Donor selection in a pediatric stem cell transplantation cohort using PIRCHE and HLA-DPB1 typing

Wiebke Stenger1 | Annette Künkele1,2,3,4 | Matthias Niemann5 | Kremena Todorova6 | Axel Pruß6 | Johannes H. Schulte1,3,4 | Angelika Eggert1,2,3,4 | Lena Oevermann1,2,3,4

1Department of Pediatric Oncology and Hematology, Charité – Universitätsmedizin Berlin, Berlin, Germany
2Berlin Institute of Health (BIH), Berlin, Germany
3German Cancer Consortium (DKTK), Berlin, Germany
4German Cancer Research Center (DKFZ), Heidelberg, Germany
5PIRCHE AG, Berlin, Germany
6Center for Transfusion Medicine and Cell Therapies Berlin, Berlin, Germany

Correspondence
Lena Oevermann, Department of Pediatric Oncology and Hematology, Charité – Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany.
Email: lena.oevermann@charite.de

W.S. and A.K. contributed equally to this work.

Abstract

Background: New strategies to optimize donor selection for hematopoietic stem cell transplantation (HSCT) have mainly been evaluated in adults, but the disease spectrum requiring HSCT differs significantly in children and has consequences for the risk of complications, such as graft-versus-host disease (GvHD).

Procedures: Here we evaluated whether HLA-DPB1 and Predicted Indirectly ReCognizable HLA-Epitope (PIRCHE) matching can improve donor selection and minimize risks specific for a pediatric cohort undergoing HSCT in Berlin between 2014 and 2016.

Results: The percentage of HLA-DPB1–mismatched HSCT in the pediatric cohort was in line with the general distribution among matched unrelated donor HSCT. Nonpermissive HLA-DPB1 mismatches were not associated with a higher incidence of GvHD, but the incidence of relapse was higher in patients undergoing HSCT from HLA-DPB1–matched transplantations. High PIRCHE-I scores were associated with a significantly higher risk for developing GvHD in patients undergoing HSCT from nine of ten matched unrelated donors. This finding persisted after including HLA-DPB1 into the PIRCHE analysis.

Conclusions: Implementing PIRCHE typing in the donor selection process for HSCT in children could particularly benefit children with nonmalignant diseases and support further validation of PIRCHE-based donor selection in a larger number of children treated at different sites.

KEYWORDS
donor selection, HLA-DPB1, HLA-typing, nonmalignant diseases, pediatric stem cell transplantation, PIRCHE

1 | INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is predominantly a curative approach in adults for high-risk malignant diseases, but provides the only chance of cure for many children with nonmalignant diseases. Graft-versus-host disease (GvHD), infections, and graft rejection remain the primary risk factors in both pediatric and adult patients. However, although GvHD provides some benefit in cancer patients known as the graft-versus-leukemia effect, it should be strictly avoided in patients with nonmalignant diseases. In acute GvHD, donor T lymphocytes attack recipient tissues to cause end-organ damage mainly affecting the skin, liver, and intestines. Severe stage IV GvHD is associated with a mortality rate of approximately 90%. The main risk factors for acute GvHD known to date are major and minor HLA disparities, preexisting infections, total body irradiation, donor/recipient sex mismatch, and use of peripheral blood stem cells as the graft...
source.\textsuperscript{5,6} Matching HLA-A, -B, -C, -DRB1, and -DQB1 alleles (10/10 match) between patient and donor is currently the gold standard for unrelated donor selection.\textsuperscript{7} However, GvHD occurs even in transplanted patients with 10 of 10 HLA matches. HLA-DPB1 has been added as an additional locus for typing in the donor and recipient. The DP subclass of HLA molecules is represented at ~10-fold lower levels on the cell surface than the DR or DQ subclasses, and their lower levels have prompted a common assumption that they are less important in the immune response.\textsuperscript{8} HLA-DPB1, however, encodes a fully functional molecule with documented specific responses in a number of settings. Based on the existence of shared T-cell epitopes expressed by HLA-DPB1 alleles, HLA-DPB1 alleles were classified according to their predicted immunogenicity into a highly immunogenic group 1 (HLA-DPB1*09:01, *10:01, and *17:01), intermediately immunogenic group 2 (HLA-DPB1 *03:01, *14:01, and *45:01), and poorly immunogenic group 3 (most other HLA-DPB1 alleles).\textsuperscript{9} This TCE algorithm was proposed for use in unrelated donor selection to identify permissive or nonpermissive HLA-DPB1 mismatches.\textsuperscript{10} The importance of HLA-DPB1 genes remains uncertain, and pretransplantation typing is not yet routinely performed. A donor-recipient HLA-DPB1 mismatch was shown to be associated with an increased risk for acute GvHD.\textsuperscript{11} In a different cohort, the incidence of relapse was higher in recipients receiving transplantations from HLA-DPB1–matched donors.\textsuperscript{12} Nevertheless, HLA-DPB1 categorization according to T-cell epitope grouping appears to allow the identification of permissive and nonpermissive mismatches relevant for survival, GvHD, and relapse occurrence.\textsuperscript{12,13}

Another recently developed matching strategy is the Predicted Indirectly ReCognizable HLA Epitopes (PIRCHE) algorithm. PIRCHE predicts the indirect recognition of mismatched HLA peptides by donor T cells that cause alloreactivity after HSCT.\textsuperscript{14} PIRCHE-I epitopes are designated as unique nonameric cores derived from the patient's mismatched HLA proteins (HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1) that do not occur in the self-peptide repertoire of the donor and which bind to matched HLA class I molecules (HLA-A, -B, and -C). PIRCHE-II epitopes, in turn, are designated as unique nonameric cores of non-self 15-mer peptides derived from the patient's mismatched HLA, and are predicted to bind to matched class II molecules (HLA-DRB1). The PIRCHE model predicts the numbers of peptides derived from the mismatched HLA molecules that can be presented on the cell surface of donor-patient shared HLA molecules.\textsuperscript{15–17} Thus, the PIRCHE score equals the number of HLA-derived T-cell epitopes. Higher PIRCHE scores for HLA classes I or II (PIRCHE-I or -II) have been correlated to acute GVHD development in adult patients.\textsuperscript{15,16,18}

Little is known about the impact of donor-recipient matching using HLA-DPB1 and PIRCHE for HSCT in children. The disease spectrum underlying a recommendation for HSCT differs between children and adults. A variety of malignant and nonmalignant diseases occur in children, including hemoglobinopathies, immunodeficiencies, and genetic disorders, which require HSCT as their only chance for cure. These diseases are accompanied by disease-inherent risk factors that do not usually occur in adult patients with leukemia,\textsuperscript{19–21} the primary reason for HSCT in adults. Chronic colitis or autoimmune disorders accompanying the underlying diseases in children raises their risk to develop GvHD. GvHD should also be avoided completely in children undergoing HSCT for nonmalignant diseases, because they receive no benefit from the GvHD accompanying, graft-versus-leukemia effect.\textsuperscript{6,22} How well risk for GvHD can be defined using HLA-DPB1 and PIRCHE to match donors to pediatric recipients remains unknown. Here we retrospectively evaluated HLA-DPB1 matching and PIRCHE matching to select donors in a cohort of 105 children who underwent HSCT in Berlin between January 2014 and December 2016.

## 2 | MATERIALS AND METHODS

### 2.1 Patient cohort

The Department of Pediatric Oncology and Hematology at the Charité – Universitätsmedizin Berlin performed a total of 134 HSCTs in 127 patients between January 2014 and December 2016. Of the 127 patients, five children were transplanted twice and one child three times. Ten patients undergoing haploidentical HSCT from a mismatched related donor and 19 patients receiving autologous grafts were not included in this evaluation. HLA typing was performed for all patients and donor pairs for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1. Donors for the 105 transplanted patients included in the analysis matched for 9 of 10 (n = 20) HLA alleles or 10 of 10 (n = 85) HLA alleles. Median age at HSCT was 7.8 years (range, 0.3-26.3 years) and median follow-up was 1.8 years (range, 0.1-4.3 years) for the 105 patients included in the analysis. The underlying diagnoses, treatment information, and detailed patient characteristics for these 105 patients are presented in Table 1.

### 2.2 HLA typing

All donor-patient pairs were prospectively genotyped for the HLA-A, -B, -C, -DRB1, -DQB1 allelic profiles using sequencing-based typing (Protrans GmbH, Hockenheim, Germany), a previously described method\textsuperscript{23} based on Sanger chain-termination sequencing. HLA-DPB1 typing was retrospectively performed on all donor-patient pairs using the LAB-Type SSO-Kit (One Lambda, Austin, TX, USA). Briefly, target DNA was amplified using PCR and biotinylated HLA group-specific primers, then denaturated and bound to complementary DNA probes conjugated to microspheres. Microspheres were labeled with streptavidin-R-phycocerythrin and flow cytometrically detected.

### 2.3 DPB1 T-cell epitope matching

Classifying HLA-DPB1 mismatches into permissive and nonpermissive T-cell epitope groups was performed using the calculator (DPB1 T-Cell Epitope Algorithm v2.0) provided on the EMBL-EBI Hinxton website (https://www.ebi.ac.uk/ipd/imgt/hla/dpb.html), and implemented as part of the DPB1 T-Cell Epitope tools in collaboration with K. Fleischhauer (University Hospital Essen, Germany) and B. Shaw (CIBMTR, USA).\textsuperscript{9,24}
TABLE 1  Patient characteristics of the pediatric HSCT cohort

| Variable                                      | 9/10 HLA match (20 patients) | 10/10 HLA match (MSD + MUD, 85 patients) |
|-----------------------------------------------|------------------------------|------------------------------------------|
| Median age at HSCT, years (range)             | 8.3 (0.3-17.5)               | 7.7 (0.7-26.3)                           |
| Graft source                                  |                              |                                          |
| PBSC (%)                                      | 2 (10.0)                     | 6 (7.0)                                  |
| Bone marrow (%)                               | 18 (90.0)                    | 78 (91.8)                                |
| Bone marrow + cord blood (%)                  | 0 (0)                        | 1 (1.2)                                  |
| Patient sex                                   |                              |                                          |
| Female (%)                                    | 12 (60.0)                    | 30 (35.3)                                |
| Male (%)                                      | 8 (40.0)                     | 55 (64.7)                                |
| Underlying diagnosis                          |                              |                                          |
| Acute lymphoblastic leukemia (%)              | 6 (30.0)                     | 26 (30.7)                                |
| Acute myeloid leukemia (%)                    | 0 (0)                        | 11 (12.9)                                |
| Lymphoma (%)                                  | 1 (5.0)                      | 4 (4.7)                                  |
| Myelodysplastic syndrome (%)                  | 4 (20.0)                     | 7 (8.2)                                  |
| Fanconi anemia (%)                            | 2 (10.0)                     | 1 (1.2)                                  |
| Immune deficiency (%)                         | 2 (10.0)                     | 11 (12.9)                                |
| Septic granulomatosis (%)                     | 1 (5.0)                      | 8 (9.4)                                  |
| B-Thalassemia (%)                             | 3 (15.0)                     | 1 (1.2)                                  |
| Sickle cell disease (%)                       | 1 (5.0)                      | 11 (12.9)                                |
| X-linked adrenoleukodystrophy (%)             | 0 (0)                        | 5 (5.9)                                  |
| Conditioning regimen                          |                              |                                          |
| Total body irradiation, etoposide (%)         | 3 (15.0)                     | 17 (20.0)                                |
| Busulfan, cyclophosphamid, (melphalan) (%)    | 3 (15.0)                     | 12 (14.1)                                |
| Fludarabin, busulfan, (clofarabin) (%)        | 3 (15.0)                     | 15 (17.6)                                |
| Fludarabin, thiotepa, (melphalan) (%)         | 1 (5.0)                      | 17 (20.0)                                |
| Treosulfan-based (%)                          | 4 (20.0)                     | 11 (12.9)                                |
| Others (%)                                    | 6 (30.0)                     | 13 (15.3)                                |
| GvHD prophylaxis                              |                              |                                          |
| Ciclosporine                                  | 19 (95)                      | 84 (98.8)                                |
| Mycophenolate mofetil                         | 10 (50)                      | 38 (44.7)                                |
| Methotrexate                                  | 14 (70)                      | 43 (50.6)                                |
| Serotherapy                                   |                              |                                          |
| ATG                                           | 14 (70)                      | 53 (62.4)                                |
| Mab Campath                                   | 6 (30)                       | 21 (24.7)                                |
| none                                          | 0 (0)                        | 11 (12.9)                                |
| Donor relationship                            |                              |                                          |
| Related (%)                                   | 1 (5.0)                      | 34 (40.0)                                |
| Unrelated (%)                                 | 19 (95.0)                    | 51 (60.0)                                |
| Sex mismatch                                  |                              |                                          |
| Female to male (%)                            | 2 (10)                       | 15 (17.6)                                |
| Male to female (%)                            | 9 (45)                       | 19 (22.4)                                |

GvHD, graft-versus-host disease; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; MSD, matched sibling donor; MUD, match unrelated donor; PBSC, peripheral blood stem cells.

2.4 | The PIRCHE algorithm

PIRCHÉ epitopes were determined as previously described,15,16,25 and PIRCHÉ scores were calculated using the PIRCHÉ algorithm (version 2.4, available at https://pirche.com), which relies on the netchop predictor for proteasomal cleavage (version 3.1),26 netMHCpan to predict peptide binding to HLA class I molecules (version 2.4)27 and netMHCIIpan to predict peptide binding to HLA class II molecules (version 3.0).28 Two-field HLA typing for patients and donors were translated into amino acid sequences of the mature HLA protein using the IPD-IMGT/HLA database (version 3.24). Incompletely filled sequences were completed using nearest-neighbor extrapolation as previously described.29 PIRCHÉ-I epitopes were considered relevant if the cleavage threshold was ≥0.5 and binding affinity (IC50) was ≤500 nM. PIRCHÉ-II epitopes were considered relevant if the IC50 was ≤1000 nM.

2.5 | Statistical analysis

Data were retrospectively analyzed. The differences in PIRCHÉ scores between patient subgroups (with and without diagnosed acute GvHD) were assessed by Mann-Whitney U test. Pearson chi-square test was used to assess categorical variables such as T-cell epitope permissiveness, HLA-DPB1 disparity, and relapse in patients with and without diagnosed acute GvHD. P values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS Statistics Software version 25.0 (SPSS Inc., Chicago, IL, USA).

2.6 | Ethics

Written informed consent was obtained from all patients before HSCT.

3 | RESULTS

3.1 | Clinical outcomes and GvHD occurrence in the cohort

Successful engraftment was achieved in 96.2% of our pediatric patients, with immediate graft rejections occurring only in 4 of 105 transplantations. After the median follow-up of 1.8 years, 84.6% overall survival was achieved. Relapses occurred in 22.9% (n = 11) of the 59 patients receiving HSCT for underlying malignant diseases. Treatment-related mortality was 9.5% in the total cohort (n = 10), with eight patients succumbing to infectious complications and two to GvHD. The overall incidence of acute GvHD was 66.7% (n = 70/105). Although all patients except one displayed skin GvHD, GvHD affected the liver in eight patients and the gut in eight patients. The incidence of grades II-IV GvHD was higher when donors had one HLA mismatch (P = 0.533; Table 2).

3.2 | GvHD incidence was not raised by nonpermissive HLA-DPB1 mismatches

Additional HLA-DPB1 typing was conducted for the 84 patients who received HSCT from a sibling (n = 34) or unrelated donor (n = 50)
TABLE 2  Incidences of GvHD in the total cohort, in patients transplanted from mismatched unrelated and matched unrelated donor grafts

| Acute GvHD | In total cohort incl. MSD | 10/10 MUD | 9/10 MUD |
|------------|---------------------------|-----------|-----------|
| 0          | 35 (33.3%)                | 57 (81.4%)| 15 (75%)  |
| I          | 47 (44.8%)                |           |           |
| II         | 10 (9.5%)                 | 13 (18.6%)| 5 (25%)   |
| III-IV     | 13 (12.4%)                |           |           |

GvHD, graft-versus-host disease; MUD, match unrelated donor.

TABLE 3  Numbers of patients in cohort transplanted from donor grafts with HLA-DPB1 mismatches and incidence of GvHD

| # HLA-DPB1 mismatches (%) | MUD   | MSD   | GvHD I   | GvHD II-IV |
|---------------------------|-------|-------|----------|------------|
| 0                         | 14 (28) | 31 (91.2) | 34 (77.3) | 10 (22.7) |
| 1                         | 29 (58) | 3 (8.8)   | 23 (79.3) | 6 (20.7)  |
| 2                         | 7 (14)  | 0 (0)     | 5 (71.4)  | 2 (28.6)  |

GvHD, graft-versus-host disease; MSD, matched sibling donor; MUD, match unrelated donor.

3.3 HLA-DPB1–matched transplantations show a high incidence of relapse

We performed T-cell epitope matching for all recipients, who received grafts unmatched for at least one of the two HLA-DPB1 alleles from unrelated donors to define patients with permissive and nonpermissive HLA-DPB1 mismatches. In our study group, 64.7% (n = 22) of patients had permissive and 35.3% (n = 12) had nonpermissive HLA-DPB1 mismatches, similar to the distribution in the cohort investigated by Fleischhauer et al.11 Having a nonpermissive HLA mismatch was not associated with a higher risk to develop GvHD (permissive: 18.2% n = 4/22, nonpermissive: 25%, n = 3/12, P > 0.05) in our cohort. We next assessed the incidence of relapse in patients with either permissive or nonpermissive HLA-DPB1 mismatches in our cohort using DPB1 T-cell Epitope matching. Interestingly, 10 of the 11 patients who relapsed had only one or no HLA-DPB1 mismatch. Six patients relapsed after HSCT with one HLA-DPB1 mismatch (19.3%, n = 6/31), of whom three had permissive and three nonpermissive HLA-DPB1 mismatches. Four patients, all of whom had a permissive HLA-DPB1 match, relapsed after HSCT from a donor without HLA-DPB1 mismatch (9.1%, n = 4/44). Only one patient relapsed who underwent transplantation with two HLA-DPB1 mismatches.

3.4 Low PIRCHE-I score is associated with reduced GvHD incidence in mismatch transplants

PIRCHE analysis was performed in the 20 patients who received transplants from unrelated donors with only 9 of 10 matched HLA alleles. Patients who experienced acute grade II-IV GvHD had significantly higher PIRCHE-I scores (P = 0.015) compared with patients experiencing acute grade I or no GVHD (Figure 1A). PIRCHE-II scores were higher in the patients who experienced acute grade II-IV GvHD than in patients who experienced acute grade I or no GVHD, but this
FIGURE 2  HLA-DPB1 match influences PIRCHE-I score. The distribution of PIRCHE-I scores for patients transplanted from matched unrelated donors with either one HLA-DPB1 (red) or two HLA-DPB1 (blue) mismatches are presented as box-and-whisker plots comparing patients who did not develop severe acute GvHD (none or only grade I) with patients who developed more severe forms of GvHD (grades II-IV). Boxes include the 25th to 75th percentiles divided by the median and whiskers indicating minimal and maximal PIRCHE scores. The correlation of PIRCHE scores with GvHD was tested using the Mann-Whitney U test. The correlation did not reach statistical significance ($P = 0.14$; Figure 1B).

Patients were divided into groups based on PIRCHE-I scores (divided into quartiles from lowest to highest, with PIRCHE-I scores of 0-1 deemed low and PIRCHE-I scores ≥2 as high). We then evaluated the difference in alloreactive potential between mismatched and matched unrelated transplants by comparing patients who received mismatched unrelated donor transplants with a low PIRCHE-I score to all patients who received transplants from a 10 of 10-matched unrelated donor. Interestingly, the incidence of acute GvHD was significantly higher in the patient subgroup with matched related donors (19.6%, $n = 10/51$ vs 0%, $n = 0/7$; $P = 0.03$). When we used the same analysis to compare patients who received transplants from matched unrelated donors with patients transplanted from mismatched unrelated donors with high PIRCHE-II scores, the difference did not reach significance. We then reanalyzed the 50 patients who received transplants from 10/10 matched unrelated donors, but incorporated HLA-DPB1 typing information in the PIRCHE algorithm to evaluate the potential of this extended matching. Interestingly, patients who received transplants from a donor with one or two mismatched HLA-DPB1 alleles and grade I or no GvHD had lower PIRCHE-I scores than the same patient subgroup with grade II-IV GvHD, even though this correlation did not reach statistical significance for PIRCHE-I ($P > 0.05$; Figure 2) or PIRCHE-II ($P > 0.05$). Our data indicate that GvHD may be predictable using PIRCHE-I scores. Whether the trend observed here that PIRCHE matching extended by HLA-DBP1 typing provides a superior predictor for patient-donor matching is confirmed will need analysis in larger patient cohorts.

4 | DISCUSSION

Here we retrospectively analyzed the applicability of PIRCHE scoring and HLA-DPB1 typing for GvHD prediction in a pediatric HSCT cohort. HLA-DPB1 mismatches were not associated with higher GvHD incidences in the investigated cohort, but HLA-DPB1 matches and permissive mismatches associated with higher incidences of disease relapse after HSCT. Moreover, PIRCHE-I score was significantly correlated with GvHD incidence in patients who received transplants from mismatched unrelated donors. Our results support implementing PIRCHE typing in the donor selection process for HSCT in children.

Studies investigating HLA-DPB1 and T-cell epitope typing show different results for overall survival, disease relapse and GvHD development after HSCT. Nonpermissive mismatches in transplantations using grafts from mismatched unrelated donors were associated with lower overall survival and severe GvHD in a study by Fleischhauer et al.$^{11}$ Another study by Gagne et al. identified only an effect of mismatched HLA-DPB1 alleles on acute GvHD incidence but not on overall survival or chronic GvHD incidence.$^{31}$ In our study cohort, HLA-DPB1 mismatches or nonpermissive mismatches determined by T-cell epitope were not associated with a higher incidence of acute GvHD, whereas permissive HLA-DPB1 mismatches were associated with a higher incidence of relapse in patients undergoing transplantations, indicating a lack of alloreactivity in these cases. The absence of alloreactivity is associated with a lack of graft-versus-leukemia effect. A strong graft-versus-leukemia effect is necessary for patients with high-risk leukemias in order to control the underlying disease. Alloreactivity is mediated by different surface proteins mainly expressed by T and natural killer cells.$^{32}$ The HLA molecules presented to T and natural killer cells are recognized as foreign or self, resulting in different responses according to the degree of (mis-)matches.$^{1,3}$ Donor-recipient HLA-matching strategies for HSCT must take the underlying disease into account, and estimate the expected graft-versus-leukemia effect without causing an overshooting graft-versus-host reaction. This main goal of donor selection has not yet been fully achieved, and is constantly complicated by emerging new graft manipulation
Killer cell immunoglobulin receptor profile analysis has been validated as an additional method to estimate natural killer cell-mediated alloreactivity after HSCT for acute leukemias.\(^3^4,\)\(^3^5\) The situation can be even more complicated in pediatric HSCT to treat nonmalignant diseases. Children with chronic inflammatory conditions accompanying the underlying disease have an elevated risk to develop GvHD that cannot be estimated by HLA-matching strategies. Two children in our cohort developed severe grade IV GvHD after transplantation from siblings with 12 of 12 matched HLA alleles. Both children suffered from chronic inflammation resulting from underlying genetic disorders. Further studies in larger pediatric cohorts are needed to evaluate the potential of HLA-DPB1 matching in pediatric HSCT for different malignant and nonmalignant diseases. The variety of underlying diseases in children undergoing HSCT makes a special prioritization in donor selection different from adult HSCT likely.

PIRCHE matching was first introduced for kidney transplantation.\(^1^7\) Only one study to date has analyzed PIRCHE typing in a pediatric cohort undergoing HSCT.\(^3^6\) Only patients receiving umbilical cord blood as grafts to treat leukemia were included, and higher PIRCHE-I scores were associated with higher antileukemic responses and improved overall survival, but not with higher incidence of GvHD development.\(^3^6\) In our study, higher PIRCHE-I scores significantly correlated with a higher incidence of GvHD for patients receiving stem cell grafts from unrelated donors with 9 of 10 mismatched HLA alleles. Cord blood contains fewer natural killer and T cells than bone marrow or peripheral stem cell grafts,\(^3^7\) explaining the stronger graft-mediated alloreactive effects with bone marrow or peripheral stem cell grafts from matched unrelated donors compared with umbilical cord blood transplantation. These findings illustrate that PIRCHE scores can have different impacts in different transplantation settings. PIRCHE scores appear to mirror expected alloreactivity in our cohort, and can effectively predict the risk for GvHD development in children receiving grafts from 9 of 10 HLA mismatched unrelated donors.

In conclusion, our data support implementing PIRCHE matching in the donor selection process for pediatric HSCT. Further studies in larger pediatric cohorts are needed to evaluate the potential of HLA-DPB1 matching in pediatric HSCT for different malignant and nonmalignant diseases. Due to the diversity of pediatric diseases with an indication for HSCT and their additional comorbidities, it is likely that different donor-recipient matching strategies will be necessary depending on the disease, graft source, and donor availability to optimize transplant settings in pediatric HSCT. Moreover, additional strategies for GvHD prevention will be needed to completely avoid GvHD when treating nonmalignant diseases.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ACKNOWLEDGMENTS
LO and AK are fellows in the BIH-Charité Clinician-Scientist Program funded by the Charité – Universitätsmedizin Berlin and the Berliner Institut für Gesundheitsforschung (BIH). This work was supported by a grant to LO from Bild Hilft e.V. – Ein Herz für Kinder and DKT Berlin. The authors wish to thank Kathy Aastrahantseff for proofreading of the manuscript.

CONFLICTS OF INTEREST
MN is employed by PIRCHE AG to develop and maintain the PIRCHE web portal. All other authors declare no conflict of interest.

ORCID
Lena Oevermann
https://orcid.org/0000-0001-6828-1783

REFERENCES
1. Harris AC, Ferrara JL, Levine JE. Advances in predicting acute GvHD. Br J Haematol. 2013;160(3):288-302.
2. Negrin RS. Graft-versus-host disease versus graft-versus-leukemia. Hematology Am Soc Hematol Educ Program. 2015;2015:225-230.
3. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009;373(9674):1550-1561.
4. Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. Nat Rev Immunol. 2012;12(6):443-458.
5. Dignan PL, Clark A, Amrolia P, et al. Diagnosis and management of acute graft-versus-host disease. Br J Haematol. 2012;158(1):30-45.
6. Ball LM, Egeler RM. Acute GvHD: pathogenesis and classification. Bone Marrow Transplant. 2008;41(Suppl 2):S58-64.
7. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood. 2007;110(13):4576-4583.
8. Hauber I, Gulle H, Wolf HM, Maris M, Eggenbauer H, Eibl MM. Molecular characterization of major histocompatibility complex class II gene expression and demonstration of antigen-specific T cell response indicate a new phenotype in class II-deficient patients. J Exp Med. 1995;181(4):1411-1423.
9. Zino E, Frumento G, Marktel S, et al. A T-cell epitope encoded by a subset of HLA-DPB1 alleles determines nonpermissive mismatches for hematologic stem cell transplantation. Blood. 2004;103(4):1417-1424.
10. Crivello P, Zito L, Sizzano F, et al. The impact of amino acid variability on alloreactivity defines a functional distance predictive of permissive HLA-DPB1 mismatches in hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2015;21(2):233-241.
11. Fleischhauer K, Shaw BE, Gooley T, et al. Effect of T-cell epitope matching at HLA-DPB1 in recipients of unrelated-donor haemopoietic-cell transplantation: a retrospective study. Lancet Oncol. 2012;13(4):366-374.
12. Burek Kamencic M, Maskalan M, Grubic Z, et al. HLA-DPB1 matching in unrelated hematopoietic stem cell transplantation program contributes to a higher incidence of disease relapse. Hum Immunol. 2017;78(11-12):665-671.
13. Loiseau P, Busson M, Balere ML, et al. HLA association with hematopoietic stem cell transplantation outcome: the number of mismatches at HLA-A,-B,-C,-DQB1, or -DRBI is strongly associated with overall survival. Biol Blood Marrow Transplant. 2007;13(8):965-974.
14. Thus KA, de Weger RA, de Hoop TA, Boers Trilles VE, Kuball J, Spierings E. Complete donor chimerism is a prerequisite for the effect of Predicted Indirectly Recognizable HLA Epitopes (PIRCHE) on acute graft-versus-host disease. Chimerism. 2014;5(3-4):94-98.
15. Thus KA, Ruizendaal MT, de Hoop TA, et al. Refinement of the definition of permissible HLA-DPB1 mismatches with predicted
indirectly recognizable HLA-DPB1 epitopes. Biol Blood Marrow Transplant. 2014;20(11):1705-1710.

16. Thus KA, Te Boome L, Kuball J, Spierings E. Indirectly recognized HLA-C mismatches and their potential role in transplant outcome. Front Immunol. 2014;5:210.

17. Otten HG, Calis JJ, Kesmir C, van Zuilen AD, Spierings E. Predicted indirectly recognizable HLA-DPB1 epitopes presented by HLA-DR correlate with the de novo development of donor-specific HLA IgG antibodies after kidney transplantation. Hum Immunol. 2013;74(3):290-296.

18. Geneugelij K, Thus KA, van Deutekom HWM, et al. Exploratory study of predicted indirectly recognizable HLA epitopes in mismatched hematopoietic cell transplants. Front Immunol. 2019;10:880.

19. Hsieh MM, Kang EM, Fitzhugh CD, et al. Allogeneic hematopoietic stem-cell transplantation for sickle cell disease. N Engl J Med. 2009;361(24):2309-2317.

20. Saif MA, Borrill R, Bigger BW, et al. In vivo T-cell depletion using alemtuzumab in family and unrelated donor transplantation for pediatric non-malignant disease achieves engraftment with low incidence of graft vs. host disease. Pediatr Transplant. 2015;19(2):211-218.

21. Angelucci E, Barontini D, Allogeneic stem cell transplantation for thalassemia major. Haematologica. 2008;93(12):1780-1784.

22. Zeiser R, Blazar BR. Acute graft-versus-host disease. N Engl J Med. 2018;378(6):586.

23. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A. 1977;74(12):5463-5467.

24. Zino E, Vago L, Di Terlizzi S, et al. Frequency and targeted detection of HLA-DPB1 T cell epitope disparities relevant in unrelated hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2007;13(9):1031-1040.

25. Geneugelij K, Spierings E. Matching donor and recipient based on predicted indirectly recognizable human leucocyte antigen epitopes. Int J Immunogenet. 2018;45(2):41-53.

26. Nielsen M, Lundegaard C, Lund O, Kesmir C. The role of the peptidase in generating cytotoxic T-cell epitopes: insights obtained from improved predictions of proteasomal cleavage. Immunogenetics. 2005;57(1-2):33-41.

27. Hoof I, Peters B, Sidney J, et al. NetMHCpan, a method for MHC class I binding prediction beyond humans. Immunogenetics. 2009;61(1):1-13.

28. Andreatta M, Karosiene E, Rasmussen M, Stryhn A, Buus S, Nielsen M. Accurate pan-specific prediction of peptide-MHC class II binding affinity with improved binding core identification. Immunogenetics. 2015;67(11-12):641-650.

29. Geneugelij K, Niemann M, de Hoop T, Spierings E. Completion of HLA protein sequences by automated homology-based nearest-neighbor extrapolation of HLA database sequences. Hum Immunol. 2016;77(11):1030-1036.

30. Fleischhauer K, Fernandez-Vina MA, Wang T, et al. Risk associations between HLA-DPB1 T-cell epitope matching and outcome of unrelated hematopoietic cell transplantation are independent of HLA-DPA1. Bone Marrow Transplant. 2014;49(9):1176-1183.

31. Gagne K, Loiseau P, Dubois V, et al. Is there any impact of HLA-DPB1 disparity in 10/10 HLA-matched unrelated hematopoietic SCT? Results of a French multicentric retrospective study. Bone Marrow Transplant. 2015;50(2):232-236.

32. Weisdorf D, Cooley S, Devine S, et al. T cell-depleted partial matched unrelated donor transplant for advanced myeloid malignancy: KIR ligand mismatch and outcome. Biol Blood Marrow Transplant. 2012;18(6):937-943.

33. Fleischhauer K, Hsu KC, Shaw BE. Prevention of relapse after alloge- neic hematopoietic cell transplantation by donor and cell source selection. Bone Marrow Transplant. 2018;53(12):1498-1507.

34. Oevermann L, Michaelis SU, Mezger M, et al. KIR B haplotype donors confer a reduced risk for relapse after haploidentical transplantation in children with ALL. Blood. 2014;124(17):2744-2747.

35. Cooley S, Trachtenberg E, Bergemann TL, et al. Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia. Blood. 2009;113(3):726-732.

36. Thus KA, de Hoop TA, de Weger RA, Bierings MB, Boelens JJ, Spierings E. Predicted indirectly recognizable HLA epitopes class I promote antileukemia responses after cord blood transplantation: indications for a potential novel donor selection tool. Biol Blood Marrow Transplant. 2016;22(1):170-173.

37. Hordyjewska A, Popiolek L, Horecka A. Characteristics of hematopoietic stem cells of umbilical cord blood. Cytotechnology. 2015;67(3):387-396.

How to cite this article: Stenger W, Künkele A, Niemann M, et al. Donor selection in a pediatric stem cell transplantation cohort using PIRCHE and HLA-DPB1 typing. Pediatr Blood Cancer. 2020;67:e28127. https://doi.org/10.1002/pbc.28127