Q2 – Q4**
	N	**% ± 2SE%**	**N**	**% ± 2SE%**	**P-value**
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

FITC – Fluorescein isothiocyanate; HLA – Human leukocyte antigen; HSCT – Hematopoietic stem cell transplant; MFI – Mean fluorescent intensity; OS – Overall survival; Q – Quartile; RFS – Relapse free survival; RR – Relapse risk; TRM – Transplant related mortality

Table 3: **Multivariable analysis for impact of HLA-DR expression quartiles on HSCT outcomes**

		OS from HSCT		RFS from HSCT		RR from HSCT		TRM	
		HR	P	HR	P	HR	P	HR	P
HLA DR FITC log MFI quartiles									
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

FITC – Fluorescein isothiocyanate; HLA – Human leukocyte antigen; HR – Hazard ratio; HSCT – Hematopoietic stem cell transplant; MFI – Mean fluorescent intensity; OS – Overall survival; P – P-value; Q – Quartile; RFS – Relapse free survival; RR – Relapse risk; TRM – Transplant related mortality