PREDICTED INDIRECT T CELL ALLOREACTIVITY AND OUTCOME FOLLOWING PEDIATRIC HEMATOPOIETIC CELL TRANSPLANTATION

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Aim: For children with high-risk hematological malignancies who lack a suitable HLA matched related or unrelated donor, haploidentical hematopoietic cell transplantation (haploHCT) is a potential curative treatment.

- Incompatibility between recipient and donor may lead to graft versus leukemia (GVL), yet direct T cell allorecognition of HLA allele mismatches (**Figure 1A**) are known risks for graft-versus-host disease (GVHD).
- Identifying and quantifying HLA mismatches (MM) involved in risk of GVHD and optimization of GVL has become an area of renewed interest.
- The **P**redicted **I**ndirectly **ReC**ognizable **H**LA **E**pitopes (**PIRCHE**) algorithm estimates indirect recognition **(Figure 1B)** of unique peptides derived from mismatched HLA that can be presented on the cell surface of shared class I (PIRCHE-T1) and class II (PIRCHE-T2) HLA molecules **(Figure 1C).**

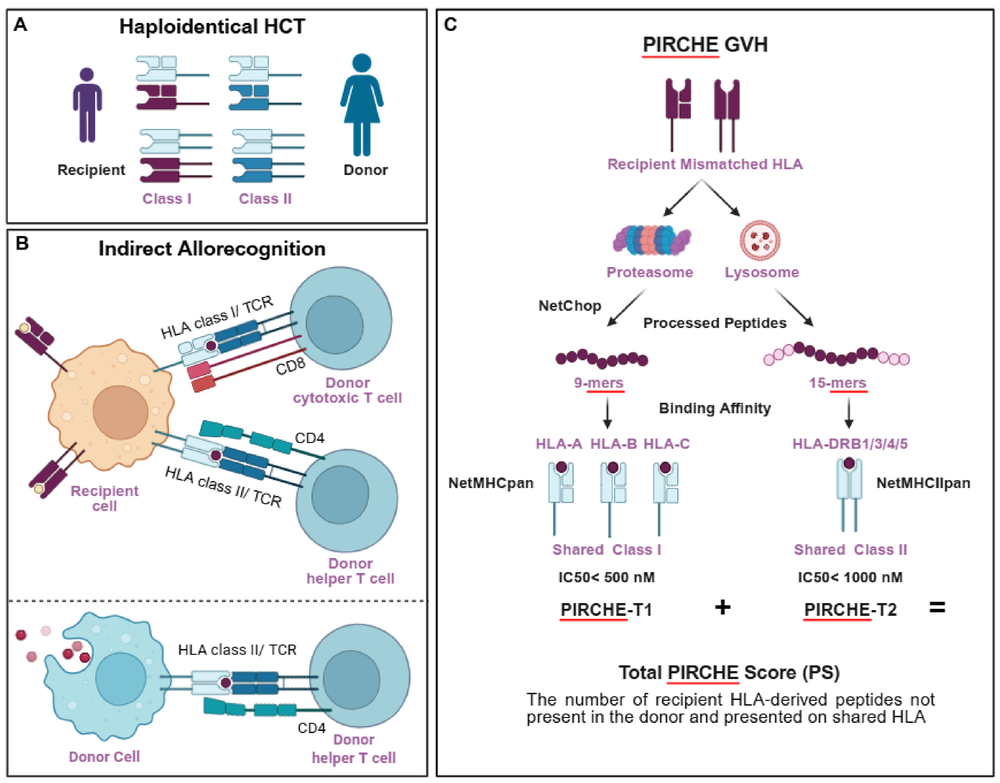
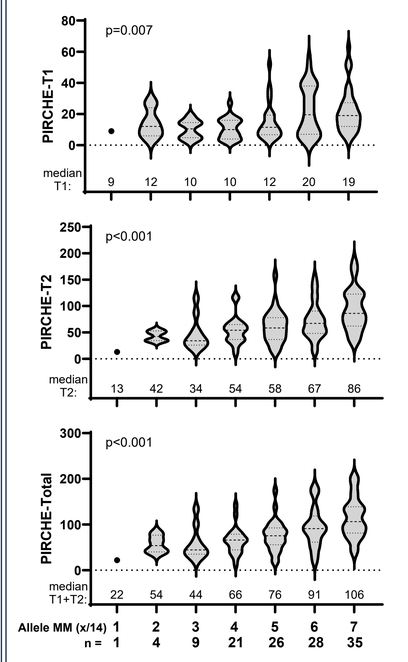


Figure 1. Predicted Indirectly ReCognizable HLA Epitopes (PIRCHE). Traditional allele matching based on direct T cell allorecognition does not quantify the number or immunogenicity of mismatched epitopes. **(A)** As shown in this haploidentical patient/parent pair, **(B)** indirect allorecognition describes T cell recognition of peptides derived from mismatched HLA molecules that are presented by shared HLA. **(C)** The PIRCHE algorithm estimates the number of unique peptides derived from recipient mismatched HLA molecules that can be processed and bound to shared class I HLA-A, B, and C (PIRCHE-T1) and class II HLA-DRB1 and DRB3/4/5 (PIRCHE-T2). The Total PIRCHE Score is the sum of the T1 and T2 scores. Figures created by Biorender.com. HCT, hematopoietic cell transplant; TCR, T cell receptor.

A

Patient and Transplant Characteristics	
Total Transplants	N=128 (100%)
Sex	
Female	65 (51%)
Male	63 (49%)
Age at HCT (years)	9.3 [0.5,9.6,21.6]
Transplant Number	
1	94 (73%)
2	34 (27%)
Donor Relationship	
Mother	64 (50%)
Father	52 (41%)
Sibling/other	12 (9%)
Donor Age (years)	37.9 [12.4,10.8,57.6]
Donor Sex	
Female	72 (56%)
Male	56 (44%)
Total HLA MM GvH (x/14)	
0-4	36 (28%)
5	26 (20%)
6	28 (22%)
7	38 (30%)
PIRCHE-T1 GvH	14.0 [2.0,16.5,63.0]
PIRCHE-T2 GvH	62.5 [10.0,46.5,186.0]
PIRCHE-Total GvH	82.0 [13.0,49.0,203.0]
ATG (Y/N)	
No	24 (19%)
Yes	104 (81%)
Graft T Cell Depletion Status	
TCRαβ+ depleted	65 (51%)
TCRαβ+ depleted; CD45RA+ depleted	39 (30%)
CD34+ enriched; CD45RA+ depleted	24 (19%)
Graft CD3 Cell Dose (x10^6/kg)	52.2 [0.0,96.9,685.4]
Graft CD34 Cell Total Dose (x10^6/kg)	24.2 [4.8,17.5,101.5]
Number of DLI	
0	49 (38%)
1+	79 (62%)
CD3 T Cell Total Dose (DLI)	10.0 [0.0,9.0,29.0]
Acute GvHD (Max Grade)	
None	87 (68%)
I	13 (10%)
II-IV	28 (22%)
Chronic GvHD (Max Grade)	
None	117 (91%)
Mild	4 (3%)
Moderate-Severe	7 (5%)

B



C

PIRCHE Score GVH	mean	median	Min, IQR, Max
T1	17.4	14.0	2.0, 16.5, 63.0
T2	69.9	62.5	10.0, 46.5, 186.0
Total	87.3	82.0	13.0, 49.0, 203.0

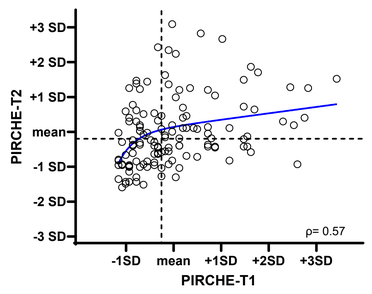


Figure 2. Cohort Recipient/Donor Transplant Pairs. (A) Patient and Transplant Characteristics. Cells represent the median [Min, IQR, Max] for continuous data and frequency (%) for categorical data. **(B)** Comparison of conventional HLA-A, B, C, DRB1, DRB3/4/5, DQB1, and DPB1 (x/14) allele MM to -T1, -T2, and Total PS. **(C)** Cohort -T1, -T2, and Total PS (left panel) and comparison of PS-T1 to PS-T2 in recipient/donor pairs (right panel). Dotted lines indicate median PS. **P**redicted **I**ndirectly **ReC**ognized **H**LA **E**pitope; MM, mismatch; SD, standard deviation, GVH, graft-versus-host; IQR, interquartile range; PS, PIRCHE Score.

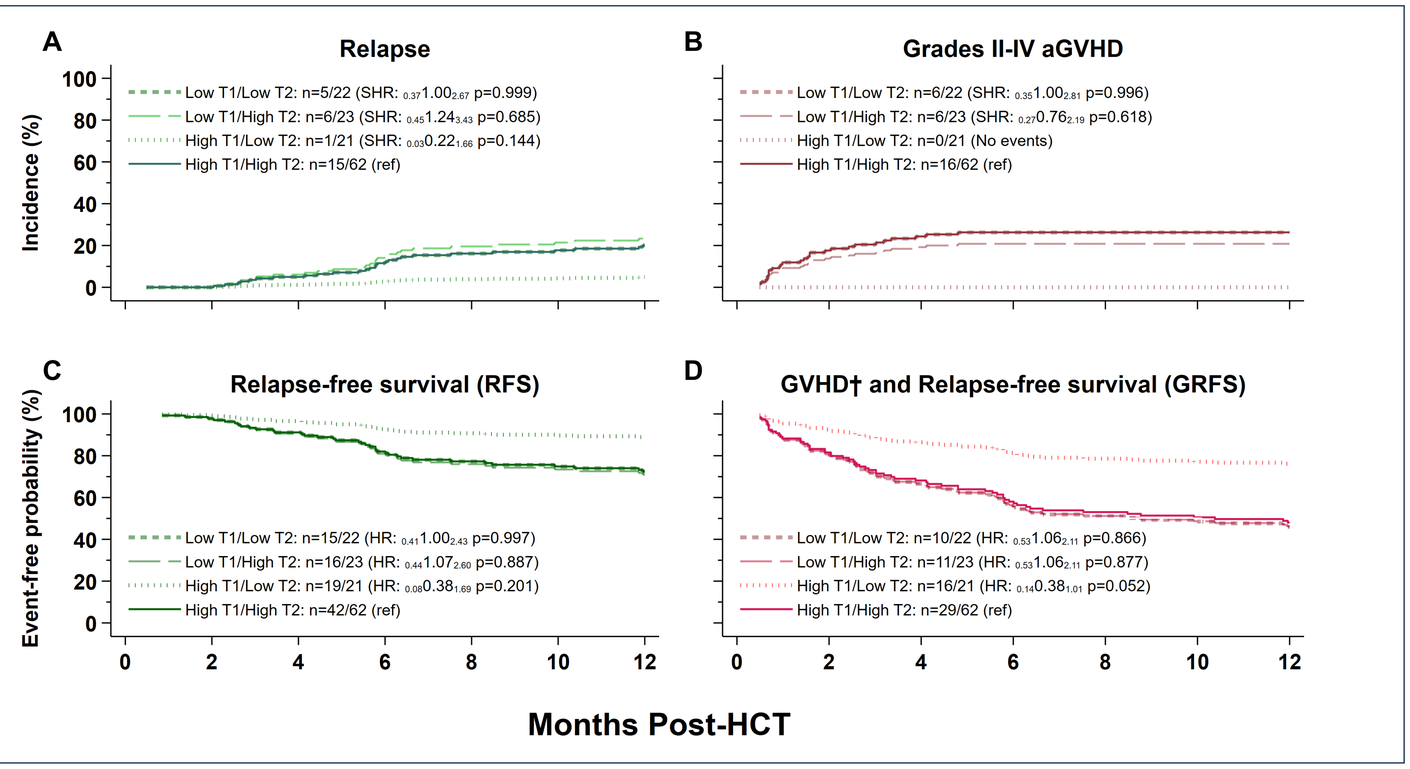


Figure 3. Relapse, aGVHD, and Survival Probabilities Associated with Indirect Allorecognition. (A) Cumulative incidence of relapse and **(B)** Grades II-IV acute GVHD. **(C)** Relapse-free survival (RFS) and **(D)** GVHD and Relapse-free survival (GRFS) in cohort recipient/donor pairs in tandem high/low groupings based on median -T1 and -T2 PIRCHE scores (PS).

Patients/Methods:

- We describe baseline, post-transplant characteristics, and patient outcomes in a retrospective T cell epitope mismatch analysis of 128 haploHCTs performed between 2017-2024 **(Figure 2A)**. All patients received HCT in complete remission using reduced intensity conditioning and a T-cell selected peripheral blood stem cell graft for acute leukemias or myelodysplastic syndrome.
- Allele-level HLA typing was performed by hybrid capture NGS (CareDx). PIRCHE scores were determined using the v4.4 PIRCHE algorithm with netchop 3.1, netMHCpan 2.4, netMHCIIpan 3.0, and IPD-IMGT-HLA database release 3.62 (pirche.com).
- Associations between PIRCHE groupings and outcomes were assessed using Wald tests from the corresponding regression models. Cumulative incidence of relapse (with competing risks for GVHD and death) and acute (a)GVHD (with competing risks for relapse and death) were analyzed by Fine-Gray competing risks models. Relapse-free survival (RFS; time until all-cause mortality [ACM], relapse or censoring at 12-months of follow-up), and GVHD-free, relapse-free survival (GRFS; time until ACM, relapse, aGVHD grade II-IV, chronic (c)GVHD grade moderate-severe, or censoring) were estimated using Cox models. All models adjusted for transplant number, donor and recipient gender, and age at transplant.

Results/Conclusions:

- Recipient/donor HLA MM in the GVH direction based on indirect alloreactivity as calculated by PS-T1, PS-T2 and total PS were highly correlated with HLA GVH allele MM (all p<0.01) **(Figure 2B)**. PS-T1 and PS-T2 were weakly correlated in recipient/donor transplant pairs (r=0.57) **(Figure 2C)**.
- In transplants with high PS-T1/ low PS-T2, incidences of relapse [sub-distribution hazard ratio (SHR)=0.22, p=0.144] **(Figure 3A)** and aGVHD (no events) **(Figure 3B)** were lower and, although not statistically supported, the best outcomes for RFS **(Figure 3C)** and GRFS **(Figure 3D)** were associated with high-T1/low-T2 PIRCHE scores (RFS HR=0.38, p=0.201 and GRFS HR=0.38, p=0.052).
- These data suggest an interplay between indirect T cell recognition of peptide epitope MM presented with HLA class I and II, which may balance GVHD and GVL in T-cell selected haploHCT. The impact of molecular MM algorithms in prediction of indirect T cell alloreactivity following HCT warrants further prospective and larger multi-center studies.

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