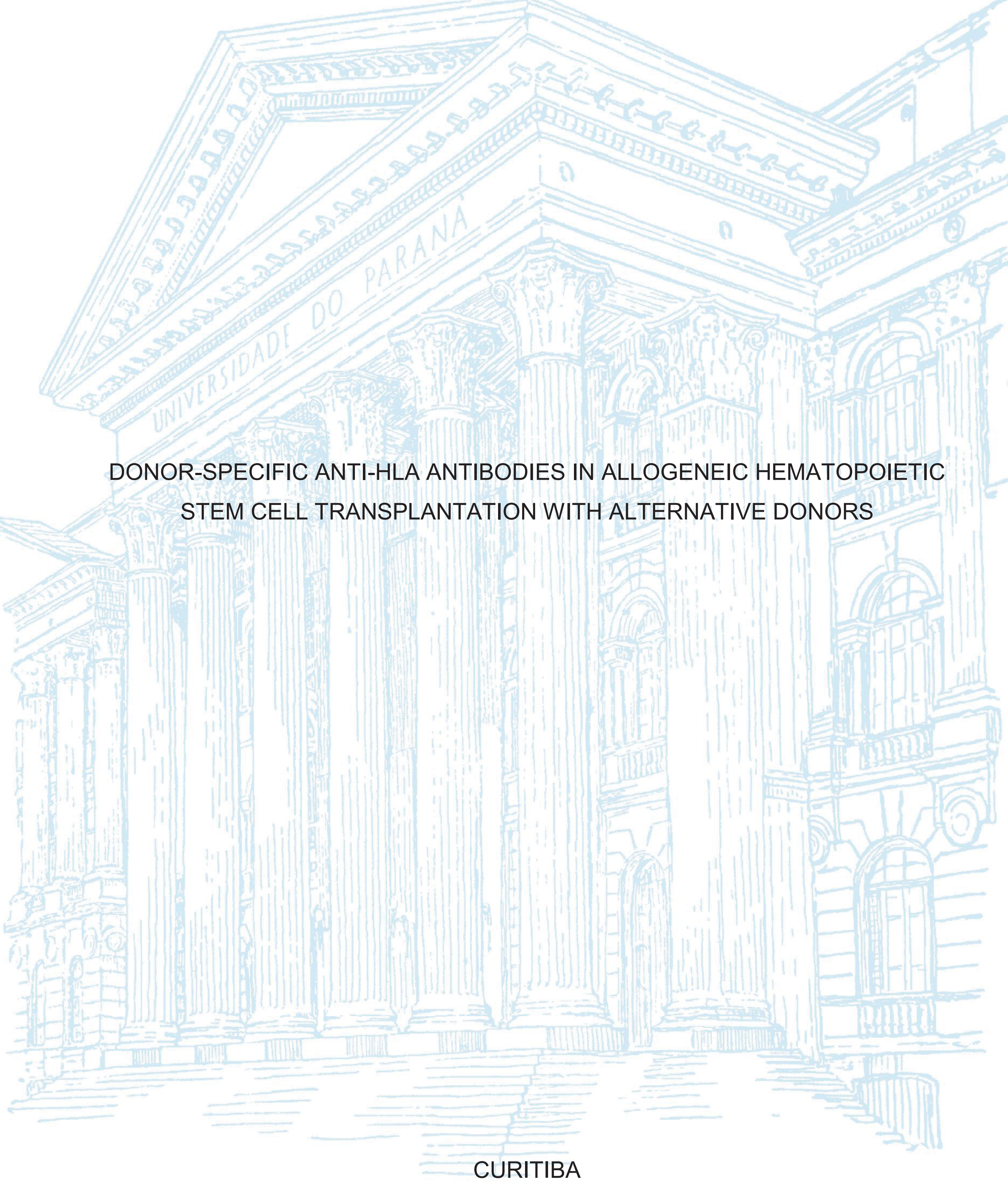
UNIVERSIDADE FEDERAL DO PARANÁ

ALBERTO CARDOSO MARTINS LIMA



DONOR-SPECIFIC ANTI-HLA ANTIBODIES IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH ALTERNATIVE DONORS

CURITIBA

2023

ALBERTO CARDOSO MARTINS LIMA

DONOR-SPECIFIC ANTI-HLA ANTIBODIES IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH ALTERNATIVE DONORS

Tese apresentada ao curso de Pós-Graduação em Medicina Interna e Ciências da Saúde, Setor de Ciências da Saúde, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Medicina Interna e Ciências da Saúde.

Orientador: Prof. Dr. Ricardo Pasquini
Coorientador: Prof. Dr. Carmem Maria Sales Bonfim

CURITIBA

2023

L732 Lima, Alberto Cardoso Martins
Donor-specific anti-hla antibodies in allogeneic hematopoietic stem cell transplantation with alternative donors. [recurso eletrônico] / Alberto Cardoso Martins Lima. – Curitiba, 2023.

Tese (doutorado) – Programa de Pós-Graduação em Medicina Interna e Ciências da Saúde. Setor de Ciências da Saúde. Universidade Federal do Paraná.
Orientador: Prof. Dr. Ricardo Pasquini
Coorientadora: Profª. Drª. Carmem Maria Sales Bonfim

1. Transplante de Células-Tronco Hematopoéticas. 2. Doadores não relacionados. 3. Antígenos Comuns de Leucócito. 4. Anticorpos. I. Pasquini, Ricardo. II. Bonfim, Carmem Maria Sales. III. Programa de Pós-Graduação em Medicina Interna e Ciências da Saúde. Setor de Ciências da Saúde. Universidade Federal do Paraná. IV. Título.

FICHA CATALOGRÁFICA ELABORADA PELO SISTEMA DE BIBLIOTECAS/UFPR
BIBLIOTECA DE CIÊNCIAS DA SAÚDE, BIBLIOTECÁRIA: RAQUEL PINHEIRO COSTA JORDÃO CRB 9/991

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS DA SAÚDE
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO MEDICINA INTERNA E CIÊNCIAS DA SAÚDE - 40001016012P1

ATA Nº018

MINUTES OF PUBLIC DEFENSE SESSION OF DISSERTATION TO OBTAIN THE DEGREE OF PHILOSOPHY DOCTOR IN INTERNAL MEDICINE POS GRADUATE PROGRAM

On 31/03/2023 at 10:00, in MICROSOFT TEAMS plataform, Virtual Room: https://bit.ly/3lJAd31 of the Federal University of Paraná, the activities pertaining to the rite of defense of work entitled: **"DONOR-SPECIFIC ANTI-HLA ANTIBODIES IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH ALTERNATIVE DONORS."** by ALBERTO CARDOSO MARTINS LIMA, under the supervision of Dr. RICARDO PASQUINI, were undertaken. The Examining Board, designated by the Faculty of the Graduate Program of the Federal University of Paraná in INTERNAL MEDICINE POS GRADUATE PROGRAM was composed of the following Members: RICARDO PASQUINI (UNIVERSIDADE FEDERAL DO PARANÁ), DANIELLI CRISTINA MUNIZ DE OLIVEIRA (INSTITUTO NACIONAL DE CÂNCER), MARIA DE LA PAZ BETTINOTTI (JOHNS HOPKINS UNIVERSITY - BALTIMORE), VANDERSON GERALDO ROCHA (FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO), ROBERT LIWSKI (DALHOUSIE UNIVERSITY), CARMEM MARIA SALES BONFIM (UNIVERSIDADE FEDERAL DO PARANÁ), . The Board Chair initiated the rites as defined by the Faculty of the Program and, after the remarks of the members of the examining commitee and the respective responses were addressed, the final evaluation of the examining board was read, wich was decided by APPROVAL. This result should be made official by the Faculty of the Program, through the fulfillment of all recommendation and corrections requested by the examining board within the procedural deadlines defined by the program. The granting of the title of philosophy doctor is contigent upon the fulfillment of all the requirements and terms determined int the regulations of the Graduate Program. There being no further business to transact, the Chair adjourned the session, of which, I, RICARDO PASQUINI, drew up these minutes, which are signed by me and the other members of the Examining Board.

Curitiba, March 31th, 2023.

Eletronic Signature
04/04/2023 09:57:20.0
RICARDO PASQUINI
President of the Examining Board

Eletronic Signature
31/03/2023 15:26:16.0
DANIELLI CRISTINA MUNIZ DE OLIVEIRA
External Member (INSTITUTO NACIONAL DE CÂNCER)

Eletronic Signature
01/04/2023 11:51:29.0
MARIA DE LA PAZ BETTINOTTI
xternal Member (JOHNS HOPKINS UNIVERSITY - BALTIMORE)

Eletronic Signature
04/04/2023 12:23:19.0
VANDERSON GERALDO ROCHA
External Member (FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO)

Eletronic Signature
01/04/2023 12:19:48.0
ROBERT LIWSKI
External Member (DALHOUSIE UNIVERSITY)

Eletronic Signature
31/03/2023 14:45:23.0
CARMEM MARIA SALES BONFIM
Co-Advisor

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS DA SAÚDE
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO MEDICINA INTERNA E
CIÊNCIAS DA SAÚDE - 40001016012P1

APPROVAL MINUTE

The Examining Board is designated by the Faculty of the Graduate Program of the Federal University of Paraná in INTERNAL MEDICINE POS GRADUATE PROGRAM where invited to argue the DISSERTATION of PHILOSOPHY DOCTOR by **ALBERTO CARDOSO MARTINS LIMA** , entitled: **"DONOR-SPECIFIC ANTI-HLA ANTIBODIES IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH ALTERNATIVE DONORS."**, under the supervision of Dr. RICARDO PASQUINI, which and after assessment of the candidate and the work, the Examining Board decided for the APPROVAL in the present rite.
The granting of the title of philosophy doctor is contingent upon the fulfillment of all the requirements indicated by the Examining Board and terms determined in the regulation of the Graduate Program.

Curitiba, March 31th, 2023.

Eletronic Signature
04/04/2023 09:57:20.0
RICARDO PASQUINI
President of the Examining Board

Eletronic Signature
31/03/2023 15:26:16.0
DANIELLI CRISTINA MUNIZ DE OLIVEIRA
External Member (INSTITUTO NACIONAL DE CÂNCER)

Eletronic Signature
01/04/2023 11:51:29.0
MARIA DE LA PAZ BETTINOTTI
External Member (JOHNS HOPKINS UNIVERSITY - BALTIMORE)

Eletronic Signature
04/04/2023 12:23:19.0
VANDERSON GERALDO ROCHA
External Member (FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO)

Eletronic Signature
01/04/2023 12:19:48.0
ROBERT LIWSKI
External Member (DALHOUSIE UNIVERSITY)

Eletronic Signature
31/03/2023 14:45:23.0
CARMEM MARIA SALES BONFIM
Co-Advisor

Documento assinado eletronicamente de acordo com o disposto na legislação federal Decreto 8539 de 08 de outubro de 2015.
Gerado e autenticado pelo SIGA-UFPR, com a seguinte identificação única: 271156
Para autenticar este documento/assinatura, acesse https://www.prppg.ufpr.br/siga/visitante/autenticacaoassinaturas.jsp e insira o codigo 271156

I dedicate the present Thesis to my beloved children, Helena and Samuel, and especially to my wife Michele, who supported me unconditionally throughout the past four years when we went through hard times with the Covid-19 pandemic. I sincerely appreciate your endless patience while I was highly occupied with this Ph.D. project.

ACKNOWLEDGEMENTS

This Ph.D. thesis would not have been possible without the support and help of many special people. Thereby, I would like to extend my appreciation to you all.

I want to express my sincere appreciation to my advisors, Ricardo Pasquini and Carmem Bonfim. Thank you for the independence you have given me to develop this project and for all the relevant advice and constructive criticism over the past four years. Dr. Carmem Bonfim is especially recognized for "challenging" me to learn and master the complex statistical methods used in hematopoietic cell transplantation (HCT). It has been simply amazing to discuss this topic with you these years.

I want to thank everyone from the Histocompatibility Laboratory of the CHC/UFPR, especially my dear friend and faithful proofreader, Joselito Getz. It is a privilege to work with you. I also like to express my gratitude to Noemi Farah Pereira, former director of our HLA Lab, who participated in the inception of this Ph.D. project and the one who introduced me to this fantastic world of HLA antibodies in allogeneic HCT.

I am grateful to several notable people and outstanding HLA experts with that I have had the privilege to discuss all aspects of HLA antibodies throughout the years. Renato de Marco, Maria Gerbase de Lima, Sandra Tafulo, Robert Liwski, Maria Bettinotti, and Marcelo Fernández-Viña, your feedback and constructive criticisms were of utmost importance to improving my knowledge on this topic and ultimately refined this research project.

Geovana Borsato do Amaral, Sheila Meneguette, and Diego Hatschbach Ferreira are highly acknowledged for all technical, logistical, and financial support with the PRONON project.

I am grateful to the statistician Ricardo Rasmussen Petterle for all relevant discussions on regression assumptions and survival analysis techniques that improved this project. I want to thank the data manager Heliz Regina Neves, who assisted me with the CHC/UFPR's HCT Unit database.

My wife Michele and our beloved children Helena and Samuel have the most beautiful and special place in my life. They bore four years of my absence, stress, and hard work until I finally completed this Ph.D. project. Now, we will have much time of joy to compensate for the past four years.

I also sincerely appreciate all support from my parents, Luis Alberto and Lenilza, and my parents-in-law, Marcos and Marli. This research project could not have been fulfilled without the substantial help in looking after my kids when I performed the experiments and wrote the papers and the present Thesis.

And most importantly, my deepest thanks are dedicated to all patients needing an allogeneic HCT, who are the primary reason for this research endeavor.

"The scientist is not a person who gives the right answers,
he is one who asks the right questions."

Claude Levi-Strauss

RESUMO

O presente projeto de pesquisa teve como objetivo investigar o impacto dos anticorpos HLA específicos contra o doador (DSA) no transplante alogênico de células hematopoiéticas (TCH) com doadores apresentando incompatibilidades HLA (doadores alternativos). Assim, este estudo incluiu 362 pacientes sem um irmão HLA-idêntico e a maioria com doenças não malignas, que receberam primeiro TCH alogênico no CHC/UFPR de janeiro de 2008 a dezembro de 2017. Entre tais pacientes, 303 realizaram primeiro TCH com doadores não aparentados (NAP), e 59 pacientes pediátricos com doenças não malignas receberam primeiro transplante com doador haploidênticos com protocolo da ciclofosfamida pós-transplante (haplo-PTCy). Também avaliamos 22 pacientes com doenças não malignas que foram submetidos a um segundo haplo-PTCy para resgatar uma falha de enxerto anterior. Os desfechos do estudo foram recuperação hematológica, falha do enxerto e sobrevivência após transplante com doadores alternativos. Realizamos uma avaliação DSA aprofundada, incluindo teste DSA com 2 painéis de antígenos HLA isolados (SAB, *Single Antigen Beads*), ensaio de ligação C1q, titulação do DSA com diluições 1:2, 1:8 e 1:32, painéis SAB expandidos para análise de epítopos HLA e protocolo de absorção/eluição para investigar possíveis reações falso-positivas. Análises multivariadas foram realizadas usando modelos de regressão de risco competitivo Fine-Gray ou modelos de regressão de riscos proporcionais de Cox. No geral, observamos que 28 pacientes apresentaram reatividade DSA verdadeira, incluindo 11 no primeiro haplo-PTCy, 6 no segundo haplo-PTCy e 11 no TCH com doadores NAP. Nas análises multivariadas, os pacientes com DSA tiveram menor recuperação de neutrófilos ($P = 0{,}001$) e plaquetas ($P < 0{,}001$), aumento de falha de enxertia ($P < 0{,}001$), sobrevida livre de eventos inferior ($P < 0{,}001$) e sobrevida global reduzida ($P = 0{,}002$) após o primeiro haplo-PTCy. Em relação ao haplo-PTCy de resgate, 3/4 (75%) pacientes com DSA apresentaram falha de enxertia em comparação a 2/16 (12,5%) pacientes DSA-negativos ($P = 0{,}032$). A sobrevida livre de eventos em um ano foi significativamente menor em pacientes DSA-positivos do que naqueles sem DSAs (16,7% vs. 62,5%, $P = 0{,}002$), e uma tendência para menor sobrevida global em 1 ano foi observada em pacientes DSA-positivos do que em pacientes sem DSAs (33,3% vs. 62,5%; $P = 0{,}076$). Na coorte de TCH com doadores NAP, observamos que os pacientes DSA-positivos tiveram recuperação significativamente menor e mais arrastada de neutrófilos ($P = 0{,}006$) e plaquetas ($P = 0{,}0003$) na regressão de Fine-Gray. Além disso, a presença de DSAs foi fortemente associada a uma maior incidência de falha de enxertia ($P < 0{,}0001$) na análise multivariada. Em conclusão, nossos resultados validam a presença de DSA como um fator de risco significativo para falha da enxertia e recuperação hematológica arrastada após haplo-PTCy e TCH com doadores não aparentados. Assim, a avaliação cuidadosa do DSA pré-transplante pode otimizar a seleção de doadores alternativos e melhorar os resultados do TCH alogênico.

Palavras-chave: Anticorpos anti-HLA específicos contra o doador (DSA); doador haploidêntico; doador não aparentado; transplante de células-tronco hematopoiéticas; falha da enxertia.

ABSTRACT

The current research project aimed to investigate the impact of donor-specific HLA antibodies (DSA) in allogeneic hematopoietic cell transplantation (HCT) with HLA-mismatched donors (alternative donors). Thus, it included 362 patients lacking an HLA-matched related sibling donor and mostly with nonmalignant disorders who underwent their first allo-HCT at CHC/UFPR from January 2008 to December 2017. Among them, 303 underwent their first unrelated donor (URD) transplantation, and 59 pediatric patients with nonmalignant disorders received upfront haploidentical donor transplantation with post-transplant cyclophosphamide (haplo-PTCy). We also assessed 22 patients with nonmalignant disorders who underwent second haplo-PTCy to rescue a previous engraftment failure. The study endpoints were hematologic recovery, graft failure, and survival following alternative donor transplantation. We performed an in-depth DSA assessment, including DSA testing with 2 Single Antigen Beads (SAB) panels, C1q-binding assay, DSA titration with 1:2, 1:8, and 1:32 dilutions, expanded SAB panels for HLA epitope analysis, and absorption/elution protocol to investigate possible false-positive DSA reactions. Multivariable analyses were performed using Fine-Gray competing risk regression or Cox proportional hazards regression models. Overall, we observed that 28 patients had true DSA reactivity, including 11 in first haplo-PTCy, 6 in second haplo-PTCy, and 11 in URD transplantation. In the multivariable analyses, patients with DSA had lower neutrophil recovery (P = .001) and platelet recovery (P <. 001), increased graft failure (P < .001), inferior event-free survival (P < .001), and poor overall survival (P = .002) after the first haplo-PTCy. Regarding salvage haplo-PTCy, 3 of 4 (75%) patients with DSAs experienced GF versus 2 of 16 (12.5%) DSA-negative patients (P= .032). One-year event-free survival was significantly lower in DSA-positive patients than those without DSAs (16.7% vs. 62.5%, P = .002), and a trend for lower 1-year OS was observed in DSA-positive patients than in patients with no DSAs (33.3% vs. 62.5%; P = .076). In the URD setting, DSA-positive patients had significantly lower and delayed neutrophil (P = .006) and platelet recovery (P = .0003) in the Fine-Gray regression. In addition, the presence of DSAs was strongly associated with a higher incidence of GF (P = .0002) in the multivariable analyses. In conclusion, our results validate the presence of DSAs as a significant risk factor for engraftment failure and delayed hematologic recovery after haplo-PTCy and unrelated donor transplantation. Thus, careful pre-transplant DSA evaluation may optimize alternative donor selection and improve allogeneic HCT outcomes.

Keywords: Donor-specific anti-HLA antibodies (DSA); haploidentical donor; unrelated donor; hematopoietic stem cell transplantation; graft failure.

LIST OF FIGURES

MANUSCRIPT 1

FIGURE 1 - Cumulative incidence of neutrophil (A) and platelet (B) recovery by DSA status. Impact of desensitization therapy on neutrophil (C) and platelet (D) recovery……………………………………………………………...….58

FIGURE 2 - Cumulative incidence of graft failure by DSA status (A) and desensitization therapy (B)…………………………………….………..59

FIGURE 3 - Probability of event-free survival (A) and overall survival (B) by DSA status. Event-free survival (C) and overall survival (D) according to desensitization therapy……………………………………….………...60

SUPPLEMENTARY FIGURE S1 - False-positive DSA reactivity was evaluated by absorption and elution protocol and LIFECODES class II SAB panel…………………………………………………………….……………66

MANUSCRIPT 2

FIGURE 1 - Probability of survival by DSA status. (A) One-year EFS. (B) One-year OS………………………………………………………………………….92

SUPPLEMENTARY FIGURE S1 – DSA assessment using C1q-assay...…………….93

SUPPLEMENTARY FIGURE S2 – DSA assessment and HLA epitope analysis using Single Antigen ExPlex…………………………………………………...96

MANUSCRIPT 3

FIGURE 1 - Adjusted cumulative incidence of neutrophil recovery (A), platelet recovery (B), and graft failure (C) by DSA status……………………...124

FIGURE 2 - Median MFI values among DSA-positive patients with and without graft failure. LIFECODES SAB with neat serum (A); LABScreen SAB with

neat serum (B), 1:2 dilution (C), 1:8 dilution (D), and 1:32 dilution (E)…………………………………………………………..……………125

SUPPLEMENTARY FIGURE S1 – Absorption/elution protocol results with surrogate donor cells expressing DQA1*05:01/DQB1*03:01 (A) and DQB1*06:03 (B). …………………………………………………….…132

LIST OF TABLES

MANUSCRIPT 1

TABLE 1 - Baseline characteristics by DSA status…………………………………..61

TABLE 2 - DSA characteristics and haplo-PTCy outcomes……………………...62

TABLE 3 - Multivariable models for the haplo-PTCy outcomes...…………………63

SUPPLEMENTAL TABLE 1 - Univariable analyses for neutrophil and platelet recovery and graft failure……………………………………………..64

SUPPLEMENTAL TABLE 2 - Univariable analyses for event-free survival and overall survival………………………………………………………………65

MANUSCRIPT 2

TABLE 1 - Patients, donor, and transplant series characteristics………………….88

TABLE 2 - Donor-specific anti-HLA antibodies in patients who underwent salvage haploidentical transplantation with PTCy………...…………...……….....89

TABLE 3 - Univariable analysis for graft failure……………………………………....90

TABLE 4 - Univariable analyses for EFS and OS…………………………………....91

MANUSCRIPT 3

TABLE 1 - Baseline characteristics in the overall cohort and by DSA status….....120

TABLE 2 - DSA characteristics and URD transplantation outcomes……………..122

TABLE 3 - Multivariable analyses for hematologic recovery and graft failure…...122

SUPPLEMENTARY TABLE 1 - Unadjusted cumulative incidence of neutrophil and platelet recovery…………………………………………………...….126

SUPPLEMENTARY TABLE 2 - Unadjusted cumulative incidence of graft failure…128

SUPPLEMENTARY TABLE 3 - Unadjusted probabilities of overall survival……….130

LIST OF ABBREVIATIONS

ADCC	Antibody-dependent cell-mediated cytotoxicity
AHG	Anti-human IgG
Allo-HCT	Allogeneic hematopoietic cell transplantation
ANC	Absolute neutrophil count
BMT	Bone marrow transplant
CDC	Complement-dependent cytotoxicity
CI	Confidence interval
Cy	Cyclophosphamide
CMV	Cytomegalovirus
DSA	Donor-specific antibodies
EBMT	European Society for Blood and Marrow Transplantation
EDTA	Ethylenediaminetetraacetic acid
EFS	Event-free survival
FC	Flow cytometry
Flu	Fludarabine
GF	Graft failure
GVHD	Graft-versus-host disease
GvH	Graft-versus-host
Haplo-PTCy	Haploidentical transplant with post-transplant cyclophosphamide
HCT	Hematopoietic cell transplantation
HHV6	Human herpesvirus 6
HLA	Human leukocyte antigen
HR	Hazard ratio
HvG	Host-versus-graft
MFI	Mean fluorescence intensity
MMUD	Mismatched unrelated donor
MUD	Matched unrelated donor
NMDP	National Marrow Donor Program

NMDs	Nonmalignant disorders
OS	Overall survival
PGF	Poor graft function
PRONON	Programa nacional de apoio à atenção oncológica
PTCy	Post-transplant cyclophosphamide
rATG	Rabbit anti-thymocyte globulin
RIC	Reduced intensity conditioning
SAA	Severe aplastic anemia
SAB	Single antigen beads
SBT	Sequence-based typing
SHR	Subdistribution hazard ratio
TBI	Total body irradiation
TNC	Total nucleated cell

LIST OF CONTENTS

1 INTRODUCTION 20
1.1 LITERATURE REVIEW 20
1.1.1 Allogeneic hematopoietic cell transplantation 20
1.1.2 Hematopoietic recovery and graft failure 21
1.1.3 Donor-specific HLA antibodies 24
1.2 JUSTIFICATION OF THE STUDY 31
2 OBJECTIVES 31
2.1 GENERAL OBJECTIVE 31
2.2 SPECIFIC OBJECTIVES 32
3 METHODS 32
3.1 ETHICAL APPROVAL 32
3.2 PATIENTS 32
3.3 HLA TYPING AND HLA ANTIBODY TESTING 33
3.4 ENDPOINTS AND DEFINITIONS 34
3.5 STATISTICAL ANALYSIS 34
4 RESULTS AND DISCUSSION 36
4.1 MANUSCRIPT 1 36
4.1.1 Introduction 39
4.1.2 Methods 40
4.1.3 Results 43
4.1.4 Discussion 47
4.1.5 Acknowledgments 52
4.1.6 Author contributions 52
4.1.7 Conflict of interest 52
4.1.8 References 53
4.2 MANUSCRIPT 2 71
4.2.1 Introduction 73
4.2.2 Methods 74
4.2.3 Results 76
4.2.4 Discussion 79
4.2.5 Acknowledgments 82
4.2.6 Author contributions 83

4.2.7 Conflict of interest 83
4.2.8 References....................................83
4.3 MANUSCRIPT 3....................................99
4.3.1 Introduction 102
4.3.2 Methods 103
4.3.3 Results 105
4.3.4 Discussion....................................109
4.3.5 Acknowledgments 114
4.3.6 Author contributions 115
4.3.7 Conflict of interest 115
4.3.8 References....................................115
5 CONCLUSIONS....................................133
5.1 FIRST HAPLOIDENTICAL DONOR TRANSPLANTATION 133
5.2 SALVAGE HAPLOIDENTICAL DONOR TRANSPLANTATION....................................133
5.3 UNRELATED DONOR TRANSPLANTATION....................................133
5.4 FUTURE PERSPECTIVES 134
REFERENCES....................................135
APPENDIX 1. CHC/UFPR institutional review board approval**....................................148**
APPENDIX 2. List of awards, other publications, scientific presentations, and educational activities during the Ph.D. (2019-2023)**....................................157**

1 INTRODUCTION

1.1 LITERATURE REVIEW

1.1.1 Allogeneic hematopoietic cell transplantation

Allogeneic hematopoietic cell transplantation (allo-HCT) is a unique curative therapy for various life-threatening hematological and non-hematological diseases (COPELAN et al., 2019). Previously referred to as bone marrow transplantation (BMT), the allo-HCT is primarily used to treat malignant hematological diseases, such as acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, and myeloproliferative neoplasms (D'SOUZA et al., 2020). Nevertheless, allo-HCT can also treat several nonmalignant disorders, including acquired severe aplastic anemia, inherited bone marrow failure syndromes, primary immunodeficiencies, inherited metabolic disorders, and hemoglobinopathies (HAYASHI, 2020; UMEDA, 2022).

Prior to allo-HCT, the patient receives a conditioning regimen consisting of chemotherapy and/or radiotherapy, which may be classified as myeloablative, nonmyeloablative, or reduced intensity conditioning (RIC), and that varies depending on the patient's disease status and the transplant modality (BACIGALUPO et al., 2009). The preparatory regimen has three primary aims, including (1) the ablation of the patient's hematopoietic system and the creation of space in the bone marrow niche to receive hematopoietic progenitor cells, (2) the reduction of the malignant burden in hematological cancers, and (3) the provision of adequate immunosuppression against host-versus-graft (HvG) alloreactivity (VRIESENDORP, 2003).

After conditioning, the donor hematopoietic progenitor cells are infused to restore the patient's hematopoiesis. These hematopoietic progenitor cells may be obtained from different sources, including bone marrow, peripheral blood stem cells after granulocyte-colony stimulating factor mobilization, or umbilical cord blood (JURIC et al., 2016). These stem cell sources differ substantially concerning engraftment rate, infection incidence, graft-versus-host disease (GVHD) occurrence, and relapse risk. Another critical component of allo-HCT is the immunosuppressive treatment to prevent graft-versus-host (GvH) alloreactivity. This immunological

complication may be divided into acute and chronic GvHD and is associated with significant morbidity and mortality after allo-HCT. Generally, the most common GVHD prophylaxis includes a calcineurin inhibitor, either cyclosporine or tacrolimus, associated with methotrexate (MARTINEZ-CIBRIAN; ZEISER; PEREZ-SIMON, 2021). Remarkably, the introduction of post-transplant cyclophosphamide as GVHD prophylaxis in the haploidentical transplant (Haplo-PTCy) has revolutionized the allo-HCT field (LUZNIK et al., 2008). The PTCy's effectiveness in minimizing severe immunological complications after allo-HCT promptly expanded its use to other alternative donor sources, such as mismatched unrelated donors (AL MALKI et al., 2021; SHAW et al., 2021).

From an immunological perspective of allo-HCT, it is well-established that the Major Histocompatibility Complex genetic disparity between patient/donor pairs triggers an intense bidirectional alloreactive response in both HvG and GvH directions, thus presenting clinically as graft rejection and acute GVHD, respectively (PERKEY; MAILLARD, 2018). Therefore, the best donor option to minimize these severe immunological complications following allo-HCT is a 12/12 human leukocyte antigen (HLA) matched sibling donor (SHOUVAL et al., 2019). However, depending on the patient's age and ethnicity, a matched related sibling is available for 13% to 51% of patients eligible for allo-HCT (BESSE et al., 2016); for the remaining patients lacking HLA-matched siblings, alternative donor options are available, including matched unrelated donors (MUD), mismatched unrelated donors (MMUD), haploidentical donors, and umbilical cord blood units (SPELLMAN, 2022). Despite the continuous improvements of alternative donor allo-HCT outcomes throughout the past years, graft failure (GF) remains a significant hurdle associated with poor prognosis after transplant (OLSSON et al., 2013, 2015; SCHRIBER et al., 2010).

1.1.2 Hematopoietic recovery and graft failure

1.1.2.1 Definitions

The engraftment after allo-HCT comprises two verifications, including the assessment of neutrophil and platelet counts post-graft infusion and followed by the confirmation of the donor-derived hematopoietic recovery by chimerism testing on days +28 or +30 (KHARFAN-DABAJA et al., 2021). Importantly, the hematopoietic

recovery post-HCT may be of recipient origin by chimerism testing and is called autologous recovery.

GF is a complex event after allo-HCT that can be characterized by the timing of its onset and the underlying immunological mechanism. Thus, GF may occur early (primary) or late (secondary) after graft infusion and includes two distinct immunological entities, graft rejection and poor graft function, that can be distinguished by chimerism testing (MASOURIDI-LEVRAT; SIMONETTA; CHALANDON, 2016). In graft rejection, the donor chimerism is absent or mixed, whereas complete donor chimerism is present in poor graft function (OZDEMIR; CIVRIZ BOZDAĞ, 2018).

Currently, the working definitions of hematopoietic recovery, GF, graft rejection, and poor graft function have been standardized as follows (KHARFAN-DABAJA et al., 2021):

Neutrophil recovery is defined as the first of three consecutive days with an absolute neutrophil count (ANC) $\geq .5\times10^9$/L.

Platelet recovery is defined as the first of three consecutive days at $\geq 20 \times 10^9$/L and at least seven days without platelet transfusion support.

Primary graft rejection was defined as the failure to achieve an ANC of $.5\times10^9$/L by day +28 and the absence of donor-derived hematopoiesis (<5% donor cells) using chimerism analysis.

Secondary graft failure is defined as achieving ANC of $.5\times10^9$/L with subsequent loss of donor chimerism (<5% donor cells)

Poor graft function is defined as the presence of 2 or 3 cytopenic counts (ANC$\leq 0.5\times10^9$/L, platelets$\leq 20\times10^9$/L, or hemoglobin $\leq$ 70 g/L) for at least 3 consecutive days beyond day 28 post-transplantation with a transfusion requirement associated with hypoplastic bone marrow in the presence of complete donor chimerism (>95% donor cells).

1.1.2.2 Risk factors for graft failure after allo-HCT

The GF pathogenesis is likely multifactorial, with several known patient-, donor-, and transplant-related risk factors (DAVIES et al., 2000; OLSSON et al., 2013, 2015; OZDEMIR; CIVRIZ BOZDAĞ, 2018).

In a retrospective cohort with 5246 unrelated donor BMT, Davies and colleagues showed that HLA-A, -B, and -DRB1 matching, higher cell dose, male donor, younger patient age, recipient seronegativity for cytomegalovirus (CMV), no methotrexate for GVHD prophylaxis, and BMT in more recent years were associated with a lower likelihood of primary GF (DAVIES et al., 2000).

In a single-center study, Olsson et al. also assessed the risk factors for failed engraftment in 967 consecutive patients undergoing allo-HCT. It was reported that HLA-mismatched donors, diagnosis of non-malignant disorders, low cell dose (<2.5×10^8 cells/kg), RIC or non-myeloablative conditioning, and *ex vivo* T-cell depletion were independent predictors of engraftment failure after allo-HCT (OLSSON et al., 2013).

Moreover, Olsson et al. conducted a retrospective analysis to identify predictors of primary GF in 23,272 patients who underwent myeloablative allo-HCT for malignancies. They observed that bone marrow as the source, HLA-mismatched donors, diagnosis of myeloproliferative disorders, ABO major incompatibility, recipient age <30 years, male recipients of female donor grafts, absence of granulocyte colony-stimulating factor, low total nucleated cell dose, busulfan/cyclophosphamide conditioning, and cryopreservation were all significant risk factors for early failed engraftment (OLSSON et al., 2015).

The pathogenesis of poor graft function after allo-HCT is complex and likely associated with an immunological dysfunction of the marrow microenvironment (KONG, 2019). To date, several risk factors of poor graft function have been described, including CMV reactivation, GVHD occurrence, older age, low stem cell dose, hyperferritinemia, ABO incompatibility, nonsibling donor, early intensive care unit admission, and blood culture positivity (PRABAHRAN et al., 2022).

More recently, the impact of donor-specific HLA antibodies (DSA) in engraftment failure has been addressed. Several studies have reported that DSA is significantly associated with an increased risk of GF after alternative donor transplants, including matched and mismatched unrelated donors (CIUREA et al., 2011; SPELLMAN et al., 2010), umbilical cord blood units (CUTLER et al., 2011; FUJI et al., 2020; RUGGERI et al., 2013), and haploidentical donor transplantation, either with the haplo-PTCy platform (CIUREA et al., 2015; ZOU et al., 2017), T-cell depleted grafts (CIUREA et al., 2009) or Beijing protocol (CHANG et al., 2015; ZHAO et al., 2017). Indeed, a recent systematic review and meta-analysis, considering 15

studies with 2436 patients, reported that the presence of DSA was associated with a 7.47 increased risk of primary GF compared with patients without DSAs (XIE et al., 2021).

1.1.3 Donor-specific HLA antibodies

1.1.3.1 Immunobiology

The *HLA* complex is located within the 6p21.3 region of the short arm of chromosome 6 and contains several genes with immunological function. The HLA gene complex is divided into three classes (I, II, and III), and the classical histocompatibility loci include *HLA-A*, *HLA-B*, and *HLA-C* for class I and *HLA-DRB1*, *HLA-DRB3/4/5, HLA-DQA1, HLA-DQB1*, *HLA-DPA1, and HLA-DPB1* for class II (MANGUM; CAYWOOD, 2022).

Patients eligible for allo-HCT may become sensitized against HLA after exposure to non-self HLA antigens in foreign cells. The most common causes of HLA allosensitization include pregnancy, blood and platelet transfusion, and previous allogeneic transplantation (JORDAN et al., 2016). Furthermore, HLA antibodies may also be detected in patients without sensitized events (MORALES-BUENROSTRO et al., 2008). These so-called "natural antibodies" have been detected in non-sensitized healthy males and appear clinically irrelevant (CAI et al., 2009). Importantly, the HLA antibodies directed to the donor's HLA-mismatched antigens are defined as donor-specific HLA antibodies, or more commonly, DSA (CIUREA et al., 2018).

1.1.3.2 HLA antibody detection methods

In general, HLA antibody detection methods are divided into cell-based assays and solid-phase assays (ENG; LEFFELL, 2011). These two methods differ significantly in their assay sensitivities and test limitations (LIWSKI; GEBEL, 2018).

Cell-based assays are performed by incubating serum with T and B lymphocytes. The crossmatch assay may be carried out by complement-dependent cytotoxicity (CDC) or flow cytometry (FC) (BETTINOTTI, 2022). The CDC was the first test used for crossmatching in allogeneic transplants and is designed to detect complement-activating antibodies lysing the donor's T and B lymphocytes. The low

sensitivity of CDC-XM may be improved by adding a secondary antibody (anti-human IgG, AHG CDC) (TAIT et al., 2013). Crossmatch testing may also be performed by flow cytometry. This technique detects DSA binding to the target donor's T and B lymphocytes using a fluorescent secondary antibody and quantification using flow cytometer equipment. Of note, the FC crossmatch has some advantages over CDC, such as improved sensitivity to detect low-level DSAs, less subjective interpretation, and less labor-intensive, especially with current optimized FC crossmatch protocols (LIWSKI et al., 2018). However, cell-based assays, either CDC or FC, present some drawbacks (LIWSKI; GEBEL, 2018; TAIT et al., 2013) that include the detection of non-HLA antibodies and the requirement of fresh donor cells, which may be unfeasible in some alternative donor settings (i.e., umbilical cord blood or unrelated donors from abroad).

On the other hand, HLA antibodies may be detected and characterized by solid-phase assays, including enzyme-linked immunosorbent assays and, most commonly, multiplex bead-based testing in the Luminex platform (BETTINOTTI, 2022). The Luminex bead assays have several advantages over cell-based assays, including increased sensitivity and specificity, high-throughput, and multiplex capacity (CIUREA et al., 2018; TAIT, 2016).

The HLA antibody assays using Luminex-based technology are available in three different formats (BETTINOTTI, 2022). The screening beads contain pooled HLA antigens and indicate the absence or presence of HLA antibodies. The phenotype (PRA) beads contain individual class I or class II HLA phenotypes. This test can also serve as a screening assay and provide data on the patient's allosensitization broadness. In contrast, the single antigen beads (SAB) assays are designed to identify and characterize HLA antibodies. The SAB assays consist of microbead panels in which each bead is coated with a single class I or class II HLA recombinant antigen. This assay shows the highest sensitivity to detect HLA antibodies, and its results are expressed as mean fluorescence intensity (MFI) (SULLIVAN; GEBEL; BRAY, 2017). Of note, MFI values may be used as a semi-quantitative parameter of antibody strength. It should be noted that SAB assays also present important limitations that include false-negative (i.e., complement-inhibition effect) or false-positive (i.e., cryptic epitopes in denatured HLA antigens and peptide-free/β2-microglobulin-free HLA-I heavy chains on the beads) reactivity (GEBEL; BRAY, 2014; LIWSKI; GEBEL, 2018; WEHMEIER; HÖNGER; SCHAUB, 2020).

These SAB drawbacks may compromise DSA assignments and donor selection if not properly mitigated by the HLA laboratory. Moreover, the C1q and C4d assays are modified SAB tests designed to distinguish complement-fixing from non-complement-fixing antibodies (TYAN, 2017).

The widespread adoption of the Luminex bead assays enabled the implementation of virtual crossmatch (VXM) assessment as a surrogate for conventional crossmatch testing in the allo-HCT setting (CIUREA et al., 2018). The VXM is an *in silico* evaluation that compares the recipient's HLA antibodies profile to the donor's HLA-mismatched antigens (MORRIS et al., 2019). To obtain the maximum accuracy, VXM analysis should use an updated serum sample and consider the complete donor's high-resolution HLA typing, preferentially by next-generation sequencing and including *HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1*, and *-DPB1* (BHASKARAN; HEIDT; MUTHUKUMAR, 2022). Nevertheless, the accuracy of VXM may be jeopardized by the SAB limitations mentioned above (LIWSKI; GEBEL, 2018; WEHMEIER; HÖNGER; SCHAUB, 2020).

Thus, emerging evidence highlights that the concomitant use of solid-phase and cell-based assays, specifically FCXM, provides a more accurate DSA strength assessment and ultimately informs clinical risk of engraftment failure and desensitization feasibility (BETTINOTTI, 2022; LEFFELL; JONES; GLADSTONE, 2015).

1.1.3.3 Impact of DSAs in allo-HCT

During the early era of BMT, few case reports have described the impact of humoral alloreactivity in engraftment failure, assessed either by CDC or antibody-dependent cell-mediated cytotoxicity (ADCC) assays (BARGE et al., 1989; ELKINS et al., 1981; SCORNIK et al., 1989; TING et al., 1986).

In 1981, Elkins et al. reported a patient with acute myeloid leukemia receiving a marrow graft from a matched sibling with an HLA-A1 recombinant gene antigen. Interestingly, the CDC was negative in the pre-transplant sample, while the ADCC assay was positive. After the BMT, the CDC became positive against the sibling donor and several surrogate cells expressing HLA-A1. This patient ultimately failed to engraft and died due to systemic candidiasis (ELKINS et al., 1981). Later, Ting and colleagues described two cases of patients with malignancies who underwent

haploidentical BMT with a positive CDC crossmatch. Both patients experienced primary graft rejection and eventually died of infection. Noteworthy, in one patient, the CDC became positive only in post-BMT samples, thus indicating a cytotoxic DSA rebound (TING et al., 1986).

In 1989, Scornik also reported one patient diagnosed with chronic myeloid leukemia who received T-cell depleted BMT. The patient had cytotoxic DSAs and, thus, underwent nine sessions of plasma exchange before the transplant. This sensitized patient also received two plasma exchanges after the graft infusion, but the CDC remained positive, and the patient experienced engraftment failure and died early (SCORNIK et al., 1989). Likewise, Barge et al. detailed one patient with acute lymphoblastic leukemia who underwent a haploidentical bone marrow transplant with cytotoxic donor-directed antibodies. This patient had primary graft rejection, despite desensitization with two sessions of plasmapheresis, and died 60 days after the transplant. This paper also described that anti-donor antibodies to ABO antigens were associated with poor erythroid engraftment after allo-HCT (BARGE et al., 1989).

The first strong evidence that donor-directed cytotoxic antibodies were associated with graft rejection was provided by Anasetti and colleagues. In a cohort of 269 patients with malignancies submitted to BMT with haploidentical donors, the Seattle group showed that a positive CDC crossmatch was strongly associated with increased risks of failed engraftment (relative risk = 2.3; 95% confidence interval [CI] = 1.8-2.8; P = .0038) (ANASETTI et al., 1989). The same research group later reassessed the association between positive CDC and unacceptable risks of graft rejection in a cohort of 528 patients receiving allo-HCT from haploidentical donors (MICKELSON et al., 2000). Remarkably, it was shown that patients with a positive CDC had a significantly higher risk of graft rejection than those with negative CDC crossmatch (62% versus 6%, P < .0000001).

Ottinger and colleagues validated these previous findings in a matched-pair analysis, which showed that the risk of engraftment failure was significantly increased in patients with positive CDC-XM compared with controls (P = .04) (OTTINGER et al., 2002). In addition, Mattsson et al. investigated the impact of CDC-XM in 157 patients receiving allo-HCT from matched or mismatched unrelated donors. It was observed that T-cell CDC-XM had a sensitivity of 9% and a specificity of 97%, whereas B-cell CDC-XM showed a sensitivity of 36% and a specificity of 86%. In the adjusted logistic regression analysis, only a positive B-cell crossmatch was significantly

associated with failure to engraft (odds ratio = 8.9; 95% CI = 1.25–63.3; *P* = .027) (MATTSSON et al., 2010).

More recently, the solid-phase assays with Luminex technology have become the gold standard for HLA antibody detection and identification and considerably replaced cell-based assays in DSA assessment to support allo-HCT donor selection (CIUREA et al., 2018).

In the haploidentical donor context, the presence of DSA has been reported as a significant predictor of engraftment failure in different haploidentical platforms, including T-cell depletion, PTCy, Beijing protocol, and nonmyeloablative conditioning and GVHD prophylaxis with tacrolimus and methylprednisolone.

In a group of 24 consecutive patients receiving T-cell-depleted haploidentical transplants with megadoses of CD34+ stem cells, Ciurea et al. observed that three out of 4 (75%) patients with DSAs experienced graft failure, compared with only 1 of 20 (5%) patients without DSAs (*P* = .008) (CIUREA et al., 2009). Later, Yoshihara reported the impact of DSAs on patients with hematologic malignancies undergoing haploidentical transplants with nonmyeloablative conditioning and with tacrolimus and methylprednisolone as GVHD prophylaxis (YOSHIHARA et al., 2012). It was found that DSA-positive patients had a significantly lower incidence of neutrophil recovery than DSA-negative patients (61.9 vs. 94.4%, *P* = .026).

The MD Anderson group also investigated the impact of DSAs in a cohort of 122 patients with malignant diseases receiving haploidentical transplants with PTCy as GVHD prophylaxis. Notably, 7 of 22 (32%) DSA-positive patients experienced GF compared with 4 of 98 (4%) patients without DSAs (*P* < .001). In addition, all patients with C1q-positive DSAs failed to engraft in this landmark study (CIUREA et al., 2015). These findings were later validated by Zou and colleagues, which showed that DSAs were significantly associated with GF, engraftment delay, transplant-related mortality, and inferior overall survival after peripheral blood haplo-PTCy to treat mostly malignancies (ZOU et al., 2017).

The deleterious impact of DSAs was also reported in the Beijing haploidentical platform, considering 345 patients prospectively recruited and randomly divided into a training group (n = 173) and a validation group (n = 172). Multivariate regression showed that DSAs with MFI ≥ 10,000 were associated with primary graft rejection (*P* < .001), and DSAs with MFI ≥ 2000 were strongly correlated with early poor graft function (*P* = .005) in both training and validation groups

(CHANG et al., 2015). In a subsequent study with 394 patients undergoing haploidentical transplants with the Beijing platform, Zhao et al. reported that low-level DSAs, with MFI values between 1000 to 2000, were significantly associated with prolonged isolated thrombocytopenia and transplant-related mortality (ZHAO et al., 2017).

In the umbilical cord blood transplantation setting, the presence of DSA has been associated with an increased risk of GF in most (CUTLER et al., 2011; FUJI et al., 2020; RUGGERI et al., 2013) but not all (BRUNSTEIN et al., 2011) studies. However, the role of DSAs after unrelated donor allo-HCT is currently controversial. In 2010, Spellman and colleagues conducted a matched-pair study in the unrelated donor setting. They found that 9 of 37 (24%) graft failure cases had DSAs compared with only 1 of 78 (1%) matched controls with sustained engraftment ($P < .001$) (SPELLMAN et al., 2010). This finding was later replicated by Ciurea et al., who showed that DSA-positive patients had significantly higher graft failure incidence than DSA-negative patients (37.5% versus 2.7%; $P = .0014$) after matched unrelated donor transplants. Importantly, it was reported that preformed DSA was the only significant predictor of GF in multivariable regression (CIUREA et al., 2011). Nevertheless, three recent reports have failed to corroborate the association between DSAs and an increased risk of engraftment failure in patients undergoing unrelated donor transplantation (CLUZEAU et al., 2016; CUADRADO et al., 2020; WOOLFREY et al., 2019).

To date, the effect of DSAs in GF after alternative donor transplantation for nonmalignant disorders has been poorly reported. In a cohort of pediatric patients with hemoglobinopathies undergoing TCRαβ/CD19-depleted grafts, Gaziev et al. showed that all three patients with DSAs had rejection compared to 4 of 10 DSA-negative patients ($P = .19$) (GAZIEV et al., 2018). In turn, Arcuri et al. reported that DSAs were significantly associated with poor event-free survival in patients with relapsed/refractory severe aplastic anemia receiving haplo-PTCy (ARCURI et al., 2020). Kurzay et al. described that four patients with DSAs were desensitized with rituximab and plasmapheresis and fully engrafted after haplo-PTCy for inborn errors (KURZAY et al., 2019). More recently, Hashem et al. reported that three severe aplastic anemia (SAA) patients with high-level DSAs failed to engraft, despite a desensitization treatment with plasmapheresis, rituximab, and intravenous immunoglobulin (HASHEM et al., 2022).

1.1.3.4 Desensitization protocols in allo-HCT

When DSA-negative donors are unavailable, desensitization therapy is necessary to facilitate donor engraftment across the DSA barrier and mitigate the poor survival prognosis associated with failed engraftment. In the allo-HCT scenario, several desensitization protocols have been reported (BAILÉN et al., 2021; CIUREA et al., 2021; LEFFELL; JONES; GLADSTONE, 2015). These DSA mitigating strategies are often multimodality and include (1) the removal through plasma exchange, (2) inhibition of DSA production by targeting B-cell lymphocytes with rituximab or plasma cells with proteasome inhibitor bortezomib or anti-CD38 monoclonal antibody daratumumab, (3) DSA neutralization by donor buffy coat or HLA incompatible platelets, and (4) treatment with intravenous immune globulin (IVIG) and immunosuppressive drugs such as mofetil mycophenolate and tacrolimus (FILE et al., 2022; KRUMMEY; GAREAU, 2022).

Based on a previous desensitization experience in solid organ transplantation, the Johns Hopkins group developed a desensitization protocol consisting of alternate plasmapheresis, IVIg, tacrolimus, and mycophenolate mofetil to reduce DSA levels below FCXM positivity. Using this protocol, Leffell and colleagues described that 14 DSA-positive patients experienced a mean reduction in DSA level of 64.4% after the desensitization treatment. Notably, all 14 patients achieved sustained donor engraftment by day +60 (LEFFELL; JONES; GLADSTONE, 2015).

Similarly, Bailen et al. reported 19 DSA-positive patients who received an aggressive multimodality desensitization treatment, including plasma exchange, rituximab, immunoglobulins, incompatible platelets, buffy coat, and immunosuppression with mofetil mycophenolate, tacrolimus, and steroids before haploidentical allo-HCT. It was observed that all patients had MFI decrease following the desensitization treatment, with a mean reduction of 74%. Noteworthy, 17 of 19 (90%) patients achieved neutrophil recovery (BAILÉN et al., 2021).

More recently, Ciurea et al. described 37 DSA-positive patients who underwent haplo-PTCy and were treated with a multimodality desensitization strategy, including plasma exchange, rituximab, and IVIg and an irradiated donor buffy coat infusion on day -1 from graft infusion. It was demonstrated that DSAs <20,000 MFI and those with C1q-negative results after desensitization showed

similar engraftment and survival compared to controls with DSAs. In contrast, those patients with DSA > 20,000 MFI and persistent C1q-positivity after desensitization experienced a significantly lower engraftment recovery, leading to higher non-relapse mortality and dismal overall survival compared to controls (CIUREA et al., 2021).

1.2 JUSTIFICATION OF THE STUDY

Graft failure remains a significant cause of morbidity and mortality after allo-HCT. Despite current evidence indicating that preformed DSAs are a substantial risk factor for engraftment failure, several gaps still exist regarding the role of DSAs after allo-HCT with alternative donors.

Even though DSAs have been recognized as a major predictor of failed engraftment in adult patients with malignancies undergoing haplo-PTCy, the impact of DSAs after pediatric haplo-PTCy for nonmalignant disorders has been scarcely reported in current HCT literature. Furthermore, the role of DSAs in patients with nonmalignant disorders receiving second haplo-PTCy for rescuing a previous graft failure had not been reported before this research project.

Additionally, the impact of DSAs in GF after unrelated donor transplantation is controversial. Although two earlier reports have shown that DSAs were significantly associated with a higher risk of GF after MUD and MMUD transplantation, recent studies have failed to confirm the association between DSAs and a higher incidence of GF after transplantation with unrelated donors.

Remarkably, most studies in haploidentical and unrelated donor settings have not thoroughly assessed relevant features for DSA risk assessment (TAMBUR et al., 2018), including C1q-binding, DSA titer, DSA strength (meaning MFI level), donor-specific epitope patterns, and possible false-positive DSA reactivity.

2 OBJECTIVES

2.1 GENERAL OBJECTIVE

To perform a thorough DSA characterization and evaluate the clinical impact of DSAs in allogeneic hematopoietic stem cell transplantation with alternative donors.

2.2 SPECIFIC OBJECTIVES

- To analyze the impact of DSA on hematologic recovery, graft failure, and survival in pediatric patients with nonmalignant disorders undergoing their first haploidentical donor transplantation with PTCy.

- To evaluate the influence of DSA on hematologic recovery, graft failure, and survival in pediatric patients with nonmalignant disorders receiving salvage haploidentical donor transplantation with PTCy to treat a previous engraftment failure.

- To investigate the effect of DSAs on hematologic recovery, graft failure, and survival after unrelated donor transplantation.

3 METHODS

3.1 ETHICAL APPROVAL

The institutional review board of the Complexo Hospital de Clínicas, Federal University of Paraná (CHC/UFPR) approved this retrospective research project (CAAE nº 96085718.6.0000.0096, final amendment; Appendix 1), which was conducted following the Declaration of Helsinki.

3.2 PATIENTS

In total, we retrospectively assessed 362 patients lacking an HLA-matched related sibling donor and mostly with nonmalignant disorders who underwent their first allo-HCT at CHC/UFPR from January 2008 to December 2017. Among them, 59 were pediatric patients with nonmalignant disorders receiving upfront haploidentical donor transplantation with PTCy, and 303 were patients undergoing their first unrelated donor transplantation. We also evaluated 22 patients with nonmalignant disorders who received second haploidentical donor transplantation with PTCy to rescue a previous engraftment failure. Clinical and laboratory data concerning the

current project were obtained from medical records and the CHC/UFPR's HCT Unit database.

3.3 HLA TYPING AND HLA ANTIBODY TESTING

Patients and donors were routinely typed for *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*, and *HLA-DQB1* loci at medium resolution by reverse sequence-specific oligonucleotide method (LABType, One Lambda Inc., Thermo Fisher Scientific, West Hills, California) and high-resolution sequence-based typing (SeCore SBT, One Lambda). Patients with antibodies against HLA-DP, HLA-DR52, HLA-DR53, and HLA-DR51 were subsequently typed for *HLA-DPA1/DPB1*, *HLA-DRB3*, *HLA-DRB4*, and *HLA-DRB5* loci, respectively (LABType, One Lambda Inc.).

HLA antibody identification testing was performed with LABScreen SAB kits (One Lambda Inc.) and with LIFECODES SAB kits (Immucor, Inc., Norcross, GA). We used Ethylenediaminetetraacetic acid (EDTA) treatment or heat inactivation of the sera to avoid the complement inhibition effect (DE MARCO; DE LIMA, 2014; WANG et al., 2017). Briefly, EDTA treatment consisted of the addition of 5 µL of 100 mM EDTA, diluted in PBS, in 95 µL of sera for a final concentration of 5 mM EDTA (WANG et al., 2017), while heat inactivation of sera was carried out in the Luminex plate heated for 1 min at 56°C in a thermocycler (DE MARCO; DE LIMA, 2014). MFI > 1000 was the cutoff for positivity, and MFI values between 1000 and 500 were considered potentially positive based on HLA epitope patterns and cross-reactive groups (BETTINOTTI; ZACHARY; LEFFELL, 2016; ELLIS, 2013). Epitope analyses were conducted using expanded SAB panels (LABScreen ExPlex, One Lambda Inc.) with the HLA-Fusion MatchMaker software (Version 4.4). We used the LIFECODES SAB panels (Immucor, Inc.) to confirm true-positive DSA reactivities (RAVINDRANATH et al., 2021). Additionally, DSA-positive patients with divergent LABScreen and LIFECODES results or suspected false-positive patterns in the epitope analysis were tested with absorption/elution protocol using cryopreserved donor cells or surrogate donor cells expressing the DSA's target (GRENZI et al., 2013) or with SAB beads with HLA molecules denatured by acid treatment (EL-AWAR et al., 2009).

DSA-positive patients were retrospectively evaluated using the LABScreen SAB assay with 3 different dilutions (1:2, 1:8, and 1:32) to estimate the DSA titer

(TAMBUR; WIEBE, 2018). The C1q-binding assay (C1qScreen, One Lambda Inc.) was also retrospectively performed, with MFI >500 as the cutoff (CIUREA et al., 2015). All laboratory tests were carried out according to the manufacturer's instructions. Cell-based crossmatches were not routinely performed during the study period.

3.4 ENDPOINTS AND DEFINITIONS

The primary endpoints of the current thesis were neutrophil and platelet recovery and GF after alternative donor transplantation (KHARFAN-DABAJA et al., 2021). Secondary endpoints included event-free survival (EFS) and overall survival (OS) (KANATE; NAGLER; SAVANI, 2019). Briefly, *neutrophil recovery* was defined as the first of three consecutive days with an absolute neutrophil count (ANC) $\geq .5\times10^9$/L. *Platelet recovery* was defined as the first of three consecutive days at $\geq 20 \times 10^9$/L and at least seven days without platelet transfusion support. The GF endpoint included primary graft rejection, secondary GF, and poor graft function as a single outcome. *Primary graft rejection* was defined as the failure to achieve an ANC of $.5\times10^9$/L by day +28 and the absence of donor-derived hematopoiesis (<5% donor cells) using short tandem repeat chimerism analysis. *Secondary GF* was defined as achieving ANC of $.5\times10^9$/L with subsequent loss of donor chimerism (<5% donor cells). *Poor graft function* was defined as the presence of 2 or 3 cytopenic counts (ANC $\leq 0.5\times10^9$/L, platelets $\leq 20\times10^9$/L, or hemoglobin $\leq$ 70g/L) for at least 3 consecutive days beyond day 28 post-transplantation with a transfusion requirement associated with hypoplastic bone marrow in the presence of complete donor chimerism. EFS was defined as the time from alternative donor allo-HCT to primary graft rejection, secondary GF, poor graft function, or death, whichever came first, or last follow-up (censored). OS was defined as the time from alternative donor allo-HCT to death from any cause or last follow-up (censored) (KANATE; NAGLER; SAVANI, 2019).

3.5 STATISTICAL ANALYSIS

All statistical analyses were carried out according to the suggestions of the European Group for Blood and Marrow Transplantation (EBMT) Statistical Committee (IACOBELLI, 2013).

The presence of DSAs was considered the main effect in all analyses. Baseline characteristics of the DSA groups were compared with Fisher's exact test and Mann–Whitney U test for categorical and continuous covariates, respectively. The MFI levels among DSA-positive patients with and without GF were compared using the Mann–Whitney U test. In this analysis, the DSAs with the highest MFI values were considered in patients with 2 or more DSAs. For univariable analyses, continuous variables were transformed into categorical variables by treating the median value as a threshold. The median follow-up was calculated using the reverse Kaplan–Meier method. Estimates of neutrophil and platelet recovery and GF were calculated using cumulative incidence curves to accommodate competing risks. Death without hematologic recovery was considered a competing risk for neutrophil and platelet recovery, while death with sustained engraftment was a competing risk for GF. The Gray test was used for univariable comparisons of cumulative incidence curves. The probabilities of EFS and OS were estimated using the Kaplan–Meier method and compared between groups using the log-rank test. Multivariable analyses for neutrophil and platelet recovery and GF were performed using Fine-Gray subdistribution hazard models, whereas multivariable analyses for EFS and OS were performed using Cox proportional hazards regression models. Covariates that reached a P value ≤ .10 in the univariable analyses were included in the initial multivariable models and were eliminated one at a time using the backward stepwise method. The final adjusted models held only covariates that attained a P value ≤ .05 (Wald test). In the salvage haplo-PTCy cohort, the multivariable analyses were not performed due to the limited sample size. Moreover, the prognostic effect of GF on OS was assessed by a Cox regression model, using GF as a time-dependent variable. Regression results are expressed as the hazard ratio (HR) or subdistribution hazard ratio (SHR) with a 95% CI. A two-sided P value ≤ .05 was considered statistically significant (IACOBELLI, 2013; KANATE; NAGLER; SAVANI, 2019). Statistical analysis was performed using EZR versions 1.53 and 1.54 (Saitama Medical Centre, Jichi Medical University) (KANDA, 2013).

4 RESULTS AND DISCUSSION

4.1 MANUSCRIPT 1

Untreated Donor-Specific HLA Antibodies Are Associated With Graft Failure And Poor Survival After Haploidentical Transplantation With Post-Transplantation Cyclophosphamide in Pediatric Patients With Nonmalignant Disorders.

Published in *Transplantation and Cellular Therapy*. 2022 Oct;28(10): 698.e1-e11. (DOI: 10.1016/j.jtct.2022.07.019)

Impact Factor: 5.6

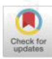
Transplantation and Cellular Therapy 28 (2022) 698.e1−698.e11

Transplantation and Cellular Therapy

journal homepage: www.tctjournal.org

Full Length Article
Pediatric

Untreated Donor-Specific HLA Antibodies Are Associated With Graft Failure and Poor Survival After Haploidentical Transplantation With Post-Transplantation Cyclophosphamide in Pediatric Patients With Nonmalignant Disorders



Alberto Cardoso Martins Lima[a,*], Carmem Bonfim[b,c], Joselito Getz[a], Geovana Borsato do Amaral[a], Ricardo Rasmussen Petterle[d], Gisele Loth[b,c], Samir Kanaan Nabhan[b], Renato de Marco[e], Maria Gerbase-DeLima[e], Noemi Farah Pereira[a], Ricardo Pasquini[b]

[a] *Immunogenetics Laboratory* – *Complexo Hospital de Clínicas, Federal University of Paraná, Curitiba, PR, Brazil*
[b] *Bone Marrow Transplantation Unit* – *Complexo Hospital de Clínicas, Federal University of Paraná, Curitiba, PR, Brazil*
[c] *Instituto de Pesquisa Pelé Pequeno Príncipe, Hospital Pequeno Príncipe, Curitiba, PR, Brazil*
[d] *Departament of Integrative Medicine, Federal University of Paraná, Curitiba, PR, Brazil*
[e] *Immunogenetics Institute/ Associação Fundo de Incentivo à Pesquisa, São Paulo, SP, Brazil*

Article history:
Received 12 March 2022
Accepted 18 July 2022

Key Words:
Donor-specific anti-HLA antibodies
Nonmalignant disorders
Graft failure
Haploidentical

A B S T R A C T

Donor-specific HLA antibodies (DSAs) have been recognized as a major risk factor for graft failure (GF) in adult patients with malignancies undergoing haploidentical transplantation with post-transplantation cyclophosphamide (haplo-PTCy). However, the impact of DSAs after pediatric haplo-PTCy for nonmalignant disorders (NMDs) has been poorly reported. We sought to investigate whether preexisting DSAs adversely affect pediatric haplo-PTCy outcomes. We retrospectively analyzed 59 pediatric patients (≤21 years) who received their first haplo-PTCy for NMDs from January 2008 to December 2017. DSA testing was performed using single antigen beads, and mean fluorescence intensity (MFI) >1000 was considered positive, and MFI <1000 and >500 was considered potentially positive, based on HLA epitope reactivity patterns. Primary endpoints were neutrophil and platelet recovery and GF, whereas secondary endpoints included event-free and overall survival. Multivariable analyses were performed using Fine-Gray competing risk regression or Cox proportional hazards regression models. The median age was 10 years, and 66.1% were male. Main indications for haplo-PTCy were Fanconi anemia (n = 33) and severe aplastic anemia (n = 11). All patients received bone marrow as the graft source, and most patients (91.5%) received fludarabine-based conditioning. Overall, 15 patients (25.4%) had DSAs >500 MFI. Four patients had false-positive DSAs with median MFI of 1762. Of the 11 patients with true-positive DSA reactivity, 5 had 1 DSA, 5 had 2 DSAs, and 1 had 3 DSAs, with median MFI of 2372 (range 527-24,200). Four patients received desensitization therapy with rituximab and plasmapheresis, whereas 7 patients were untreated. All patients with treated DSAs achieved donor engraftment. In the multivariable analyses, untreated DSAs were associated with lower neutrophil recovery (subdistribution hazard ratio [SHR] = 0.15; 95% confidence interval [CI], 0.03-0.63; *P* = .001), increased GF (SHR = 20.57; 95% CI, 6.57-64.43; *P* < .001), inferior event-free survival (hazard ratio [HR] = 10.09; 95% CI, 3.37-30.22; *P* < .001), and poor overall survival (HR 5.56; 95% CI, 1.92-16.12; *P* = .002). Both treated DSAs (SHR = 0.26; 95% CI, 0.10-0.68; *P* = .006) and untreated DSAs (SHR = 0.13; 95% CI, 0.04-0.37; *P* < .001) adversely affected platelet recovery. Our results indicate that the presence of DSAs is an independent predictor of poor outcomes after pediatric haplo-PTCy for NMDs. Therefore DSA-positive donors should be avoided whenever possible, and when a DSA-negative donor is unavailable, desensitization therapy must be performed to enhance the likelihood of donor engraftment and improve transplantation outcomes.

© 2022 The American Society for Transplantation and Cellular Therapy. Published by Elsevier Inc. All rights reserved.

Hematopoietic cell transplantation with haploidentical donors using post-transplant cyclophosphamide (haplo-PTCy) as graft-versus-host disease (GVHD) prophylaxis has expanded the donor options for patients lacking an HLA-matched sibling or matched unrelated donors [1]. This platform has been

Financial disclosure: See Acknowledgments on page 698.e.10

*Correspondence and reprint requests: Alberto Cardoso Martins Lima, M.Sc., Immunogenetics Laboratory – Complexo Hospital de Clínicas, Federal University of Paraná, Rua Padre Camargo, 280 – 1o Andar, CEP 80060-240, Curitiba, PR, Brazil.

E-mail address: acml@ufpr.br (A.C.M. Lima).

https://doi.org/10.1016/j.jtct.2022.07.019
2666-6367/© 2022 The American Society for Transplantation and Cellular Therapy. Published by Elsevier Inc. All rights reserved.

STRUCTURED ABSTRACT

Background: Donor-specific HLA antibodies (DSAs) have been recognized as a major risk factor for graft failure (GF) in adult patients with malignancies undergoing haploidentical transplantation with post-transplant cyclophosphamide (haplo-PTCy). However, the impact of DSAs after pediatric haplo-PTCy for nonmalignant disorders (NMDs) has been poorly reported.

Objective: We sought to investigate whether preexisting DSAs adversely affect pediatric haplo-PTCy outcomes.

Study Design: We retrospectively analyzed 59 pediatric patients (≤21 years) who received their first haplo-PTCy for NMDs from January 2008 to December 2017. DSA testing was performed using single antigen beads, and mean fluorescence intensity (MFI) >1000 was considered positive and MFI <1000 and >500 was considered potentially positive, based on HLA epitope reactivity patterns. Primary endpoints were neutrophil and platelet recovery and GF, whereas secondary endpoints included event-free and overall survival. Multivariable analyses were performed using Fine-Gray competing risk regression or Cox proportional hazards regression models.

Results: The median age was 10 years, and 66.1% were male. Main indications for haplo-PTCy were Fanconi anemia (n=33) and severe aplastic anemia (n=11). All patients received bone marrow as the graft source, and most patients (91.5%) received fludarabine-based conditioning. Overall, 15 patients (25.4%) had DSAs >500 MFI. Four patients had false-positive DSAs with median MFI of 1762. Of the 11 patients with true-positive DSA reactivity, 5 had 1 DSA, 5 had 2 DSAs, and one had 3 DSAs, with median MFI of 2372 (range, 527-24200). Four patients received desensitization therapy with rituximab and plasmapheresis, whereas 7 patients were untreated. All patients with treated DSAs achieved donor engraftment. In the multivariable analyses, untreated DSAs were associated with lower neutrophil recovery (SHR=0.15; 95% CI, 0.03-0.63; *P*=.001), increased GF (SHR=11.90; 95% CI, 3.56-39.81; *P*<.001), inferior event-free survival (HR=10.09; 95%CI, 3.37-30.22; *P*<.001), and poor overall survival (HR 5.56; 95% CI, 1.92-16.12; *P*=.002). Both treated DSAs (SHR=0.26; 95% CI, 0.10-0.68; *P*=.006) and untreated DSAs (SHR=0.13; 95% CI, 0.04-0.37; *P*<.001) adversely affected platelet recovery.

Conclusions: Our results indicate that the presence of DSAs is an independent predictor of poor outcomes following pediatric haplo-PTCy for NMDs. Therefore, DSA-positive donors should be avoided whenever possible, and when a DSA-negative donor is unavailable, desensitization therapy must be performed to enhance the likelihood of donor engraftment and improve transplant outcomes.

Keywords: Donor-specific anti-HLA antibodies, nonmalignant disorders, graft failure, haploidentical

4.1.1 Introduction

Hematopoietic cell transplantation with haploidentical donors using post-transplant cyclophosphamide (haplo-PTCy) as graft-versus-host disease (GVHD) prophylaxis has expanded the donor options for patients lacking an HLA-matched sibling or matched unrelated donors[1]. This platform has been increasingly used to treat nonmalignant disorders (NMDs), including inherited bone marrow failure syndromes[2], acquired severe aplastic anemia (SAA)[3], primary immunodeficiency diseases[4], and hemoglobinopathies[5, 6]. Nevertheless, graft failure (GF) remains a major complication following haplo-PTCy for NMDs[7, 8].

Previous observational studies have consistently demonstrated that the presence of donor-specific anti-HLA antibodies (DSA) is significantly associated with higher risks of graft failure after alternative donor transplants, including matched unrelated donors[9], cord blood units[10], and haploidentical donor transplantation, either with Beijing protocol[11] or haplo-PTCy platform[12, 13]. Indeed, a recent meta-analysis, including 15 studies with 2,436 patients, mostly with malignancies, indicated that patients with DSA had an odds ratio of 7.47 for primary graft failure, regardless of graft source, alternative donor type, and primary disease[14]. However, most previous studies regarding DSAs as predictors of GF after haploidentical transplants have been conducted in adult patients with malignant diseases, and current evidence regarding the role of DSAs following pediatric haplo-PTCy for NMDs is scarce. Kurzay et al. reported that 4 DSA-positive patients treated with rituximab and plasmapheresis had complete engraftment following haplo-PTCy for inborn errors[15]. In addition, Arcuri et al. recently showed that DSAs were associated with poor event-free survival (EFS) in patients undergoing haplo-PTCy for relapsed/refractory SAA[3]. Importantly, such studies have not addressed relevant DSA characteristics such as C1q-binding, antibody titer, donor-epitope specificity, and false-positive DSA reactivity[16].

As we previously demonstrated that untreated DSAs were related to high risk of GF and inferior EFS in pediatric patients with NMDs undergoing salvage haplo-PTCy for engraftment failure[17], we hypothesized that preformed DSAs would also be associated with poor outcomes following the first pediatric haplo-PTCy for NMDs. Therefore, the present retrospective single-center study sought to perform a

comprehensive DSA characterization and investigate whether preexisting DSAs would adversely affect transplantation outcomes.

4.1.2 Methods

4.1.2.1 Patient data

In total, 66 pediatric patients (≤21 years) who lacked an HLA-matched related or matched unrelated donor received their first haplo-PTCy for NMDs from January 2008 to December 2017. We excluded seven patients who lacked serum samples for DSA testing. Thus, 59 patients were analyzed in our study. This retrospective study was conducted following the Declaration of Helsinki and was approved by our institutional review board (number 96085718.6.0000.0096).

4.1.2.2 HLA typing and HLA antibody detection

Patients and haploidentical donors were routinely typed for *HLA-A, HLA-B, HLA-C, HLA-DRB1,* and *HLA-DQB1 loci* at medium resolution by reverse sequence-specific oligonucleotide method (LABType, One Lambda Inc., Canoga Park, California) and/or high-resolution sequence-based typing (SeCore SBT, One Lambda). Patients with antibodies against HLA-DP, HLA-DR52, HLA-DR53, and HLA-DR51 were subsequently typed for *HLA-DPA1/DPB1*, *HLA-DRB3*, *HLA-DRB4*, and *HLA-DRB5* loci, respectively.

HLA antibody testing was performed using LABScreen (One Lambda Inc.) Single Antigen Beads (SAB) kits on a FlexMap 3D instrument (Luminex, Austin, Texas). All SAB testing used EDTA-treated sera to prevent the complement inhibition effect[18]. Reactions with mean fluorescence intensity (MFI) >1000 were considered positive and with MFI <1000 and >500 were considered potentially positive, based on HLA epitope reactivity patterns and cross-reactive groups[19]. HLA epitope analyses were done with HLA-Fusion MatchMaker software (Version 4.4) using expanded SAB panels (LABScreen ExPlex, One Lambda Inc.). True-positive DSA reactions were confirmed with LIFECODES SAB kits (Immucor, Norcross, GA)[20]. Furthermore, DSA-positive patients with divergent SAB results in LABScreen and LIFECODES assays or suspected false-positive DSA reactivity were tested with beads with HLA

molecules denatured by acid treatment or absorption/elution protocol, using cryopreserved donor cells[21-23]. DSA-positive samples were retrospectively tested with the C1q-binding assay (C1qScreen, One Lambda Inc.) and with 1:2, 1:8, and 1:32 dilutions to estimate DSA titer. C1q assay used MFI>500 as the cutoff to assign positivity. All tests were carried out according to the manufacturer's instructions. Cell-based crossmatches were not routinely performed during the study period.

4.1.2.3 Preparatory regimen and GVHD prophylaxis

Reduced-intensity preparatory regimens included fludarabine (Flu) (130–160 mg/m2) combined with total body irradiation (200-400 cGy) with or without cyclophosphamide (Cy) (29-50 mg/kg). Myeloablative regimens were busulfan-based (12-16 mg/kg), combined with Flu (160 mg/m^2) or Cy (30 mg/kg). The choice of the conditioning regimen was determined at the physician's discretion. Conditioning regimens and dose-reduced PTCy approach used for patients with Fanconi anemia are described elsewhere[2]. GVHD prophylaxis for all other patients consisted of PTCy (50 mg/kg/day on days +3 and +4), cyclosporine A (target trough levels of 150–200 ng/ml), and mycophenolate mofetil (45 mg/kg/day with a maximum of 3 g/day) starting on day +5.

4.1.2.4 Definitions and endpoints

The primary endpoints were neutrophil and platelet recovery and GF after haplo-PTCy. Secondary endpoints included EFS and overall survival (OS)[24]. Neutrophil recovery was defined as the first of three consecutive days with an absolute neutrophil count (ANC) $\geq .5\times10^9/L$. Platelet recovery was defined as the first of three consecutive days at $\geq 20 \times 10^9/L$ and at least seven days without platelet transfusion support. The primary graft rejection was defined as the failure to achieve an ANC of $.5\times10^9/L$ by day +28 and absence of donor-derived hematopoiesis (<5% donor cells) using short tandem repeat chimerism analysis. Secondary GF was defined as having achieved ANC of $.5\times10^9/L$ with subsequent loss of donor chimerism. Poor graft function was defined as the presence of 2 or 3 cytopenic counts (ANC$\leq 0.5\times10^9/L$, platelets$\leq 20\times10^9/L$, or hemoglobin≤70 g/L) for at least 3 consecutive days beyond day 28 post-transplantation with a transfusion requirement

associated with hypoplastic bone marrow in the presence of complete donor chimerism[25]. Primary graft rejection, secondary GF, and poor graft function were considered together as a single outcome. EFS was defined as the time from haplo-PTCy to primary graft rejection, secondary GF, poor graft function, or death, whichever came first, or last follow-up (censored). OS was defined as the time from haplo-PTCy to death from any cause or last follow-up (censored)[24].

4.1.2.5 Statistical analysis

Patient and transplantation characteristics by DSA status were compared with the Fisher's exact test for categorical variables and the Mann–Whitney *U* test for continuous variables. For univariable analyses, continuous variables were transformed into categorical variables by treating the median value as a threshold. The median follow-up time was calculated using the reverse Kaplan–Meier method. Estimates of neutrophil and platelet recovery and GF were calculated using cumulative incidence curves to accommodate competing risks. Death without hematologic recovery was considered a competing risk for neutrophil and platelet recovery, while death with sustained engraftment was a competing risk for GF. The Gray test was used for univariable comparisons of cumulative incidence curves. The probabilities of EFS and OS were estimated using the Kaplan–Meier method and compared between groups using the log-rank test. Multivariable analyses for neutrophil and platelet recovery and GF were performed using Fine-Gray subdistribution hazard models, whereas multivariable analyses for EFS and OS were performed using Cox proportional hazards regression models. The presence of DSAs was included in all models as the main effect. To estimate the prognostic influence of DSAs on haplo-PTCy outcomes, we fit two distinct multivariable models. First, we examined the role of preexisting DSAs, regardless of desensitization therapy (DSA-negative vs. DSA-positive). In the second model, we evaluated the impact of desensitization treatment (DSA-negative vs. treated DSAs vs. untreated DSAs). Covariates that reached a P-value ≤ .10 in the univariable analyses were included in the initial multivariable models and were eliminated one at a time using the backward stepwise method. Only covariates that attained a P-value ≤.05 (Wald test) were held in the final adjusted models. Results are expressed as the hazard ratio (HR) or subdistribution hazard ratio (SHR) with a 95% confidence interval (95% CI). A two-

sided *P*-value ≤.05 was considered statistically significant[24]. Statistical analysis was performed using EZR version 1.53 (Saitama Medical Centre, Jichi Medical University)[26].

4.1.3 Results

4.1.3.1 Patient-, donor-, and transplantation-related characteristics

Baseline characteristics of the 59 patients are presented in Table 1. The median age was 10 years (range, 1-20) at haplo-PTCy, and 66.1% were male. The main indications for haplo-PTCy were Fanconi anemia (n=33), acquired SAA (n=11), and Wiskott–Aldrich syndrome (n=6). Most patients (91.5%) received fludarabine-based reduced-intensity conditioning. Unmanipulated marrow was used as a graft source for all patients, and median total nucleated cell (TNC) and $CD34^+$ cell doses were 7×10^8/kg (range, 2.07-14.93$\times10^8$/kg) and 4.09$\times10^6$/kg (range, 0.63-19.42$\times10^6$/kg), respectively. Patients with and without DSA had equivalent patient and donor characteristics, except for a trend (*P* =.077) regarding ABO matching (Table 1).

4.1.3.2 HLA Allosensitization and DSA Assessment

Among all patients, 36 (61%) showed alloantibodies to HLA antigens. Among these patients, 8 (13.6%) had antibodies against class I HLA antigens, 10 (16.9%) against class II HLA antigens, and 18 (30.5%) against both HLA classes. The degree of HLA allosensitization was not associated with patient age (*P*=.60), diagnosis (*P*=1.0), or gender (*P*=.16). Overall, 15 patients (25.4%) had DSAs >500 MFI. Four patients showed DSAs with suspected reactivity patterns (DP1/DP5; Pan-DR reactivity; DR4/DR16/DP19; DQA1*01:03/DQB1*06:03) and median MFI of 1762 (range, 544-2483). These DSAs were confirmed as false-positive reactions in the absorption/elution protocol (Supplementary figure S1) and therefore were disregarded of the DSA-positive group. Of the 11 patients (18.6%) with true-positive DSA reactivity, 5 had 1 DSA, 5 had 2 DSAs, and one had 3 DSAs, with median MFI of 2372 (range, 527-24200) before the bone marrow infusion. Four patients with 7 DSAs (median MFI= 12351; range, 1549–27405) in the first DSA testing underwent

desensitization therapy with three sessions of plasma exchange, performed on days -15, -12, and -9, and one single dose of rituximab (375 mg/m^2) on day -8. After desensitization, the treated DSAs had a median MFI of 8337 (range, 527–24200). In contrast, seven patients with 11 DSAs (median MFI=1752; range, 562-16117) were untreated. All DSAs were directed to public epitopes, with several shared HLA antigens, and most of them had low-titers, with median MFI values of 1641, 405, and 41.5 at 1:2, 1:8, and 1:32 dilutions, respectively. All but one DSA were C1q-negative. The detailed information on the DSAs is shown in Table 2.

4.1.3.3 Neutrophil and Platelet Recovery

In the entire cohort, the cumulative incidence of neutrophil recovery at day +28 was 89.8% (95% CI, 78.3-95.4%), with a median time to neutrophil recovery of 16 days (range, 9-31). The cumulative incidence of platelet recovery at day +28 was 72.9% (95% CI, 59.3-82.5%), with a median time to platelet recovery of 20 days (range, 10-142 days).

The unadjusted cumulative incidences of neutrophil recovery at day +28 were 95.8% (95% CI, 81.3-99.1%) in patients without DSAs and 63.6% (95% CI, 26.6-85.7%) in patients with DSAs (*P*=.043; Figure 1a). The unadjusted cumulative incidences of platelet recovery at day +28 were 85.4% (95% CI, 71.3-92.9%) and 18.2% (95% CI, 2.5–45.6%) in DSA-negative and DSA-positive groups, respectively (*P*<.001; Figure 1b). Concerning desensitization therapy, the cumulative incidences of neutrophil recovery at day +28 were 95.8% (95% CI, 81.3-99.1%) in the DSA-negative group, 100% in the treated DSA group, and 42.9% (95% CI, 7.7-75.6%) in the untreated DSA group (*P*=.024; Fig. 1c). Alternatively, the cumulative incidence of platelet recovery at day +28 in the DSA-negative group was significantly higher (85.4%; 95% CI, 71.3-92.9%) than those in the treated DSA (25.0%; 95% CI: 0.2-73.2%) and untreated DSA groups (14.3%; 95% CI, 0.5-49.1%) (*P*=.001; Fig. 1d). Considering only the DSA-negative group, we observed that major ABO incompatibility was not correlated with neutrophil (*P*=.695) and platelet recovery (*P*>.88). Univariate cumulative incidences of neutrophil and platelet recovery of the other covariates are shown in supplemental Table 1.

In the adjusted multivariable regression, the presence of DSA was significantly associated with a lower incidence of neutrophil (SHR=0.28; 95% CI,

0.12-0.7; *P*=.006) and platelet recovery (SHR=0.18; 95% CI, 0.09-0.38; *P*<.001) (Table 3). When stratified by desensitization status, only untreated DSAs were associated with lower neutrophil recovery (SHR=0.15; 95% CI, 0.03-0.63; *P*=.001), while treated DSA and DSA-negative groups had similar neutrophil recovery (SHR=0.73; 95% CI, 0.3-1.77; *P*=.48). Moreover, compared with the DSA-negative cohort, both the treated DSA (SHR=0.26; 95% CI, 0.10-0.68; *P*=.006) and untreated DSA (SHR=0.13; 95% CI, 0.04-0.37; *P*<.001) groups were associated with lower platelet recovery in multivariable analyses (Table 3). TNC<7×10^8/kg (SHR=0.46; *P*=.001) and diagnosis of acquired SAA (SHR=0.23; *P*<.001) or primary immunodeficiency/metabolic diseases (SHR=0.38; *P*=.001) were also identified as independent predictors of poor neutrophil recovery, whereas the absence of T-cell depletion with rabbit anti-thymocyte globulin (rATG) was an independent risk factor for lower platelet recovery (SHR=0.44; *P*=.003).

4.1.3.4 Graft Failure

Overall, 11 patients experienced engraftment failure after haplo-PTCy. Among them, 3 had primary graft rejection, 5 had secondary GF, and 3 had poor graft function. The median time to GF onset was 35 days (range, 28-312 days). Notably, the 4 patients with engraftment failure at day +28, either primary graft rejection or poor graft function, had untreated DSAs prior haplo-PTCy. On the other hand, the six GF cases in the DSA-negative group consisted of five secondary GF, with a median of 85 days (range, 35-312) to the graft loss onset and one poor graft function on day +31. Data on HHV6 reactivation was available only for 12 symptomatic patients. These patients had median reactivation of 61 days (range, 10-193), and only three experienced GF. Among the 11 DSA-positive patients, the median MFI for patients with GF was 1468 (range, 562-10150) versus 5665 (range, 527– 24200) for those who have engrafted (*P*=.36). Also, the 4 patients with confirmed false-positive DSAs were assigned as DSA-negative at the time of haplo-PTCy, and no desensitization therapy was performed. These patients fully engrafted and were alive and disease-free at the last follow-up. Of the 11 patients with GF, 7 underwent a second transplant using distinct haploidentical donors. Details on the outcomes of salvage haplo-PTCy are described elsewhere[17].

For the whole cohort, the cumulative incidence of GF at 100 days and 1-year were 15.3% (95% CI, 7.4-25.6%) and 18.6% (95% CI, 9.9-29.6%), respectively. The unadjusted cumulative incidences of GF at 100 days were 8.3% (95%CI, 2.6-18.3%) in the DSA-negative group and 45.5% (95% CI, 4.9-72.1%) in the DSA-positive group (*P*=.006; Figure 2a). All four DSA-positive patients who underwent desensitization therapy achieved donor engraftment. Of them, one patient with two DSAs achieved neutrophil recovery at day +15, with 100% donor cells in the chimerism testing, but had early death at day +24 due to thrombotic microangiopathy. With regard to desensitization, the unadjusted cumulative incidences of GF at day +100 were 8.3% (95%CI, 2.6-18.3%) in the DSA-negative group, 0% in the treated DSA, and 71.4% (95%CI, 15.7-94.1%) in the untreated DSA group (*P*<.0001; Figure 2b). Supplemental Table 1 contains the unadjusted cumulative incidences of GF for the other covariates.

Multivariable regression showed that the presence of DSAs was associated with an increased risk of GF (SHR=11.90; 95% CI, 3.56-39.81; *P*<.001). Additionally, compared with the DSA-negative and treated DSA cohorts, the untreated DSA group was strongly associated with a higher incidence of GF (SHR=20.57; 95% CI, 6.57-64.43; *P*<.001) in the adjusted multivariable analysis (Table 3). The only other independent risk factors for GF were diagnosis of acquired SAA (SHR=6.23; *P*=.026) and primary immunodeficiency or metabolic diseases (SHR=13.08; *P*=.002).

4.1.3.5 Event-Free Survival and Overall Survival

Overall, 19 out of 59 patients died. Causes of death included infection (n=5), rejection (n=4), GVHD (n=4), organ failure (n=4), pulmonary hemorrhage (n=1), and thrombotic microangiopathy (n=1). The median follow-up was 4.7 years (range, 0.04-9.4 years), based on the reverse Kaplan–Meier method. Considering the entire cohort, the probabilities of EFS and OS at 1-year were 69.5% (95% CI, 56.0–79.6%) and 74.6% (95% CI, 61.4-83.8%), respectively. The unadjusted 1-year EFS was significantly lower in patients with DSAs (36.4%; 95% CI, 11.2-62.7%) than in those without DSAs (77.1%; 95% CI, 62.5-86.6%) (*P*<.001; Figure 3a). Similarly, the unadjusted 1-year OS rates in the DSA-positive and DSA-negative groups were 45.5% (95% CI, 16.7-70.7%) and 81.2% (95% CI, 67.1-89.8%), respectively (*P*=.003; Figure 3b). Moreover, the unadjusted EFS and OS probabilities were 77.1% (95% CI,

62.5-86.6%) and 81.2% (95% CI, 67.1-89.8%) in the DSA-negative group, 75.0% (95% CI, 12.8-96.1%) and 75.0% (95% CI, 12.8-96.1%) in the treated DSA group, and 14.3% (95% CI, 0.7-46.5%) and 28.6% (95% CI, 4.1-61.2%) in the untreated DSA group (EFS: $P<.001$, Figure 3c; OS: $P=.002$, Figure 3d). In the DSA-negative group, major ABO incompatibility was marginally associated with EFS ($P=.045$) or OS ($P=.07$). The unadjusted EFS and OS probabilities for other variables are shown in supplemental Table 2.

Multivariable analysis showed that DSA-positive patients had significantly lower EFS (HR=4.07; 95%CI, 1.69-9.81; $P=.002$) than DSA-negative patients. Further, when stratified by desensitization status, we observed that patients with untreated DSAs had poorer EFS (HR=10.09; 95%CI, 3.37-30.22; $P<.001$) than patients without DSA (Table 3). In contrast, EFS was similar between the treated DSA and DSA-negative groups (HR=1.57; 95% CI, 0.35-6.92; $P=.56$). The absence of T-cell depletion with rATG was also predictive of inferior EFS after haplo-PTCy (HR=3.25; P=.018).

In the Cox regression model for OS, including preexisting DSAs as the main effect, we observed a violation in the proportional hazard assumption ($P=.033$). Therefore, by assessing Schoenfeld-residuals, follow-up time was split considering day +365, and the proportional hazard assumption was held both before and after day +365 ($P=.06$ and $P=.19$, respectively). Thus, in the multivariable Cox regression considering the period before day +365, positivity for DSAs was the only significant adverse factor for OS (HR=9.32; 95%CI, 1.75-49.6; $P=.009$). Conversely, after day +365, no significant difference was observed for patients with and without DSAs (HR=0.57; 95%, 0.04-8.77; $P=.69$) (Table 3). Additionally, compared with the DSA-negative cohort, only patients with untreated DSAs were significantly associated with worse OS (Untreated DSA: HR 5.56; 95% CI, 1.92-16.12; $P=.002$; Treated DSA: HR=2.19; 95% CI, 0.49-9.81; $P=.31$) (Table 3). No other patient-related or donor-related variables were associated with poor survival.

4.1.4 Discussion

The current study demonstrates that preformed DSAs were independent predictors of poor hematologic recovery, high incidence of graft failure, and worse EFS and OS after pediatric haplo-PTCy for NMDs. These novel findings agree with

previous studies in adult patients undergoing haplo-PTCy for malignancies[12, 13]. Our data are also consistent with the association of DSAs with poorer EFS described by Arcuri et al.[3] and our previous study in pediatric patients with NMDs who underwent salvage haplo-PTCy for GF, which showed that untreated DSAs were associated with higher GF incidence and worse EFS[17]. Remarkably, we observed that patients without DSAs had an excellent 1-year OS of 81.2%, and this finding is similar to the survival outcomes reported in previous studies[7, 27, 28].

When stratified by the desensitization status, we observed a significantly higher GF likelihood among patients with untreated DSAs in univariable and multivariable competing risk regression. However, we acknowledge that the few cases in the treated and untreated DSA groups limit our ability to evaluate the effect of desensitization in our cohort. Importantly, we observed an overlap between patients with untreated DSAs with SAA and Wiskott–Aldrich diseases and between the treated DSA group and Fanconi anemia. Since acquired SAA and immune regulatory diagnoses were also independent predictors of GF compared to patients with Fanconi anemia, one might consider that the beneficial effect of desensitization therapy is confounded by the Fanconi diagnosis. Due to this relevant limitation, our study's apparent impact of desensitization therapy on graft failure should be interpreted cautiously. This point warrants further exploration in studies with appropriate power in the haplo-PTCy context for NMDs.

Our report also indicates that DSAs were strongly associated with delayed platelet recovery, regardless of desensitization therapy. This finding is consistent with those reported by Yoshihara et al.[29] and Zhao et al.[30], using distinct haploidentical transplant approaches. In contrast, previous studies in the haplo-PTCy setting showed that DSAs were not associated with impaired platelet recovery[13, 31].

The occurrence of poor graft function has been related to dismal survival following haplo-PTCy[31]. In our study, the three patients who experienced poor graft function had early mortality. To date, the role of DSA in poor graft function pathogenesis is controversial[25]. Chang et al. showed that low-level DSAs (MFI ≥2000) were strongly associated with primary poor graft function after haploidentical transplants with the Beijing protocol in both training and validation sets[11]. In turn, Bramanti et al. failed to validate DSAs as a risk factor for poor graft function following haplo-PTCy for adults with malignancies[31]. To our knowledge, the potential role of DSAs on poor graft function after haplo-PTCy for NMDs has not yet been evaluated.

In our report, two of 7 untreated DSA-positive patients experienced poor graft function compared with only one of 48 patients without DSAs. However, this observation should be interpreted with caution due to the few graft dysfunction events.

The criteria that define a tolerable DSA after haplo-PTCy are currently unknown. Nevertheless, a body of data indicates that higher DSA levels are associated with an increased likelihood of GF[12], but this is not always the case. In this report, we observed sustained donor engraftment despite a high-titer, C1q-positive anti-DQ DSA. In contrast, primary graft failure has been documented in the presence of low-level DSAs against HLA-A and -B antigens. One possible explanation for this contradictory finding is the differential HLA antigen expression on $CD34^+$ cells. Bettinotti and colleagues have recently shown that HLA-A, -B, and -DR molecules are highly expressed on $CD34^+$ cells, whereas HLA-DQ antigens are detected at lower levels[32]. Interestingly, the assumption that DSAs directed to highly expressed HLA antigens are more pathogenic than DSAs to low-expressed HLA molecules has been previously suggested[33]. Hence, this hypothesis should be validated in future studies to improve DSA risk stratification eventually.

It is well established that anti-HLA antibodies are specific for epitopes rather than to the whole HLA antigens[22, 34]. Using SAB Explex panels, we verified that all low-level DSAs in our cohort were directed to public epitopes, with several HLA cross-reactive antigens. This so-called "shared epitope phenomenon" has been described as a SAB assay drawback since the antibody MFI signal is diluted among beads expressing the donor-specific epitope, thus underestimating the actual DSA MFI strength[35]. In this sense, Ciurea et al. reported that low-level DSAs against HLA-DP public epitopes were associated with an increased risk of GF after matched unrelated donor transplantation[9]. Currently, the impact of low-level DSAs directed to public epitopes and the contribution of the "shared epitope phenomenon" on the GF incidence after pediatric haplo-PTCy has not yet been reported. Thus, more studies are needed to clarify this issue.

Ciurea et al. have previously demonstrated that C1q-positive DSAs are strongly associated with GF after haplo-PTCy and that C1q-negativity should be the goal of desensitization therapy[12, 36]. Only one DSA against HLA-DQ was C1q-positive in our cohort, with strong reactivity at a 1:32 dilution. In countries with limited resources, the use of solid-phase complement binding assays is restricted due to the

high costs associated with this additional testing. However, by performing standard SAB with EDTA-treated sera to prevent complement inhibition, Wiebe et al. showed that HLA antibodies with MFI higher than 15,000 perfectly correlated with C1q-binding results, with 99% sensitivity and 100% specificity and an area under the curve of 0.99[37]. Moreover, emerging evidence shows that factors other than C1q-binding may affect the DSA pathogenicity, including IgG3 subclass[38], IgG glycosylation[39, 40], and IgG affinity/avidity assessed by surface plasmon resonance[41]. To date, none of these DSA features have been investigated in the haplo-PTCy setting, and further studies are needed to elucidate the role of these characteristics on transplant outcomes.

Although SAB panels have revolutionized HLA antibody identification, such assays still present limitations that jeopardize DSA assignment. Indeed, it has been shown that several HLA antibodies above a given cutoff (e.g., MFI>1000) may represent false-positive reactivity to denatured HLA on the beads[42]. In our report, four patients (6,8%) had false-positive DSAs, with median MFI of 1762 (range, 544-2483). They were not desensitized and fully engrafted after haplo-PTCy. While the issue of spurious reactions to denatured HLA has been addressed in solid organ transplantation[43, 44], evidence on the incidence of false-positive DSAs in pediatric haplo-PTCy is currently lacking. In the kidney transplant setting, previous reports indicate that these false-positive DSAs are clinically irrelevant[43]. Indeed, reporting a false-positive DSA may yield an unnecessary exclusion of a suitable donor (e.g., the younger donor) or the desensitization treatment of an irrelevant DSA. Of note, investigating spurious DSA reactivity is costly and time-consuming since several additional tests are needed to obtain an accurate DSA assignment[20].

Ideally, when only DSA-positive donors are available, desensitization therapy is necessary to facilitate donor engraftment across the DSA barrier[45]. Our results showed that patients with untreated DSAs experienced higher GF incidence and had worse survival. In low/middle-income countries, desensitization treatment may not be widely available due to the elevated costs of the procedure, thereby jeopardizing the transplantation outcomes. Although current desensitization strategies after haplo-PTCy effectively reduce DSA levels and promote donor engraftment, the results for patients with strong DSAs (MFI>25,000) are still sub-optimal and associated with poor outcomes[36, 46]. In the NMD setting, Kurzay et al. successfully treated four DSA-positive patients with inborn errors using rituximab and plasmapheresis, and all

achieved donor engraftment[15]. In contrast, Hasan et al. recently reported that three SAA patients with strong DSAs experienced GF, despite desensitization with plasmapheresis, intravenous immunoglobulin, and rituximab[7]. In this sense, some reports have shown that bortezomib or anti-CD38 monoclonal antibody daratumumab may be valuable additional tools for desensitization therapy. Choe and colleagues have described their promising results of using bortezomib combined with IVIG, rituximab, and plasmapheresis to treat DSA-positive patients prior to haplo-cord transplants[47]. Likewise, the use of daratumumab has been recently described in a case report of successful desensitization of a strong DSA before unrelated donor transplantation[48]. Furthermore, the use of imlifidase, carfilzomib, and tocilizumab has shown promising results in the solid organ transplantation setting, especially for highly HLA-sensitized patients[49-51]. To date, none of these drugs have been evaluated in the haplo-PTCy, and whether these new agents would improve the current desensitization protocols used in haploidentical transplants remains to be determined.

The optimal DSA's MFI level following desensitization therapy that allows sustained engraftment is currently unknown. Ciurea et al. reported that C1q-negativity should be the goal of desensitization therapy and that DSAs with MFI<5000 were associated with improved engraftment rates[12]. Other groups considered the reduction below the 2000 MFI cutoff after desensitization intervention as clinically significant[13, 47]. In contrast, our center applies a more conservative goal, targeting a DSA strength reduction below 1000 MFI following desensitization. Still, we use a "donor-specific epitope tracking"[52] approach to assess the DSA response following the antibody depletion therapy. This analysis monitors the overall reduction of the DSA's MFI strength and the MFI of non-DSA antigens sharing the donor-specific epitope[19, 34, 53]. In our experience, such a monitoring approach provides more accurate data on desensitization treatment efficiency.

This study has some limitations. First, it is a single-center retrospective study with heterogeneous NMDs. Second, the impact of DSA on primary graft rejection, secondary GF, and poor graft function was not analyzed separately, owing to the small number of events in our cohort. Third, all patients lacked data on DSA post-transplantation monitoring, which limited our ability to assess DSA's MFI kinetics after haplo-PTCy and its effect on transplant outcomes. Despite these limitations, our

report has the merit of providing novel evidence on the role of preformed DSAs in pediatric haplo-PTCy for NMDs.

In conclusion, our results showed that preformed DSAs are significantly associated with poor hematologic recovery, high incidence of graft failure, and worse survival outcomes in pediatric patients with nonmalignant disorders undergoing haplo-PTCy. Hence, when a DSA-negative donor is unavailable, desensitization therapy must be performed to increase the likelihood of donor engraftment, thus improving transplant outcomes.

4.1.5 Acknowledgments

The authors thank all HCT physicians and HLA laboratory staff who collaborated in this study. We also thank the data manager Heliz Regina Neves. Carmen Lucia Kuniyoshi Rebelatto is acknowledged for helping with the donor cells for the absorption/elution experiments. This work was supported by Programa Nacional de Apoio à Atenção Oncológica (PRONON) [grant number 25.000.001142/2017-71]. We are grateful for the support provided by Associação dos Amigos do Hospital de Clínicas for this project.

4.1.6 Author contributions

ACML, CB, NFP, and RP designed the study. ACML and RRP contributed to statistical design and performed the statistical analysis. ACML, JG, RM, and MGL performed and interpreted HLA antibody testing. ACML, CB, and NFP analyzed and interpreted the data. ACML and CB wrote the paper. All the other authors contributed to data collection and patient care. All authors critically reviewed the paper and approved the final version of the manuscript.

4.1.7 Conflict of interest

The authors have declared no conflicting interests.

4.1.8 References

1. Bashey A, Solomon SR. T-cell replete haploidentical donor transplantation using post-transplant CY: an emerging standard-of-care option for patients who lack an HLA-identical sibling donor. *Bone Marrow Transplant.* 2014;49:999-1008.

2. Bonfim C, Ribeiro L, Nichele S, et al. Haploidentical Bone Marrow Transplantation with Post-Transplant Cyclophosphamide for Children and Adolescents with Fanconi Anemia. *Biol Blood Marrow Transplant.* 2017;23:310-317.

3. Arcuri LJ, Nabhan SK, Cunha R, et al. Impact of CD34 Cell Dose and Conditioning Regimen on Outcomes after Haploidentical Donor Hematopoietic Stem Cell Transplantation with Post-Transplantation Cyclophosphamide for Relapsed/Refractory Severe Aplastic Anemia. *Biol Blood Marrow Transplant.* 2020;26:2311-2317.

4. Fernandes JF, Nichele S, Arcuri LJ, et al. Outcomes after Haploidentical Stem Cell Transplantation with Post-Transplantation Cyclophosphamide in Patients with Primary Immunodeficiency Diseases. *Biol Blood Marrow Transplant.* 2020;26:1923-1929.

5. de la Fuente J, Dhedin N, Koyama T, et al. Haploidentical Bone Marrow Transplantation with Post-Transplantation Cyclophosphamide Plus Thiotepa Improves Donor Engraftment in Patients with Sickle Cell Anemia: Results of an International Learning Collaborative. *Biol Blood Marrow Transplant.* 2019;25:1197-1209.

6. Vellaichamy Swaminathan V, Ravichandran N, Ramanan KM, et al. Augmented immunosuppression and PTCY-based haploidentical hematopoietic stem cell transplantation for thalassemia major. *Pediatr Transplant.* 2021;25:e13893.

7. Hashem H, Najjar R, Abu-Shanap M, et al. Haploidentical Hematopoietic Cell Transplantation Using Post-transplant Cyclophosphamide for Children with Non-malignant Diseases. *J Clin Immunol.* 2021;41:1754-1761.

8. George B, Kulkarni U, Lionel S, et al. Haploidentical transplantation is feasible and associated with reasonable outcomes despite major infective complications-A single center experience from India. *Transplant Cell Ther.* 2021.

9. Ciurea SO, Thall PF, Wang X, et al. Donor-specific anti-HLA Abs and graft failure in matched unrelated donor hematopoietic stem cell transplantation. *Blood.* 2011;118:5957-5964.

10. Fuji S, Oshima K, Ohashi K, et al. Impact of pretransplant donor-specific anti-HLA antibodies on cord blood transplantation on behalf of the Transplant Complications Working Group of Japan Society for Hematopoietic Cell Transplantation. *Bone Marrow Transplant.* 2020;55:722-728.

11. Chang YJ, Zhao XY, Xu LP, et al. Donor-specific anti-human leukocyte antigen antibodies were associated with primary graft failure after unmanipulated

haploidentical blood and marrow transplantation: a prospective study with randomly assigned training and validation sets. *J Hematol Oncol.* 2015;8:84.

12. Ciurea SO, Thall PF, Milton DR, et al. Complement-Binding Donor-Specific Anti-HLA Antibodies and Risk of Primary Graft Failure in Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant.* 2015;21:1392-1398.

13. Zou J, Romee R, Slade M, et al. Untreated donor specific antibodies against HLA are associated with poor outcomes in peripheral blood haploidentical hematopoietic cell transplantation. *Bone Marrow Transplant.* 2017;52:898-901.

14. Xie Y, Parekh J, Tang Z, Wu D, Wu X. Donor-Specific Antibodies and Primary Graft Failure in Allogeneic Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. *Transplant Cell Ther.* 2021;27:687 e681-687 e687.

15. Kurzay M, Hauck F, Schmid I, et al. T-cell replete haploidentical bone marrow transplantation and post-transplant cyclophosphamide for patients with inborn errors. *Haematologica.* 2019;104:e478-e482.

16. Tambur AR, Campbell P, Claas FH, et al. Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group Meeting Report. *Am J Transplant.* 2018;18:1604-1614.

17. Lima ACM, Bonfim C, Getz J, et al. The impact of donor-specific anti-human leukocyte antigen antibodies in salvage haploidentical hematopoietic cell transplantation with posttransplant cyclophosphamide in patients with nonmalignant disorders. *HLA.* 2021;97:493-504.

18. Weinstock C, Schnaidt M. The complement-mediated prozone effect in the Luminex single-antigen bead assay and its impact on HLA antibody determination in patient sera. *Int J Immunogenet.* 2013;40:171-177.

19. Ellis TM. Interpretation of HLA single antigen bead assays. *Transplant Rev (Orlando).* 2013;27:108-111.

20. Ravindranath MH, Filippone EJ, Amato-Menker CJ, et al. Antibodies to cryptic epitopes on HLA class I and class II heavy chains bound to single antigen beads: Clinically relevant? *Transpl Immunol.* 2021;69:101482.
21. El-Awar N, Terasaki PI, Nguyen A, et al. Epitopes of human leukocyte antigen class I antibodies found in sera of normal healthy males and cord blood. *Hum Immunol.* 2009;70:844-853.

22. El-Awar N, Jucaud V, Nguyen A. HLA Epitopes: The Targets of Monoclonal and Alloantibodies Defined. *J Immunol Res.* 2017;2017:3406230.

23. Grenzi PC, de Marco R, Silva RZ, Campos EF, Gerbase-DeLima M. Antibodies against denatured HLA class II molecules detected in luminex-single antigen assay. *Hum Immunol.* 2013;74:1300-1303.

24. Kanate AS, Nagler A, Savani B. Summary of Scientific and Statistical Methods, Study Endpoints and Definitions for Observational and Registry-Based Studies in Hematopoietic Cell Transplantation. *Clin Hematol Int.* 2020;2:2-4.

25. Kong Y. Poor graft function after allogeneic hematopoietic stem cell transplantation-an old complication with new insights(). *Semin Hematol.* 2019;56:215-220.

26. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant.* 2013;48:452-458.

27. Neven B, Diana JS, Castelle M, et al. Haploidentical Hematopoietic Stem Cell Transplantation with Post-Transplant Cyclophosphamide for Primary Immunodeficiencies and Inherited Disorders in Children. *Biol Blood Marrow Transplant.* 2019;25:1363-1373.

28. Mallhi KK, Srikanthan MA, Baker KK, et al. HLA-Haploidentical Hematopoietic Cell Transplantation for Treatment of Nonmalignant Diseases Using Nonmyeloablative Conditioning and Post-Transplant Cyclophosphamide. *Biol Blood Marrow Transplant.* 2020;26:1332-1341.

29. Yoshihara S, Maruya E, Taniguchi K, et al. Risk and prevention of graft failure in patients with preexisting donor-specific HLA antibodies undergoing unmanipulated haploidentical SCT. *Bone Marrow Transplant.* 2012;47:508-515.

30. Zhao X, Zhao X, Huo M, et al. Donor-Specific Anti-Human Leukocyte Antigen Antibodies Predict Prolonged Isolated Thrombocytopenia and Inferior Outcomes of Haploidentical Hematopoietic Stem Cell Transplantation. *J Immunol Res.* 2017;2017:1043836.

31. Bramanti S, Calafiore V, Longhi E, et al. Donor-Specific Anti-HLA Antibodies in Haploidentical Stem Cell Transplantation with Post-Transplantation Cyclophosphamide: Risk of Graft Failure, Poor Graft Function, and Impact on Outcomes. *Biol Blood Marrow Transplant.* 2019;25:1395-1406.

32. Bettinotti MP, Lucas DP, Ghiaur G, Jackson AM, Gladstone DE. OR30. HLA expression on CD34+ stem cells: is a flow cytometric crossmatch a good gatekeeper for mismatched hematopoietic cell transplantion? *Human Immunology.* 2018;79:35.

33. Fernandez Vina M, Heslop HE, Barker JN. New Approaches in Alternative Donor Transplantation. *Biology of Blood and Marrow Transplantation.* 2013;19:S91-S96.

34. Tambur AR, Claas FH. HLA epitopes as viewed by antibodies: what is it all about? *Am J Transplant.* 2015;15:1148-1154.

35. Garcia-Sanchez C, Usenko CY, Herrera ND, Tambur AR. The shared epitope phenomenon-A potential impediment to virtual crossmatch accuracy. *Clin Transplant.* 2020;34:e13906.

36. Ciurea SO, Al Malki MM, Kongtim P, et al. Treatment of allosensitized patients receiving allogeneic transplantation. *Blood Adv.* 2021;5:4031-4043.

37. Wiebe C, Gareau AJ, Pochinco D, et al. Evaluation of C1q Status and Titer of De Novo Donor-Specific Antibodies as Predictors of Allograft Survival. 2017;17:703-711.

38. Pernin V, Beyze A, Szwarc I, et al. Distribution of de novo Donor-Specific Antibody Subclasses Quantified by Mass Spectrometry: High IgG3 Proportion Is Associated With Antibody-Mediated Rejection Occurrence and Severity. *Front Immunol.* 2020;11:919.

39. Pernin V, Bec N, Beyze A, et al. IgG3 donor-specific antibodies with a proinflammatory glycosylation profile may be associated with the risk of antibody-mediated rejection after kidney transplantation. *Am J Transplant.* 2021.

40. Malard-Castagnet S, Dugast E, Degauque N, et al. Sialylation of antibodies in kidney recipients with de novo donor specific antibody, with or without antibody mediated rejection. *Hum Immunol.* 2016;77:1076-1083.

41. Visentin J, Leu DL, Mulder A, et al. Measuring anti-HLA antibody active concentration and affinity by surface plasmon resonance: Comparison with the luminex single antigen flow beads and T-cell flow cytometry crossmatch results. *Mol Immunol.* 2019;108:34-44.

42. Wehmeier C, Honger G, Schaub S. Caveats of HLA antibody detection by solid-phase assays. *Transpl Int.* 2020;33:18-29.

43. Cai J, Terasaki PI, Anderson N, Lachmann N, Schonemann C. Intact HLA not beta2m-free heavy chain-specific HLA class I antibodies are predictive of graft failure. *Transplantation.* 2009;88:226-230.

44. Oaks M, Michel K, Sulemanjee NZ, Thohan V, Downey FX. Practical value of identifying antibodies to cryptic HLA epitopes in cardiac transplantation. *J Heart Lung Transplant.* 2014;33:713-720.

45. Leffell MS, Jones RJ, Gladstone DE. Donor HLA-specific Abs: to BMT or not to BMT? *Bone Marrow Transplant.* 2015;50:751-758.

46. Bailen R, Vicario JL, Solan L, et al. Management of Donor-Specific Antibodies in Haploidentical Transplant: Multicenter Experience From the Madrid Group of Hematopoietic Transplant. *Front Immunol.* 2021;12:674658.

47. Choe H, Gergis U, Hsu J, et al. Bortezomib and Immune Globulin Have Limited Effects on Donor-Specific HLA Antibodies in Haploidentical Cord Blood Stem Cell Transplantation: Detrimental Effect of Persistent Haploidentical Donor-Specific HLA Antibodies. *Biol Blood Marrow Transplant.* 2019;25:e60-e64.

48. Ibrahim U, Keyzner A. Daratumumab for donor-specific anti-HLA antibody desensitization in a case of HLA-mismatched allogeneic stem cell transplantation. *Hematol Transfus Cell Ther.* 2022.

49. Vo AA, Choi J, Kim I, et al. A Phase I/II Trial of the Interleukin-6 Receptor-Specific Humanized Monoclonal (Tocilizumab) + Intravenous Immunoglobulin in Difficult to Desensitize Patients. *Transplantation.* 2015;99:2356-2363.

50. Jordan SC, Lorant T, Choi J, et al. IgG Endopeptidase in Highly Sensitized Patients Undergoing Transplantation. *N Engl J Med.* 2017;377:442-453.

51. Tremblay S, Driscoll JJ, Rike-Shields A, et al. A prospective, iterative, adaptive trial of carfilzomib-based desensitization. *Am J Transplant.* 2020;20:411-421.

52. Lowe D, Shabir S, Buckels J, et al. HLA incompatible combined liver-kidney transplantation: dynamics of antibody modulation revealed by a novel approach to HLA antibody characterisation. *Transpl Immunol.* 2014;30:30-33.

53. Bettinotti MP, Zachary AA, Leffell MS. Clinically relevant interpretation of solid phase assays for HLA antibody. *Curr Opin Organ Transplant.* 2016;21:453-458.

FIGURE 1. CUMULATIVE INCIDENCE OF NEUTROPHIL (A) AND PLATELET (B) RECOVERY BY DSA STATUS. IMPACT OF DESENSITIZATION THERAPY ON NEUTROPHIL (C) AND PLATELET (D) RECOVERY.

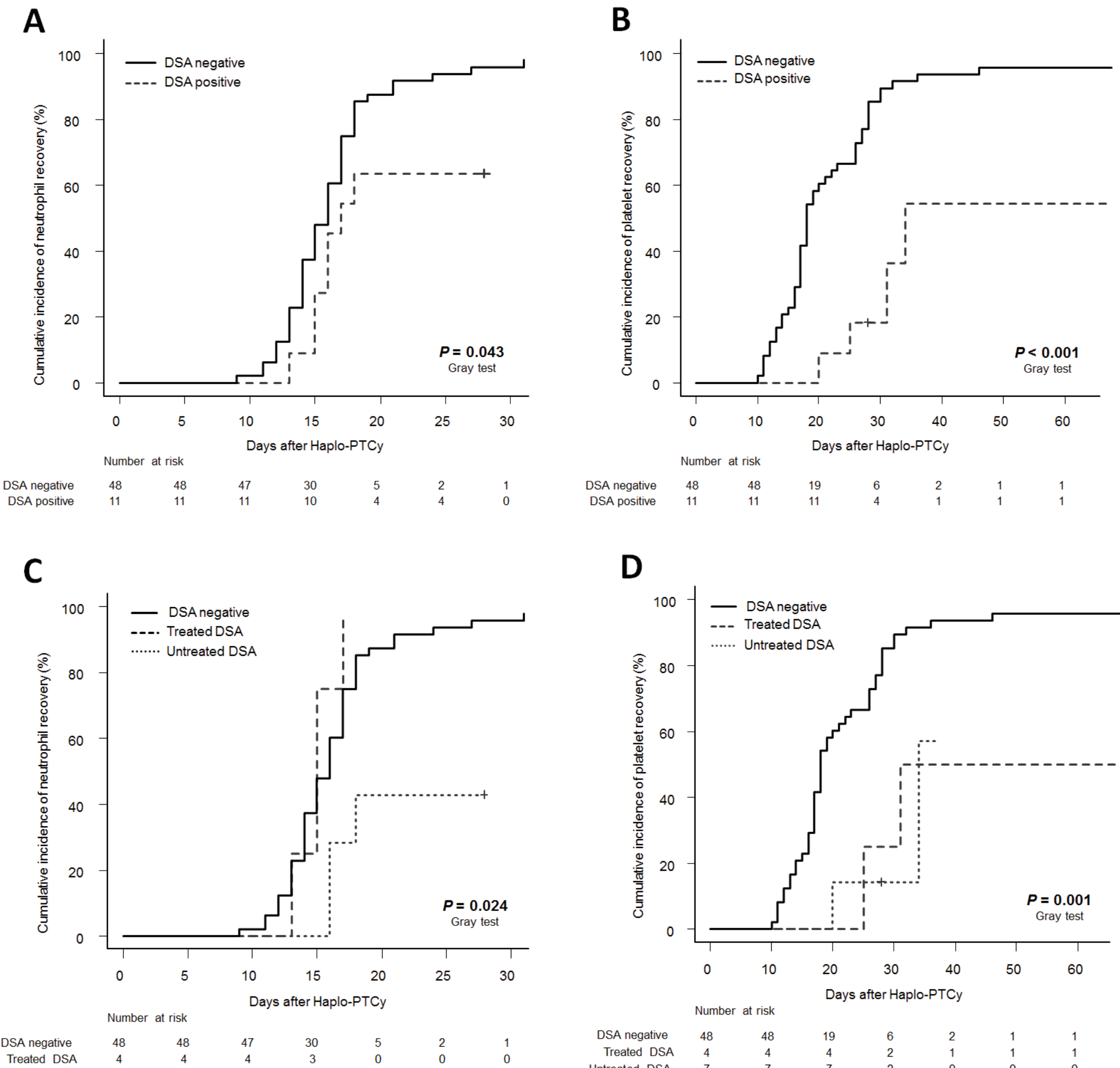


FIGURE 2. CUMULATIVE INCIDENCE OF GRAFT FAILURE BY DSA STATUS (A) AND DESENSITIZATION THERAPY (B).

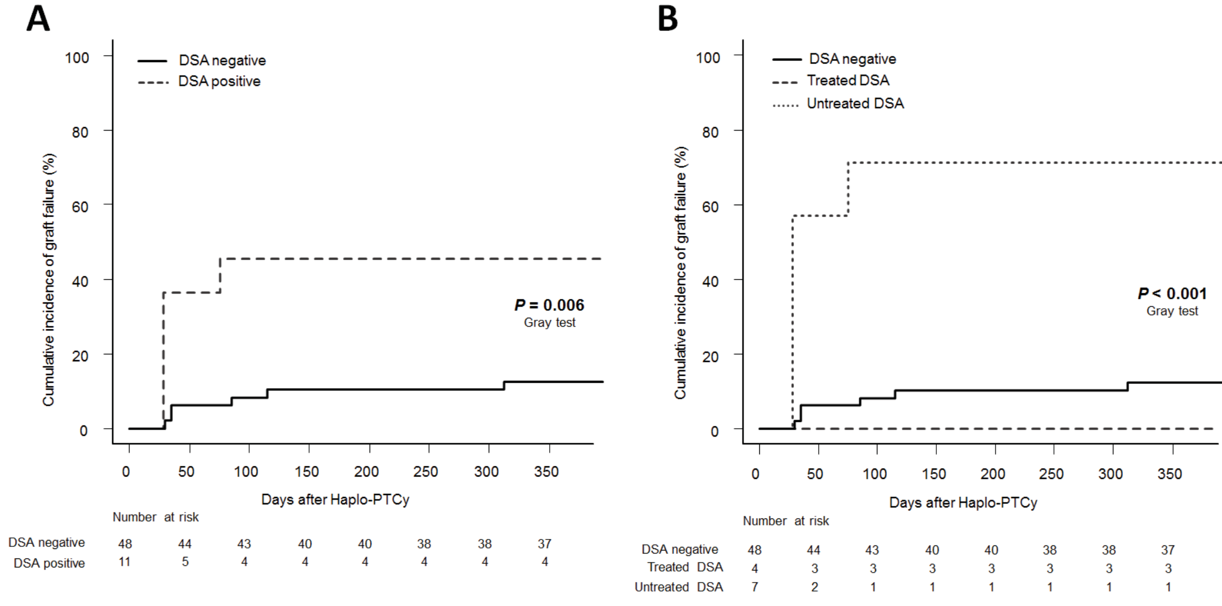


FIGURE 3. PROBABILITY OF EVENT-FREE SURVIVAL (A) AND OVERALL SURVIVAL (B) BY DSA STATUS. EVENT-FREE SURVIVAL (C) AND OVERALL SURVIVAL (D) ACCORDING TO DESENSITIZATION THERAPY.

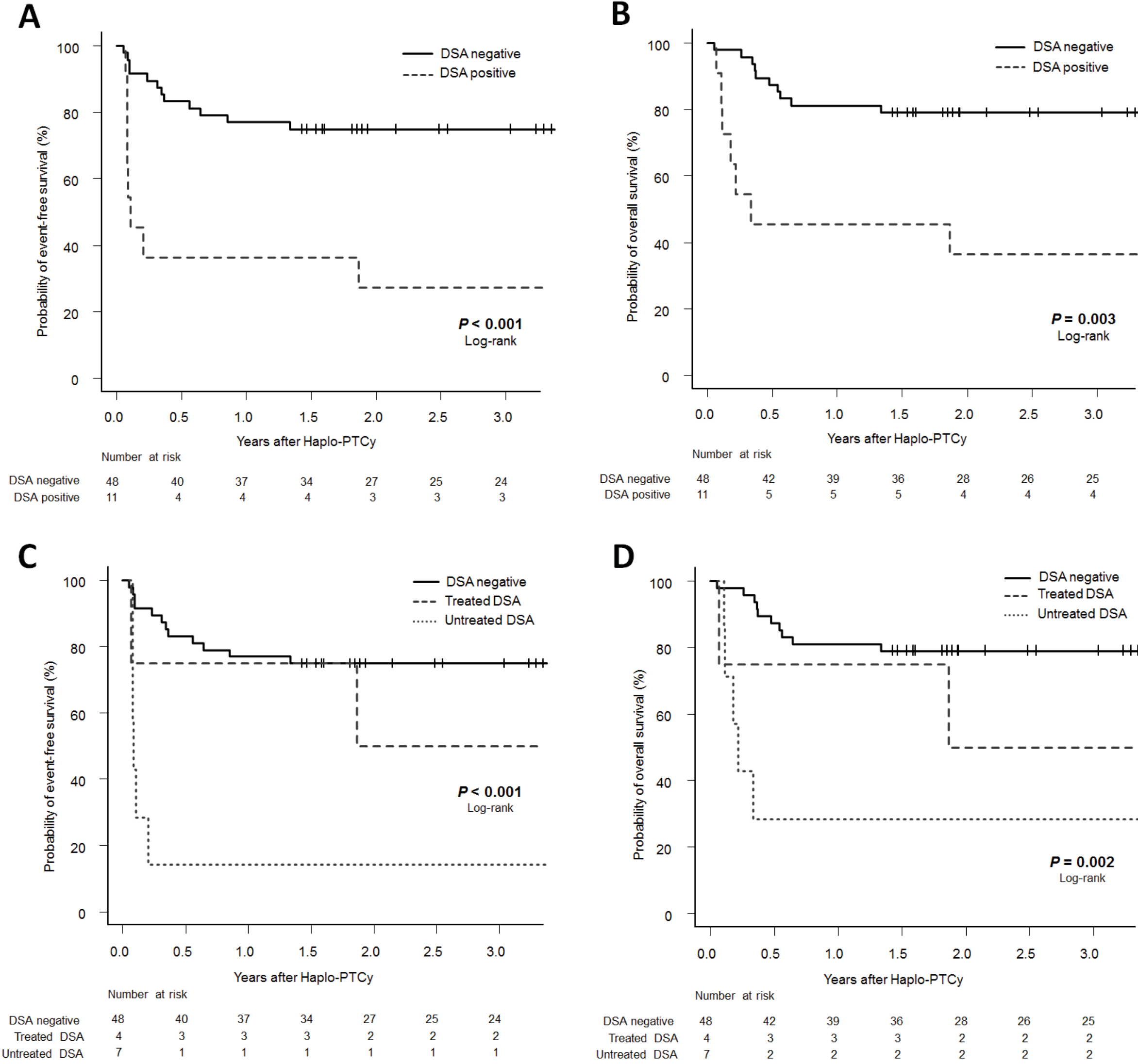


TABLE 1. BASELINE CHARACTERISTICS BY DSA STATUS.

Variables	Overall (n=59)	DSA negative (n=48)	DSA positive (n=11)	*P*-value
ABO, n (%)				**0.077**
ABO Match	35 (59.3)	25 (52.1)	10 (90.9)	
ABO Minor mismatch	11 (18.6)	11 (22.9)	0 (0.0)	
ABO Major mismatch	13 (22.1)	12 (25.0)	1 (9.1)	
Recipient/donor CMV serostatus, n (%)				0.582
R+/D+	53 (89.8)	42 (87.5)	11 (100.0)	
Others	6 (10.2)	6 (12.5)	0 (0.0)	
Conditioning regimen, n (%)				0.572
RIC	54 (91.5)	43 (89.6)	11 (100.0)	
MAC	5 (8.5)	5 (10.4)	0 (0.0)	
***In vivo* T-cell depletion with rATG, n (%)**				0.316
Yes	26 (44.1)	23 (47.9)	3 (27.3)	
No	33 (55.9)	25 (52.1)	8 (72.7)	
Diagnosis, n (%)				0.877
FA	33 (55.9)	27 (56.2)	6 (54.5)	
SAA	11 (18.6)	9 (18.8)	2 (18.2)	
WAS	6 (10.2)	5 (10.4)	1 (9.1)	
DC	5 (8.5)	3 (6.2)	2 (18.2)	
ALD	2 (3.4)	2 (4.2)	0 (0.0)	
CAMT	2 (3.4)	2 (4.2)	0 (0.0)	
Desensitization (Only patients with DSA)				NA
Treated DSA	-	-	4 (36.4)	
Untreated DSA	-	-	7 (63.6)	
Donor type, n (%)				0.838
Father	21 (35.6)	16 (33.3)	5 (45.5)	
Mother	26 (44.1)	22 (45.8)	4 (36.4)	
Sibling	10 (16.9)	8 (16.7)	2 (18.2)	
Other donors	2 (3.4)	2 (4.2)	0 (0.0)	
HLA mismatches in HvG direction, n (%)				1
0-3	18 (30.5)	15 (31.2)	3 (27.3)	
4-5	41 (69.5)	33 (68.8)	8 (72.7)	
KIR-ligand mismatch in HvG direction, n(%)				0.269
Match	45 (76.3)	35 (72.9)	10 (90.9)	
HvG mismatch	14 (23.7)	13 (27.1)	1 (9.1)	
Patient gender, n (%)				1
Male	39 (66.1)	32 (66.7)	7 (63.6)	
Female	20 (33.9)	16 (33.3)	4 (36.4)	
Donor gender, n (%)				0.741
Male	28 (47.5)	22 (45.8)	6 (54.5)	
Female	31 (52.5)	26 (54.2)	5 (45.5)	
Number of transfusions, n (%)				0.140
<20 transfusions	25 (42.4)	22 (45.8)	3 (27.3)	
>20 transfusions	17 (28.8)	11 (22.9)	6 (54.5)	
Missing	17 (28.8)	15 (31.2)	2 (18.2)	
Time from diagnosis to transplant, median (range), months	32 (1, 95)	40 (1, 93)	30 (5, 95)	0.690
Patient age, median (range), years	10 (1, 20)	9.50 (1, 20)	11 (3, 18)	0.488
Donor age, median (range), years	35 (5, 54)	35 (5, 54)	36 (17, 52)	0.778
TNC, median (range), x10^8/kg	7 (2.07, 14.93)	6.92 (2.07, 14.93)	7.07 (2.21, 14.40)	0.884
CD34$^+$, median (range), x10^6/kg	4.09 (0.63, 19.42)	4.10 (0.63, 11.16)	3.40 (1.80, 19.42)	0.823
Year of BMT, median (range)	2015 (2008 - 2017)	2015 (2009 - 2017)	2014 (2008 - 2017)	0.186

Abbreviations: DSA, donor-specific anti-HLA antibodies; CMV, cytomegalovirus; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; rATG, rabbit anti-thymocyte globulin; FA, Fanconi anemia; SAA, Acquired severe aplastic anemia; ALD, Adrenoleukodystrophy; CAMT, Congenital amegakaryocytic thrombocytopenia; DC, Dyskeratosis Congenita; WAS, Wiskott–Aldrich syndrome; HvG, host-versus-graft; KIR, killer immunoglobulin-like receptor; TNC, total nucleated cell; BMT, Bone marrow transplant. Significant values are in bold.

TABLE 2. DSA CHARACTERISTICS AND HAPLO-PTCY OUTCOMES

Diagnosis	Number of DSAs	DSA specificity	Epitope pattern*	MFI (Neat)‡	MFI (1:2 dilution)	MFI (1:8 dilution)	MFI (1:32 dilution)	C1q assay	Desensitization therapy	Neutrophil recovery (day)	Platelet recovery (day)	Graft failure	Second BMT	Survival (days after BMT)
FA	2	C*03:04	173K	2993	2207	552	48	Negative	No	16	NR	Engraftment	-	Deceased (37)
		DRB1*14:01	96HK	16117	12462	6136	2178	Negative						
FA	1	A*02:01	62GE/144TKH	1076	692	145	35	Negative	Yes	13	142	Engraftment	-	Deceased (681)
FA	3	DRB1*13:01	70D+74A	8337	5260	1645	398	Negative	Yes	17	31	Engraftment	-	Alive (2566)
		DRB3*02:02	51R$_2$/98Q	11269	7361	2745	935	Negative						
		DQB1*03:03	55PP	24200	22402	20606	19531	Positive						
WAS	1	C*04:01	80K	1184	848	91	0	Negative	No	NR	NR	Primary PGF	No	Deceased (41)
DC	1	DQB1*03:03	55PP	5331	4478	848	107	Negative	No	NR	NR	Primary GR	Yes	Alive (1986)
SAA	2	A*02:01	62GE/144TKH	562	352	112	28	Negative	No	NR	NR	Primary GR	Yes	Deceased (122)
		B*44:03	41T	1149	761	208	0	Negative						
SAA	2	DRB1*13:02	149H	10150	7059	2909	748	Negative	No	NR	NR	Primary GR	Yes	Deceased (65)
		DRB3*03:01	149H	10057	7208	3202	867	Negative						
FA	1	DQB1*06:02	52PQ$_2$	10820	8108	3606	1054	Negative	Yes	15	25	Engraftment	-	Alive (1558)
FA	1	A*25:01	145RT	1665	1076	210	7	Negative	No	16	20	Engraftment	-	Alive (1445)
DC	2	A*29:01	62LQ	1128	709	116	0	Negative	Yes	15	NR	Engraftment	-	Deceased (24)
		DRB1*13:01	70D	527	436	123	13	Negative						
FA	2	B*44:03	163LS/G	901	603	110	0	Negative	No	18	34	Secondary PGF	Yes	Deceased (79)
		DQB1*03:01	55PP	1752	707	259	0	Negative						

Abbreviations: DSA, donor-specific anti-HLA antibodies; MFI, mean fluorescence intensity; FA, Fanconi anemia; WAS, Wiskott–Aldrich syndrome; DC, Dyskeratosis congenita; SAA, Acquired severe aplastic anemia; NR, Not reached; PGF; Poor graft function; GR; Graft rejection; BMT, Bone marrow transplantation.

*Putative amino acid residues in donor HLA antigens recognized by patient's DSA.

‡DSA's MFI before bone marrow infusion.

TABLE 3. MULTIVARIABLE MODELS FOR THE HAPLO-PTCY OUTCOMES.

Neutrophil recovery[‡]	**Adjusted SHR (95% CI)**	***P*-value**
Model 1: DSA status		
DSA negative	1 (Reference)	-
DSA positive	0.28 (0.12-0.7)	0.006
Model 2: DSA desensitization		
DSA negative	1 (Reference)	-
Treated DSA	0.73 (0.3-1.77)	0.48
Untreated DSA	0.15 (0.03-0.63)	0.001
Platelet recovery[¥]	**Adjusted SHR (95% CI)**	***P*-value**
Model 1: DSA status		
DSA negative	1 (Reference)	-
DSA positive	0.18 (0.09-0.38)	<0.001
Model 2: DSA desensitization		
DSA negative	1 (Reference)	-
Treated DSA	0.26 (0.10-0.68)	0.006
Untreated DSA	0.13 (0.04-0.37)	<0.001
Graft failure[§]	**Adjusted SHR (95% CI)**	***P*-value**
Model 1: DSA status		
DSA negative	1 (Reference)	-
DSA positive	11.90 (3.56-39.81)	<0.001
Model 2: DSA desensitization		
DSA negative/Treated DSA	1 (Reference)	-
Untreated DSA	20.57 (6.57-64.43)	<0.001
Event-free survival*	**Adjusted HR (95% CI)**	***P*-value**
Model 1: DSA status		
DSA negative	1 (Reference)	-
DSA positive	4.07 (1.69-9.81)	0.002
Model 2: DSA desensitization		
DSA negative	1 (Reference)	-
Treated DSA	1.57 (0.35-6.92)	0.56
Untreated DSA	10.09 (3.37-30.22)	<0.001
Overall survival	**Adjusted HR (95% CI)**	***P*-value**
Model 1: DSA status[#]		
Before day +360		
DSA negative	1 (Reference)	-
DSA positive	9.32 (1.75-49.6)	0.009
After day +360		
DSA negative	1 (Reference)	-
DSA positive	0.57 (0.04-8.77)	0.69
Model 2: DSA desensitization		
DSA negative	1 (Reference)	-
Treated DSA	2.19 (0.49-9.81)	0.31
Untreated DSA	5.56 (1.92-16.12)	0.002

Abbreviations: SHR, subdistribution hazard ratio; HR, hazard ratio

[‡]Neutrophil recovery: Final model adjusted for diagnosis and TNC.

[¥]Platelet recovery: Final model adjusted for *In vivo* T-cell depletion with rATG.

[§]Graft failure: Final model adjusted for diagnosis.

[*]Event-free survival: Final model adjusted for *In vivo* T-cell depletion with rATG.

[#]Overall survival: We observed a violation in the proportional hazard assumption (*P*=.033), and thus, follow-up time was split considering day +365, and the proportional hazard assumption was held both before and after day +365; DSA was the only significant predictor for OS in final models 1 and 2.

SUPPLEMENTAL TABLE 1. UNIVARIABLE ANALYSES FOR NEUTROPHIL AND PLATELET RECOVERY AND GRAFT FAILURE.

Variable	n	28-day NR (95% CI)	*P*-value	28-day PR (95% CI)	*P*-value	100-day GF (95% CI)	*P*-value
ABO							
ABO Match	35	0.857 (0.680-0.940)	0.721	0.600 (0.416-0.743)	0.088	0.200 (0.087-0.347)	0.349
ABO Minor mismatch	11	0.909 (0.294-0.993)		0.909 (0.292-0.993)		0.182 (0.025-0.455)	
ABO Major mismatch	13	NA (NA-NA)		0.923 (0.360-0.994)		NA (NA-NA)	
Patient age (Median)							
<10 years	28	0.964 (0.638-0.997)	0.161	0.821 (0.608-0.925)	0.072	0.179 (0.063-0.341)	0.869
≥10 years	31	0.839 (0.643-0.932)		0.645 (0.445-0.789)		0.129 (0.040-0.273)	
Donor age (Median)							
<35 years	28	0.893 (0.678-0.967)	**0.036**	0.714 (0.499-0.850)	0.503	0.107 (0.026-0.254)	0.429
≥35 years	31	0.903 (0.708-0.970)		0.742 (0.541-0.865)		0.194 (0.077-0.349)	
***In vivo* T-cell depletion with rATG**							
Yes	27	0.963 (0.625-0.997)	0.059	0.852 (0.631-0.946)	**0.003**	0.111 (0.027-0.262)	0.468
No	32	0.844 (0.654-0.934)		0.625 (0.430-0.770)		0.188 (0.075-0.340)	
Recipient/donor CMV serostatus							
R+/D+	53	0.925 (0.800-0.973)	0.407	0.717 (0.572-0.820)	0.877	0.170 (0.083-0.283)	0.848
Others	6	0.667 (0.125-0.924)		0.833 (0.020-0.992)		0.000 (0.000-0.000)	
Conditioning regimen							
RIC	54	0.889 (0.764-0.950)	0.945	0.704 (0.560-0.808)	0.532	0.167 (0.081-0.278)	0.99
MAC	5	NA (NA-NA)		1.000 (0.000-1.000)		0.000 (0.000-0.000)	
Diagnosis							
Inherited BMF	40	0.975 (0.732-0.998)	**0.005**	0.725 (0.553-0.840)	0.29	0.100 (0.031-0.217)	**0.026**
Acquired SAA	11	0.636 (0.270-0.855)		0.636 (0.272-0.855)		0.182 (0.025-0.456)	
Other NMDs	8	NA (NA-NA)		0.875 (0.172-0.990)		0.375 (0.072-0.694)	
Donor type							
Father	21	0.905 (0.615-0.980)	0.082	0.714 (0.456-0.866)	0.644	0.190 (0.057-0.383)	0.488
Mother	26	0.885 (0.664-0.964)		0.769 (0.544-0.893)		0.115 (0.028-0.271)	
Others	12	0.917 (0.328-0.993)		0.667 (0.304-0.871)		0.167 (0.023-0.426)	
DSA status							
DSA negative	48	0.958 (0.813-0.991)	**0.043**	0.854 (0.713-0.929)	**<0.001**	0.083 (0.026-0.183)	**0.006**
DSA positive	11	0.636 (0.266-0.857)		0.182 (0.025-0.456)		0.455 (0.149-0.721)	
DSA desensitization							
DSA negative	48	0.958 (0.813-0.991)	**0.024**	0.854 (0.713-0.929)	**0.001**	0.083 (0.026-0.183)	**<0.001**
Treated DSA	4	NA (NA-NA)		0.250 (0.002-0.732)		0.000 (0.000-0.000)	
Untreated DSA	7	0.429 (0.077-0.756)		0.143 (0.005-0.491)		0.714 (0.157-0.941)	
HLA mismatches in HvG direction							
2-3	18	0.833 (0.531-0.949)	0.844	0.667 (0.388-0.840)	0.427	0.056 (0.003-0.231)	0.327
4-5	41	0.927 (0.770-0.978)		0.756 (0.588-0.863)		0.195 (0.090-0.329)	
KIR-ligand mismatch in HvG direction							
Match	45	0.889 (0.746-0.954)	0.821	0.689 (0.528-0.804)	0.528	0.178 (0.082-0.303)	0.848
HvG Mismatch	14	0.929 (0.389-0.994)		0.857 (0.480-0.968)		0.071 (0.004-0.285)	
Patient gender							
Male	39	0.949 (0.775-0.989)	0.540	0.744 (0.569-0.856)	0.798	0.154 (0.061-0.285)	0.816
Female	20	0.800 (0.528-0.925)		0.700 (0.437-0.858)		0.150 (0.035-0.340)	
Donor gender							
Male	28	0.893 (0.677-0.968)	0.829	0.750 (0.535-0.876)	0.891	0.143 (0.044-0.298)	0.641
Female	31	0.903 (0.715-0.970)		0.710 (0.509-0.840)		0.161 (0.057-0.312)	
TNC (Median)							
≥7x10^8/kg	30	0.900 (0.695-0.970)	**0.027**	0.667 (0.462-0.808)	0.888	0.200 (0.079-0.360)	0.728
<7x10^8/kg	29	0.897 (0.691-0.968)		0.793 (0.586-0.904)		0.103 (0.025-0.246)	
CD34$^+$ (Median)							
≥4.09x10^6/kg	30	0.933 (0.715-0.986)	**0.026**	0.767 (0.561-0.885)	0.315	0.100 (0.025-0.239)	0.662
<4.09x10^6/kg	29	0.862 (0.660-0.948)		0.690 (0.481-0.828)		0.207 (0.082-0.370)	
Year of BMT (Median)							
≥2015	33	0.939 (0.740-0.987)	0.428	0.788 (0.596-0.896)	0.333	0.152 (0.054-0.295)	0.617
<2015	26	0.846 (0.625-0.942)		0.654 (0.432-0.806)		0.154 (0.047-0.318)	

Abbreviations: NR, neutrophil recovery; PR, platelet recovery; GF, graft failure; CI, confidence interval; rATG, rabbit anti-thymocyte globulin; CMV, cytomegalovirus; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; BMF, Bone marrow failure; SAA, acquired severe aplastic anemia; NMD, nonmaligant disorders; DSA, donor-specific anti-HLA antibodies; NA, not applicable; HvG, host-versus-graft; KIR, killer immunoglobulin-like receptor; TNC, total nucleated cell; BMT, Bone marrow transplant. Significant values are in bold.

SUPPLEMENTAL TABLE 2. UNIVARIABLE ANALYSES FOR EVENT-FREE SURVIVAL AND OVERALL SURVIVAL.

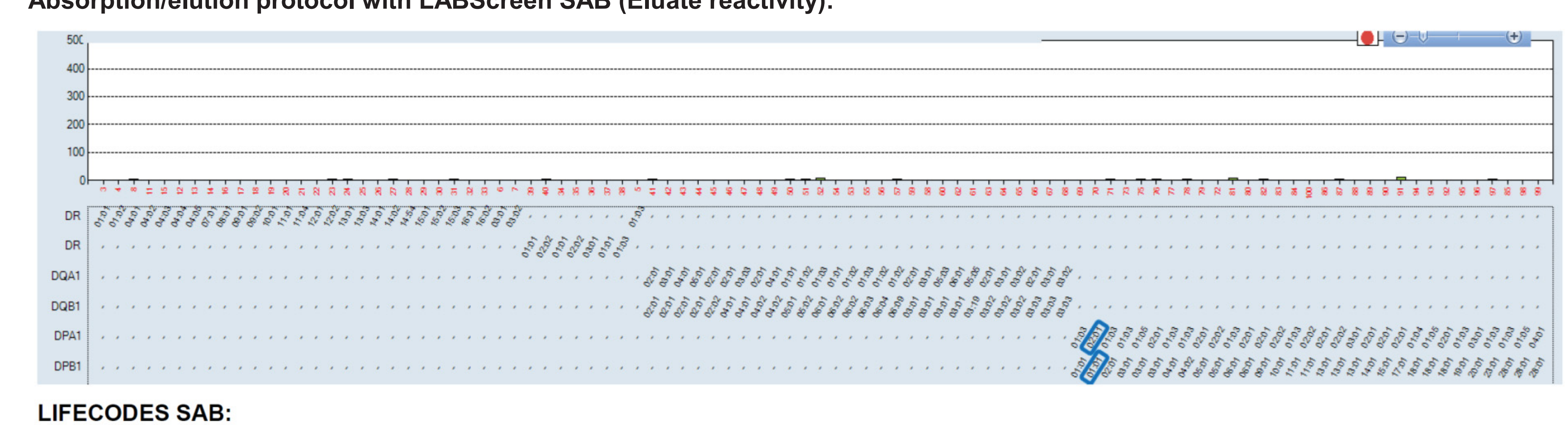
Variable	n	1-year EFS (95% CI)	*P*-value	1-year OS (95% CI)	*P*-value
ABO					
ABO Match	35	0.600 (0.420-0.740)	**0.013**	0.629 (0.448-0.765)	**0.012**
ABO Minor mismatch	11	0.636 (0.297-0.845)		0.818 (0.447-0.951)	
ABO Major mismatch	13	NA (NA-NA)		NA (NA-NA)	
Patient age (Median)					
<10 years	28	0.750 (0.546-0.872)	0.436	0.786 (0.584-0.898)	0.593
≥10 years	31	0.645 (0.452-0.785)		0.710 (0.516-0.837)	
Donor age (Median)					
<35 years	28	0.714 (0.509-0.846)	0.913	0.750 (0.546-0.872)	0.939
≥35 years	31	0.677 (0.484-0.812)		0.742 (0.550-0.862)	
***In vivo* T-cell depletion with rATG**					
Yes	27	0.815 (0.611-0.918)	**0.033**	0.852 (0.652-0.942)	0.052
No	32	0.594 (0.405-0.740)		0.656 (0.466-0.793)	
Recipient/donor CMV serostatus					
R+/D+	53	0.698 (0.555-0.803)	0.386	0.755 (0.615-0.849)	0.221
Others	6	0.667 (0.195-0.904)		0.667 (0.195-0.904)	
Conditioning regimen					
RIC	54	0.685 (0.543-0.791)	0.445	0.722 (0.582-0.822)	0.168
MAC	5	0.800 (0.204-0.969)		NA (NA-NA)	
Diagnosis					
Inherited BMF	40	0.750 (0.585-0.857)	0.586	0.775 (0.612-0.876)	0.883
Acquired SAA	11	0.636 (0.297-0.845)		0.636 (0.297-0.845)	
Other NMDs	8	0.500 (0.152-0.775)		0.750 (0.315-0.931)	
Donor type					
Father	21	0.714 (0.472-0.860)	0.552	0.810 (0.569-0.924)	0.337
Mother	26	0.731 (0.517-0.862)		0.769 (0.557-0.889)	
Others	12	0.583 (0.270-0.801)		0.583 (0.270-0.801)	
DSA status					
DSA negative	48	0.771 (0.625-0.866)	**<0.001**	0.812 (0.671-0.898)	**0.003**
DSA positive	11	0.364 (0.112-0.627)		0.455 (0.167-0.707)	
DSA desensitization					
DSA negative	48	0.771 (0.625-0.866)	**<0.001**	0.812 (0.671-0.898)	**0.002**
Treated DSA	4	0.750 (0.128-0.961)		0.750 (0.128-0.961)	
Untreated DSA	7	0.143 (0.007-0.465)		0.286 (0.041-0.612)	
HLA mismatches in HvG direction					
2-3	18	0.722 (0.456-0.874)	0.639	0.722 (0.456-0.874)	0.264
4-5	41	0.683 (0.517-0.802)		0.756 (0.594-0.861)	
KIR-ligand mismatch in HvG direction					
Match	45	0.711 (0.555-0.821)	0.777	0.733 (0.578-0.839)	0.719
HvG Mismatch	14	0.643 (0.343-0.833)		0.786 (0.472-0.925)	
Patient gender					
Male	39	0.718 (0.549-0.833)	0.674	0.769 (0.603-0.873)	0.745
Female	20	0.650 (0.403-0.815)		0.700 (0.451-0.853)	
Donor gender					
Male	28	0.714 (0.509-0.846)	0.976	0.786 (0.584-0.898)	0.811
Female	31	0.677 (0.484-0.812)		0.710 (0.516-0.837)	
TNC (Median)					
$\geq 7 \times 10^8$/kg	30	0.633 (0.436-0.778)	0.114	0.667 (0.469-0.805)	0.066
$<7 \times 10^8$/kg	29	0.759 (0.559-0.877)		0.828 (0.634-0.924)	
$CD34^+$ (Median)					
$\geq 4.09 \times 10^6$/kg	30	0.733 (0.537-0.857)	0.510	0.767 (0.572-0.881)	0.708
$<4.09 \times 10^6$/kg	29	0.655 (0.454-0.797)		0.724 (0.523-0.851)	
Year of BMT (Median)					
≥2015	33	0.727 (0.541-0.848)	0.223	0.788 (0.606-0.893)	0.134
<2015	26	0.654 (0.440-0.803)		0.692 (0.478-0.833)	

Abbreviations: EFS, event-free survival; OS, overall survival; CI, confidence interval; NA, not applicable; rATG, rabbit anti-thymocyte globulin; CMV, cytomegalovirus; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; BMF, Bone marrow failure; SAA, severe aplastic anemia; NMD, nonmaligant disorders; DSA, donor-specific anti-HLA antibodies; HvG, host-versus-graft; KIR, killer immunoglobulin-like receptor; TNC, total nucleated cell; BMT, Bone marrow transplant. Significant values are in bold.

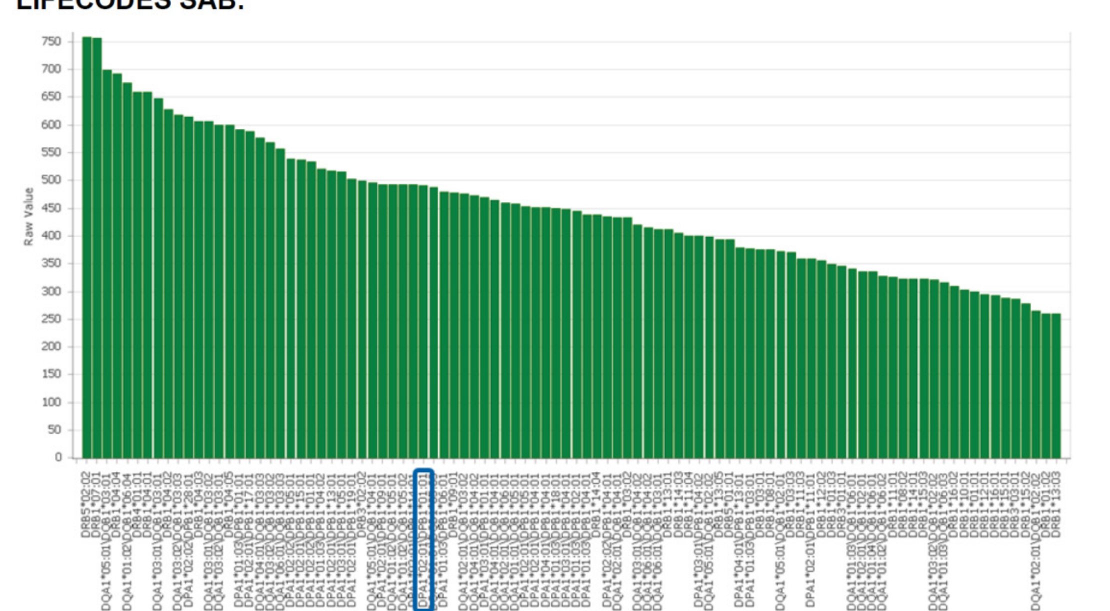
SUPPLEMENTARY FIGURE S1. FALSE-POSITIVE DSA REACTIVITY WAS EVALUATED BY ABSORPTION AND ELUTION PROTOCOL AND LIFECODES CLASS II SAB PANEL. Four patients had suspected class II DSA reactivity (Patterns: Pan-DR reactivity; DR4/DR16/DP19; DP1/DP5; DQA1*01:03/DQB1*06:03). These patterns had already been reported in males without sensitizing events and were not justified by known epitopes in the HLA pattern analysis. Three out of 4 DSAs were negative in the LIFECODES SAB testing, while the pan-DR reactivity was also observed with high reactivity to self-HLA alleles. The absorption/elution protocol was carried out to validate the LIFECODES findings and assess the DSA binding to native HLA antigens on donor cells. All four suspected DSAs were not eluted from donor cells, indicating that the LABSCREEN DSA reactivity was specific to denatured antigens on the beads. The eluate results support the false-positive DSAs assignment and the change of these antibodies for the DSA negative group.

1A. The blue rectangle indicates DSA to HLA-DP1 (DPA1*02:01/DPB1*01:01)

Absorption/elution protocol with LABScreen SAB (Eluate reactivity):

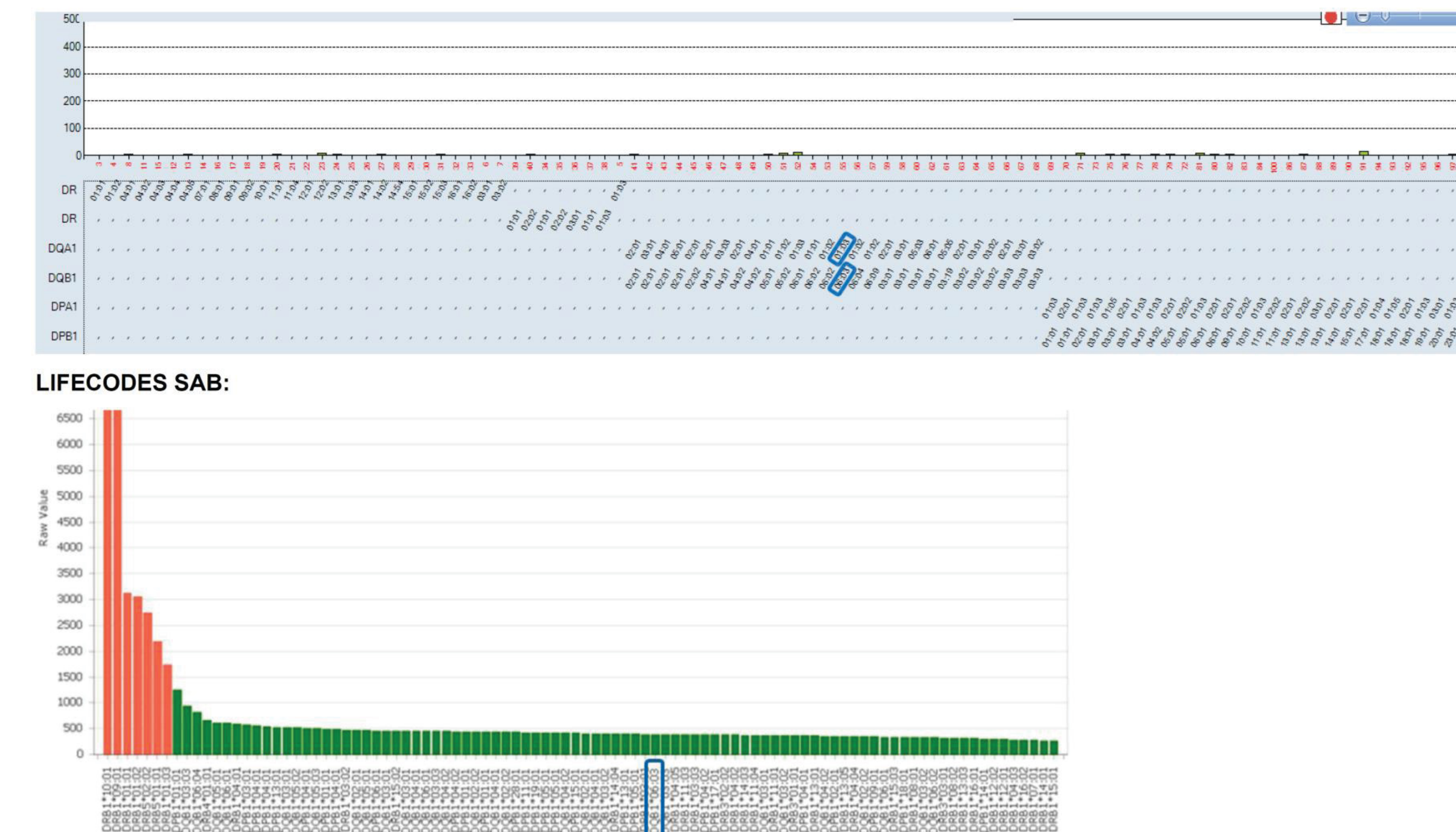


LIFECODES SAB:



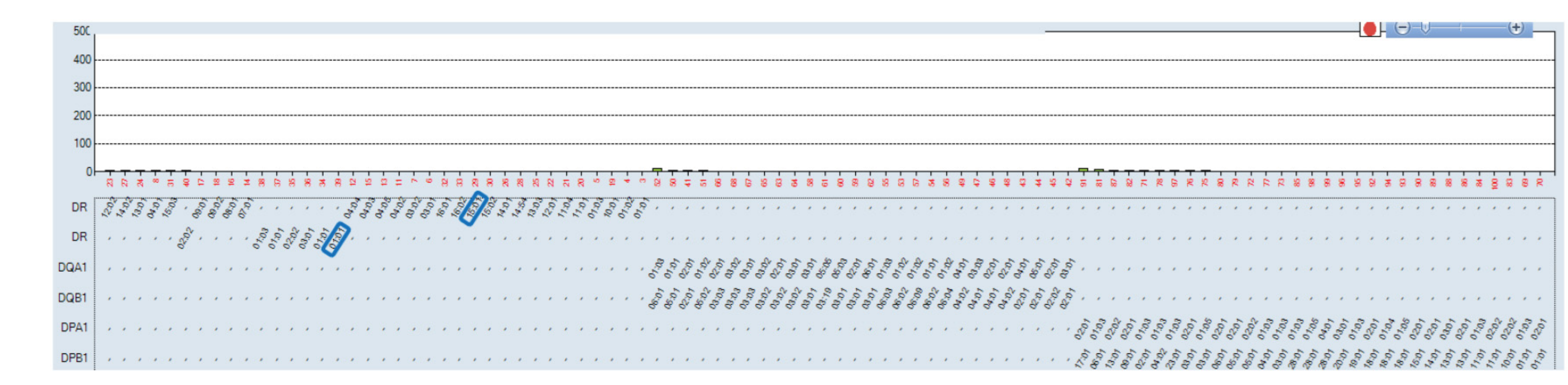
1B. The blue rectangle indicates DSA to HLA-DQ6 (DQA1*01:03/DQB1*06:03)

Absorption/elution protocol with LABScreen SAB (Eluate reactivity):

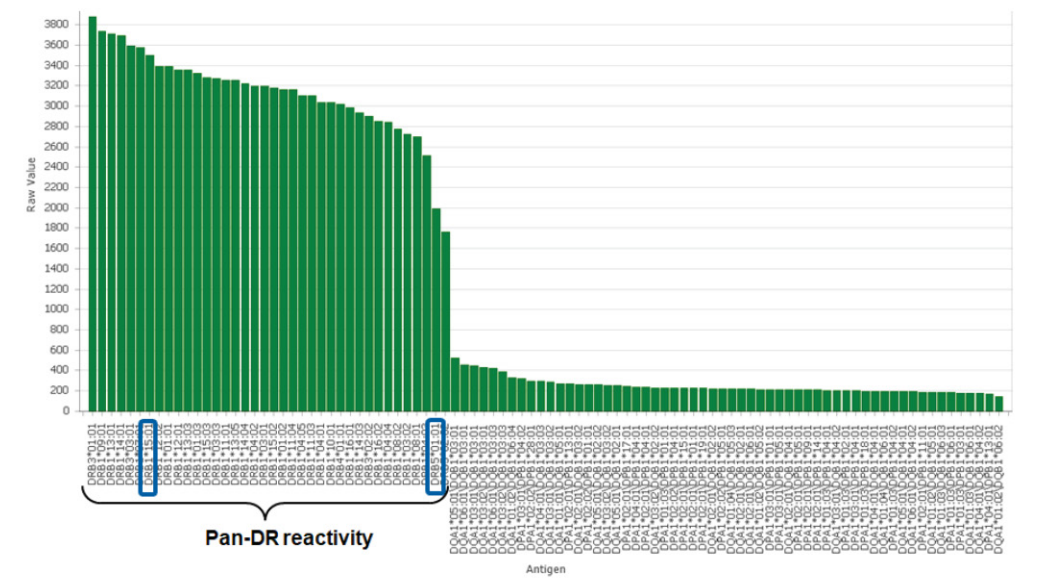


1C. The blue rectangles indicate DSAs to HLA-DR15 (DRB1*15:01) and DR51 (DRB5*01:01); Presence of pan-DR pattern in LIFECODES class II SAB panel, with reactivity to self-HLA alleles (DRB1*03:01, MFI=3590; DRB1*12:02, MFI=3391; DRB3*01:01, MFI=; DRB3*02:02, MFI=2899)

Absorption/elution protocol with LABScreen SAB (Eluate reactivity):

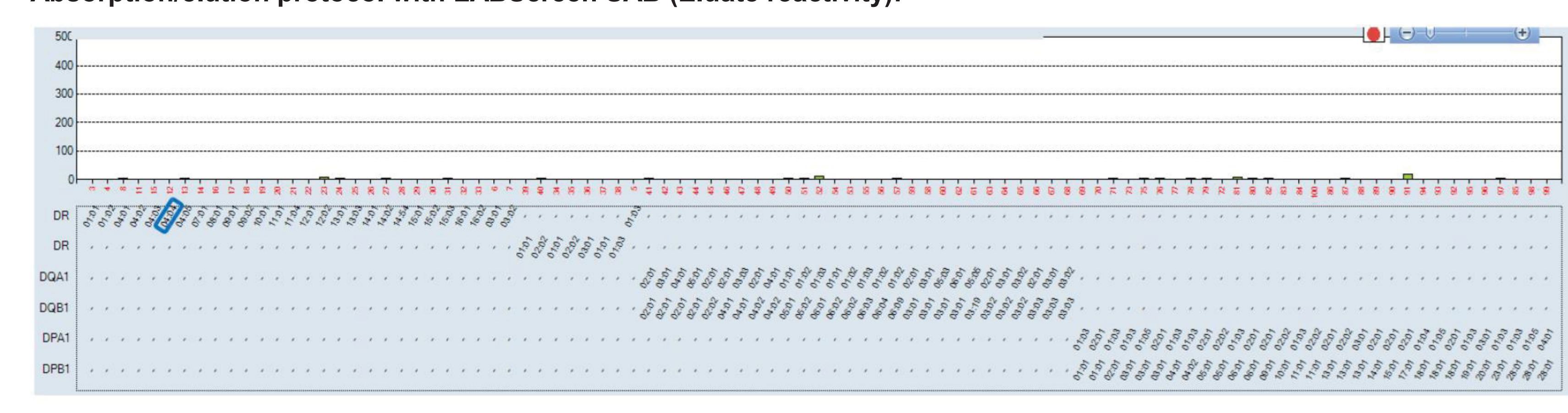


LIFECODES SAB:

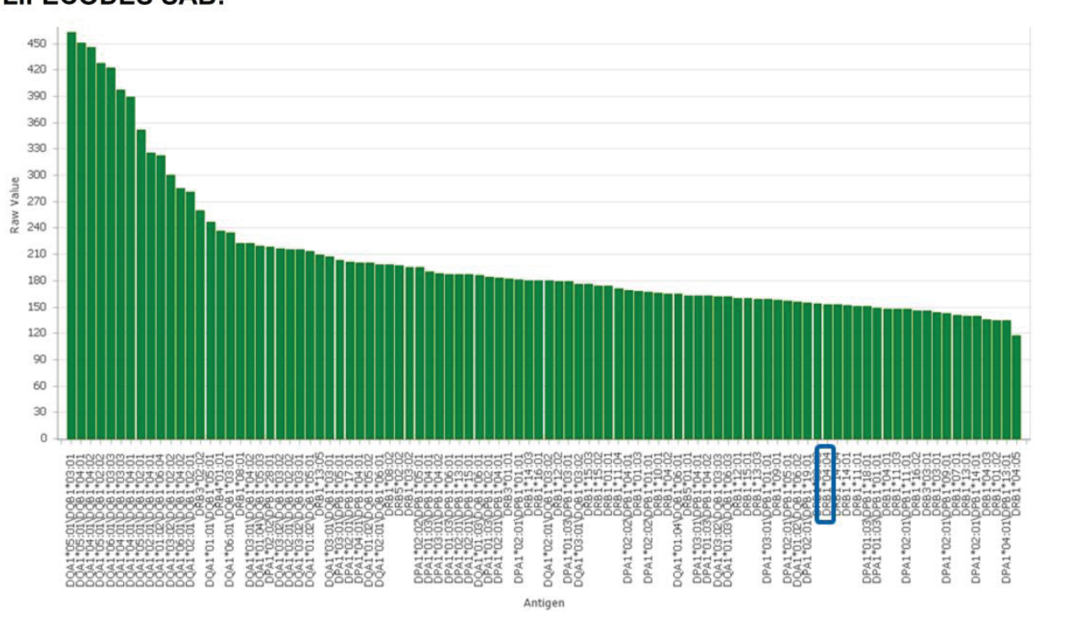


1D. The blue rectangle indicate DSA to HLA-DR4 (DRB1*04:04)

Absorption/elution protocol with LABScreen SAB (Eluate reactivity):



LIFECODES SAB:



4.2 MANUSCRIPT 2

The impact of donor-specific anti-human leukocyte antigen antibodies in salvage haploidentical hematopoietic cell transplantation with posttransplant cyclophosphamide in patients with nonmalignant disorders.

Published in *HLA*. 2021 Jun;97(6):493-504. (DOI: 10.1111/Tan.14277)

Impact Factor: 9.2

Received: 10 March 2021 | Revised: 8 April 2021 | Accepted: 15 April 2021

DOI: 10.1111/tan.14277

ORIGINAL ARTICLE

HLA Immune Response Genetics WILEY

The impact of donor-specific anti-human leukocyte antigen antibodies in salvage haploidentical hematopoietic cell transplantation with posttransplant cyclophosphamide in patients with nonmalignant disorders

Alberto Cardoso Martins Lima[1] | **Carmem Bonfim[2]** | **Joselito Getz[1]** | **Luciana Nasser Dornelles[1]** | **Geovana Borsato do Amaral[1]** | **Ricardo Rasmussen Petterle[3]** | **Gisele Loth[2]** | **Samir Kanaan Nabhan[2]** | **Noemi Farah Pereira[1]** | **Ricardo Pasquini[2]**

[1]Immunogenetics Laboratory, Complexo Hospital de Clínicas, Federal University of Paraná, Curitiba, Brazil

[2]Bone Marrow Transplantation Unit, Complexo Hospital de Clínicas, Federal University of Paraná, Curitiba, Brazil

[3]Department of Integrative Medicine, Federal University of Paraná, Curitiba, Brazil

Correspondence

Alberto Cardoso Martins Lima, Immunogenetics Laboratory, Complexo Hospital de Clínicas, Federal University of Paraná, Rua Padre Camargo, 280—1 Andar, CEP 80060-240, Curitiba, PR, Brazil.

Email: acml@ufpr.br

Funding information

Programa Nacional de Apoio à Atenção Oncológica (PRONON), Grant/Award Number: 25.000.001142/2017-71

Abstract

The presence of donor-specific anti-human leukocyte antigen (HLA) antibodies (DSAs) has been recognized as a major risk factor for graft failure (GF) after haploidentical hematopoietic cell transplantation with post-transplant cyclophosphamide (haplo-PTCy). However, the role of DSAs in salvage haplo-PTCy for rescuing patients with nonmalignant disorders (NMDs) has not yet been reported. The present study retrospectively analyzed 22 patients with NMDs who underwent salvage haplo-PTCy from January 2008 to December 2017. The median age at the time of the rescue haplo-PTCy was 9 years (range, 1–26 years). Median time from the first transplant to second haplo-PTCy was 56 days (range, 37–591 days). Among all patients, six (27.3%) had DSAs, with a median DSA strength (mean fluorescence intensity [MFI]) of 5201 (range, 1412–11,543) in the first DSA testing. In addition, the median DSA MFI was 2672 (range, 832–10,498) before the bone marrow infusion. Overall, GF occurred in 5 (25%) of the 20 assessable patients. Three of four (75%) patients with DSAs experienced GF versus 2 of 16 (12.5%) DSA-negative patients ($P = 0.032$). The median DSA MFI for patients with GF was 6437 (range, 1412–10,498) versus 1845 (range, 832–2672) for those who engrafted or had early death ($P = 0.030$). One-year event-free survival was significantly lower in DSA-positive patients than in those without DSAs (16.7% vs. 62.5%, $P = 0.002$). DSA-negative patients had an acceptable 1-year survival of 62.5%. In conclusion, this study suggests that DSAs may be associated with deleterious outcomes after salvage haplo-PTCy in patients with NMDs.

KEYWORDS

donor-specific anti-HLA antibodies, graft failure, nonmalignant disorders, posttransplant cyclophosphamide, salvage haploidentical transplant

 © 2021 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

ABSTRACT

The presence of donor-specific anti-HLA antibodies (DSAs) has been recognized as a major risk factor for graft failure (GF) after haploidentical hematopoietic cell transplantation with posttransplant cyclophosphamide (haplo-PTCy). However, the role of DSAs in salvage haplo-PTCy for rescuing patients with nonmalignant disorders (NMDs) has not yet been reported. The present study retrospectively analyzed 22 patients with NMDs who underwent salvage haplo-PTCy from January 2008 to December 2017. The median age at the time of the rescue haplo-PTCy was 9 years (range, 1-26 years). Median time from the first transplant to second haplo-PTCy was 56 days (range, 37-591 days). Among all patients, six (27.3%) had DSAs, with a median DSA strength (mean fluorescence intensity - MFI) of 5201 (range, 1412-11543) in the first DSA testing. In addition, the median DSA MFI was 2672 (range, 832-10498) before the bone marrow infusion. Overall, GF occurred in five (25%) of the 20 assessable patients. Three of four (75%) patients with DSAs experienced GF versus two of 16 (12.5%) DSA-negative patients (*P*=0.032). The median DSA MFI for patients with GF was 6437 (range, 1412-10498) versus 1845 (range, 832-2672) for those who engrafted or had early death (*P*=0.030). One-year event-free survival was significantly lower in DSA-positive patients than in those without DSAs (16.7% vs. 62.5%, *P*=0.002). DSA-negative patients had an acceptable one-year survival of 62.5%. In conclusion, this study suggests that DSAs may be associated with deleterious outcomes after salvage haplo-PTCy in patients with NMDs.

Keywords: Donor-specific anti-HLA antibodies, nonmalignant disorders, graft failure, salvage haploidentical transplant, posttransplant cyclophosphamide.

4.2.1 Introduction

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment for patients with nonmalignant disorders (NMDs)[1]. However, graft failure (GF) remains an important cause of morbidity and mortality after allogeneic HCT in these patients. Indeed, some reports have shown that patients with NMDs had a three times higher risk of GF than those with malignancies[2, 3]. Additionally, GF occurs more frequently in patients with NMDs undergoing alternative donor transplantation[4, 5].

The pathogenesis of GF is likely multifactorial, with several known risk factors[6]. Recently, the presence of donor-specific anti-HLA antibodies (DSAs) has been recognized as a major risk factor for GF after alternative donor HCT for malignant diseases[7-9]. Nonetheless, data on preformed DSAs and GF occurrence are still limited for patients with NMDs undergoing alternative donor HCT[10, 11].

Without autologous recovery, patients with GF need urgent rescue HCT, as prolonged cytopenia is associated with significant risks of life-threatening infections and hemorrhage. Presently, there is no consensus regarding the optimal treatment for patients with GF[12]. In this sense, haploidentical HCT with posttransplant cyclophosphamide (haplo-PTCy) as graft-versus-host disease (GVHD) prophylaxis may be a valuable option for rescue transplants[13]. Most patients in need of an urgent transplant have at least one haploidentical donor readily available and highly motivated for donation.

Currently, the role of DSAs on salvage haplo-PTCy outcomes is still debatable. Srour and colleagues described that DSAs were associated with high nonrelapse mortality and poor survival in 29 patients with malignancies who underwent a second haplo-PTCy for relapse[14]. On the other hand, Giammarco et al. showed that the presence of DSAs had not affected engraftment in 19 patients receiving salvage haplo-PTCy for primary GF[15]. To our knowledge, the impact of DSAs in salvage haplo-PTCy for rescuing patients with NMDs has not yet been reported.

To address this gap, we retrospectively investigated the influence of DSAs on the occurrence of GF in patients with NMDs who underwent salvage haplo-PTCy. We also evaluated whether the presence of DSAs influenced survival outcomes.

4.2.2 Methods

4.2.2.1 Patient data

The present study included 22 children, adolescents, and young adults diagnosed with NMDs who underwent salvage haplo-PTCy at the Federal University of Paraná, Curitiba, Brazil from January 2008 to December 2017. This retrospective study was approved by the institutional review board (number 96085718.6.0000.0096).

4.2.2.2 *HLA* typing

Patients and haploidentical donors were routinely typed for *HLA-A*, *-B*, *-C*, *-DRB1*, and *-DQB1* loci at medium resolution level by reverse sequence-specific oligonucleotides LABType (One Lambda Inc., Canoga Park, CA, USA) and/or sequence-based typing method (SeCore® SBT, One Lambda). Patients with HLA-DP, -DR52, -DR53, and -DR51 antibodies were also typed for *HLA-DPB1, -DRB3, -DRB4, and -DRB5* loci, respectively.

4.2.2.3 Anti-HLA antibody detection

Anti-HLA antibody evaluation was performed by bead-based technology using LABScreen Single Antigen Beads (SAB) panels (One Lambda, Inc., Canoga Park, CA). Given that several lots of SAB reagents were used from 2008 to 2017 and protocol to mitigate the complement-mediated interference was not performed before 2014, we have repeated the HLA antibody assessment on ethylenediaminetetraacetic acid (EDTA)-treated sera using current LABScreen SAB class I (LS1A04, lot 12) and class II (LS2A01, lot 13) panels on FlexMap 3D instrument (Luminex, Austin, TX). DSA-positive samples were also tested with the C1q binding assay (C1qScreen™, One Lambda Inc., Canoga Park, CA) and LABScreen Single Antigen ExPlex (One Lambda, Inc., Canoga Park, CA). All tests were performed according to the manufacturer's instructions. Mean fluorescence intensity (MFI) >1000 was considered positive, and MFI <1000 and >500 was considered potentially positive, based on epitope reactivity patterns and cross-reactive groups[16, 17]. HLA epitope pattern analyses were retrospectively performed

with HLA Fusion MatchMaker software (Version 4.4). Cell-based crossmatches were not performed during the study period.

4.2.2.4 Preparatory regimen and GVHD prophylaxis

All recipients received a reduced intensity conditioning regimen, 95.4% of which were fludarabine-based[18]. Fourteen out of 22 patients (63.6%) received a Baltimore conditioning regimen consisting of fludarabine (150 mg/m2), low-dose cyclophosphamide (29 mg/kg), and low-dose total body irradiation (TBI) (200 cGy), 2 of them associated with anti-thymocyte globulin (4.5 mg/kg). The regimen was selected at the physician's discretion. All patients received unmanipulated bone marrow as graft source. GVHD prophylaxis consisted of cyclophosphamide (50 mg/kg/day on days +3 and +4) and cyclosporine A (trough targeted level of 150 to 200 mg/L) plus mycophenolate mofetil (45mg/kg/day with a maximum of 3g/day) starting on day +5. Patients with Fanconi anemia received a dose-adapted PTCy approach, as previously described[19].

4.2.2.5 Definitions and endpoints

The primary endpoint of the study was GF. The secondary endpoints were event-free survival (EFS) and overall survival (OS). GF was defined as either lack of engraftment of donor cells by day +28 (primary GF) or loss of donor cells after initial engraftment (secondary GF). Poor graft function (PGF) was defined as the presence of three cytopenic counts (absolute neutrophil count ≤ 0.5 $\times 10^9$/L, platelet count ≤ 20×10^9/L, and hemoglobin ≤80 g/L) with complete donor chimerism[20]. Primary GF, secondary GF, and PGF were considered together as a single outcome. Early death was defined as death before day +21 posttransplant[21]. Complete donor chimerism was defined as ≥95% leukocytes of donor origin in peripheral blood samples. Mixed chimerism was defined as more than 5% but less than 95% leukocytes of donor origin. EFS was defined as survival with sustained engraftment. For this endpoint, either death, GF, or PGF were considered an event. OS was defined as the time from rescue transplantation to death from any cause, and surviving patients were censored at the last follow-up[22].

4.2.2.6 Statistical analysis

Categorical variables were compared between groups utilizing Fisher's exact test, and continuous variables were compared by the Mann-Whitney U test. GF was expressed as the number and percentage of evaluable patients and compared with Fisher's exact test. The probabilities of EFS and OS were estimated according to the Kaplan-Meier method and compared among groups by the log-rank test. The prognostic effect of GF on OS was assessed by a univariable Cox regression model, using GF as a time-dependent variable. In our analyses, continuous variables were dichotomized into categorical variables by treating the median value as a threshold. The following covariates were considered for univariable analyses: ABO (match vs. mismatch), conditioning regimen (fludarabine, TBI, and cyclophosphamide vs. others), *in vivo* T-cell depletion with antithymocyte globulin (no vs. yes), diagnosis (acquired severe aplastic anemia vs. others), DSA (negative vs. positive), HLA mismatch in the host-versus-graft (HvG) direction (2-3 vs. 4-5), killer immunoglobulin-like receptor-ligand mismatch in the HvG direction (match vs. mismatch), $CD34^+$ cells (< median vs. ≥ median), total nucleated cell dose (< median vs. ≥ median), patient age (< median vs. ≥ median), donor age (< median vs. ≥ median), and time from first transplant to salvage haplo-PTCy (< median vs. ≥ median). Multivariable analyses were not performed in this cohort due to the limited sample size. P values < 0.05 were considered statistically significant. All statistical analyses were performed with EZR version 1.53 (Saitama Medical Centre, Jichi Medical University)[22, 23].

4.2.3 Results

4.2.3.1 Patient-, donor-, and transplantation-related characteristics

Patient, donor, and transplant series characteristics are summarized in Table 1. The donor types of the first HCT included 9 unrelated donors, 6 haploidentical donors, 6 umbilical cord blood units, and one HLA-identical sibling. All patients were rescued from either primary GF (13/22, 59.1%) or secondary GF (9/22, 40.9%). The median time from first transplant to salvage haplo-PTCy was 56 days (range, 37-591 days). The median age of patients at the salvage haplo-PTCy was 9 years (range, 1-26), and 95.4% of the patients were under or equal to 18 years. The most frequent

NMDs were acquired severe aplastic anemia (SAA) (8/22, 36.4%) and Fanconi anemia (7/22, 31.8%). All second transplants were performed with a different donor from the first HCT. The median doses of infused total nucleated cells and CD34+ cells were 7.04 × 10^8/kg (range, 2.34-12.80 × 10^8/kg) and 3.05 × 10^6/kg (range, 1.45-7.63 × 10^6/kg), respectively.

4.2.3.2 HLA allosensitization and DSA assessment

Among all patients studied, 17 (77.3%) had anti-HLA antibodies. Five of them (22.7%) had anti-HLA class I, one (4.6%) had anti-HLA class II, and 11 (50%) had antibodies against both HLA classes. Six patients (27.3%) were DSA-positive. Of them, three patients (P13, P16, and P22) had one DSA, one patient (P14) had two DSAs, and two patients (P1 and P10) had three DSAs, with a median DSA MFI of 5201 (range, 1412-11543) in the first SAB assessment (Table 2). After repetition with EDTA-treated samples, the DSAs strength demonstrated either an increase (P14, DR52 MFI: from 484 to 10498; P16, B13 MFI: from 2452 to 7673) or no change in MFI values. All DSAs were C1q-negative (Supplementary Figure S1). Only two patients with DSAs (P1 and P13) were desensitized with rituximab and plasma exchange. Following the desensitization therapy and before the bone marrow infusion, the median MFI was 2672 (range, 832-10498) among the 11 DSAs. Two patients (P10 and P14) had donor HLA allele incompatibilities not represented in the SAB class II panel (P10: DRB1*11:02 and DQB1*03:19; P14: DRB1*13:02) used at second haplo-PTCy. Retrospective epitope analysis with SAB ExPlex confirmed the presence of donor epitope-specific reactivity against these missing HLA mismatches (Supplementary Figure S2). Moreover, P10 had an additional low-level DSA against HLA-DP17 mismatch (MFI=1412). Epitope analysis showed that this DSA was specific for a public epitope 56E, which was shared by 19 cross-reactive HLA-DP antigens (median MFI=1723). This so-called “shared-epitope phenomenon” diluted the MFI signal among all DP molecules sharing the donor-specific epitope, thus leading to an underestimation of the actual anti-DP17 DSA strength.

4.2.3.3 Engraftment, graft failure and chimerism

Overall, the cumulative incidence of neutrophil engraftment at day +28 was 72.7%, with a median engraftment time of 18.5 days (range, 12-29). The cumulative incidence of platelet engraftment at day +28 was 65.7%, with a median engraftment time of 25 days (range, 14-34). Two DSA-positive patients died early at days +3 and +11 and were not evaluated for engraftment failure. Overall, GF occurred in 5 (25%) of the 20 assessable patients. Among the five patients with GF, three had primary GF, one had secondary GF at day +34, and one had secondary PGF at day +41. Three of four (75%) DSA-positive patients experienced GF versus two of 16 (12.5%) patients without DSAs (*P*=0.032; Table 3). Notably, one DSA-positive patient who engrafted (P14) was desensitized with rituximab and two sessions of plasma exchange. This patient achieved neutrophil and platelet engraftment at days +13 and +14, respectively. The median DSA MFI for patients with GF was 6437 (range, 1412-10498) versus 1845 (range, 832-2672) for those who engrafted or had early death (*P*=0.030; Mann-Whitney U test). In addition, patients with acquired SAA had a higher incidence of GF (4/8, 50%) compared to other NMDs (1/12, 8.3%), although this difference was not statistically significant (*P*=0.109). No other patient-, donor-, or transplant-related variables were associated with engraftment failure (*P* >.05; Table 3).

Data on chimerism at day +30 were available from 19 patients (95%) of the 20 assessable patients. Fourteen patients with sustained hematologic recovery had complete donor chimerism, with 100% donor cells. One DSA-positive patient (P10), who achieved neutrophil engraftment at day +16 and platelet engraftment at day +21, showed mixed chimerism with 21% donor cells at day +30. The chimerism analysis was repeated at day +34 and confirmed secondary GF with undetectable donor cells. One patient without DSAs (P6), with previous neutrophil and platelet engraftment at days +16 and +19, developed pancytopenia after CMV reactivation at day +28. Chimerism testing at days +30 and +41 showed 100% donor cells in the context of persistent pancytopenia, thereby confirming secondary PGF. All three patients with primary GF had 0% donor cells at day +30.

Among the five patients with GF, only two underwent a third transplant. Both had DSAs before the second haplo-HCT. Patient P10 found a 12/12 identical cousin in the extended family search. This child fully engrafted and was alive and disease-free at the last follow-up. Patient P14 underwent desensitization (rituximab and three plasma exchange sessions) before the third transplant with peripheral blood cells

from the mother. A significant MFI reduction was obtained after desensitization (DSA's MFI=0). This patient had neutrophil engraftment at day +14 but died of fungal infection at day +18.

4.2.3.4 Survival outcomes

The EFS and OS of the entire cohort at one year were 50.0% (95% CI, 28.2%-68.4%), and 54.5% (95% CI, 32.1%-72.4%), respectively. The median follow-up among the survivors was 1399 days (range, 448-2752 days). The 1-year EFS was significantly lower in DSA-positive patients than in those without DSAs (16.7% vs. 62.5%, *P*=0.002; Figure 1A). A trend for lower 1-year OS was observed in patients with DSAs than in patients with no DSAs (33.3% vs. 62.5%; *P*=0.076; Figure 1B). Moreover, patients with acquired SAA showed a trend for worse EFS (*P*=0.093) and a marginally significant difference for poor OS (*P*=0.049). None of the other covariates were associated with EFS or OS (*P*>0.05; Table 4). Four patients with GF died within a median time of 62 days after rescue transplant. Thus, GF as a time-dependent variable was significantly associated with inferior OS (HR 5.46; 95% CI: 1.18-25.85; *P*=0.029).

4.2.4 Discussion

In this retrospective single-center study, we evaluated the contribution of DSAs on the incidence of GF, EFS, and OS following second haplo-PTCy in patients with nonmalignant disorders. Our findings suggest that the presence of DSAs was associated with an increased risk of GF and poor EFS after second haplo-PTCy. To the best of our knowledge, the present study is the first to address the impact of DSAs in patients with NMDs undergoing a rescue haploidentical transplant with PTCy platform.

Pretransplant DSAs have been recognized as a major risk factor for GF in distinct haploidentical platforms for malignant diseases. In the context of T-cell replete haplo-PTCy, DSAs were associated with deleterious outcomes in some[7, 24] but not all studies[25]. Nevertheless, the role of DSAs in haplo-PTCy for NMDs is not well established. Kurzay et al.[26] showed complete engraftment in four patients with moderate-level DSAs undergoing Haplo-PTCy for inborn errors. In contrast, Clay et

al.[10] described two DSA-mediated GF in a small case series of refractory SAA patients treated with peripheral blood haplo-PTCy. In this study, we observed a nonsignificant trend of increased incidence of GF in patients with acquired SAA after rescue haplo-PTCy. However, due to the small GF number in our cohort, we could not fit a logistic regression model to assess whether the DSAs and acquired SAA diagnosis were independent GF predictors after salvage haplo-PTCy. Therefore, our results should be interpreted with caution.

Evidence on the role of DSAs after second haplo-PTCy is scarce[14, 15]. Although Srour et al. showed that DSAs were associated with poor survival outcomes, Giammarco et al. failed to confirm the deleterious effects of preexisting DSAs after rescue transplant. However, data on DSA strength and specificity were not detailed in those reports. In salvage haplo-PTCy for NMDs, the impact of DSAs has not yet been reported. Fernandes et al.[27] described the results of 12 pediatric patients with NMDs who received salvage HCT from haploidentical grafts with PTCy prophylaxis. All but one who died early had sustained engraftment, with complete donor chimerism. Information on DSA status was not reported in this small case series. Likewise, Prata et al.[28] published the outcomes of 24 patients rescued with haploidentical transplants with the PTCy platform. However, only four patients had NMDs, and data on DSAs were not available.

Several studies in the haploidentical donor setting have reported distinct cutoff values for HLA antibody determination, ranging from 500 to 5000 MFI[24, 25, 29]. As indicated by the European Society for Blood and Marrow Transplantation consensus guidelines for the detection and treatment of DSAs, a threshold of 1000 MFI is used to determine HLA antibody positivity[30]. Nonetheless, some authors have argued that using a strict MFI value may jeopardize HLA antibody detection[16, 17]. Currently, it is well recognized that SAB panels have some intrinsic limitations that may lead to false-positive and false-negative results[31]. Indeed, some DSAs above the cutoff may represent false-positive reactions specific to the denatured HLA antigens on the beads[32]. Conversely, DSA reactivity may be below a given cutoff due to the "shared epitope phenomenon"[33] or complement-mediated inhibition. A false-negative DSA assessment may also occur when donor alleles are not present in the SAB panel. Importantly, the GFs experienced by P10 and P14 underscore how a false-negative assignment may adversely affect DSA risk stratification. Therefore, a thorough SAB

analysis, considering not only a fixed cutoff but also epitope pattern analysis, may provide a more accurate DSA characterization.

It has been suggested that DSAs against high expression *HLA* loci (i.e., *HLA-A*, *-B*, and *-DRB1*) may be more detrimental compared with DSAs directed to low-expression loci[34]. In our case-series, three patients with GF had at least one DSA directed to HLA-B or -DR antigens. Interestingly, Bettinotti et al. have shown that HLA-A, -B, and -DR are expressed in higher levels on CD34+ cells[35], and therefore may be more pathogenic in the context of HLA-mismatched transplants. We speculate that even low/moderate level DSAs against highly expressed HLA antigens may be associated with poor outcomes. Thus, whether DSAs directed to high-expression HLA antigens are more deleterious after the haplo-PTCy platform remains to be established.

The exact mechanism of DSA-mediated GF is not entirely understood. Pre-clinical studies[36, 37] and earlier clinical reports[38, 39] support that DSA-mediated rejection may occur either by antibody-dependent cell-mediated cytotoxicity or complement-mediated cytotoxicity. Currently, it is well established that the likelihood of GF increases with higher DSA levels and that high MFI values on SAB assays are correlated with C1q positivity[40, 41]. In this sense, Ciurea et al.[7] have reported that C1q-positive DSAs were significantly associated with a higher risk of GF after first haplo-PTCy. In our study, three DSA-positive patients who experienced GF were C1q-negative, with DSA MFI varying from 1412 to 10498 in the regular SAB assay. Thus, the influence of moderate-level, C1q-negative DSAs on GF incidence after second haplo-PTCy merits further investigation.

Since a second GF after rescue transplant is associated with poor survival, selecting a DSA-negative donor is paramount to maximizing the haplo-PTCy outcomes. When only DSA-positive donors are available, a desensitization therapy should be performed to favor engraftment after the salvage transplant[30]. Although an optimal desensitization strategy has not yet been established, the MD Anderson and Johns Hopkins protocols[7, 42] have shown promising results. Nevertheless, in low-income countries, including Brazil, access to desensitization therapies may be limited because of the high costs associated with the procedure. In our study, the only DSA-positive patient with sustained engraftment was treated with plasmapheresis and rituximab. Notably, three patients with untreated DSAs failed to engraft, which is consistent with previous studies[24].

The occurrence of a second GF after rescue HCT has been associated with dismal overall survival[43, 44]. In the present study, four out of 5 patients who failed to engraft died within a median time of 62 days after rescue haplo-PTCy. The entire cohort's OS at one year was 54.5%, which is similar to that reported by other studies with a PTCy platform[14, 28]. On the other hand, Fernandes et al.[27] reported a higher survival probability, with 10 out of 12 (83.3%) patients alive at a median follow-up of 45 months. In our study, DSA-negative patients had an acceptable 1-year OS of 62.5%. Based on our findings and recent recommendations by the European Society for Blood and Marrow Transplantation consensus for donor selection in haplo-HCT[45], a DSA-negative donor should be chosen for patients with NMDs to avoid a second GF and poor survival after second haplo-HCT.

This study has some limitations. First, it is a single-center retrospective study. Second, our study included a small sample size with heterogeneous nonmalignant disorders. Third, multivariable analyses were not performed because of the small number of events. Nevertheless, it remains the largest series of children, adolescents, and young adults with NMDs who have undergone salvage haplo-PTCy for GF.

In conclusion, this study showed that the presence of DSAs appears to be associated with increased risks of GF and inferior EFS after second haplo-PTCy in patients with NMDs. Consequently, DSA-positive donors should be avoided for rescue haploidentical transplants, and when not possible, desensitization therapy should be performed in patients with DSAs. Further studies are warranted to validate our results.

4.2.5 Acknowledgments

The authors thank all HCT physicians and HLA laboratory staff who collaborated in this study. We also thank the data manager Heliz Regina Neves. Renato de Marco is gratefully acknowledged for proofreading the final version of the manuscript. This work was supported by Programa Nacional de Apoio à Atenção Oncológica (PRONON) [grant number 25.000.001142/2017-71]. We are grateful for the support provided by Associação dos Amigos do Hospital de Clínicas (AAHC) for this project.

4.2.6 Author contributions

ACML, CB, NFP, and RP designed the study. ACML and RRP performed the statistical analysis. ACML, CB, and NFP analyzed and interpreted the data. ACML and CB wrote the paper. All the other authors contributed to data collection and patient care and critically reviewed the paper. All authors approved the final version of the manuscript.

4.2.7 Conflict of interest

The authors have declared no conflicting interests.

4.2.8 References

1. Tolar J, Sodani P, Symons H. Alternative donor transplant of benign primary hematologic disorders. Bone Marrow Transplant. May 2015;50(5):619-27. doi:10.1038/bmt.2015.1

2. Woodard P, Tong X, Richardson S, et al. Etiology and outcome of graft failure in pediatric hematopoietic stem cell transplant recipients. J Pediatr Hematol Oncol. Dec 2003;25(12):955-9. doi:10.1097/00043426-200312000-00010

3. Olsson R, Remberger M, Schaffer M, et al. Graft failure in the modern era of allogeneic hematopoietic SCT. Bone Marrow Transplant. Apr 2013;48(4):537-43. doi:10.1038/bmt.2012.239

4. Horan J, Wang T, Haagenson M, et al. Evaluation of HLA matching in unrelated hematopoietic stem cell transplantation for nonmalignant disorders. Blood. Oct 4 2012;120(14):2918-24. doi:10.1182/blood-2012-03-417758

5. Eapen M, Wang T, Veys PA, et al. Allele-level HLA matching for umbilical cord blood transplantation for non-malignant diseases in children: a retrospective analysis. The Lancet Haematology. 2017;4(7):e325-e333. doi:10.1016/s2352-3026(17)30104-7

6. Ozdemir ZN, Civriz Bozdag S. Graft failure after allogeneic hematopoietic stem cell transplantation. Transfus Apher Sci. Apr 2018;57(2):163-167. doi:10.1016/j.transci.2018.04.014

7. Ciurea SO, Thall PF, Milton DR, et al. Complement-Binding Donor-Specific Anti-HLA Antibodies and Risk of Primary Graft Failure in Hematopoietic Stem Cell Transplantation. Biol Blood Marrow Transplant. Aug 2015;21(8):1392-8. doi:10.1016/j.bbmt.2015.05.001

8. Chang YJ, Zhao XY, Xu LP, et al. Donor-specific anti-human leukocyte antigen antibodies were associated with primary graft failure after unmanipulated haploidentical blood and marrow transplantation: a prospective study with randomly assigned training and validation sets. J Hematol Oncol. Jul 10 2015;8:84. doi:10.1186/s13045-015-0182-9

9. Ciurea SO, Thall PF, Wang X, et al. Donor-specific anti-HLA Abs and graft failure in matched unrelated donor hematopoietic stem cell transplantation. Blood. Nov 24 2011;118(22):5957-64. doi:10.1182/blood-2011-06-362111

10. Clay J, Kulasekararaj AG, Potter V, et al. Nonmyeloablative peripheral blood haploidentical stem cell transplantation for refractory severe aplastic anemia. Biol Blood Marrow Transplant. Nov 2014;20(11):1711-6. doi:10.1016/j.bbmt.2014.06.028

11. Woolfrey A, Wang T, Lee SJ, et al. Donor-specific anti-HLA antibodies in unrelated hematopoietic cell transplantation for non-malignant disorders. Bone Marrow Transplant. Mar 2019;54(3):494-496. doi:10.1038/s41409-018-0334-y

12. Ferra C, Sanz J, Diaz-Perez MA, et al. Outcome of graft failure after allogeneic stem cell transplant: study of 89 patients. Leuk Lymphoma. Mar 2015;56(3):656-62. doi:10.3109/10428194.2014.930849

13. Fuchs EJ. Related haploidentical donors are a better choice than matched unrelated donors: Point. Blood Adv. Feb 14 2017;1(6):397-400. doi:10.1182/bloodadvances.2016002196

14. Srour SA, Kongtim P, Rondon G, et al. Haploidentical transplants for patients with relapse after the first allograft. Am J Hematol. Jul 3 2020;doi:10.1002/ajh.25924

15. Giammarco S, Raiola AM, Di Grazia C, et al. Second haploidentical stem cell transplantation for primary graft failure. Bone Marrow Transplant. Dec 16 2020;doi:10.1038/s41409-020-01183-9

16. Ellis TM. Interpretation of HLA single antigen bead assays. Transplant Rev (Orlando). Oct 2013;27(4):108-11. doi:10.1016/j.trre.2013.07.001

17. Sullivan HC, Gebel HM, Bray RA. Understanding solid-phase HLA antibody assays and the value of MFI. Hum Immunol. Jul - Aug 2017;78(7-8):471-480. doi:10.1016/j.humimm.2017.05.007

18. Bacigalupo A, Ballen K, Rizzo D, et al. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant. Dec 2009;15(12):1628-33. doi:10.1016/j.bbmt.2009.07.004

19. Bonfim C, Ribeiro L, Nichele S, et al. Haploidentical Bone Marrow Transplantation with Post-Transplant Cyclophosphamide for Children and Adolescents with Fanconi Anemia. Biol Blood Marrow Transplant. Feb 2017;23(2):310-317. doi:10.1016/j.bbmt.2016.11.006

20. Sun YQ, He GL, Chang YJ, et al. The incidence, risk factors, and outcomes of primary poor graft function after unmanipulated haploidentical stem cell transplantation. Ann Hematol. Oct 2015;94(10):1699-705. doi:10.1007/s00277-015-2440-x

21. Champlin RE, Horowitz MM, van Bekkum DW, et al. Graft failure following bone marrow transplantation for severe aplastic anemia: risk factors and treatment results. Blood. 1989;73(2):606-613. doi:10.1182/blood.V73.2.606.606

22. Savani B, Nagler A, Kanate AS. Summary of Scientific and Statistical Methods, Study Endpoints and Definitions for Observational and Registry-Based Studies in Hematopoietic Cell Transplantation. Clinical Hematology International. 2019;doi:10.2991/chi.d.191207.001

23. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. Mar 2013;48(3):452-8. doi:10.1038/bmt.2012.244

24. Zou J, Romee R, Slade M, et al. Untreated donor specific antibodies against HLA are associated with poor outcomes in peripheral blood haploidentical hematopoietic cell transplantation. Bone Marrow Transplant. Jun 2017;52(6):898-901. doi:10.1038/bmt.2017.7

25. Bramanti S, Calafiore V, Longhi E, et al. Donor-Specific Anti-HLA Antibodies in Haploidentical Stem Cell Transplantation with Post-Transplantation Cyclophosphamide: Risk of Graft Failure, Poor Graft Function, and Impact on Outcomes. Biol Blood Marrow Transplant. Jul 2019;25(7):1395-1406. doi:10.1016/j.bbmt.2019.02.020

26. Kurzay M, Hauck F, Schmid I, et al. T-cell replete haploidentical bone marrow transplantation and post-transplant cyclophosphamide for patients with inborn errors. Haematologica. Oct 2019;104(10):e478-e482. doi:10.3324/haematol.2018.215285

27. Fernandes JF, Ribeiro AAF, Kerbauy FR, et al. Salvage Haploidentical Stem Cell Transplantation with Post-Transplant Cyclophosphamide for graft failure in children with non-malignant diseases. Bone Marrow Transplantation. 2019;53(S1):720-21. doi:10.1038/s41409-018-0354-7

28. Prata PH, Resche-Rigon M, Blaise D, et al. Outcomes of Salvage Haploidentical Transplant with Post-Transplant Cyclophosphamide for Rescuing Graft Failure Patients: a Report on Behalf of the Francophone Society of Bone Marrow Transplantation and Cellular Therapy. Biol Blood Marrow Transplant. Sep 2019;25(9):1798-1802. doi:10.1016/j.bbmt.2019.05.013

29. Ciurea SO, de Lima M, Cano P, et al. High risk of graft failure in patients with anti-HLA antibodies undergoing haploidentical stem-cell transplantation. Transplantation. Oct 27 2009;88(8):1019-24. doi:10.1097/TP.0b013e3181b9d710

30. Yoshihara S, Maruya E, Taniguchi K, et al. Risk and prevention of graft failure in patients with preexisting donor-specific HLA antibodies undergoing unmanipulated

haploidentical SCT. Bone Marrow Transplant. Apr 2012;47(4):508-15. doi:10.1038/bmt.2011.131

31. Ciurea SO, Cao K, Fernandez-Vina M, et al. The European Society for Blood and Marrow Transplantation (EBMT) Consensus Guidelines for the Detection and Treatment of Donor-specific Anti-HLA Antibodies (DSA) in Haploidentical Hematopoietic Cell Transplantation. Bone Marrow Transplant. May 2018;53(5):521-534. doi:10.1038/s41409-017-0062-8

32. Wehmeier C, Honger G, Schaub S. Caveats of HLA antibody detection by solid-phase assays. Transpl Int. Jan 2020;33(1):18-29. doi:10.1111/tri.13484

33. El-Awar N, Terasaki PI, Nguyen A, et al. Epitopes of human leukocyte antigen class I antibodies found in sera of normal healthy males and cord blood. Hum Immunol. Oct 2009;70(10):844-53. doi:10.1016/j.humimm.2009.06.020

34. Garcia-Sanchez C, Usenko CY, Herrera ND, Tambur AR. The shared epitope phenomenon-A potential impediment to virtual crossmatch accuracy. Clin Transplant. May 17 2020:e13906. doi:10.1111/ctr.13906

35. Weinstock C, Schnaidt M. The complement-mediated prozone effect in the Luminex single-antigen bead assay and its impact on HLA antibody determination in patient sera. 2013;40(3):171-177. doi:https://doi.org/10.1111/j.1744-313X.2012.01147.x

36. Fernandez Vina M, Heslop HE, Barker JN. New Approaches in Alternative Donor Transplantation. Biology of Blood and Marrow Transplantation. 2013/01/01/ 2013;19(1, Supplement):S91-S96. doi:https://doi.org/10.1016/j.bbmt.2012.10.027

37. Bettinotti MP, Lucas DP, Ghiaur G, Jackson AM, Gladstone DE. OR30. HLA expression on CD34+ stem cells: is a flow cytometric crossmatch a good gatekeeper for mismatched hematopoietic cell transplantion? Human Immunology. 2018/10/01/ 2018;79:35. doi:https://doi.org/10.1016/j.humimm.2018.07.035

38. Xu H, Chilton PM, Tanner MK, et al. Humoral immunity is the dominant barrier for allogeneic bone marrow engraftment in sensitized recipients. Blood. 2006;108(10):3611-3619. doi:10.1182/blood-2006-04-017467 %J Blood

39. Taylor PA, Ehrhardt MJ, Roforth MM, et al. Preformed antibody, not primed T cells, is the initial and major barrier to bone marrow engraftment in allosensitized recipients. Blood. 2006;109(3):1307-1315. doi:10.1182/blood-2006-05-022772 %J Blood

40. Elkins WL, Pierson GR, Zmijewski CM, Koch P, Serota F, August CS. Rejection of sibling bone marrow expressing foreign HLA-A1 by a leukemia patient detection of prior sensitization by antibody-dependent cell-mediated cytotoxicity. 1981;31(4):306-307.

41. Anasetti C, Amos D, Beatty PG, et al. Effect of HLA Compatibility on Engraftment of Bone Marrow Transplants in Patients with Leukemia or Lymphoma. 1989;320(4):197-204. doi:10.1056/nejm198901263200401

42. Schaub S, Hönger G, Koller MT, Liwski R, Amico P. Determinants of C1q Binding in the Single Antigen Bead Assay. Transplantation. 2014;98(4)

43. Wiebe C, Gareau AJ, Pochinco D, et al. Evaluation of C1q Status and Titer of De Novo Donor-Specific Antibodies as Predictors of Allograft Survival. 2017;17(3):703-711. doi:https://doi.org/10.1111/ajt.14015

44. Leffell MS, Jones RJ, Gladstone DE. Donor HLA-specific Abs: to BMT or not to BMT? Bone Marrow Transplant. Jun 2015;50(6):751-8. doi:10.1038/bmt.2014.331

45. Schriber J, Agovi MA, Ho V, et al. Second unrelated donor hematopoietic cell transplantation for primary graft failure. Biol Blood Marrow Transplant. Aug 2010;16(8):1099-106. doi:10.1016/j.bbmt.2010.02.013

46. Lund TC, Liegel J, Bejanyan N, et al. Second allogeneic hematopoietic cell transplantation for graft failure: poor outcomes for neutropenic graft failure. Am J Hematol. Oct 2015;90(10):892-6. doi:10.1002/ajh.24111

47. Ciurea SO, Al Malki MM, Kongtim P, et al. The European Society for Blood and Marrow Transplantation (EBMT) consensus recommendations for donor selection in haploidentical hematopoietic cell transplantation. Bone Marrow Transplant. Jan 2020;55(1):12-24. doi:10.1038/s41409-019-0499-z

TABLE 1. PATIENTS, DONOR, AND TRANSPLANT SERIES CHARACTERISTICS

UPN	Age/Gender	Disease	First allogeneic HCT		Second haploidentical HCT								
			Donor / Type of GF	Interval (d) between 1st/2nd HCT	Donor type/Age	HLA Match*	Presence of DSA	ABO (Patient/Donor)	TNC dose ($\times 10^8$/kg)	CD34+ cells ($\times 10^6$/kg)	Conditioning regimen‡	GF	Survival (Days after 2nd HCT)
1	10 / M	FA	UCB / Primary	91	Cousin / 5	8/10	Yes	O-/A+	5.49	4.39	Flu150+Cy29+TBI200+ATG4.5	Not evaluated	Deceased (11)
2	6 / F	FA	UCB / Primary	48	Father / 35	6/10	No	A-/A+	11.28	3.38	Flu150+TBI200	No	Alive (2246)
3	17 / F	SAA	UCB / Primary	55	Mother / 51	7/10	No	B+/B+	5.28	4.74	Flu150+Cy29+TBI200	Primary GF	Deceased (53)
4	10 / M	SAA	URD / Primary	56	Mother / 29	5/10	No	O+/A+	3.02	1.7	Flu150+Cy29+TBI200	No	Alive (1413)
5	11 / F	FA	MRD / Secondary	213	Mother / 43	7/10	No	A+/A+	2.34	1.57	Flu150+TBI200	No	Alive (1272)
6	3 / M	SAA	URD / Secondary	447	Mother / 43	7/10	No	O+/A+	8.32	2.83	Flu150+Cy29+TBI200	Secondary PGF	Deceased (71)
7	1 / M	WAS	UCB / Secondary	101	Father / 27	6/10	No	O+/O+	7.89	3.08	Flu150+Cy29+TBI200	No	Alive (1385)
8	2 / M	HS	UCB / Primary	44	Mother / 27	5/10	No	A+/A+	5.47	3.5	Flu150+Cy29+TBI200	No	Deceased (58)
9	10 / F	DC	Mother / Primary	49	Aunt / 47	8/10	No	O+/O-	7.88	1.97	Flu150+Cy29+TBI200+ATG4.5	No	Alive (1462)
10	3 / M	DBA	URD / Secondary	133	Father / 25	5/10	Yes	A+/A+	5.21	2.71	Flu150+Cy29+TBI200	Secondary GF	Alive (2058)
11	3 / M	WAS	URD / Secondary	591	Mother / 24	8/10	No	A+/A-	9.56	3.53	Flu150+Cy29+TBI200	No	Alive (2752)
12	8 / M	FA	URD / Primary	97	Mother / 49	6/10	No	O+/O-	6.58	1.45	Flu150+TBI200+ATG4.5	No	Alive (1931)
13	9 / F	FA	URD / Primary	55	Mother / 34	5/10	Yes	O+/O-	9.89	1.51	Flu150+TBI200+ATG4.5	No	Alive (1034)
14	13 / F	SAA	Mother / Primary	55	Aunt / 44	7/10	Yes	O-/B-	5.48	4.5	Flu150+Cy29+TBI200	Primary GF	Deceased (74)
15	6 / M	WAS	Father / Secondary	74	Mother / 36	6/10	No	A+/A+	7.51	1.65	Flu130+Bu16	No	Alive (849)
16	18 / M	SAA	Father / Primary	43	Mother / 37	6/10	Yes	A+/O+	10.8	3.03	Cy30+Bu12	Primary GF	Deceased (24)
17	8 / M	FA	Sister / Secondary	54	Brother / 21	6/10	No	B+/O+	12.3	7.26	Flu150+TBI200	No	Deceased (144)
18	2 / M	WAS	UCB / Secondary	84	Father / 30	5/10	No	AB+/B+	10.6	7.63	Flu150+Cy29+TBI200	No	Alive (1327)
19	14 / M	SAA	URD / Primary	37	Father / 39	6/10	No	A+/A-	12.8	3.07	Flu150+Cy29+TBI200	No	Deceased (94)
20	26 / M	SAA	URD / Primary	40	Mother / 57	5/10	No	O+/A+	5.17	2.95	Flu150+Cy29+TBI200	No	Deceased (83)
21	17 / M	SAA	URD / Primary	37	Father / 45	6/10	No	O-/B+	3.65	3.25	Flu150+Cy29+TBI200	No	Alive (448)
22	9 / M	FA	Father / Secondary	75	Mother / 34	5/10	Yes	O+/O+	2.63	1.97	Flu120+Bu1.6	Not evaluated	Deceased (4)

All patients received bone marrow as graft source and had posttransplant cyclophosphamide, cyclosporine A and mycophenolate mofetil as graft-versus-host disease prophylaxis.

UPN indicates unique patient number; M, male; F, female; FA, Fanconi anemia; SAA, severe aplastic anemia; WAS, Wiskott–Aldrich syndrome; HS, Hurler syndrome; DC, dyskeratosis congenita; DBA, Diamond-Blackfan anemia; UCB, umbilical cord blood; MRD, matched related donor; URD, unrelated donor; GF, graft failure; 1st, first; 2nd, second; HCT, hematopoietic cell transplantation; DSA, donor-specific anti-HLA antibodies; TNC, total nucleated cells; Flu, fludarabine; Cy, cyclophosphamide; TBI, total body irradiation; ATG, antithymocyte globulin; Bu, busulfan; PGF, poor graft function.

*HLA match for HLA-A, -B, -C, -DRB1 and -DQB1 in host-versus-graft direction

‡Total doses, ATG, Bu, and Cy doses in mg/kg, Flu doses in mg/m^2, TBI in cGy.

TABLE 2. DONOR-SPECIFIC ANTI-HLA ANTIBODIES IN PATIENTS WHO UNDERWENT SALVAGE HAPLOIDENTICAL TRANSPLANTATION WITH PTCY

Pat.	Number of DSAs	DSA specificity (MFI)§	Donor-specific epitopes¥	Desensitization protocol	Post-Desensitization DSA Strength (MFI)	GF	Third HCT Donor/Source	Engraftment after Third HCT	Survival (Days after 2nd HCT)
P1	3	DRB1*07:01 (11543) DRB4*01:01 (9411) DQB1*02:01 (4443)	98E 4Q 52LL	Rituximab + 3 PEX	DRB1*07:01 (2394) DRB4*01:01 (1845) DQB1*02:01 (832)	Not evaluated	-	-	Early death (11)
P10	3	DRB1*11:02‡ (5186) DQB1*03:19 (7948) DPB1*17:01 (1412)	70D 55PP 56E	NP	-	Secondary GF (Day +34)	HLA-identical cousin/BM	Yes	Alive (2058)
P13	1	DQB1*04:02 (2698)	56L	Rituximab + 2 PEX	1248	Engraftment	-	-	Alive (1034)
P14	2	DRB1*13:02 (5201) DRB3*03:01 (10498)	149H	NP	-	Primary GF	Mother/PB	Yes	Deceased (74)
P16	1	B*13:02 (7673)	163EW+73TE	NP	-	Primary GF	-	-	Deceased (24)
P22	1	DQB1*03:01 (2672)	55PP	NP	-	Not evaluated	-	-	Early death (4)

Pat. indicates patient number; DSA, donor-specific anti-HLA antibodies; MFI, median fluorescence intensity; PEX, plasma exchange; NP, Not performed; GF, graft failure; HCT, hematopoietic cell transplantation; BM, bone marrow; PB, peripheral blood; 2nd, second

§ DSA strength in the first LABScreen Single Antigen Beads assessment

‡ Donor HLA alleles not represented in the LABScreen Single Antigen Beads panel used at the time of second haplo-PTCy

* MFI value of a surrogate HLA allele which shares the donor-specific epitope; P10: MFI of DRB1*11:01.

¥ Putative amino acid residues in donor HLA antigens recognized by DSAs.

TABLE 3. UNIVARIABLE ANALYSIS FOR GRAFT FAILURE

Variables	GF*		P-value‡
	No (n=15)	Yes (n=5)	
ABO			
Match	10 (83.3)	2 (16.7)	0.347
Mismatch	5 (62.5)	3 (37.5)	
Patient age			
< 9 years	8 (80.0)	2 (20.0)	1
≥ 9 years	7 (70.0)	3 (30.0)	
Donor age			
< 36 years	8 (88.9)	1 (11.1)	0.319
≥ 36 years	7 (63.6)	4 (36.4)	
Conditioning regimen			
Flu + Cy + TBI	9 (69.2)	4 (30.8)	0.613
Others	6 (85.7)	1 (14.3)	
***In vivo* T-cell depletion with ATG**			
No	12 (70.6)	5 (29.4)	0.539
Yes	3 (100.0)	0 (0.0)	
Diagnosis			
Other NMDs	11 (91.7)	1 (8.3)	0.109
Acquired SAA	4 (50.0)	4 (50.0)	
DSA			
Negative	14 (87.5)	2 (12.5)	**0.032**
Positive	1 (25.0)	3 (75.0)	
HLA mismatches in HvG direction			
2-3 HLA mismatches	3 (50.0)	3 (50.0)	0.131
4-5 HLA mismatches	12 (85.7)	2 (14.3)	
KIR-Ligand mismatch in HvG direction			
Match	10 (71.4)	4 (28.6)	1
HvG Mismatch	5 (83.3)	1 (16.7)	
TNC ($\times 10^8$/kg)			
< 7.04	6 (66.7)	3 (33.3)	0.617
≥ 7.04	9 (81.8)	2 (18.2)	
CD34 ($\times 10^6$/kg)			
< 3.05	7 (70.0)	3 (30.0)	1
≥ 3.05	8 (80.0)	2 (20.0)	
Time between 1st and 2nd HCT (days)			
< 56 days	8 (72.7)	3 (27.3)	1
≥ 56 days	7 (77.8)	2 (22.2)	

GF indicates graft failure; Flu, fludarabine; Cy, cyclophosphamide; TBI, total body irradiation; ATG, antithymocyte globulin; SAA, severe aplastic anemia; NMDs, nonmalignant diseases; DSA, donor-specific anti-HLA antibodies; HvG, host-versus-graft; KIR, Killer immunoglobulin-like receptor; TNC, total nucleated cells; 1st, first; 2nd, second; HCT, hematopoietic cell transplantation.

*Two patients with early death were not evaluated for graft failure

‡Fisher's exact test; Significant values are in bold

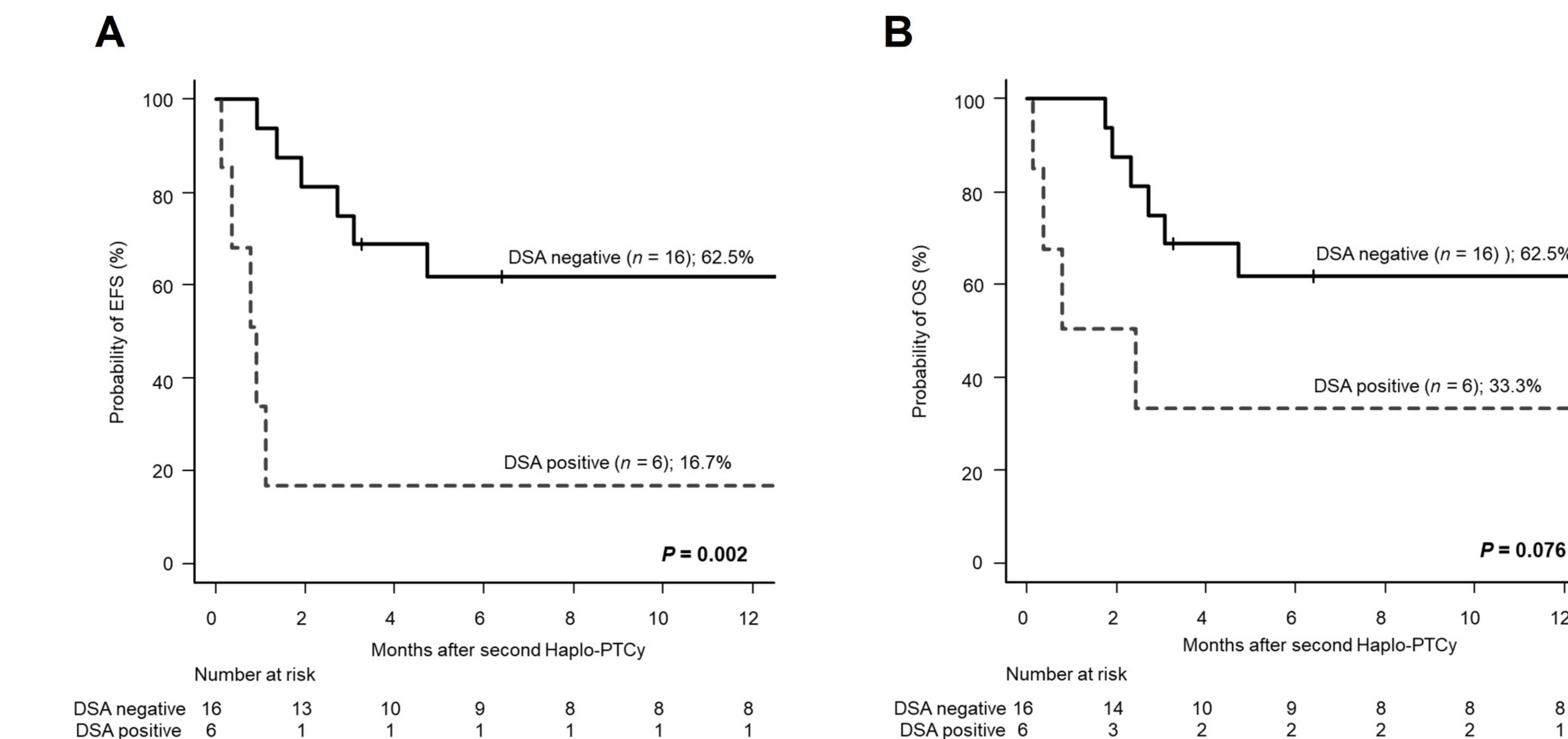
TABLE 4. UNIVARIABLE ANALYSES FOR EFS AND OS

Variables	*n*	EFS	*P*-value[‡]	OS	*P*-value[‡]
ABO					
Match	13	0.615 (0.308-0.818)	0.215	0.692 (0.373-0.872)	0.132
Mismatch	9	0.333 (0.078-0.623)		0.333 (0.078-0.623)	
Patient age					
< 9 years	10	0.600 (0.253-0.827)	0.276	0.700 (0.329-0.892)	0.150
≥ 9 years	12	0.417 (0.152-0.665)		0.417 (0.152-0.665)	
Donor age					
< 36 years	11	0.545 (0.229-0.780)	0.734	0.636 (0.297-0.845)	0.488
≥ 36 years	11	0.455 (0.167-0.707)		0.455 (0.167-0.707)	
Conditioning regimen					
Flu + Cy + TBI	14	0.429 (0.177-0.660)	0.452	0.500 (0.229-0.722)	0.639
Others	8	0.625 (0.229-0.861)		0.625 (0.229-0.861)	
***In vivo* T-cell depletion with ATG**					
No	18	0.444 (0.216-0.651)	0.396	0.500 (0.259-0.701)	0.486
Yes	4	0.750 (0.128-0.961)		0.750 (0.128-0.961)	
Diagnosis					
Other NMDs	14	0.635 (0.331-0.830)	0.093	0.714 (0.406-0.882)	**0.049**
Acquired SAA	8	0.250 (0.037-0.558)		0.250 (0.037-0.558)	
DSA					
Negative	16	0.625 (0.349-0.811)	**0.002**	0.625 (0.349-0.811)	0.074
Positive	6	0.167 (0.008-0.517)		0.333 (0.046-0.676)	
HLA mismatches in HvG direction					
2-3 HLA mismatches	7	0.429 (0.098-0.734)	0.476	0.429 (0.098-0.734)	0.367
4-5 HLA mismatches	15	0.533 (0.263-0.744)		0.600 (0.318-0.797)	
KIR-Ligand mismatch in HvG direction					
Match	16	0.492 (0.236-0.706)	0.809	0.562 (0.295-0.762)	0.949
HvG Mismatch	6	0.500 (0.111-0.804)		0.500 (0.111-0.804)	
TNC (×10^8/kg)					
< 7.04	11	0.364 (0.112-0.627)	0.130	0.455 (0.167-0.707)	0.286
≥ 7.04	11	0.636 (0.297-0.845)		0.636 (0.297-0.845)	
CD34 (×10^6/kg)					
< 3.05	11	0.545 (0.229-0.780)	0.817	0.636 (0.297-0.845)	0.506
≥ 3.05	11	0.455 (0.167-0.707)		0.455 (0.167-0.707)	
Time between 1st and 2nd HCT (days)					
< 56 days	11	0.364 (0.112-0.627)	0.365	0.364 (0.112-0.627)	0.172
≥ 56 days	11	0.636 (0.297-0.845)		0.727 (0.371-0.903)	

EFS indicates event-free survival; OS, overall survival; Flu, fludarabine; Cy, cyclophosphamide; TBI, total body irradiation; ATG, antithymocyte globulin; SAA, severe aplastic anemia; NMDs, nonmalignant diseases; DSA, donor-specific anti-HLA antibodies; HvG, host-versus-graft; KIR, Killer immunoglobulin-like receptor; TNC, total nucleated cells; 1st, first; 2nd, second; HCT, hematopoietic cell transplantation; NA, not applicable.

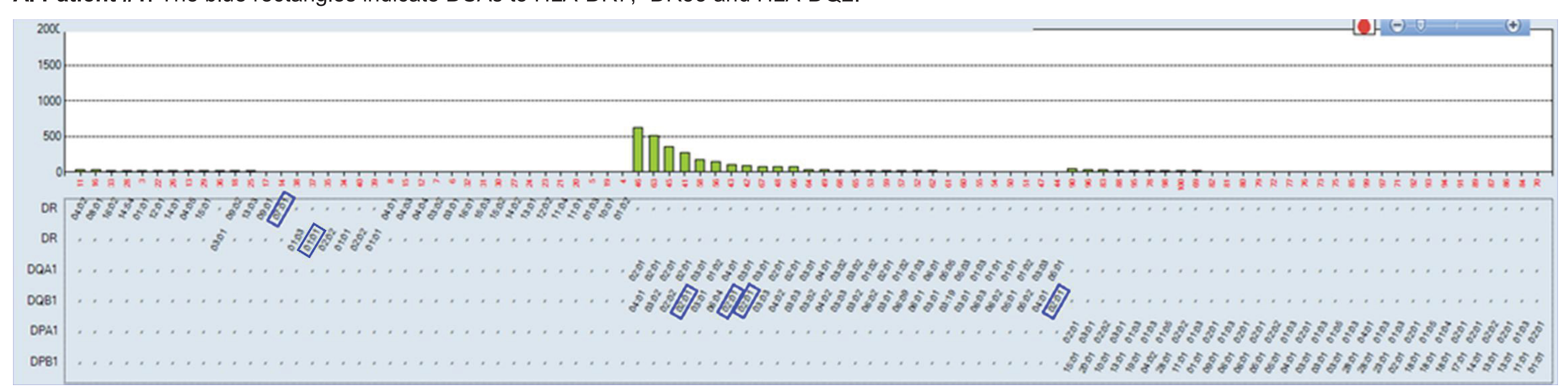
[‡]Log-rank; Significant values are in bold

FIGURE 1. PROBABILITY OF SURVIVAL BY DSA STATUS. (A) ONE-YEAR EFS. (B) ONE-YEAR OS.

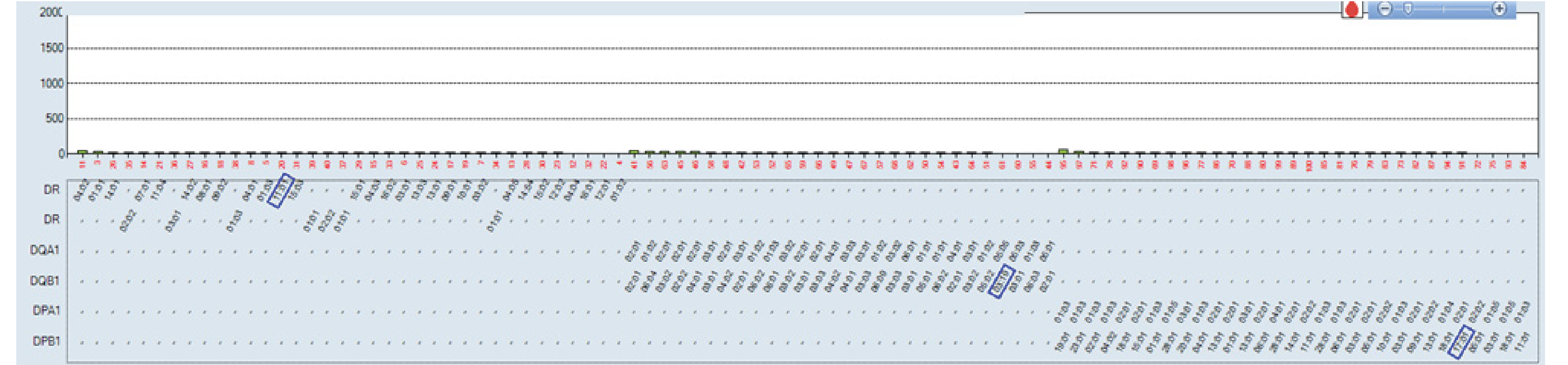


SUPPLEMENTARY FIGURE S1. DSA ASSESSMENT USING C1Q-BINDING ASSAY.

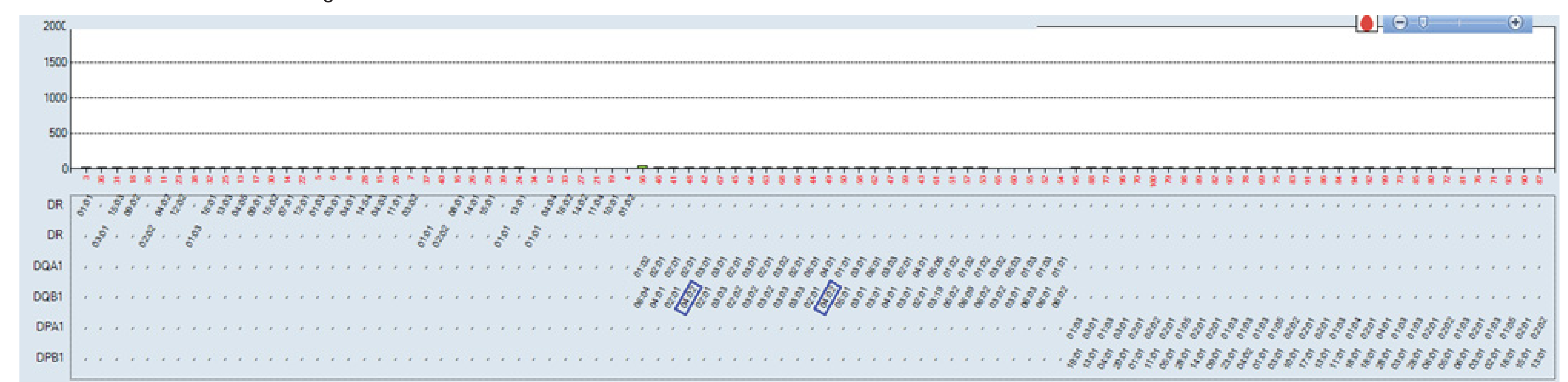
A. Patient #1. The blue rectangles indicate DSAs to HLA-DR7, -DR53 and HLA-DQ2.



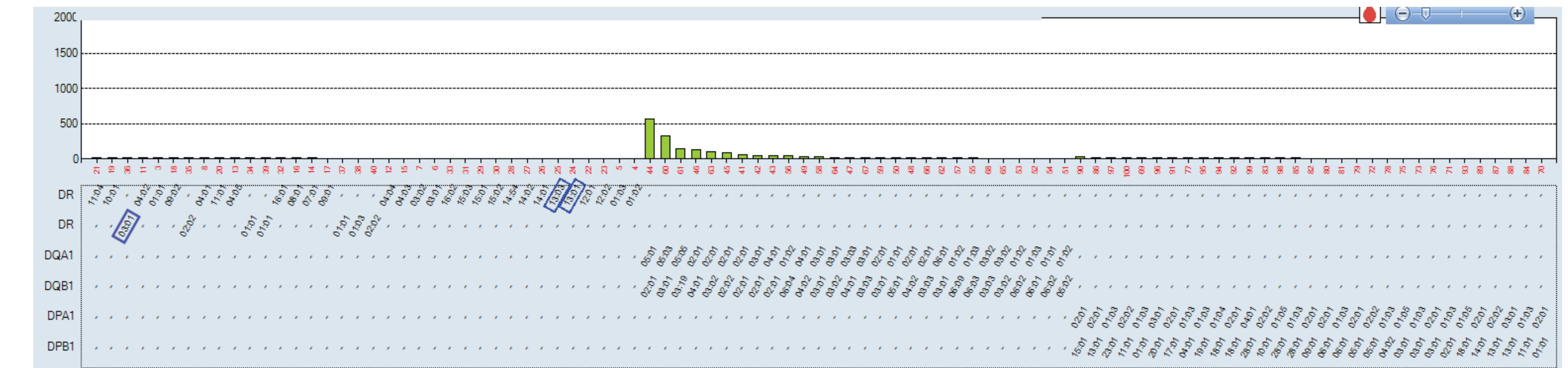
B. Patient #10. The blue rectangles indicate DSAs to HLA-DR11, -DQ7, and -DP17. DRB1*11:01 is highlighted as a surrogate allele since donor DRB1*11:02 mismatch is not represented in SAB class **II** panel used for C1q assay.



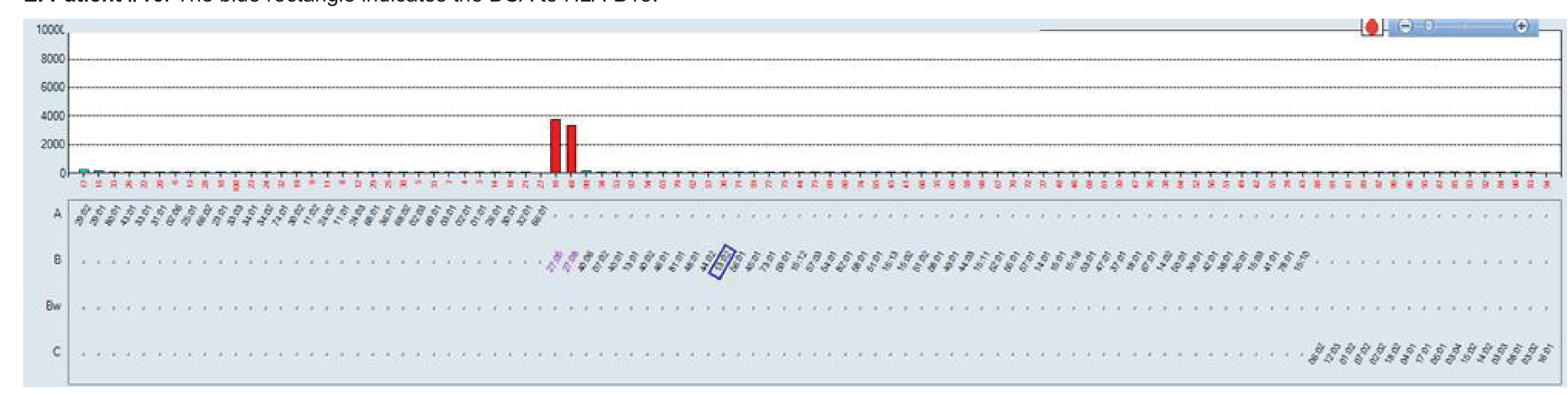
C. Patient #13. The blue rectangle indicates the DSA to HLA-DQ4.



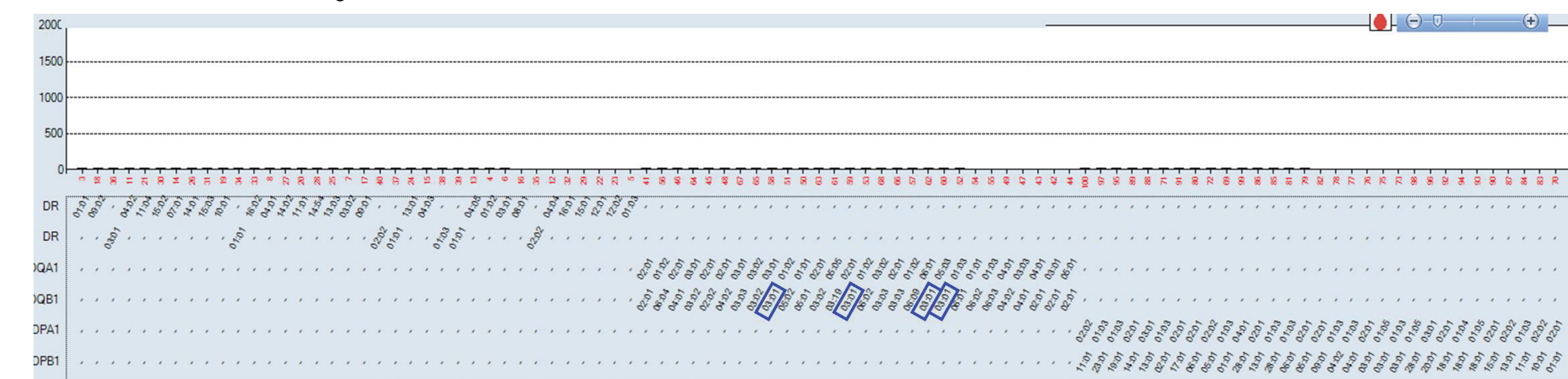
D. Patient #14. The blue rectangles indicate DSAs to HLA-DR13 and -DR52. DRB1*13:01 and DRB1*13:03 are highlighted as surrogates alleles since donor DRB1*13:02 mismatch is not represented in SAB class **II** panel used for C1q assay.



E. Patient #16. The blue rectangle indicates the DSA to HLA-B13.

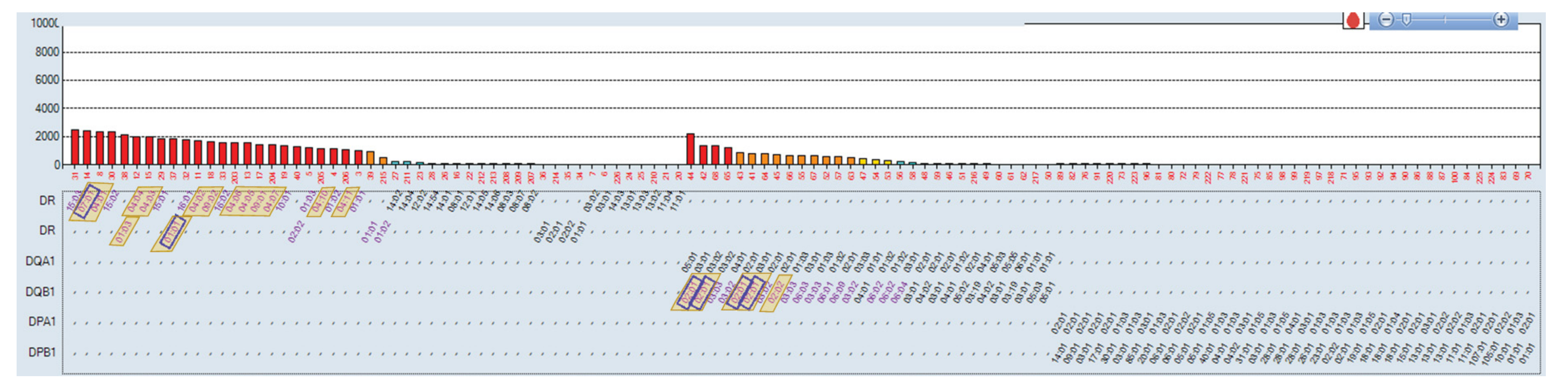


F. Patient #22. The blue rectangle indicates the DSA to HLA-DQ7.

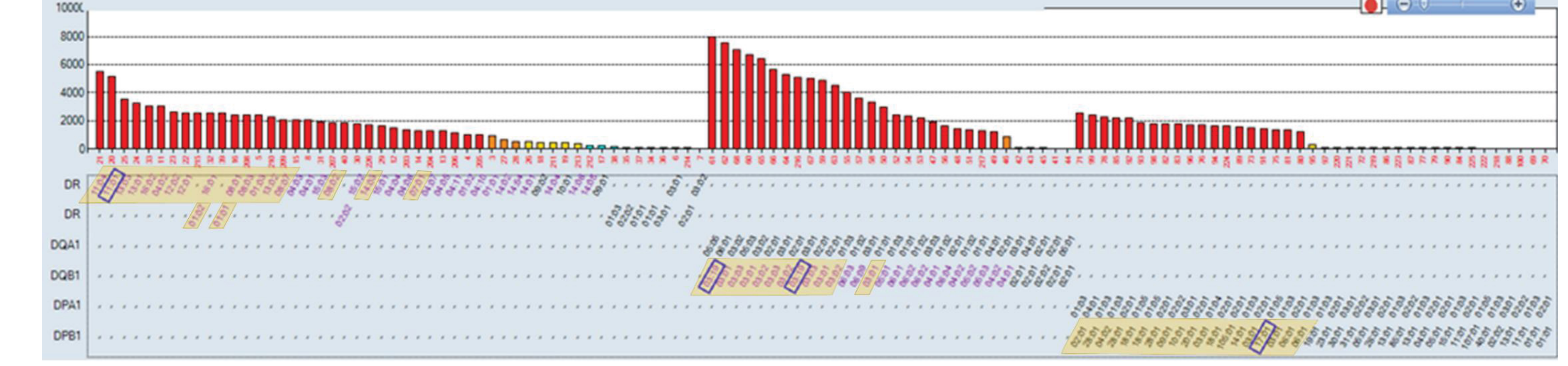


SUPPLEMENTARY FIGURE S2. DSA ASSESSMENT AND HLA EPITOPE ANALYSIS USING SINGLE ANTIGEN EXPLEX.

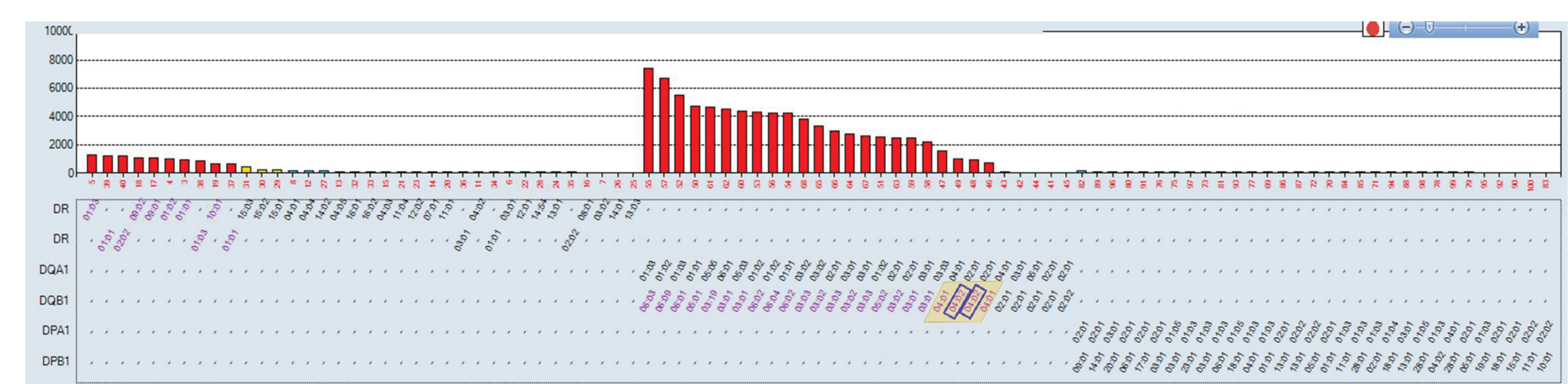
A. Patient #1. DSAs to HLA-DR7, -DR53, and -DQ2 (blue rectangles), and the presumed 98E, 4Q, and 52LL donor-specific epitopes (light yellow), respectively



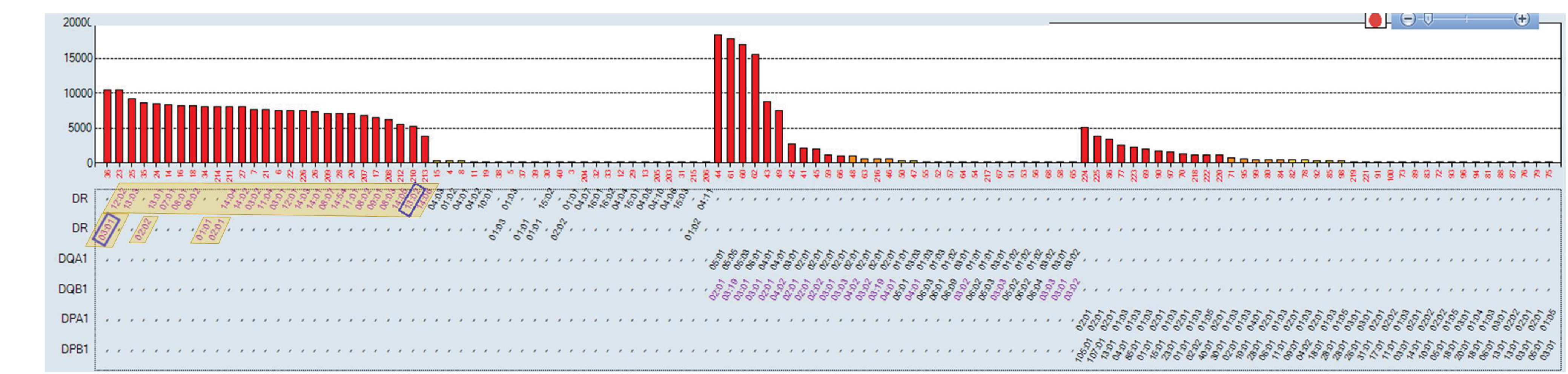
B. Patient #10. DSAs to HLA-DR11, -DQ7, and -DP17 (blue rectangles), and the presumed 70D, 55PP, and 56E donor-specific epitopes (light yellow), respectively. DRB1*11:01 is highlighted as a surrogate allele since donor DRB1*11:02 mismatch is not represented in the SAB Explex class **II** panel.



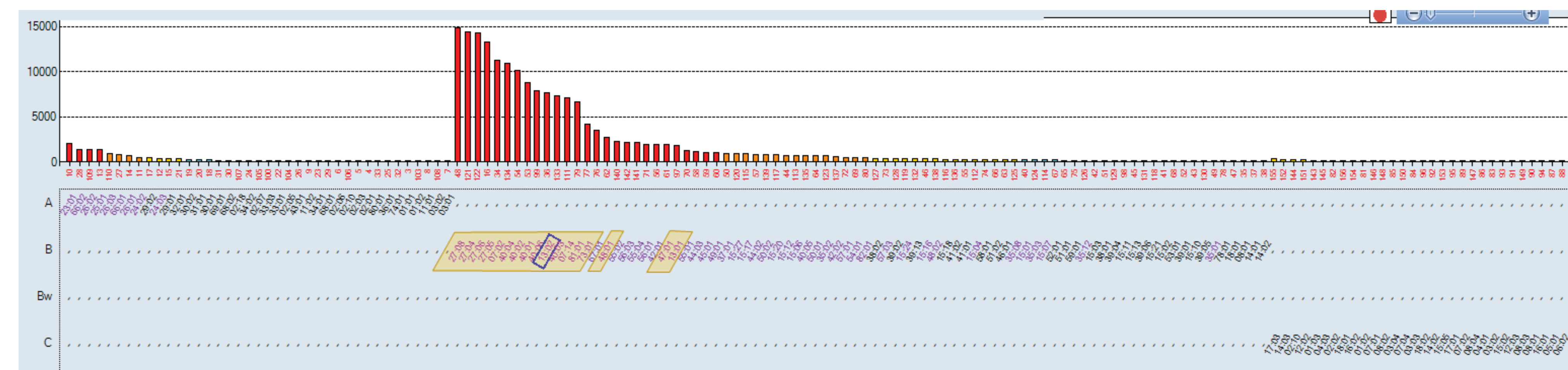
C. Patient #13. DSA to HLA-DQ4 (blue rectangle) and the presumed 55RL donor-specific epitope (light yellow).



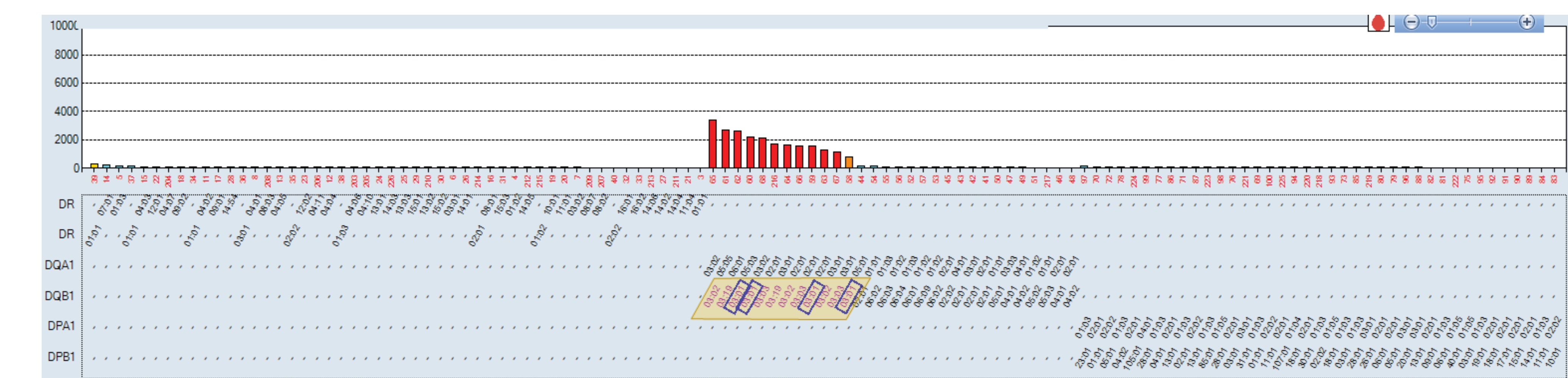
D. Patient #14. DSAs to HLA-DR13 and -DR52 (blue rectangles) and the presumed 149H donor-specific epitope (light yellow)



E. Patient #16. DSA to HLA-B13 (blue rectangle) and the presumed 163EW+138T donor-specific epitope (light yellow)



F. Patient #22. DSA to HLA-DQ7 (blue rectangle) and the presumed 55PP donor-specific epitope (light yellow).



4.3 MANUSCRIPT 3

Donor-specific HLA antibodies are associated with graft failure and delayed hematologic recovery after unrelated donor hematopoietic cell transplantation.

Submitted to *Transplantation and Cellular Therapy* on February 12, 2023; Minor revisions after peer-review on April 17, 2023

Impact Factor: 5.6

Transplantation and Cellular Therapy

Donor-specific HLA antibodies are associated with graft failure and delayed hematologic recovery after unrelated donor hematopoietic cell transplantation

--Manuscript Draft--

Manuscript Number:	TCT-D-23-00089
Article Type:	Full Length Article
Section/Category:	Histocompatibility
Keywords:	Donor-specific anti-HLA antibodies; unrelated donor transplantation; graft failure; delayed hematologic recovery
Corresponding Author:	Alberto Cardoso Martins Lima, M.Sc. Federal University of Parana Curitiba, Paraná BRAZIL
First Author:	Alberto Cardoso Martins Lima, MSc
Order of Authors:	Alberto Cardoso Martins Lima, MSc
	Joselito Getz, MSc
	Geovana Borsato do Amaral, BSc
	Gisele Loth, MD
	Vaneuza Araújo Moreira Funke, MD
	Samir Kanaan Nabhan, MD
	Ricardo Rasmussen Petterle, MSc
	Renato de Marco, MSc
	Maria Gerbase-DeLima, MD, PhD
	Noemi Farah Pereira, PhD
	Carmem Bonfim, MD, PhD
	Ricardo Pasquini, MD
Abstract:	Background: Graft failure (GF) is one of the major concerns after allogeneic hematopoietic cell transplantation (allo-HCT) and remains a significant cause of morbidity and mortality. Although earlier reports have associated the presence of donor-specific HLA antibodies (DSAs) with increased risk of GF after unrelated donor allo-HCT, recent studies have failed to confirm this association. Objective: We sought to validate the presence of DSAs as a risk factor for GF and hematologic recovery in the unrelated donor allo-HCT setting. Study Design: We retrospectively evaluated 303 consecutive patients who underwent their first unrelated donor allo-HCT at our institution from January 2008 to December 2017. DSA evaluation was performed using 2 Single Antigen Beads (SAB) assays, DSA titration with 1:2, 1:8, and 1:32 dilutions, C1q-binding assay, and absorption/elution protocol to assess possible false-positive DSA reactivity. The primary endpoints were neutrophil and platelet recovery and GF, whereas the secondary endpoint was overall survival. Multivariable analyses were performed using Fine-Gray competing risks regression or Cox proportional hazards regression models. Results: The median patient age was 14 years (range, 0-61 years), and 56.1% were male. Eleven patients (3.63%) were DSA-positive. Of them, 10 had preexisting DSAs, and one showed post-transplant DSA rebound. Nine patients had 1 DSA, 1 had 2 DSAs, and 1 had 3 DSAs, with a median MFI of 4334 (range, 588–20,456) and 3581 (range, 227–12,266) in LABScreen and LIFECODES SAB assays, respectively. Overall, 21 patients experienced GF. Of them, 12 had primary graft rejection, 8 had secondary graft rejection, and 1 had primary poor graft function. The cumulative incidences of GF at 100 days and 1 year were 6.6% (95% CI, 4.2%-9.8%) and 6.9% (95% CI, 4.4%-10.2%), respectively. In the multivariable analyses, DSA-positive patients had significantly lower and delayed neutrophil (subdistribution hazard ratio

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

STRUCTURED ABSTRACT

Background: Graft failure (GF) is one of the major concerns after allogeneic hematopoietic cell transplantation (allo-HCT) and remains a significant cause of morbidity and mortality. Although earlier reports have associated the presence of donor-specific HLA antibodies (DSAs) with increased risk of GF after unrelated donor allo-HCT, recent studies have failed to confirm this association.

Objective: We sought to validate the presence of DSAs as a risk factor for GF and hematologic recovery in the unrelated donor allo-HCT setting.

Study Design: We retrospectively evaluated 303 consecutive patients who underwent their first unrelated donor allo-HCT at our institution from January 2008 to December 2017. DSA evaluation was performed using 2 Single Antigen Beads (SAB) assays, DSA titration with 1:2, 1:8, and 1:32 dilutions, C1q-binding assay, and absorption/elution protocol to assess possible false-positive DSA reactivity. The primary endpoints were neutrophil and platelet recovery and GF, whereas the secondary endpoint was overall survival. Multivariable analyses were performed using Fine-Gray competing risks regression or Cox proportional hazards regression models.

Results: The median patient age was 14 years (range, 0-61 years), and 56.1% were male. Eleven patients (3.63%) were DSA-positive. Of them, 10 had preexisting DSAs, and one showed post-transplant DSA rebound. Nine patients had 1 DSA, 1 had 2 DSAs, and 1 had 3 DSAs, with a median MFI of 4334 (range, 588–20,456) and 3581 (range, 227–12,266) in LABScreen and LIFECODES SAB assays, respectively. Overall, 21 patients experienced GF. Of them, 12 had primary graft rejection, 8 had secondary graft rejection, and 1 had primary poor graft function. The cumulative incidences of GF at 100 days and 1 year were 6.6% (95% CI, 4.2%-9.8%) and 6.9% (95% CI, 4.4%-10.2%), respectively. In the multivariable analyses, DSA-positive patients had significantly lower and delayed neutrophil (subdistribution hazard ratio [SHR] = 0.48; 95% CI, 0.29–0.81; *P* = .006) and platelet recovery (SHR = 0.51; 95% CI, 0.35–0.74; *P* = .0003) than patients without DSAs. The Fine-Gray regression demonstrated that the presence of DSAs was strongly associated with a higher

incidence of GF (SHR = 7.60; 95%CI, 2.61–22.14; *P* = .0002). DSA-positive patients with GF had higher median MFI values than DSA-positive patients who achieved engraftment, both in neat serum (LABScreen, 13,751 vs. 3322; *P* = .073; LIFECODES, 10,334 vs. 1250; *P* = .006) and with 3 different dilutions (1:2 dilution, 11,527 vs. 2254, *P* = .073; 1:8 dilution, 5438 vs. 502, *P* = .012; 1:32 dilution, 1627 vs. 61; *P* = .006). All 3 patients with C1q-positive DSAs failed to engraft. DSAs were not predictive of inferior survival (hazard ratio = 0.50; 95% CI, 0.20–1.26, *P* = .14).

Conclusions: Our results validate the presence of DSAs as a significant risk factor for GF and poor hematologic recovery after unrelated donor allo-HCT. Thus, careful pre-transplant DSA evaluation may optimize unrelated donor selection and improve allo-HCT outcomes.

Keywords: Donor-specific anti-HLA antibodies, unrelated donor transplantation, graft failure, delayed hematologic recovery

4.3.1 Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is an established therapeutic option for treating several malignant and nonmalignant hematologic diseases. An HLA-matched sibling donor is considered the first option for allo-HCT and is associated with the best post-transplant outcomes[1]. However, depending on the patient's age and ethnicity, a matched related sibling is available for 13% to 51% of patients needing an allo-HCT[2]. In this sense, a matched unrelated donor (MUD) or mismatched unrelated donor (MMUD) may be acceptable donor options for patients lacking an HLA-matched related donor[2]. Despite the continuous improvement of the MUD or MMUD outcomes throughout the years, graft failure (GF) is still a significant hurdle associated with poor prognosis after allo-HCT[3–5]. To date, several risk factors for GF have been described, including donor/recipient HLA mismatch, use of alternative donors, T-cell depletion of the graft, diagnosis of nonmalignant disorders, use of reduced-intensity conditioning regimen, use of bone marrow or cord blood as the stem cell source, low number of infused cells, and the presence of donor-specific HLA antibodies (DSA)[6,7].

The role of preexisting DSAs in GF occurrence has already been reported in the unrelated donor setting. Two earlier reports have shown that DSAs were significantly associated with higher GF incidence after MUD and MMUD transplantation[8,9]. Thus, the current National Marrow Donor Program (NMDP) guideline for unrelated donor selection includes the presence of DSAs as a relevant selection parameter[10]. Nevertheless, recent reports have failed to confirm the association between DSAs and higher risk of GF after unrelated donor transplants[11–13]. Notably, some relevant features for DSA risk assessment[14], such as C1q-binding, DSA titer, donor-specific epitope patterns, and false-positive DSA reactivity[15], have not been thoroughly assessed in most previous studies regarding DSA in the MUD/MMUD context.

Therefore, due to the conflicting evidence regarding the impact of DSA following MUD or MMUD transplantation, we sought to perform in-depth DSA characterization and retrospectively investigate the clinical impact of DSAs on hematologic recovery and GF in a cohort of 303 patients who underwent unrelated donor transplantation at our center.

4.3.2 Methods

4.3.2.1 Patient data

This study included 303 consecutive patients who lacked an HLA-matched related sibling and received their first MUD or MMUD allo-HCT at our center from January 2008 to December 2017. Our institutional review board approved this retrospective study (nº. 96085718.6.0000.0096) which was conducted following the Declaration of Helsinki.

4.3.2.2 Preparatory regimen and graft-versus-host disease prophylaxis

Preparatory regimens were classified into myeloablative and reduced-intensity regimens according to Bacigalupo et al[16]. The conditioning regimen and graft-versus-host disease (GVHD) prophylaxis were selected at the physician's discretion and varied according to the underlying disease, graft source, and degree of HLA matching.

4.3.2.3 HLA typing, HLA antibody testing, and DSA characterization

High-resolution HLA typing and the HLA antibody characterization strategy were performed as previously described[15]. Briefly, HLA antibody identification testing was performed with LABScreen Single Antigen Beads (SAB) kits (One Lambda Inc., West Hills, California). Mean fluorescence intensity (MFI)>1000 was the cutoff for positivity, and MFI values between 1000 and 500 were considered potentially positive based on HLA epitope patterns[17]. To avoid the complement inhibition effect, we used EDTA treatment or heat inactivation of the sera[18]. Epitope analyses were carried out using expanded SAB panels (LABScreen ExPlex, One Lambda Inc.) with the HLA-Fusion MatchMaker software (Version 4.4). Next, we used the LIFECODES SAB panels (Immucor, Norcross, GA) to confirm true-positive DSA reactivities[19]. Additionally, DSA-positive patients with divergent LABScreen and LIFECODES results or suspected false-positive patterns in the epitope analysis were tested with absorption/elution protocol, using surrogate donor cells expressing the DSA's target[20]. DSA-positive patients were also retrospectively evaluated using the LABScreen SAB assay with 3 different dilutions (1:2, 1:8, and 1:32) to estimate the

DSA titer. The C1q-binding assay (C1qScreen, One Lambda Inc.) was also retrospectively performed, with MFI >500 as the cutoff. All laboratory tests were carried out according to the manufacturer's instructions.

4.3.2.4 Endpoints and Definitions

The primary endpoints included neutrophil and platelet recovery and GF[21]. Overall survival was the secondary endpoint. All time to events were calculated from the date of graft infusion. *Neutrophil recovery* was defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) $\geq .5\times10^9$/L. *Platelet recovery* was defined as the first of 3 consecutive days at $\geq 20 \times 10^9$/L and at least 7 days without platelet transfusion support. The GF endpoint included primary and secondary graft rejection and poor graft function as a single outcome. *Primary graft rejection* was defined as the failure to achieve an ANC of $.5\times10^9$/L by day +28 and the absence of donor-derived hematopoiesis (<5% donor cells) using short tandem repeat chimerism analysis. *Secondary graft rejection* was defined as achieving an ANC of $.5\times10^9$/L with subsequent loss of donor chimerism (<5% donor cells). *Poor graft function* was defined as the presence of 2 or 3 cytopenic counts (ANC $\leq .5\times10^9$/L, platelets $\leq 20\times10^9$/L, or hemoglobin ≤70g/L) for at least 3 consecutive days after transplantation with a transfusion requirement associated with hypoplastic bone marrow in the presence of complete donor chimerism (>95% donor cells). Overall survival (OS) was defined as the time from transplantation to death from any cause, and surviving patients were censored at the last follow-up[22].

4.3.2.5 Statistical analysis

Baseline characteristics of the DSA groups were compared with Fisher's exact test and Mann–Whitney U test for categorical and continuous covariates, respectively. The MFI levels among DSA-positive patients with and without GF were compared using the Mann–Whitney U test. In this analysis, the DSAs with the highest MFI values were considered in patients with 2 or more DSAs. The median follow-up was calculated using the reverse Kaplan–Meier method. The unadjusted estimates of neutrophil and platelet recovery and GF were calculated using cumulative incidence curves to accommodate competing risks and compared among groups with Gray's

test. Death or relapse before neutrophil and platelet recovery or GF were the competing events. The probability of OS was estimated with the Kaplan–Meier method and compared among groups by the log-rank test. In our analyses, continuous covariates were transformed into categorical covariates by treating the median value as a threshold. Fine-Gray regression models were used to identify the risk factors in the multivariable analyses for neutrophil recovery, platelet recovery, and GF. The Cox proportional hazards regression was used for OS. The prognostic effect of GF on OS was assessed through a Cox regression model, using GF as a time-dependent covariate. The DSA covariate was included in all models as the main effect, regardless of the significance level in unadjusted analyses. Other covariates that reached a *P* value ≤.10 in the unadjusted analyses were included in the initial multivariable models and were eliminated one by one using a stepwise selection method. The final adjusted models held only covariates with a *P* value ≤.05. Regression results were expressed as subdistribution hazard ratio (SHR) or hazard ratio (HR) with a 95% confidence interval (95% CI). All tests were two-sided, and *P* values ≤.05 were considered statistically significant[22]. All statistical analyses were performed using EZR version 1.54 (Saitama Medical Center, Jichi Medical University, Saitama, Japan)[23].

4.3.3 Results

4.3.3.1 Patients and transplant characteristics

Patient and transplant characteristics of the entire cohort and by DSA groups are summarized in Table 1. The median patient age was 14 years (range, 0–61 years), and 56.1% were male. The most common indications for allo-HCT were acute leukemia (35.6%), inherited bone marrow failure syndromes (22.8%), and acquired severe aplastic anemia (19.8%). Regarding HLA matching, 201 (66.3%) patients received a 10/10 MUD transplantation, whereas 102 (33.7%) underwent a 9/10 MMUD transplant. The conditioning regimen was myeloablative in 73.3% of cases. Most patients received GVHD prophylaxis with cyclosporine and methotrexate (90.1%), and bone marrow was the predominant graft source (88.1%). The median infused total nucleated cell dose (TNC), and CD34+ cell doses were 4.94×10^{8}/kg (range, 1.24–20.2×10^{8}/kg) and 2.76×10^{6}/kg (range, 0.29–19.8×10^{6}/kg), respectively.

Baseline characteristics were similar in patients with and without DSAs, except by patient age (Table 1).

4.3.3.2 DSA Assessment

HLA antibodies were detected in 117 patients (38.6%). Among them, 29 (9.6%) had antibodies against class I HLA antigens, 44 (14.5%) against class II HLA antigens, and 44 (14.5%) against both HLA classes. The presence of HLA antibodies was not correlated with patient age (P = .099), gender (P = .91), or disease type (P = .25).

Overall, 11 patients (3.63%) were DSA-positive (Table 2). Of them, 10 had preexisting DSAs, and one showed post-transplant DSA rebound. Most DSAs were against HLA-DP and mainly directed to public epitopes. Two patients showed divergent results in LABScreen SAB (DQB1*06:03, 3322 MFI; DQA1*05:01P/DQB1*03:01, 2914 MFI) and LIFECODES SAB (DQB1*06:03, 227 MFI; DQA1*05:01/DQB1*03:01, 272 MFI) assays (Table 2). Although the DSAs directed to DQB1*06:03 and DQA1*05:01/DQB1*03:01 were not justified by verified epitopes in the HLA pattern analysis and had already been reported in males without sensitizing events, these DSAs were confirmed as true-positive reactions in the absorption/elution protocol with surrogate donor cells (Supplementary Figure S1) and, thus, were included in the DSA-positive group. Nine patients had 1 DSA, 1 had 2 DSAs, and 1 had 3 DSAs, with a median MFI of 4334 (range, 588–20,456) and 3581 (range, 227–12,266) in LABScreen and LIFECODES SAB assays, respectively. Additionally, the median DSA MFI values at 1:2, 1:8, and 1:32 dilutions were 2397 (range, 380–20,874), 979 (range, 60–21,294), and 188 (range, 0–18,332), respectively. We observed that 3 DSAs were C1q-positive. Regarding desensitization therapy, 4 DSA-positive patients were treated with 3 sessions of plasmapheresis, performed on days -15, -12, and -9, and 1 single dose of rituximab (375 mg/m^2) on day -8. In contrast, 7 DSA-positive patients were untreated.

4.3.3.3 Neutrophil and Platelet Recovery

In the whole cohort, the cumulative incidence of neutrophil recovery at day +28 was 89.1% (95% CI, 85.0%–92.1%), with a median time of 21 days to neutrophil

recovery. The cumulative incidence of platelet recovery at days +28 and +60 were 69.6% (95% CI, 64.1%–74.5%) and 90.4% (95% CI, 86.2%–93.3%), respectively. The median time for platelet recovery was 24 days. In unadjusted analysis, the cumulative incidence of neutrophil recovery at day +28 was lower in patients with DSA (72.7%; 95% CI, 32.6%–91.3%; median, 26 days) than in those without DSAs (89.7%; 95% CI, 85.6%–92.7%; median, 21 days) (*P* = .072). The unadjusted cumulative incidence of platelet recovery at +28 days was 36.4% (95% CI, 10.2%–63.9%) and 70,9% (95% CI, 65.3%–75.8%) in the DSA-positive and the DSA-negative groups, respectively (*P* = .095). The median time to platelet recovery was delayed in the DSA-positive group compared with the DSA-negative group (32 days vs. 24 days), albeit not statistically significant (*P* = .095). The unadjusted cumulative incidences of neutrophil and platelet recovery of other covariates are described in Supplemental Table S1.

In the adjusted multivariable regression, DSA-positive patients had significantly lower and delayed neutrophil (SHR = 0.48; 95% CI, 0.29–0.81; *P* = .006) and platelet recovery (SHR = 0.51; 95% CI, 0.35–0.74; *P* = .0003) than patients without DSAs (Table 3; Figure 1a and 1b). Other independent predictors of lower neutrophil recovery included TNC < 4.94×10^8/kg (SHR = 0.71; *P* =.003), allo-HCT before 2013 (SHR = 0.58; *P* <.0001), and sex mismatch (male recipient of female donor [SHR = 0.58; *P* = .0006]; female recipients of female donor [SHR = 0.67; *P* = .016]). On the other hand, $CD34^+$ cell dose <2.76×10^6/kg (SHR = 0.68; *P* =.0012), allo-HCT before 2013 (SHR = 0.62; *P* <.0001), and patient age ⩾ 14 years (SHR = 0.62; *P* <.0001) were independently associated with poorer platelet recovery in the multivariable regression.

4.3.3.4 Graft Failure

In total, 21 patients experienced GF following unrelated donor allo-HCT. Of them, 12 had primary graft rejection, 8 had secondary graft rejection, and 1 had primary poor graft function. Among the cases of secondary graft rejection, the median time to the graft loss was 61 days (range 38–126). Thus, the cumulative incidences of GF at 100 days and 1 year were 6.6% (95% CI, 4.2%–9.8%) and 6.9% (95% CI, 4.4%–10.2%), respectively. The unadjusted cumulative incidence of GF in DSA-positive patients (36.4%; 95%CI, 10.2%–63.9%) was significantly higher than

that in DSA-negative patients (5.5%; 95%CI, 3.3%–8.5%) (*P* < .0001). Supplemental Table S2 includes the univariate cumulative incidence of GF for the other covariates.

Using the LABScreen SAB assay, we observed that DSA-positive patients with GF had higher median MFI values than DSA-positive patients with sustained engraftment, both in neat serum (13,751 vs. 3322; *P* = .073) and with 3 different dilutions (1:2 dilution, 11,527 vs. 2254, *P* = .073; 1:8 dilution, 5438 vs. 502, *P* = .012; 1:32 dilution, 1627 vs. 61; *P* = .006) (Figure 2). Similarly, with the LIFECODES SAB assay, the median MFI among the DSA-positive patients who experienced GF was 10,334 versus 1250 in the DSA-positive patients who achieved engraftment (*P* = .006) (Figure 2). All 3 patients with C1q-positive DSAs failed to engraft. Regarding desensitization, only 4 patients were treated with 3 sessions of plasmapheresis and rituximab. Since post-desensitization samples were unavailable, the MFI values after the procedure were not evaluated. Two patients with C1q-positive, high-titer DSAs were desensitized but ultimately experienced GF. In contrast, 2 patients with C1q-negative, moderate-level DSAs were treated, and both achieved engraftment (Table 2). Moreover, 18 of the 21 patients with graft failure underwent a second HCT with different donors.

Six of 11 DSA-positive patients were assessed in post-HCT samples at days +28 or +31 (Table 2). Post-transplant median MFI was 993 (range, 226-13,825). DSA patients with GF had post-HCT MFI levels above the cutoff (MFI of 13,825, 9263, and 1667), while DSA-positive patients who achieved engraftment showed post-transplant reactivity below the established threshold (MFI of 319, 306, and 226). Interestingly, one patient without DSA against DPB1*04:01 in the pre-transplant samples (57 MFI at day -36; 56 MFI at day -13) showed a DSA rebound (2922 MFI) at day +21 after a 10/10 unrelated donor allo-HCT (Table 2). The post-transplant DSA reactivity was confirmed with the LIFECODES SAB assay (8956 MFI) and justified by the 84GGPM epitope in the Explex panel. This post-transplant DSA also showed positivity (2363 MFI) in the C1q-binding assay. Further, the anti-DPB1*04:01 DSA was detected (9263 MFI) at day +31 when the chimerism analysis showed 0% of donor cells. To investigate whether the DSA rebound was associated with a primary or memory humoral response, we tested the day +21 sample with an anti-IgM secondary antibody. We observed no DSA reactivity to 84GGPM epitope (<50 MFI) using the anti-IgM secondary antibody. Thus, these findings suggest a case of

cryptic sensitization[24] and a recall DSA response following the HLA-DPB1-mismatched transplant.

The multivariable competing risks regression demonstrated that the presence of DSAs was strongly associated with a higher incidence of GF (SHR = 7.60; 95%CI, 2.61–22.14; *P* = .0002) (Table 3; Figure 1c). The only independent predictor related to an increased risk of GF was $CD34^+$ cell dose $<2.76\times10^6$/kg (SHR = 2.65; *P* = .037). Next, we fit an alternative competing risk model for GF, including DSA and forcing HLA mismatch, conditioning regimen, graft source, and disease type in the model. Of note, DSA retained the association of increased GF (SHR = 5.73; 95%CI, 1.48–22.3; *P* = .012), while the other covariates in the alternative model were not predictive of failed engraftment (data not shown).

4.3.3.5 Overall Survival

In total, 96 of 303 patients died. Causes of death were infection (n = 27), relapse (n = 23), rejection (n = 14), GVHD (n = 12), organ failure (n = 5), and other causes (n = 13). Using the reverse Kaplan–Meier method, the median follow-up time was 2 years (95% CI, 1.7–2.3). Considering the whole cohort, the probability of OS at 1 year was 72.8% (95% CI, 67.2%–77.6%). Moreover, the 1-year unadjusted OS probabilities in the DSA-positive and DSA-negative groups were 72.7% (95% CI, 37.1%–90.3%) and 72.8% (95% CI, 67.1%–77.6%), respectively (*P* = .51). The unadjusted survival probabilities of the other variables are provided in Supplemental Table S3.

In multivariable Cox analysis, the presence of DSA was not predictive of inferior survival compared to the DSA-negative group (HR = 0.50; 95% CI, 0.20–1.26, *P* = .14). Factors independently associated with an increased risk of overall mortality were patient age ⩾ 14 years (HR = 3.2; 95% CI, 2.03-5.04; *P* < .0001), HLA mismatch (HR = 1.83; 95% CI, 1.22–2.74; *P* = 0.004), and GF as a time-dependent covariate (HR = 8.70; 95% CI, 5.55–13.63; *P* < .00001).

4.3.4 Discussion

The present study demonstrated that high-titer DSAs were significantly associated with increased risks of GF after MUD and MMUD transplantation. This

finding supports earlier studies describing the role of DSAs in GF occurrence after HLA-mismatched unrelated donor transplantation[8,9]. We also observed that patients with low-level DSAs achieved engraftment, albeit with delayed neutrophil and platelet recovery. Interestingly, the impact of weak DSAs on delayed hematologic recovery had not been detailed in previous studies investigating the influence of DSAs following MUD or MMUD transplants[8,9,11–13]. Therefore, our results underscore the importance of pre-transplant DSA assessment and validate the inclusion of DSA as a relevant unrelated donor selection parameter per the current NMDP guidelines[10].

It is well established that preformed DSAs are significant predictors of GF following allo-HCT. Indeed, a recent meta-analysis with 2436 recipients, mostly of haploidentical donors or cord blood units, showed that patients with DSAs had a 7.47-fold increased risk of primary GF compared with patients without DSAs[7]. However, the role of DSA in engraftment failure after MUD or MMUD transplantation is more controversial. An earlier NMDP case-control study demonstrated that 9 of 37 (24%) GF cases had DSAs against HLA-A, -B, and -DPB1 compared with only 1 of 78 (1%) matched controls ($P < .001$)[8]. Later, Ciurea et al. validated this initial finding in a cohort of 592 MUD transplants. The MD Anderson group showed that the GF incidence was significantly higher in DSA-positive patients than in DSA-negative patients (37.5% versus 2.7%; $P = .0014$)[9]. These prior findings align with this study, which showed that DSAs were strongly associated with GF occurrence in the competing risks regression. Nevertheless, some recent reports have failed to corroborate the association between preexisting DSAs and an increased risk of GF. In a French multicenter study with 2716 patients and 103 cases of GF, 61 pre-transplant sera were available, and DSA was identified in only five (8.2%) patients with failed engraftment[11]. Similarly, a UK's unrelated donor registry case-control study, including 538 patients with malignancies, demonstrated that DSAs were detected in 1 of 35 GF cases (3%) and 12 of 502 engrafted controls (2%)[13]. Further, Woolfrey et al. described that moderate-level DSAs and C1q-positive antibodies were not associated with GF after unrelated donor transplantation for nonmalignant diseases[12]. Of note, these studies used distinct cutoff values for DSA definition, ranging from 500 to 2000 MFI. Additionally, other important DSA characteristics were scarcely assessed in these previous reports, with only one including C1q-binding testing, one describing detailed epitope reactivity patterns, none evaluating MFI levels in different DSA titers or using a second SAB kit[14]. Therefore, more studies

with comprehensive DSA characterization are needed to clarify the conflicting data regarding DSAs and GF after unrelated donor allo-HCT.

The MFI cutoff threshold that separates permissible DSAs from prohibitive ones has not yet been established in allo-HCT[25]. In our cohort, all DSA-positive patients who achieved engraftment had DSA levels below 5000 MFI in LABScreen and LIFECODES SAB assays. This finding is consistent with Ciurea et al., who showed that DSAs with MFI <5000 were associated with improved engraftment rates after haploidentical allo-HCT with post-transplantation cyclophosphamide (PTCy)[26]. Nevertheless, data on a DSA MFI threshold that predicts GF in the unrelated donor setting are debatable. Ciurea et al. showed that DSAs with low-to-moderate MFI were associated with GF in 10/10 MUD transplantation[9]. Alternatively, Cuadrado et al. reported that DSA patients with MFI values below 8000 achieved sustained engraftment[13]. Remarkably, these previous studies have not reported SAB testing with serial dilutions to estimate DSA titers and their effect on GF incidence. While DSA titers have been extensively discussed in the kidney transplant context[27,28], this approach has been poorly described in allo-HCT literature[15]. This study showed that DSA patients experiencing GF had a higher median MFI, not only in neat sera but also in 1:2, 1:8, and 1:32 dilutions, than the median MFI of DSA patients with proper engraftment. Importantly, our center performs DSA testing with 1:8 and 1:32 dilutions in selected cases[25], including highly sensitized patients with strong DSAs against all available unrelated donors. In our experience, adding these 2 additional dilutions improve virtual crossmatch assessment and DSA risk stratification in the unrelated donor selection. One limitation of this approach is the increased costs associated with testing additional dilutions.

In our cohort, we observed that all 3 patients with C1q-positive DSAs failed to engraft. In the haploidentical donor setting with PTCy, Ciurea and colleagues demonstrated that C1q-positive DSAs were strongly associated with GF and that C1q-negativity should be the desensitization treatment goal[26,29]. In contrast, Woolfrey et al. failed to describe C1q-positive DSAs as predictors of GF in patients with nonmalignant diseases undergoing unrelated donor transplantation[12]. Additionally, our study showed that patients with C1q-positive DSAs had reactivity in 1:8 or 1:32 dilutions in the standard SAB assay. This finding is consistent with previous studies highlighting that C1q-positivity was highly correlated with high MFI values in EDTA-treated sera and with 1:16/1:32 titers[27]. While some authors argued that DSA titer

evaluation provides more granular information on antigen-antibody dissociation rates, HLA epitope patterns, and DSA strength than C1q-testing[27,28], other groups claim that C1q-assay is time-effective and offers more clear-cut information than DSA titer assessment[29]. In this sense, more studies are warranted to validate C1q-assay and DSA titers as predictors of GF following unrelated donor transplantation.

Although Luminex SAB assay is considered the gold standard for HLA antibody evaluation in allo-HCT, as recently outlined in the European Society for Blood and Marrow Transplantation (EBMT) consensus paper[30], this assay still presents limitations that may compromise DSA characterization[31]. Hence, providing accurate DSA assignments and ruling out spurious SAB reactivity may be challenging[19,20,32,33]. In this cohort, we observed two DSA-positive patients with divergent results in two different SAB assays[32]. However, the absorption/elution results ruled out spurious reactivity, thereby confirming the DSA specificity to native HLA antigens. In this sense, performing cell-based assays may elucidate these SAB inconsistencies[20]. Recently, Liwski and colleagues reported an optimized absorption/elution protocol for routine use in clinical HLA laboratories[33]. Indeed, this optimized method was designed to distinguish weak HLA antibodies against native antigens from spurious reactivity to cryptic epitopes on denatured HLA molecules. Furthermore, it is paramount to rule out spurious SAB reactivity in the unrelated donor setting. Reporting a false-positive DSA, especially one with high MFI values, may jeopardize the virtual crossmatch accuracy and ultimately affect the HLA-mismatched donor selection by excluding unrelated donors with favorable characteristics[2,10].

When only DSA-positive unrelated donors are available, desensitization treatment is recommended to facilitate donor engraftment, as recently postulated by the EBMT consensus guidelines on DSAs[30]. At present, there is little evidence concerning desensitization therapy in patients with DSA undergoing unrelated donor HCT[34]. Most studies on desensitization have been performed in the haploidentical donor context, either with PTCy[29,35] or Beijing platforms[36]. Even though the optimal strategy for DSA desensitization in allo-HCT remains unclear, current data indicate that MD Anderson and Johns Hopkins desensitization protocols successfully mitigate low-to-moderate-level DSAs[29,37,38]. In contrast, these desensitization strategies remain ineffective for patients undergoing allo-HCT with strong DSAs (>20,000 MFI) or persistent C1q-positive-DSAs, which are associated with dismal transplant

outcomes[29]. In our cohort, 2 patients with C1q-positive, high-titer DSAs failed to engraft despite the desensitization treatment with 3 sessions of plasmapheresis and one dose of rituximab. Conversely, 2 patients with moderate-level DSAs were treated with the same protocol, and both achieved sustained engraftment. Moreover, it has been shown that several new desensitization drugs, including imlifidase[39], carfilzomib[40], tocilizumab[41], and daratumumab[42], effectively reduce DSA levels in highly sensitized patients undergoing solid organ transplants. Thus, further studies are required to verify whether these drugs would eventually improve the current desensitization strategies used in allosensitized patients receiving allo-HCT.

The post-transplant DSA MFI levels predictive of engraftment failure after unrelated donor transplantation are currently unknown. The present study observed that DSA patients who failed to engraft had post-transplant MFI levels above our cutoff at days +28 or +31 post-HCT. In turn, those patients with DSA who achieved engraftment showed no DSA reactivity (<500 MFI) one month after transplant. Importantly, our current post-transplant DSA monitoring protocol includes SAB testing at days +2, +5, +14, and +28 for all DSA-positive patients undergoing alternative donor transplantation in our center[38]. This close DSA monitoring is aimed at checking any early DSA rebound after the graft infusion and providing prompt desensitization intervention in case of substantial post-HCT DSA rebound. Of note, one caveat of this DSA monitoring strategy is that it is costly and may not be feasible in countries with limited resources.

Another interesting finding of our study is that one patient without preexisting DSA in pre-HCT samples showed a DSA rebound at day +21 post-HCT and ultimately failed to engraft. As the post-transplant DSA showed an IgG-positive/IgM-negative pattern in the SAB assay, we speculated that this patient could present a cryptic HLA sensitization and that the observed rebound was likely associated with a memory DSA response[14,24]. Presently, evidence on DSA rebound in patients without preformed DSAs in MUD or MMUD transplantation is scarce. To our knowledge, only 2 case reports have described post-transplant DSA detection in patients without circulating donor-directed antibodies in pre-HCT samples. Hefazi et al. described a patient with failed engraftment after a 10/10 MUD transplant and without DSAs on serum specimens at days −5, −1, +6, +13, and +20 who experienced a late DSA rebound directed to HLA-DP (13,572 MFI) on day +161[43]. Likewise, Yabe et al. reported a pediatric patient with negative DSA reactivity at day -1 from an HLA-DQ

MMUD transplant who showed a substantial anti-DQA1/DQB1 DSA rebound (11,079 MFI) on day +41 and eventually rejected the unrelated donor graft[44]. Additionally, the issue of cryptic HLA sensitization has been previously reported in solid organ transplants[45]. Available assays to detect alloreactive memory B cells include tetramer staining, culture supernatant analysis, and IgG ELISpot assay, and have been applied primarily in the research context[45,46]. To date, assays to detect memory B cells have not yet been evaluated in the allo-HCT setting. Notably, such assays could be particularly interesting for DSA-negative patients with a history of known sensitized events, including multiparous women or heavily transfused patients. Hence, the clinical utility of alloreactive memory B cell assays to optimize immunological risk stratification of patients eligible for allo-HCT merits further investigations in future studies.

We acknowledge that our study has several limitations. First, it is a single-center, retrospective observational study with heterogeneous malignant and nonmalignant diseases. Second, the impact of DSA was not separately analyzed in adult and pediatric cohorts owing to the small number of DSA-positive cases. Third, data on post-desensitization DSA levels were unavailable, thus precluding a detailed assessment of the desensitization efficacy and its impact on GF. Despite these limitations, our findings shed light on the impact of DSAs in hematologic recovery and graft failure following unrelated donor allo-HCT.

In conclusion, our results corroborate some earlier studies and validate the presence of DSAs as a significant risk factor for graft failure and poor hematologic recovery after unrelated donor allo-HCT. Hence, carefully assessing pre-transplant DSA status may optimize unrelated donor selection and improve transplant outcomes.

4.3.5 Acknowledgments

The authors thank all physicians and HLA laboratory staff who collaborated with this study. We gratefully acknowledge Margareth Kleina Feitosa and Luciane Rocha Ernlund Pangracio for the unrelated donor searches throughout the study period. We also thank the data manager Heliz Regina Neves. We are grateful for the support provided by Associação dos Amigos do Hospital de Clínicas (AAHC) for this project.

4.3.6 Author contributions

ACML, NFP, CB, and RP designed the study. ACML and RRP performed the statistical analysis. ACML, JG, RM, and MGL performed and interpreted HLA antibody testing. ACML and CB interpreted the data and wrote the manuscript. All other authors contributed to data collection and patient care. All authors critically reviewed and approved the final version of the manuscript.

4.3.7 Conflict of interest

There are no conflicts of interest to report.

4.3.8 References

1. Shouval R, Fein JA, Labopin M, et al. Outcomes of allogeneic haematopoietic stem cell transplantation from HLA-matched and alternative donors: a European Society for Blood and Marrow Transplantation registry retrospective analysis. *Lancet Haematol*. 2019;6(11):e573-e584. doi:10.1016/S2352-3026(19)30158-9

2. Spellman SR. Hematology 2022—what is complete HLA match in 2022? *Hematology*. 2022;2022(1):83-89. doi:10.1182/hematology.2022000326

3. Olsson R, Remberger M, Schaffer M, et al. Graft failure in the modern era of allogeneic hematopoietic SCT. *Bone Marrow Transplant*. 2013;48(4):537-543. doi:10.1038/bmt.2012.239

4. Olsson RF, Logan BR, Chaudhury S, et al. Primary graft failure after myeloablative allogeneic hematopoietic cell transplantation for hematologic malignancies. *Leukemia*. 2015;29(8):1754-1762. doi:10.1038/leu.2015.75

5. Schriber J, Agovi MA, Ho V, et al. Second Unrelated Donor Hematopoietic Cell Transplantation for Primary Graft Failure. *Biol Blood Marrow Transplant*. 2010;16(8):1099-1106. doi:10.1016/j.bbmt.2010.02.013

6. Ozdemir ZN, Civriz Bozdağ S. Graft failure after allogeneic hematopoietic stem cell transplantation. *Transfus Apher Sci*. 2018;57(2):163-167. doi:10.1016/j.transci.2018.04.014

7. Xie Y, Parekh J, Tang Z, Wu D, Wu X. Donor-Specific Antibodies and Primary Graft Failure in Allogeneic Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. *Transplant Cell Ther*. 2021;27(8):687.e1-687.e7. doi:10.1016/j.jtct.2021.04.030

8. Spellman S, Bray R, Rosen-Bronson S, et al. The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure. *Blood*. 2010;115(13):2704-2708. doi:10.1182/blood-2009-09-244525

9. Ciurea SO, Thall PF, Wang X, et al. Donor-specific anti-HLA Abs and graft failure in matched unrelated donor hematopoietic stem cell transplantation. *Blood*. 2011;118(22):5957-5964. doi:10.1182/blood-2011-06-362111

10. Dehn J, Spellman S, Hurley CK, et al. Selection of unrelated donors and cord blood units for hematopoietic cell transplantation: guidelines from the NMDP/CIBMTR. *Blood*. 2019;134(12):924-934. doi:10.1182/blood.2019001212

11. Cluzeau T, Lambert J, Raus N, et al. Risk factors and outcome of graft failure after HLA matched and mismatched unrelated donor hematopoietic stem cell transplantation: a study on behalf of SFGM-TC and SFHI. *Bone Marrow Transplant*. 2016;51(5):687-691. doi:10.1038/bmt.2015.351

12. Woolfrey A, Wang T, Lee SJ, et al. Donor-specific anti-HLA antibodies in unrelated hematopoietic cell transplantation for non-malignant disorders. *Bone Marrow Transplant*. 2019;54(3):494-496. doi:10.1038/s41409-018-0334-y

13. Cuadrado M, Anthias C, Fernando R, et al. Impact of Donor-Directed HLA Allo-Antibodies on Primary Graft Failure After Unrelated Donor Allogeneic Haematopoietic Cell Transplantation. *Bone Marrow Transplant*. 2020;55(Suppl 1):133-134. doi:10.1038/s41409-020-01119-3

14. Tambur AR, Campbell P, Claas FH, et al. Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group Meeting Report. *Am J Transplant*. 2018;18(7):1604-1614. doi:10.1111/ajt.14752

15. Lima ACM, Bonfim C, Getz J, et al. Untreated Donor-Specific HLA Antibodies Are Associated With Graft Failure and Poor Survival After Haploidentical Transplantation With Post-Transplantation Cyclophosphamide in Pediatric Patients With Nonmalignant Disorders. *Transplant Cell Ther*. 2022;28(10):698.e1-698.e11. doi:10.1016/j.jtct.2022.07.019

16. Bacigalupo A, Ballen K, Rizzo D, et al. Defining the Intensity of Conditioning Regimens: Working Definitions. *Biol Blood Marrow Transplant*. 2009;15(12):1628-1633. doi:10.1016/j.bbmt.2009.07.004

17. Bettinotti MP, Zachary AA, Leffell MS. Clinically relevant interpretation of solid phase assays for HLA antibody. *Curr Opin Organ Transplant*. 2016;21(4):453-458. doi:10.1097/MOT.0000000000000326

18. Zhang X, Reinsmoen NL. Comprehensive assessment for serum treatment for single antigen test for detection of HLA antibodies. *Hum Immunol*. 2017;78(11-12):699-703. doi:10.1016/j.humimm.2017.09.001

19. Ravindranath MH, Filippone EJ, Amato-Menker CJ, et al. Antibodies to cryptic epitopes on HLA class I and class II heavy chains bound to single antigen beads: Clinically relevant? *Transpl Immunol*. 2021;69:101482. doi:10.1016/j.trim.2021.101482

20. Grenzi PC, de Marco R, Silva RZR, Campos ÉF, Gerbase-DeLima M. Antibodies against denatured HLA class II molecules detected in luminex-single antigen assay. *Hum Immunol*. 2013;74(10):1300-1303. doi:10.1016/j.humimm.2013.06.035

21. Kharfan-Dabaja MA, Kumar A, Ayala E, et al. Standardizing Definitions of Hematopoietic Recovery, Graft Rejection, Graft Failure, Poor Graft Function, and Donor Chimerism in Allogeneic Hematopoietic Cell Transplantation: A Report on Behalf of the American Society for Transplantation and Cellular Therapy. *Transplant Cell Ther*. 2021;27(8):642-649. doi:10.1016/j.jtct.2021.04.007

22. Kanate AS, Nagler A, Savani B. Summary of Scientific and Statistical Methods, Study Endpoints and Definitions for Observational and Registry-Based Studies in Hematopoietic Cell Transplantation: *Clin Hematol Int*. 2019;2(1):2. doi:10.2991/chi.d.191207.001

23. Kanda Y. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. *Bone Marrow Transplant*. 2013;48(3):452-458. doi:10.1038/bmt.2012.244

24. Zachary AA, Lucas DP, Montgomery RA, Leffell MS. Rituximab Prevents an Anamnestic Response in Patients With Cryptic Sensitization to HLA. *Transplantation*. 2013;95(5):701-704. doi:10.1097/TP.0b013e31827be3c1

25. Bettinotti MP. Evolution of HLA testing for hematopoietic stem cell transplantation: Importance of the candidate’s antibody profile for donor selection. *Hum Immunol*. 2022;83(10):721-729. doi:10.1016/j.humimm.2022.04.012

26. Ciurea SO, Thall PF, Milton DR, et al. Complement-Binding Donor-Specific Anti-HLA Antibodies and Risk of Primary Graft Failure in Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant*. 2015;21(8):1392-1398. doi:10.1016/j.bbmt.2015.05.001

27. Tambur AR, Herrera ND, Haarberg KMK, et al. Assessing Antibody Strength: Comparison of MFI, C1q, and Titer Information. *Am J Transplant*. 2015;15(9):2421-2430. doi:10.1111/ajt.13295

28. Tambur AR, Wiebe C. HLA Diagnostics: Evaluating DSA Strength by Titration. *Transplantation*. 2018;102(1S):S23-S30. doi:10.1097/TP.0000000000001817
29. Ciurea SO, Al Malki MM, Kongtim P, et al. Treatment of allosensitized patients receiving allogeneic transplantation. *Blood Adv*. 2021;5(20):4031-4043. doi:10.1182/bloodadvances.2021004862

30. Ciurea SO, Cao K, Fernandez-Vina M, et al. The European Society for Blood and Marrow Transplantation (EBMT) Consensus Guidelines for the Detection and Treatment of Donor-specific Anti-HLA Antibodies (DSA) in Haploidentical

Hematopoietic Cell Transplantation. *Bone Marrow Transplant*. 2018;53(5):521-534. doi:10.1038/s41409-017-0062-8

31. Wehmeier C, Hönger G, Schaub S. Caveats of HLA antibody detection by solid-phase assays. *Transpl Int*. 2020;33(1):18-29. doi:10.1111/tri.13484

32. Sullivan HC, Krummey SM, Gebel HM, Bray RA. The utility of second single antigen bead assay: Clearing the water or stirring up mud? *Hum Immunol*. 2020;81(12):663-670. doi:10.1016/j.humimm.2020.09.002

33. Liwski RS, Tafulo S, Carroll R, Lan JH, Greenshields AL. Cutting through the weeds: Evaluation of a novel adsorption with crossmatch cells and elution protocol to sharpen HLA antibody identification by the single antigen bead assay. *Front Genet*. 2022;13. doi: 10.3389/fgene.2022.1059650

34. Spriewald BM, Bach C, Zingsem J, et al. Depletion of donor-specific anti-HLA A2 alloantibodies in a hematopoietic cell transplant recipient using directed mismatched platelet transfusions. *Bone Marrow Transplant*. 2018;53(6):791-794. doi:10.1038/s41409-018-0220-7

35. Bailén R, Vicario JL, Solán L, et al. Management of Donor-Specific Antibodies in Haploidentical Transplant: Multicenter Experience From the Madrid Group of Hematopoietic Transplant. *Front Immunol*. 2021;12:674658. doi:10.3389/fimmu.2021.674658

36. Chang YJ, Xu LP, Wang Y, et al. Rituximab for desensitization during HLA-mismatched stem cell transplantation in patients with a positive donor-specific anti-HLA antibody. *Bone Marrow Transplant*. 2020;55(7):1326-1336. doi:10.1038/s41409-020-0928-z

37. Gladstone DE, Zachary AA, Fuchs EJ, et al. Partially Mismatched Transplantation and Human Leukocyte Antigen Donor-Specific Antibodies. *Biol Blood Marrow Transplant*. 2013;19(4):647-652. doi:10.1016/j.bbmt.2013.01.016

38. Leffell MS, Jones RJ, Gladstone DE. Donor HLA-specific Abs: to BMT or not to BMT? *Bone Marrow Transplant*. 2015;50(6):751-758. doi:10.1038/bmt.2014.331

39. Jordan SC, Lorant T, Choi J, et al. IgG Endopeptidase in Highly Sensitized Patients Undergoing Transplantation. *N Engl J Med*. 2017;377(5):442-453. doi:10.1056/NEJMoa1612567

40. Tremblay S, Driscoll JJ, Rike-Shields A, et al. A prospective, iterative, adaptive trial of carfilzomib-based desensitization. *Am J Transplant*. 2020;20(2):411-421. doi:10.1111/ajt.15613

41. Cabezas L, Jouve T, Malvezzi P, et al. Tocilizumab and Active Antibody-Mediated Rejection in Kidney Transplantation: A Literature Review. *Front Immunol*. 2022;13:839380. doi:10.3389/fimmu.2022.839380

42. Joher N, Matignon M, Grimbert P. HLA Desensitization in Solid Organ Transplantation: Anti-CD38 to Across the Immunological Barriers. *Front Immunol*. 2021;12:688301. doi:10.3389/fimmu.2021.688301

43. Hefazi M, Hogan WJ, Wakefield LL, Gandhi MJ. The association of de novo anti-HLA-DPB1 donor-specific antibody formation and primary graft failure after allogeneic hematopoietic cell transplantation. *Hum Immunol*. 2018;79(12):861-864. doi:10.1016/j.humimm.2018.08.009

44. Yabe H, Morimoto T, Takakura H, et al. Post-transplantation-emerging anti-HLA DQA1/DQB1 antibody possibly responsible for graft rejection after myeloablative-unrelated marrow grafting. *Bone Marrow Transplant*. 2016;51(4):601-603. doi:10.1038/bmt.2015.292

45. Wehmeier C, Karahan GE, Heidt S. HLA-specific memory B-cell detection in kidney transplantation: Insights and future challenges. *Int J Immunogenet*. 2020;47(3):227-234. doi:10.1111/iji.12493

46. Tambur AR, Campbell P, Chong AS, et al. Sensitization in transplantation: Assessment of risk (STAR) 2019 Working Group Meeting Report. *Am J Transplant*. 2020;20(10):2652-2668. doi:10.1111/ajt.15937

TABLE 1. BASELINE CHARACTERISTICS IN THE OVERALL COHORT AND BY DSA GROUPS

Variables	Total (n = 303)	DSA negative (n = 292)	DSA positive (n = 11)	*P* value
Recipient age (years), median (range)	14 (0, 61)	13 (0, 61)	32 (2, 59)	**.024**
Recipient sex, n (%)				.222
Male	170 (56.1)	166 (56.8)	4 (36.4)	
Female	133 (43.9)	126 (43.2)	7 (63.6)	
Disease types, n (%)				.101
Acute leukemia	108 (35.6)	105 (36.0)	3 (27.3)	
Inherited bone marrow failure syndromes	69 (22.8)	68 (23.3)	1 (9.1)	
Acquired severe aplastic anemia	60 (19.8)	54 (18.5)	6 (54.5)	
Other malignant diseases	36 (11.9)	35 (12.0)	1 (9.1)	
Other nonmalignant diseases	30 (9.9)	30 (10.3)	0 (0.0)	
Diagnosis, n (%)				.547
Malignant	144 (47.5)	140 (47.9)	4 (36.4)	
Nonmalignant	159 (52.5)	152 (52.1)	7 (63.6)	
ABO matching, n (%)				1
ABO Match	127 (41.9)	122 (41.8)	5 (45.5)	
Minor ABO mismatch	75 (24.8)	73 (25.0)	2 (18.2)	
Major ABO mismatch	101 (33.3)	97 (33.2)	4 (36.4)	
Recipient/URD CMV serostatus, n (%)				.803
Recipient+/URD+	178 (58.7)	172 (58.9)	6 (54.5)	
Recipient+/URD-	88 (29.0)	83 (28.4)	5 (45.5)	
Recipient-/URD-	7 (2.3)	7 (2.4)	0 (0.0)	
Recipient-/URD+	17 (5.6)	17 (5.8)	0 (0.0)	
Missing	13 (4.3)	13 (4.5)	0 (0.0)	
Conditioning regimen, n (%)				.298
MAC	222 (73.3)	212 (72.6)	10 (90.9)	
RIC	81 (26.7)	80 (27.4)	1 (9.1)	
In vivo**T-cell depletion with rATG, n (%)**				.482
Yes	78 (25.7)	74 (25.3)	4 (36.4)	
No	225 (74.3)	218 (74.7)	7 (63.6)	
Use of TBI, n (%)				.211
Yes	119 (39.3)	117 (40.1)	2 (18.2)	
No	184 (60.7)	175 (59.9)	9 (81.8)	
GVHD prophylaxis, n (%)				.468
CSA + MTX	273 (90.1)	263 (90.1)	10 (90.9)	
CSA + CY + MMF	13 (4.3)	12 (4.1)	1 (9.1)	
Others	17 (5.6)	17 (5.8)	0 (0.0)	
URD age (years), median (range)	31 (18, 58)	32 (18, 58)	25 (22, 48)	.176
URD sex, n (%)				1
Male	186 (61.4)	179 (61.3)	7 (63.6)	
Female	117 (38.6)	113 (38.7)	4 (36.4)	
Sex mismatch, n (%)				.427
Male Recipient/Male URD	109 (36.0)	107 (36.6)	2 (18.2)	
Male Recipient/Female URD	61 (20.1)	59 (20.2)	2 (18.2)	

Female Recipient/Female URD	56 (18.5)	54 (18.5)	2 (18.2)	
Female Recipient/Male URD	77 (25.4)	72 (24.7)	5 (45.5)	
URD search, n (%)				.128
National URD	180 (59.4)	176 (60.3)	4 (36.4)	
Internacional URD	123 (40.6)	116 (39.7)	7 (63.6)	
HLA matching*, n (%)				.517
10/10	201 (66.3)	195 (66.8)	6 (54.5)	
09/10	102 (33.7)	97 (33.2)	5 (45.5)	
HLA-DPB1 TCE permissiveness, n (%)				.425
Match	50 (16.5)	49 (16.8)	1 (9.1)	
Permissive	112 (37.0)	109 (37.3)	3 (27.3)	
Nonpermissive in HvG direction	31 (10.2)	28 (9.6)	3 (27.3)	
Nonpermissive in GvH direction	47 (15.5)	45 (15.4)	2 (18.2)	
Missing	63 (20.8)	61 (20.9)	2 (18.2)	
Desensitization (only DSA+ patients), n (%)				NA
Treated DSA	-	-	4 (36.4)	
Untreated DSA	-	-	7 (63.6)	
Source, n (%)				.626
Bone marrow	267 (88.1)	258 (88.4)	9 (81.8)	
Peripheral blood	36 (11.9)	34 (11.6)	2 (18.2)	
TNC ($x10^8$/kg), median (range)	4.94 (1.24, 20.2)	4.96 (1.24, 20.2)	3.55 (2.26, 11.8)	.153
$CD34^+$ ($x10^6$/kg), median (range)	2.76 (0.29, 19.8)	2.75 (0.29, 19.8)	2.94 (0.93, 11.1)	.507
Year of HCT, median (range)	2013 (2008, 2017)	2013 (2008, 2017)	2014 (2008, 2017)	.490

*High-resolution matching at HLA-A, -B, -C, -DRB1, and -DQB1 loci.

Abbreviations: DSA, donor-specific anti-HLA antibodies; URD, unrelated donor; CMV, cytomegalovirus; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; rATG, rabbit anti-thymocyte globulin; TBI, total body irradiation; GVHD, graft-versus-host disease; CSA, cyclosporine; MTX, methotrexate; CY, cyclophosphamide; MMF, mycophenolate mofetil; TCE, T-Cell Epitope; HvG, host-versus-graft; GvH, Graft-versus-Host; TNC, total nucleated cell; HCT, hematopoietic cell transplant. Significant values are in bold.

TABLE 2. DSA CHARACTERISTICS AND URD TRANSPLANTATION OUTCOMES.

Age/ Diagnosis	Number of DSAs	DSA Specificity	DSA Epitope*	LIFECODES MFI (Neat)	LABScreen MFI (Neat)	LABScreen MFI (1:2 dilution)	LABScreen MFI (1:8 dilution)	LABScreen MFI (1:32 dilution)	C1q-binding assay	Desensitization therapy	Post-HCT MFI†	NR / PR (days)	GF	Second HCT	Survival
59/MDS	1	DPB1*01:01	84DEAV	7634	12787	9596	3707	851	Negative	No	NT	Not reached	Primary GR	Yes	Deceased
38/AML	3	DQB1*04:02	56L	13,309	18,733	18,403	16,63	19,866	Positive	Yes	13,825	Not reached	Primary PGF	Yes	Deceased
		DPB1*03:01	56ED/ 84DEAV	12,266	20,456	20,874	21,294	18,332			2706				
		DPB1*14:01	56ED/ 84DEAV	11,961	19,928	21,209	20,741	16,407			5199				
34/AML	1	DQB1*06:03	30H + 70GT	227	3322	2397	706	59	Negative	No	NT	27 / 41	Engraftment	-	Deceased
32/PNH	1	DQA1*05:01/ DQB1*03:01	α75S + β55PP	272	2914	1664	502	61	Negative	No	NT	23 / 50	Engraftment	-	Deceased
2/ALL	1	DPA1*01:03/ DPB1*04:01	α31M + β85GPM + β96R	4095	4571	2254	310	31	Negative	No	NT	26 / 26	Engraftment	-	Alive
46/SAA	2	DPB1*02:01	56EE	11,712	14,716	13,459	7170	2404	Positive	Yes	1667	21 / 26	Secondary GR (Day +38)	No	Deceased
		DPB1*06:01	96K	327	894	523	250	44			265				
32/SAA	1	A*01:01	163RG	1782	4426	3139	979	189	Negative	Yes	319	19 / 52	Engraftment	-	Alive
47/SAA	1	DPB1*04:01	56A	1250	2240	1188	273	132	Negative	No	306	25 / 32	Engraftment	-	Alive
9/SAA	1	DPB1*14:01	56ED	3581	4334	3310	1114	213	Negative	Yes	226	17 / 21	Engraftment	-	Alive
13/DBA	1	DPB1*03:01	56E	788	588	380	60	0	Negative	No	NT	26 / 26	Engraftment	-	Alive
17/SAA	1	DPB1*04:01	84GGPM	8956‡	2922‡	1950‡	1064‡	226‡	Positive	No	9263	Not reached	Primary GR	Yes	Alive

Abbreviations: MDS indicates Myelodysplastic Syndrome; AML, Acute Myeloid Leukemia; ALL, Acute Lymphoblastic Leukemia; PNH, Paroxysmal Nocturnal Hemoglobinuria; SAA, Severe Aplastic Anemia; DBA, Diamond-Blackfan Anemia; MFI, mean fluorescent intensity; NT, not tested; NR, neutrophil recovery; PR, platelet recovery; GR, graft rejection; PGF, poor graft function; HCT, hematopoietic cell transplantation.

*Presumed amino acid residues in donor mismatched HLA antigens recognized by patient's DSA.

‡IgG MFI values of a DSA rebound detected on day +21 post-BMT in a patient without pretransplant DSA (cryptic sensitization).

†DSA MFI at days +28 or +31, using LIFECODES SAB assay in neat sera.

TABLE 3. MULTIVARIABLE ANALYSES FOR HEMATOLOGIC RECOVERY AND GRAFT FAILURE.

Outcome	Adjusted SHR (95% CI)	*P* value
Neutrophil Recovery*		
DSA negative	1 (Reference)	—
DSA positive	0.48 (0.29–0.81)	.006
Platelet Recovery†		
DSA negative	1 (Reference)	—
DSA positive	0.51 (0.35–0.74)	.0003
Graft Failure‡		
DSA negative	1 (Reference)	—
DSA positive	7.60 (2.61–22.14)	.0002

*Final model adjusted for TNC (SHR=0.71; *P*=.003), year of HCT (SHR=0.58; *P*<.0001), and gender mismatch (Male recipient/Female URD: SHR=0.58; *P*=.0006; Female recipient/Female URD: SHR=0.67; *P*=.016).

†Final model adjusted for patient age (SHR=0.62; *P*<.0001), $CD34^+$ cell dose (SHR=0.68; *P*=.0012), and year of HCT (SHR=0.62; *P*<.0001).

‡Final model adjusted for $CD34^+$ cell dose (SHR=2.65; *P*=.037)

FIGURE 1. ADJUSTED CUMULATIVE INCIDENCE OF NEUTROPHIL RECOVERY (A), PLATELET RECOVERY (B), AND GRAFT FAILURE (C) BY DSA STATUS.

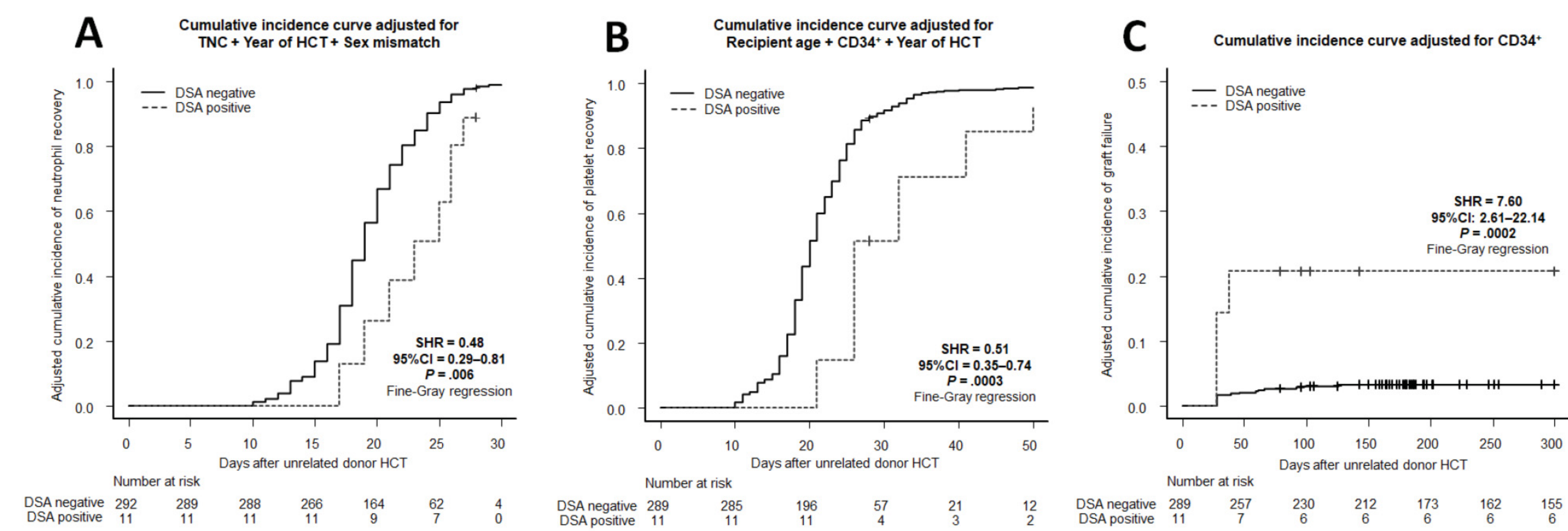
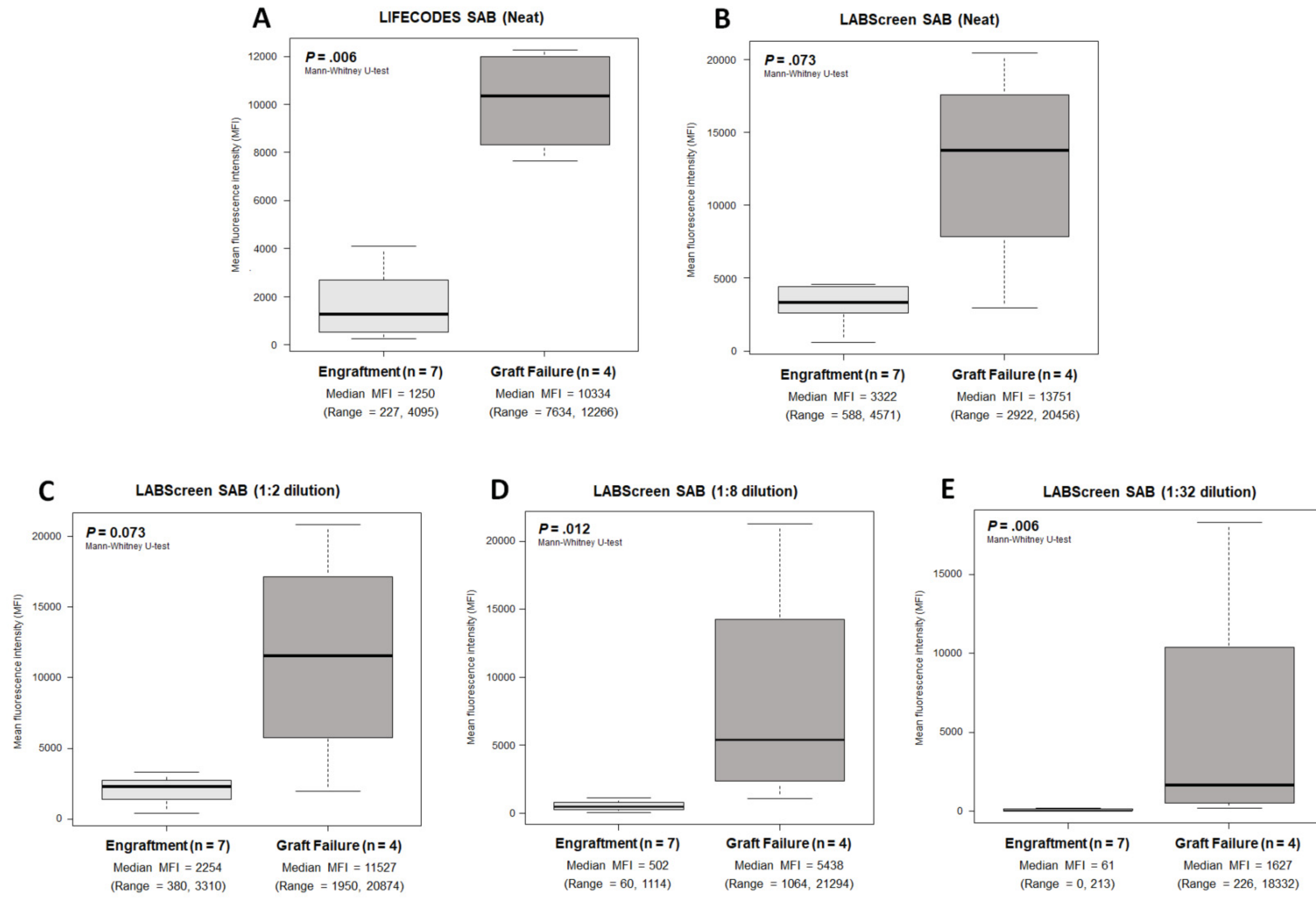


FIGURE 2. MEDIAN MFI VALUES AMONG DSA-POSITIVE PATIENTS WITH AND WITHOUT GRAFT FAILURE. LIFECODES SAB WITH NEAT SERUM (A); LABSCREEN SAB WITH NEAT SERUM (B), 1:2 DILUTION (C), 1:8 DILUTION (D), AND 1:32 DILUTION (E).



SUPPLEMENTARY TABLE 1. UNADJUSTED CUMULATIVE INCIDENCE OF NEUTROPHIL AND PLATELET RECOVERY.

Covariates	Group	n	28-day NR (95% CI)	Median time	*P* value	28-day PR (95% CI)	Median time	*P* value
ABO matching	ABO Match	127	0.929 (0.866-0.963)	20 (20-22)	.30	0.780 (0.696-0.843)	23 (21-25)	**.019**
	Minor ABO mismatch	75	0.813 (0.703-0.886)	21 (19-22)		0.667 (0.546-0.762)	24 (21-26)	
	Major ABO mismatch	101	0.901 (0.822-0.946)	22 (20-24)		0.614 (0.511-0.701)	25 (24-28)	
Recipient age (Median)	< 14 years	151	0.960 (0.911-0.982)	21 (20-22)	**.017**	0.801 (0.728-0.857)	23 (21-24)	**.00002**
	≥ 14 years	152	0.822 (0.751-0.875)	21 (20-22)		0.592 (0.509-0.666)	25 (24-28)	
Donor age (Median)	< 31 years	144	0.875 (0.808-0.920)	20 (19-22)	.725	0.674 (0.590-0.744)	25 (23-26)	.224
	≥ 31 years	159	0.906 (0.847-0.942)	21 (20-22)		0.717 (0.640-0.781)	23 (22-24)	
Recipient/URD CMV serostatus	Recipient+/URD+	178	0.882 (0.824-0.922)	21 (20-22)	.912	0.680 (0.605-0.743)	24 (22-26)	.515
	Recipient+/URD-	88	0.898 (0.809-0.946)	21 (19-23)		0.727 (0.620-0.809)	24 (22-26)	
	Recipient-/URD-	7	NA (NA-NA)	19 (17-NA)		0.857 (0.129-0.988)	21 (19-NA)	
	Recipient-/URD+	17	0.941 (0.535-0.994)	21 (18-26)		0.706 (0.411-0.873)	24 (20-33)	
	Missing	13	0.846 (0.419-0.968)	20 (19-NA)		0.615 (0.283-0.829)	24 (20-NA)	
Conditioning regimen	MAC	222	0.901 (0.853-0.934)	21 (20-22)	.588	0.694 (0.628-0.750)	24 (23-25)	.744
	RIC	81	0.864 (0.766-0.923)	21 (20-23)		0.704 (0.590-0.791)	22 (20-25)	
Use of rATG	Yes	78	0.872 (0.772-0.930)	20 (20-23)	.674	0.692 (0.575-0.783)	25 (23-27)	.554
	No	225	0.898 (0.850-0.931)	21 (20-22)		0.698 (0.633-0.753)	24 (22-25)	
Use of TBI	Yes	119	0.891 (0.818-0.936)	22 (20-24)	.069	0.664 (0.571-0.741)	25 (24-26)	.085
	No	184	0.891 (0.836-0.929)	20 (19-21)		0.717 (0.646-0.777)	22 (21-24)	
GVHD prophylaxis	CSA + MTX	273	0.890 (0.846-0.922)	21 (20-22)	.455	0.696 (0.638-0.747)	24 (23-25)	.445
	CSA + CY + MMF	13	0.923 (0.360-0.994)	16 (15-NA)		0.692 (0.345-0.881)	20 (17-77)	
	Others	17	NA (NA-NA)	21 (17-24)		0.706 (0.406-0.874)	24 (20-NA)	
Disease types	Acute Leukemia	108	0.898 (0.822-0.943)	23 (21-24)	**.0004**	0.676 (0.578-0.756)	25 (24-26)	**.015**
	Other Malignant diseases	36	0.806 (0.625-0.905)	21 (19-24)		0.667 (0.482-0.798)	24 (22-32)	
	Acquired SAA	60	0.833 (0.709-0.908)	21 (19-24)		0.650 (0.513-0.757)	24 (21-28)	
	IBMFS	69	0.971 (0.875-0.994)	19 (18-21)		0.768 (0.647-0.852)	21 (19-24)	
	Other NMDs	30	0.900 (0.688-0.971)	21 (19-25)		0.733 (0.527-0.860)	22.5 (19-27)	
HLA matching	10/10	201	0.905 (0.855-0.939)	21 (20-22)	.431	0.741 (0.675-0.796)	23 (21-24)	.122
	09/10	102	0.863 (0.778-0.917)	21 (19-23)		0.608 (0.505-0.696)	26 (24-28)	

HLA-DPB1 TCE permissiveness	Permissive	112	0.911 (0.838-0.952)	20 (19-22)	**.035**	0.741 (0.648-0.813)	24 (21-25)	.051
	Match	50	0.960 (0.817-0.992)	20 (19-22)		0.780 (0.633-0.874)	22 (20-25)	
	Nonpermissive in HvG direction	31	0.774 (0.573-0.889)	23 (20-26)		0.548 (0.354-0.706)	23 (20-33)	
	Nonpermissive in GvH direction	47	0.936 (0.800-0.981)	20 (18-22)		0.809 (0.658-0.898)	23 (20-26)	
	Missing	63	0.825 (0.703-0.901)	22 (21-24)		0.540 (0.408-0.654)	27 (25-32)	
DSA status	DSA negative	292	0.897 (0.856-0.927)	21 (20-22)	.072	0.709 (0.653-0.758)	24 (22-25)	.095
	DSA positive	11	0.727 (0.326-0.913)	26 (21-NA)		0.364 (0.102-0.639)	32 (26-NA)	
URD search	National URD	180	0.900 (0.845-0.936)	21 (20-22)	.524	0.717 (0.644-0.777)	24 (22-25)	.375
	Internacional URD	123	0.878 (0.805-0.925)	21 (20-23)		0.667 (0.575-0.743)	24 (21-26)	
Sex mismatch	Male Recipient/Male URD	109	0.917 (0.845-0.957)	20 (19-21)	**.010**	0.761 (0.669-0.832)	23 (21-25)	.363
	Male Recipient/Female URD	61	0.836 (0.713-0.910)	24 (22-26)		0.607 (0.471-0.718)	25 (22-30)	
	Female Recipient/Female URD	56	0.857 (0.730-0.927)	22 (21-24)		0.696 (0.555-0.801)	25 (23-27)	
	Female Recipient/Male URD	77	0.922 (0.829-0.966)	20 (19-21)		0.675 (0.557-0.768)	24 (21-26)	
Source	Bone marrow	267	0.903 (0.860-0.933)	21 (20-22)	.382	0.697 (0.637-0.748)	24 (23-25)	.65
	Peripheral blood	36	0.806 (0.622-0.906)	21.5 (19-24)		0.694 (0.511-0.820)	22 (21-27)	
TNC (Median)	≥ 4.94 x 10^8/kg	153	0.922 (0.865-0.955)	20 (19-21)	**.002**	0.810 (0.738-0.864)	21 (20-24)	**.001**
	< 4.94 x 10^8/kg	150	0.860 (0.792-0.907)	22 (21-23)		0.580 (0.496-0.655)	26 (25-29)	
CD34+ (Median)	≥ 2.76 x 10^6/kg	150	0.920 (0.862-0.954)	20 (20-21)	**.015**	0.800 (0.726-0.856)	21 (20-23)	**.0012**
	< 2.76 x 10^6/kg	150	0.867 (0.800-0.912)	22 (21-23)		0.600 (0.517-0.674)	26 (25-27)	
Year of HCT (Median)	≥ 2013	171	0.936 (0.886-0.964)	20 (19-21)	**.0001**	0.766 (0.695-0.823)	22 (21-24)	**.002**
	< 2013	132	0.833 (0.757-0.887)	22 (21-24)		0.606 (0.517-0.684)	26 (25-28)	

Abbreviations: NR indicates neutrophil recovery; PR, platelet recovery; CI, confidence interval; URD, unrelated donor; CMV, cytomegalovirus; NA, not applicable; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; rATG, rabbit anti-thymocyte globulin; TBI, total body irradiation; GVHD, graft-versus-host disease; CSA, cyclosporine; MTX, methotrexate; CY, cyclophosphamide; MMF, mycophenolate mofetil; SAA, severe aplastic anemia; IBMFS, Inherited bone marrow failure syndromes; NMD, nonmalignant diseases; TCE, T-Cell Epitope; HvG, host-versus-graft; GvH, Graft-versus-Host; TNC, total nucleated cell; HCT, hematopoietic cell transplant. Significant values are in bold

SUPPLEMENTARY TABLE 2. UNADJUSTED CUMULATIVE INCIDENCE OF GRAFT FAILURE

Covariates	Group	n	100-day GF (95% CI)	*P* value
ABO matching	ABO Match	127	0.063 (0.029-0.115)	.635
	Minor ABO mismatch	75	0.080 (0.032-0.156)	
	Major ABO mismatch	101	0.059 (0.024-0.117)	
Recipient age (Median)	< 14 years	151	0.046 (0.020-0.089)	.256
	≥ 14 years	152	0.086 (0.048-0.137)	
Donor age (Median)	< 31 years	144	0.063 (0.031-0.110)	.674
	≥ 31 years	159	0.069 (0.037-0.116)	
Recipient/URD CMV serostatus	Recipient+/URD+	178	0.067 (0.037-0.111)	.104
	Recipient+/URD-	88	0.045 (0.015-0.104)	
	Recipient-/URD-	7	0.143 (0.005-0.491)	
	Recipient-/URD+	17	0.000 (0.000-0.000)	
	Missing	13	0.231 (0.051-0.486)	
Conditioning regimen	MAC	222	0.063 (0.036-0.100)	.838
	RIC	81	0.074 (0.030-0.145)	
Use of rATG	Yes	78	0.090 (0.039-0.166)	.406
	No	225	0.058 (0.032-0.094)	
Use of TBI	Yes	119	0.042 (0.016-0.089)	.134
	No	184	0.082 (0.048-0.127)	
GVHD prophylaxis	CSA + MTX	273	0.070 (0.043-0.104)	.512
	CSA + CY + MMF	13	0.077 (0.004-0.303)	
	Others	17	0.000 (0.000-0.000)	
Disease types	Acute Leukemia	108	0.046 (0.017-0.098)	.182
	Other Malignant diseases	36	0.028 (0.002-0.126)	
	Acquired SAA	60	0.133 (0.062-0.233)	
	IBMFS	69	0.058 (0.019-0.131)	
	Other NMDs	30	0.067 (0.011-0.195)	
HLA matching	10/10	201	0.055 (0.029-0.092)	.156
	09/10	102	0.088 (0.043-0.153)	
HLA-DPB1 TCE permissiveness	Permissive	112	0.063 (0.027-0.118)	.0745
	Match	50	0.000 (0.000-0.000)	
	Nonpermissive in HvG direction	31	0.161 (0.057-0.312)	
	Nonpermissive in GvH direction	47	0.064 (0.016-0.159)	
	Missing	63	0.079 (0.029-0.163)	
DSA status	DSA negative	292	0.055 (0.033-0.085)	**.00007**
	DSA positive	11	0.364 (0.102-0.639)	
URD search	National URD	180	0.072 (0.040-0.116)	.487
	Internacional URD	123	0.057 (0.025-0.108)	
Sex mismatch	Male Recipient/Male URD	109	0.073 (0.034-0.133)	.499
	Male Recipient/Female URD	61	0.098 (0.040-0.189)	

	Female Recipient/Female URD	56	0.054 (0.014-0.135)	
	Female Recipient/Male URD	77	0.039 (0.010-0.100)	
Source	Bone marrow	267	0.071 (0.044-0.106)	.302
	Peripheral blood	36	0.028 (0.002-0.126)	
TNC (Median)	≥ 4.94 $x10^8$/kg	153	0.039 (0.016-0.079)	**.0371**
	< 4.94 $x10^8$/kg	150	0.093 (0.053-0.147)	
$CD34^+$ (Median)	≥ 2.76 x 10^6/kg	150	0.040 (0.016-0.080)	**.0418**
	< 2.76 x 10^6/kg	150	0.093 (0.053-0.147)	
Year of HCT (Median)	≥ 2013	171	0.059 (0.030-0.101)	.397
	< 2013	132	0.076 (0.039-0.129)	

Abbreviations: GF indicates graft failure; CI, confidence interval; URD, unrelated donor; CMV, cytomegalovirus; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; rATG, rabbit anti-thymocyte globulin; TBI, total body irradiation; GVHD, graft-versus-host disease; CSA, cyclosporine; MTX, methotrexate; CY, cyclophosphamide; MMF, mycophenolate mofetil; SAA, severe aplastic anemia; IBMFS, Inherited bone marrow failure syndromes; NMD, nonmalignant diseases; TCE, T-Cell Epitope; HvG, host-versus-graft; GvH, Graft-versus-Host; TNC, total nucleated cell; HCT, hematopoietic cell transplant. Significant values are in bold

SUPPLEMENTARY TABLE 3. UNADJUSTED PROBABILITIES OF OVERALL SURVIVAL.

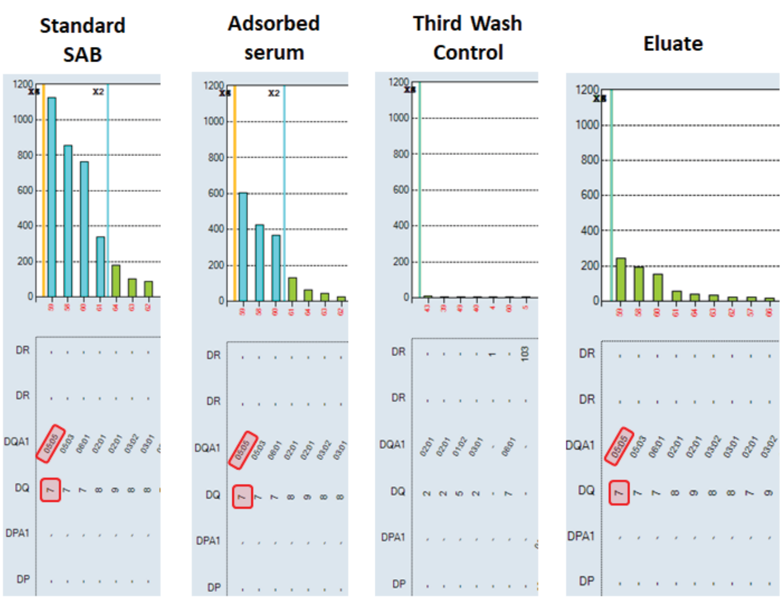
Covariates	Group	n	1-year OS (95% CI)	*P* value
ABO matching	ABO Match	127	0.784 (0.699-0.848)	.258
	Minor ABO mismatch	75	0.671 (0.549-0.767)	
	Major ABO mismatch	101	0.703 (0.601-0.783)	
Recipient age (Median)	< 14 years	151	0.864 (0.794-0.911)	**.00000007**
	≥ 14 years	152	0.593 (0.509-0.668)	
Donor age (Median)	< 31 years	144	0.681 (0.595-0.752)	.0621
	≥ 31 years	159	0.770 (0.694-0.829)	
Recipient/URD CMV serostatus	Recipient+/URD+	178	0.734 (0.660-0.795)	.363
	Recipient+/URD-	88	0.700 (0.592-0.785)	
	Recipient-/URD-	7	0.857 (0.334-0.979)	
	Recipient-/URD+	17	0.824 (0.547-0.939)	
	Missing	13	0.677 (0.349-0.865)	
Conditioning regimen	MAC	222	0.699 (0.631-0.757)	.0716
	RIC	81	0.800 (0.694-0.872)	
Use of rATG	Yes	78	0.794 (0.687-0.869)	.0831
	No	225	0.702 (0.635-0.760)	
Use of TBI	Yes	119	0.702 (0.608-0.777)	.613
	No	184	0.745 (0.674-0.804)	
GVHD prophylaxis	CSA + MTX	273	0.724 (0.665-0.774)	.831
	CSA + CY + MMF	13	0.673 (0.340-0.865)	
	Others	17	0.824 (0.547-0.939)	
Disease types	Acute Leukemia	108	0.615 (0.509-0.705)	**.0018**
	Other Malignant diseases	36	0.554 (0.379-0.698)	
	Acquired SAA	60	0.764 (0.634-0.853)	
	IBMFS	69	0.894 (0.790-0.948)	
	Other NMDs	30	0.867 (0.683-0.948)	
HLA matching	10/10	201	0.809 (0.746-0.858)	**.00009**
	09/10	102	0.570 (0.464-0.662)	
HLA-DPB1 TCE permissiveness	Permissive	112	0.774 (0.681-0.843)	.0511
	Match	50	0.784 (0.635-0.878)	
	Nonpermissive in HvG direction	31	0.705 (0.508-0.835)	
	Nonpermissive in GvH direction	47	0.794 (0.639-0.888)	
	Missing	63	0.564 (0.431-0.677)	
DSA status	DSA negative	292	0.728 (0.671-0.776)	.508
	DSA positive	11	0.727 (0.371-0.903)	
URD search	National URD	180	0.779 (0.709-0.834)	**.0159**
	Internacional URD	123	0.653 (0.559-0.732)	
Sex mismatch	Male Recipient/Male URD	109	0.786 (0.693-0.854)	.226
	Male Recipient/Female URD	61	0.701 (0.560-0.804)	

	Female Recipient/Female URD	56	0.729 (0.592-0.827)	
	Female Recipient/Male URD	77	0.663 (0.542-0.759)	
Source	Bone marrow	267	0.745 (0.687-0.795)	**.033**
	Peripheral blood	36	0.602 (0.422-0.742)	
TNC (Median)	≥ 4.94 x10^8/kg	153	0.774 (0.696-0.834)	.245
	< 4.94 x10^8/kg	150	0.682 (0.599-0.752)	
$CD34^+$ (Median)	≥ 2.76 x 10^6/kg	150	0.758 (0.680-0.820)	.646
	< 2.76 x 10^6/kg	150	0.699 (0.616-0.768)	
Year of HCT (Median)	≥ 2013	171	0.723 (0.644-0.788)	.764
	< 2013	132	0.726 (0.642-0.794)	

Abbreviations: OS indicates overall survival; CI, confidence interval; URD, unrelated donor; CMV, cytomegalovirus; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; rATG, rabbit anti-thymocyte globulin; TBI, total body irradiation; GVHD, graft-versus-host disease; CSA, cyclosporine; MTX, methotrexate; CY, cyclophosphamide; MMF, mycophenolate mofetil; SAA, severe aplastic anemia; IBMFS, Inherited bone marrow failure syndromes; NMD, nonmalignant diseases; TCE, T-Cell Epitope; HvG, host-versus-graft; GvH, Graft-versus-Host; TNC, total nucleated cell; HCT, hematopoietic cell transplant. Significant values are in bold

SUPPLEMENTARY FIGURE 1. ABSORPTION/ELUTION PROTOCOL RESULTS WITH SURROGATE DONOR CELLS EXPRESSING DQA1*05:01/DQB1*03:01 (A) AND DQB1*06:03 (B). Two patients showed DSAs (DQA1*05:01/DQB1*03:01 and DQB1*06:03) with divergent results in LABScreen and LIFECODES SAB assays. These DSAs were confirmed as true-positive reactions in the absorption/elution protocol with surrogate donor cells since both DSAs were eluted from informative cells expressing the DSA target. The eluate results were validated by observing no DSA carry-over detection in the third wash control. The red rectangles indicate the investigated DSAs.

A) Surrogate donor cells expressing DQA1*05:01/DQB1*03:01



B) Surrogate donor cells expressing DQB1*06:03

5 CONCLUSIONS

5.1 FIRST HAPLOIDENTICAL DONOR TRANSPLANTATION

- Untreated DSAs are associated a lower neutrophil recovery, increased graft failure, inferior event-free survival, and worse overall survival.

- Treated or untreated DSAs are associated with delayed platelet recovery.

- False-positive DSAs do not impact engraftment.

5.2 SALVAGE HAPLOIDENTICAL DONOR TRANSPLANTATION

- DSA positivity is associated with a higher risk of graft failure and inferior event-free survival after salvage haplo-PTCy.

- DSA positivity showed a trend of poorer overall survival after salvage haplo-PTCy.

5.3 UNRELATED DONOR TRANSPLANTATION

- High-titer DSAs are associated with graft failure after allo-HCT with unrelated donors.

- Low-level DSAs are associated with delayed neutrophil and platelet recovery.

- The presence of DSAs is not predictive of inferior overall survival.

5.4 FUTURE PERSPECTIVES

Although the findings of the current study validate the association of DSAs with engraftment failure, several relevant topics that may eventually impact the DSA pathogenicity, including differential HLA expression on $CD34^+$ cells, IgG affinity, IgG subclasses, IgG glycosylation, and cryptic HLA sensitization remain largely unexplored in the alternative donor HCT.

Current data indicate that HLA antigens are differentially expressed in $CD34^+$ cells, with HLA-A, -B, and -DR molecules highly expressed, whereas HLA-DQ antigens are detected at lower levels (BETTINOTTI et al., 2018). Additionally, HLA-C and HLA-DPB1 also present differential expression at the allelic level (PETERSDORF et al., 2014; SCHÖNE et al., 2018). Thus, future studies regarding DSA in alternative donor transplantation should address this topic to validate the hypothesis that DSAs directed to highly expressed HLA antigens are more predictive of failed engraftment (FERNANDEZ VINA; HESLOP; BARKER, 2013).

Further work is needed to elucidate the impact of IgG3 subclass, IgG glycosylation, and IgG affinity assessed by surface plasmon resonance in allo-HCT. Importantly, these specific characteristics appear to affect solid organ transplantation (MALARD-CASTAGNET et al., 2016; PERNIN et al., 2022) and have not yet been investigated in haplo-PTCy or unrelated donor allo-HCT settings.

Moreover, the incidence and clinical impact of cryptic HLA sensitization in allo-HCT with alternative donors has been scarcely reported. Thus, assays to detect alloreactive memory B cells (WEHMEIER; KARAHAN; HEIDT, 2020), such as culture supernatant analysis, tetramer staining, and IgG ELISpot assay, should be prospectively validated in the allo-HCT setting. Indeed, we anticipate that these assays could be helpful in multiparous women and heavily transfused patients without circulating DSAs undergoing alternative donor HCT.

Finally, future studies investigating the impact of DSA in haploidentical, MUD, and MMUD transplantation should adhere to minimal requirements of reporting solid phase assay results, as recently proposed by a group of histocompatibility experts (VALENZUELA et al., 2018). Therefore, using one standardized approach will permit more accurate comparisons among future studies investigating the role of DSA in allo-HCT with alternative donors.

REFERENCES

AL MALKI, M. M. et al. Posttransplant cyclophosphamide as GVHD prophylaxis for peripheral blood stem cell HLA-mismatched unrelated donor transplant. **Blood Advances**, v. 5, n. 12, p. 2650–2659, 22 jun. 2021.

ANASETTI, C. et al. Effect of HLA Compatibility on Engraftment of Bone Marrow Transplants in Patients with Leukemia or Lymphoma. **New England Journal of Medicine**, v. 320, n. 4, p. 197–204, 26 jan. 1989.

ARCURI, L. J. et al. Impact of CD34 Cell Dose and Conditioning Regimen on Outcomes after Haploidentical Donor Hematopoietic Stem Cell Transplantation with Post-Transplantation Cyclophosphamide for Relapsed/Refractory Severe Aplastic Anemia. **Biology of Blood and Marrow Transplantation**, v. 26, n. 12, p. 2311–2317, 1 dez. 2020.

BACIGALUPO, A. et al. Defining the Intensity of Conditioning Regimens: Working Definitions. **Biology of Blood and Marrow Transplantation**, v. 15, n. 12, p. 1628–1633, 1 dez. 2009.

BAILÉN, R. et al. Management of Donor-Specific Antibodies in Haploidentical Transplant: Multicenter Experience From the Madrid Group of Hematopoietic Transplant. **Frontiers in Immunology**, v. 12, p. 674658, 19 maio 2021.

BARGE, A. J. et al. Antibody-Mediated Marrow Failure After Allogeneic Bone Marrow Transplantation. **Blood**, v. 74, n. 5, p. 1477–1480, 1 out. 1989.

BASHEY, A.; SOLOMON, S. R. T-cell replete haploidentical donor transplantation using post-transplant CY: an emerging standard-of-care option for patients who lack an HLA-identical sibling donor. **Bone Marrow Transplantation**, v. 49, n. 8, p. 999–1008, ago. 2014.

BESSE, K. et al. On Modeling Human Leukocyte Antigen–Identical Sibling Match Probability for Allogeneic Hematopoietic Cell Transplantation: Estimating the Need for an Unrelated Donor Source. **Biology of Blood and Marrow Transplantation**, v. 22, n. 3, p. 410–417, 1 mar. 2016.

BETTINOTTI, M. et al. HLA expression on CD34+ stem cells: is a flow cytometric crossmatch a good gatekeeper for mismatched hematopoietic cell transplantion? **Human Immunology**, v. 79, p. 35–35, out. 2018.

BETTINOTTI, M. P. Evolution of HLA testing for hematopoietic stem cell transplantation: Importance of the candidate's antibody profile for donor selection. **Human Immunology**, v. 83, n. 10, p. 721–729, out. 2022.

BETTINOTTI, M. P.; ZACHARY, A. A.; LEFFELL, M. S. Clinically relevant interpretation of solid phase assays for HLA antibody. **Current Opinion in Organ Transplantation**, v. 21, n. 4, p. 453–458, ago. 2016.

BHASKARAN, M. C.; HEIDT, S.; MUTHUKUMAR, T. Principles of Virtual Crossmatch Testing for Kidney Transplantation. **Kidney International Reports**, v. 7, n. 6, p. 1179–1188, 1 jun. 2022.

BONFIM, C. et al. Haploidentical Bone Marrow Transplantation with Post-Transplant Cyclophosphamide for Children and Adolescents with Fanconi Anemia. **Biology of Blood and Marrow Transplantation**, v. 23, n. 2, p. 310–317, fev. 2017.

BRAMANTI, S. et al. Donor-Specific Anti-HLA Antibodies in Haploidentical Stem Cell Transplantation with Post-Transplantation Cyclophosphamide: Risk of Graft Failure, Poor Graft Function, and Impact on Outcomes. **Biology of Blood and Marrow Transplantation**, v. 25, n. 7, p. 1395–1406, jul. 2019.

BRUNSTEIN, C. G. et al. Anti-HLA Antibodies in Double Umbilical Cord Blood Transplantation. **Biology of Blood and Marrow Transplantation**, v. 17, n. 11, p. 1704–1708, nov. 2011.

CABEZAS, L. et al. Tocilizumab and Active Antibody-Mediated Rejection in Kidney Transplantation: A Literature Review. **Frontiers in Immunology**, v. 13, p. 839380, 14 abr. 2022.

CAI, J. et al. Intact HLA Not β2m-free Heavy Chain-Specific HLA Class I Antibodies Are Predictive of Graft Failure. **Transplantation**, v. 88, n. 2, 2009.

CHAMPLIN, R. E. et al. Graft Failure Following Bone Marrow Transplantation for Severe Aplastic Anemia: Risk Factors and Treatment Results. **Blood**, v. 73, n. 2, p. 606–613, 1 fev. 1989.

CHANG, Y.-J. et al. Donor-specific anti-human leukocyte antigen antibodies were associated with primary graft failure after unmanipulated haploidentical blood and marrow transplantation: a prospective study with randomly assigned training and validation sets. **Journal of Hematology & Oncology**, v. 8, n. 1, p. 84, dez. 2015.

CHANG, Y.-J. et al. Rituximab for desensitization during HLA-mismatched stem cell transplantation in patients with a positive donor-specific anti-HLA antibody. **Bone Marrow Transplantation**, v. 55, n. 7, p. 1326–1336, jul. 2020.

CHOE, H. et al. Bortezomib and Immune Globulin Have Limited Effects on Donor-Specific HLA Antibodies in Haploidentical Cord Blood Stem Cell Transplantation: Detrimental Effect of Persistent Haploidentical Donor-Specific HLA Antibodies. **Biology of Blood and Marrow Transplantation**, v. 25, n. 2, p. e60–e64, fev. 2019.

CIUREA, S. O. et al. High Risk of Graft Failure in Patients With Anti-HLA Antibodies Undergoing Haploidentical Stem-Cell Transplantation. **Transplantation**, v. 88, n. 8, p. 1019–1024, 27 out. 2009.

CIUREA, S. O. et al. Donor-specific anti-HLA Abs and graft failure in matched unrelated donor hematopoietic stem cell transplantation. **Blood**, v. 118, n. 22, p. 5957–5964, 24 nov. 2011.

CIUREA, S. O. et al. Complement-Binding Donor-Specific Anti-HLA Antibodies and Risk of Primary Graft Failure in Hematopoietic Stem Cell Transplantation. **Biology of Blood and Marrow Transplantation**, v. 21, n. 8, p. 1392–1398, ago. 2015.

CIUREA, S. O. et al. The European Society for Blood and Marrow Transplantation (EBMT) Consensus Guidelines for the Detection and Treatment of Donor-specific Anti-HLA Antibodies (DSA) in Haploidentical Hematopoietic Cell Transplantation. **Bone Marrow Transplantation**, v. 53, n. 5, p. 521–534, maio 2018.

CIUREA, S. O. et al. The European Society for Blood and Marrow Transplantation (EBMT) consensus recommendations for donor selection in haploidentical hematopoietic cell transplantation. **Bone Marrow Transplantation**, v. 55, n. 1, p. 12–24, 1 jan. 2020.

CIUREA, S. O. et al. Treatment of allosensitized patients receiving allogeneic transplantation. **Blood Advances**, v. 5, n. 20, p. 4031–4043, 26 out. 2021.

CLAY, J. et al. Nonmyeloablative Peripheral Blood Haploidentical Stem Cell Transplantation for Refractory Severe Aplastic Anemia. **Biology of Blood and Marrow Transplantation**, v. 20, n. 11, p. 1711–1716, nov. 2014.

CLUZEAU, T. et al. Risk factors and outcome of graft failure after HLA matched and mismatched unrelated donor hematopoietic stem cell transplantation: a study on behalf of SFGM-TC and SFHI. **Bone Marrow Transplantation**, v. 51, n. 5, p. 687–691, maio 2016.

COPELAN, E. A. et al. Allogeneic hematopoietic cell transplantation; the current renaissance. **Blood Reviews**, v. 34, p. 34–44, 1 mar. 2019.

CUADRADO, M. et al. Impact of Donor-Directed HLA Allo-Antibodies on Primary Graft Failure After Unrelated Donor Allogeneic Haematopoietic Cell Transplantation. **Bone Marrow Transplantation**, v. 55, n. Suppl 1, p. 133–134, dez. 2020.

CUTLER, C. et al. Donor-specific anti-HLA antibodies predict outcome in double umbilical cord blood transplantation. **Blood**, v. 118, n. 25, p. 6691–6697, 15 dez. 2011.

DAVIES, S. M. et al. Engraftment and survival after unrelated-donor bone marrow transplantation: a report from the National Marrow Donor Program. **Blood**, v. 96, n. 13, p. 4096–4102, 15 dez. 2000.

DE LA FUENTE, J. et al. Haploidentical Bone Marrow Transplantation with Post-Transplantation Cyclophosphamide Plus Thiotepa Improves Donor Engraftment in Patients with Sickle Cell Anemia: Results of an International Learning Collaborative. **Biology of Blood and Marrow Transplantation**, v. 25, n. 6, p. 1197–1209, jun. 2019.

DE MARCO, R.; DE LIMA, G. M. Comparison of Methods for Abolishing Inhibitory Serum Factors in Luminex Single Antigen Assay.: Abstract# D2330. **Transplantation**, v. 98, 2014.

DEHN, J. et al. Selection of unrelated donors and cord blood units for hematopoietic cell transplantation: guidelines from the NMDP/CIBMTR. **Blood**, v. 134, n. 12, p. 924–934, 19 set. 2019.

D'SOUZA, A. et al. Current Use of and Trends in Hematopoietic Cell Transplantation in the United States. **Biology of Blood and Marrow Transplantation**, v. 26, n. 8, p. e177–e182, 1 ago. 2020.

EAPEN, M. et al. Allele-level HLA matching for umbilical cord blood transplantation for non-malignant diseases in children: a retrospective analysis. **The Lancet. Haematology**, v. 4, n. 7, p. e325–e333, jul. 2017.

EL-AWAR, N. et al. Epitopes of human leukocyte antigen class I antibodies found in sera of normal healthy males and cord blood. **Human Immunology**, v. 70, n. 10, p. 844–853, 1 out. 2009.

EL-AWAR, N.; JUCAUD, V.; NGUYEN, A. HLA Epitopes: The Targets of Monoclonal and Alloantibodies Defined. **Journal of Immunology Research**, v. 2017, p. 3406230, 2017.

ELKINS, W. L. et al. Rejection of sibling bone marrow expressing foreign HLA-A1 by a leukemia patient. Detection of prior sensitization by antibody-dependent cell-mediated cytotoxicity. **Transplantation**, v. 31, n. 4, 1981.

ELLIS, T. M. Interpretation of HLA single antigen bead assays. **Transplantation reviews (Orlando)**, v. 27, n. 4, p. 108–111, out. 2013.

ENG, H. S.; LEFFELL, M. S. Histocompatibility testing after fifty years of transplantation. **Journal of Immunological Methods**, v. 369, n. 1–2, p. 1–21, 30 jun. 2011.

FERNANDES, J. et al. Salvage Haploidentical Stem Cell Transplantation with Post-Transplant Cyclophosphamide for graft failure in children with non-malignant diseases. **Bone Marrow Transplantation**, v. 53 (S1), p. 720–721, set.)2018.

FERNANDES, J. F. et al. Outcomes after Haploidentical Stem Cell Transplantation with Post-Transplantation Cyclophosphamide in Patients with Primary Immunodeficiency Diseases. **Biology of Blood and Marrow Transplantation**, v. 26, n. 10, p. 1923–1929, out. 2020.

FERNANDEZ VINA, M.; HESLOP, H. E.; BARKER, J. N. New approaches in alternative donor transplantation. **Biology of Blood and Marrow Transplantation**, v. 19, n. 1 Suppl, p. S91-96, jan. 2013.

FERRÀ, C. et al. Outcome of graft failure after allogeneic stem cell transplant: study of 89 patients. **Leukemia & Lymphoma**, v. 56, n. 3, p. 656–662, mar. 2015.

FILE, B. et al. The impact of HLA donor-specific antibodies on engraftment and the evolving desensitization strategies. **Bone Marrow Transplantation**, v. 57, n. 4, p. 526–531, abr. 2022.

FUCHS, E. J. Related haploidentical donors are a better choice than matched unrelated donors: Point. **Blood Advances**, v. 1, n. 6, p. 397–400, 14 fev. 2017.

FUJI, S. et al. Impact of pretransplant donor-specific anti-HLA antibodies on cord blood transplantation on behalf of the Transplant Complications Working Group of Japan Society for Hematopoietic Cell Transplantation. **Bone Marrow Transplantation**, v. 55, n. 4, p. 722–728, abr. 2020.

GARCIA-SANCHEZ, C. et al. The shared epitope phenomenon - A potential impediment to virtual crossmatch accuracy. **Clinical Transplantation**, v. 34, n. 8, p. e13906, ago. 2020.

GAZIEV, J. et al. Haploidentical HSCT for hemoglobinopathies: improved outcomes with TCRαβ+/CD19+-depleted grafts. **Blood Advances**, v. 2, n. 3, p. 263–270, 13 fev. 2018.

GEBEL, H. M.; BRAY, R. A. HLA Antibody Detection With Solid Phase Assays: Great Expectations or Expectations Too Great? **American Journal of Transplantation**, v. 14, n. 9, p. 1964–1975, 1 set. 2014.

GEORGE, B. et al. Haploidentical transplantation is feasible and associated with reasonable outcomes despite major infective complications-A single center experience from India. **Transplantation and Cellular Therapy**, v. 28, n. 1, p. 45.e1-45.e8, jan. 2022.

GIAMMARCO, S. et al. Second haploidentical stem cell transplantation for primary graft failure. **Bone Marrow Transplantation**, v. 56, n. 6, p. 1291–1296, jun. 2021.

GLADSTONE, D. E. et al. Partially Mismatched Transplantation and Human Leukocyte Antigen Donor-Specific Antibodies. **Biology of Blood and Marrow Transplantation**, v. 19, n. 4, p. 647–652, abr. 2013.

GRENZI, P. C. et al. Antibodies against denatured HLA class II molecules detected in luminex-single antigen assay. **Human Immunology**, v. 74, n. 10, p. 1300–1303, out. 2013.

HASHEM, H. et al. Haploidentical Hematopoietic Cell Transplantation Using Post-Transplant Cyclophosphamide for Children with Non-malignant Diseases. **Journal of Clinical Immunology**, v. 41, n. 8, p. 1754–1761, nov. 2021.

HASHEM, H. et al. Novel conditioning regimen for upfront haploidentical hematopoietic cell transplantation in children with severe aplastic anemia and donor-specific anti-HLA antibodies. **Bone Marrow Transplantation**, v. 57, n. 2, p. 304–305, fev. 2022.

HAYASHI, R. J. Considerations in Preparative Regimen Selection to Minimize Rejection in Pediatric Hematopoietic Transplantation in Non-Malignant Diseases. **Frontiers in Immunology**, v. 11, 2020.

HEFAZI, M. et al. The association of de novo anti-HLA-DPB1 donor-specific antibody formation and primary graft failure after allogeneic hematopoietic cell transplantation. **Human Immunology**, v. 79, n. 12, p. 861–864, dez. 2018.

HORAN, J. et al. Evaluation of HLA matching in unrelated hematopoietic stem cell transplantation for nonmalignant disorders. **Blood**, v. 120, n. 14, p. 2918–2924, 4 out. 2012.

IACOBELLI, S. Suggestions on the use of statistical methodologies in studies of the European Group for Blood and Marrow Transplantation. **Bone Marrow Transplantation**, v. 48 Suppl 1, p. S1-37, mar. 2013.

IBRAHIM, U.; KEYZNER, A. Daratumumab for donor-specific anti-HLA antibody desensitization in a case of HLA-mismatched allogeneic stem cell transplantation. **Hematology, Transfusion and Cell Therapy**, p. S2531137922000086, jan. 2022.

JOHER, N.; MATIGNON, M.; GRIMBERT, P. HLA Desensitization in Solid Organ Transplantation: Anti-CD38 to Across the Immunological Barriers. **Frontiers in Immunology**, v. 12, p. 688301, 20 maio 2021.

JORDAN, S. C. et al. Risk factors associated with the development of histocompatibility leukocyte antigen sensitization. **Current Opinion in Organ Transplantation**, v. 21, n. 4, p. 447–452, ago. 2016.

JORDAN, S. C. et al. IgG Endopeptidase in Highly Sensitized Patients Undergoing Transplantation. **New England Journal of Medicine**, v. 377, n. 5, p. 442–453, 3 ago. 2017.

JURIC, M. K. et al. Milestones of Hematopoietic Stem Cell Transplantation – From First Human Studies to Current Developments. **Frontiers in Immunology**, v. 7, 2016.

KANATE, A. S.; NAGLER, A.; SAVANI, B. Summary of Scientific and Statistical Methods, Study Endpoints and Definitions for Observational and Registry-Based Studies in Hematopoietic Cell Transplantation: **Clinical Hematology International**, v. 2, n. 1, p. 2, 2019.

KANDA, Y. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. **Bone Marrow Transplantation**, v. 48, n. 3, p. 452–458, mar. 2013.

KHARFAN-DABAJA, M. A. et al. Standardizing Definitions of Hematopoietic Recovery, Graft Rejection, Graft Failure, Poor Graft Function, and Donor Chimerism in Allogeneic Hematopoietic Cell Transplantation: A Report on Behalf of the American Society for Transplantation and Cellular Therapy. **Transplantation and Cellular Therapy**, v. 27, n. 8, p. 642–649, ago. 2021.

KONG, Y. Poor graft function after allogeneic hematopoietic stem cell transplantation-an old complication with new insights. **Seminars in Hematology**, v. 56, n. 3, p. 215–220, jul. 2019.

KRUMMEY, S. M.; GAREAU, A. J. Donor specific HLA antibody in hematopoietic stem cell transplantation: Implications for donor selection. **Frontiers in Immunology**, v. 13, p. 916200, 2022.

KURZAY, M. et al. T-cell replete haploidentical bone marrow transplantation and post-transplant cyclophosphamide for patients with inborn errors. **Haematologica**, v. 104, n. 10, p. e478–e482, 1 out. 2019.

LEFFELL, M. S.; JONES, R. J.; GLADSTONE, D. E. Donor HLA-specific Abs: to BMT or not to BMT? **Bone Marrow Transplantation**, v. 50, n. 6, p. 751–758, jun. 2015.

LIMA, A. C. M. et al. The impact of donor-specific anti-human leukocyte antigen antibodies in salvage haploidentical hematopoietic cell transplantation with posttransplant cyclophosphamide in patients with nonmalignant disorders. **HLA**, v. 97, n. 6, p. 493–504, jun. 2021.

LIMA, A. C. M. et al. Untreated Donor-Specific HLA Antibodies Are Associated With Graft Failure and Poor Survival After Haploidentical Transplantation With Post-Transplantation Cyclophosphamide in Pediatric Patients With Nonmalignant Disorders. **Transplantation and Cellular Therapy**, v. 28, n. 10, p. 698.e1-698.e11, out. 2022.

LIWSKI, R. S. et al. Rapid optimized flow cytometric crossmatch (FCXM) assays: The Halifax and Halifaster protocols. **Human Immunology**, v. 79, n. 1, p. 28–38, 1 jan. 2018.

LIWSKI, R. S. et al. Cutting through the weeds: Evaluation of a novel adsorption with crossmatch cells and elution protocol to sharpen HLA antibody identification by the single antigen bead assay. **Frontiers in Genetics**, v. 13, 2022.

LIWSKI, R. S.; GEBEL, H. M. Of Cells and Microparticles: Assets and Liabilities of HLA Antibody Detection. **Transplantation**, v. 102, n. 1S, 2018.

LOWE, D. et al. HLA incompatible combined liver-kidney transplantation: dynamics of antibody modulation revealed by a novel approach to HLA antibody characterisation. **Transplant immunology**, v. 30, n. 1, p. 30–33, jan. 2014.

LUND, T. C. et al. Second allogeneic hematopoietic cell transplantation for graft failure: poor outcomes for neutropenic graft failure. **American Journal of Hematology**, v. 90, n. 10, p. 892–896, out. 2015.

LUZNIK, L. et al. HLA-Haploidentical Bone Marrow Transplantation for Hematologic Malignancies Using Nonmyeloablative Conditioning and High-Dose, Posttransplantation Cyclophosphamide. **Biology of Blood and Marrow Transplantation**, v. 14, n. 6, p. 641–650, 1 jun. 2008.

MALARD-CASTAGNET, S. et al. Sialylation of antibodies in kidney recipients with de novo donor specific antibody, with or without antibody mediated rejection. **Human Immunology**, v. 77, n. 11, p. 1076–1083, nov. 2016.

MALLHI, K. K. et al. HLA-Haploidentical Hematopoietic Cell Transplantation for Treatment of Nonmalignant Diseases Using Nonmyeloablative Conditioning and Post-Transplant Cyclophosphamide. **Biology of Blood and Marrow Transplantation**, v. 26, n. 7, p. 1332–1341, jul. 2020.

MANGUM, D. S.; CAYWOOD, E. A clinician's guide to HLA matching in allogeneic hematopoietic stem cell transplant. **Human Immunology**, v. 83, n. 10, p. 687–694, 1 out. 2022.

MARTINEZ-CIBRIAN, N.; ZEISER, R.; PEREZ-SIMON, J. A. Graft-versus-host disease prophylaxis: Pathophysiology-based review on current approaches and future directions. **Blood Reviews**, v. 48, p. 100792, 1 jul. 2021.

MASOURIDI-LEVRAT, S.; SIMONETTA, F.; CHALANDON, Y. Immunological Basis of Bone Marrow Failure after Allogeneic Hematopoietic Stem Cell Transplantation. **Frontiers in Immunology**, v. 7, 2016.

MATTSSON, J. et al. Cytotoxic crossmatch analysis before allo-SCT is a poor diagnostic tool for prediction of rejection. **Bone Marrow Transplantation**, v. 45, n. 2, p. 235–238, fev. 2010.

MICKELSON, E. M. et al. HLA matching in hematopoietic cell transplantation. **Human Immunology**, v. 61, n. 2, p. 92–100, fev. 2000.

MORALES-BUENROSTRO, L. E. et al. "Natural" Human Leukocyte Antigen Antibodies Found in Nonalloimmunized Healthy Males. **Transplantation**, v. 86, n. 8, 2008.

MORRIS, A. B. et al. Out with the old, in with the new: Virtual versus physical crossmatching in the modern era. **HLA**, v. 94, n. 6, p. 471–481, dez. 2019.

NEVEN, B. et al. Haploidentical Hematopoietic Stem Cell Transplantation with Post-Transplant Cyclophosphamide for Primary Immunodeficiencies and Inherited Disorders in Children. **Biology of Blood and Marrow Transplantation**, v. 25, n. 7, p. 1363–1373, jul. 2019.

OAKS, M. et al. Practical value of identifying antibodies to cryptic HLA epitopes in cardiac transplantation. **The Journal of Heart and Lung Transplantation**, v. 33, n. 7, p. 713–720, jul. 2014.

OLSSON, R. et al. Graft failure in the modern era of allogeneic hematopoietic SCT. **Bone Marrow Transplantation**, v. 48, n. 4, p. 537–543, abr. 2013.

OLSSON, R. F. et al. Primary graft failure after myeloablative allogeneic hematopoietic cell transplantation for hematologic malignancies. **Leukemia**, v. 29, n. 8, p. 1754–1762, ago. 2015.

OTTINGER, H. D. et al. Positive serum crossmatch as predictor for graft failure in HLA-mismatched allogeneic blood stem cell transplantation: **Transplantation**, v. 73, n. 8, p. 1280–1285, abr. 2002.

OZDEMIR, Z. N.; CIVRIZ BOZDAĞ, S. Graft failure after allogeneic hematopoietic stem cell transplantation. **Transfusion and Apheresis Science**, v. 57, n. 2, p. 163–167, abr. 2018.

PERKEY, E.; MAILLARD, I. New Insights into Graft-Versus-Host Disease and Graft Rejection. **Annual Review of Pathology: Mechanisms of Disease**, v. 13, n. 1, p. 219–245, 24 jan. 2018.

PERNIN, V. et al. Distribution of de novo Donor-Specific Antibody Subclasses Quantified by Mass Spectrometry: High IgG3 Proportion Is Associated With Antibody-Mediated Rejection Occurrence and Severity. **Frontiers in Immunology**, v. 11, p. 919, 2020.

PERNIN, V. et al. IgG3 donor-specific antibodies with a proinflammatory glycosylation profile may be associated with the risk of antibody-mediated rejection after kidney transplantation. **American Journal of Transplantation**, v. 22, n. 3, p. 865–875, mar. 2022.

PETERSDORF, E. W. et al. HLA-C expression levels define permissible mismatches in hematopoietic cell transplantation. **Blood**, v. 124, n. 26, p. 3996–4003, 18 dez. 2014.

PRABAHRAN, A. et al. Clinical features, pathophysiology, and therapy of poor graft function post–allogeneic stem cell transplantation. **Blood Advances**, v. 6, n. 6, p. 1947–1959, 22 mar. 2022.

PRATA, P. H. et al. Outcomes of Salvage Haploidentical Transplant with Post-Transplant Cyclophosphamide for Rescuing Graft Failure Patients: a Report on Behalf of the Francophone Society of Bone Marrow Transplantation and Cellular Therapy. **Biology of Blood and Marrow Transplantation**, v. 25, n. 9, p. 1798–1802, set. 2019.

RAVINDRANATH, M. H. et al. Antibodies to cryptic epitopes on HLA class I and class II heavy chains bound to single antigen beads: Clinically relevant? **Transplant Immunology**, v. 69, p. 101482, dez. 2021.

RUGGERI, A. et al. Impact of donor-specific anti-HLA antibodies on graft failure and survival after reduced intensity conditioning-unrelated cord blood transplantation: a Eurocord, Societe Francophone d'Histocompatibilite et d'Immunogenetique (SFHI) and Societe Francaise de Greffe de Moelle et de Therapie Cellulaire (SFGM-TC) analysis. **Haematologica**, v. 98, n. 7, p. 1154–1160, 1 jul. 2013.

SCHAUB, S. et al. Determinants of C1q binding in the single antigen bead assay. **Transplantation**, v. 98, n. 4, p. 387–393, 27 ago. 2014.

SCHÖNE, B. et al. Predicting an HLA-DPB1 expression marker based on standard DPB1 genotyping: Linkage analysis of over 32,000 samples. **Human Immunology**, v. 79, n. 1, p. 20–27, jan. 2018.

SCHRIBER, J. et al. Second Unrelated Donor Hematopoietic Cell Transplantation for Primary Graft Failure. **Biology of Blood and Marrow Transplantation**, v. 16, n. 8, p. 1099–1106, ago. 2010.

SCORNIK, J. C. et al. Role of anti-donor antibodies in bone marrow transplant rejection: evaluation by flow cytometry and effect of plasma exchanges. **Transplantation Proceedings**, v. 21, n. 1 Pt 3, p. 2974–2975, fev. 1989.

SHAW, B. E. et al. National Marrow Donor Program–Sponsored Multicenter, Phase II Trial of HLA-Mismatched Unrelated Donor Bone Marrow Transplantation Using Post-Transplant Cyclophosphamide. **Journal of Clinical Oncology**, v. 39, n. 18, p. 1971–1982, 20 jun. 2021.

SHOUVAL, R. et al. Outcomes of allogeneic haematopoietic stem cell transplantation from HLA-matched and alternative donors: a European Society for Blood and Marrow Transplantation registry retrospective analysis. **The Lancet Haematology**, v. 6, n. 11, p. e573–e584, nov. 2019.

SPELLMAN, S. et al. The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure. **Blood**, v. 115, n. 13, p. 2704–2708, 1 abr. 2010.

SPELLMAN, S. R. Hematology 2022—what is complete HLA match in 2022? **Hematology**, v. 2022, n. 1, p. 83–89, 9 dez. 2022.

SPRIEWALD, B. M. et al. Depletion of donor-specific anti-HLA A2 alloantibodies in a hematopoietic cell transplant recipient using directed mismatched platelet transfusions. **Bone Marrow Transplantation**, v. 53, n. 6, p. 791–794, jun. 2018.

SROUR, S. A. et al. Haploidentical transplants for patients with relapse after the first allograft. **American Journal of Hematology**, v. 95, n. 10, p. 1187–1192, out. 2020.

SULLIVAN, H. C. et al. The utility of second single antigen bead assay: Clearing the water or stirring up mud? **Human Immunology**, v. 81, n. 12, p. 663–670, 1 dez. 2020.

SULLIVAN, H. C.; GEBEL, H. M.; BRAY, R. A. Understanding solid-phase HLA antibody assays and the value of MFI. **Human Immunology**, v. 78, n. 7, p. 471–480, 1 jul. 2017.

SUN, Y.-Q. et al. The incidence, risk factors, and outcomes of primary poor graft function after unmanipulated haploidentical stem cell transplantation. **Annals of hematology**, v. 94, n. 10, p. 1699–1705, out. 2015.

TAIT, B. D. et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. **Transplantation**, v. 95, n. 1, p. 19–47, 15 jan. 2013.

TAIT, B. D. Detection of HLA Antibodies in Organ Transplant Recipients - Triumphs and Challenges of the Solid Phase Bead Assay. **Frontiers in immunology**, v. 7, p. 570, 2016.

TAMBUR, A. R. et al. Assessing Antibody Strength: Comparison of MFI, C1q, and Titer Information. **American Journal of Transplantation**, v. 15, n. 9, p. 2421–2430, set. 2015.

TAMBUR, A. R. et al. Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group Meeting Report. **American Journal of Transplantation**, v. 18, n. 7, p. 1604–1614, jul. 2018.

TAMBUR, A. R. et al. Sensitization in transplantation: Assessment of risk (STAR) 2019 Working Group Meeting Report. **American Journal of Transplantation**, v. 20, n. 10, p. 2652–2668, 1 out. 2020.

TAMBUR, A. R.; CLAAS, F. H. J. HLA epitopes as viewed by antibodies: what is it all about? **American Journal of Transplantation**, v. 15, n. 5, p. 1148–1154, maio 2015.

TAMBUR, A. R.; WIEBE, C. HLA Diagnostics: Evaluating DSA Strength by Titration. **Transplantation**, v. 102, n. 1S, p. S23–S30, jan. 2018.

TAYLOR, P. A. et al. Preformed antibody, not primed T cells, is the initial and major barrier to bone marrow engraftment in allosensitized recipients. **Blood**, v. 109, n. 3, p. 1307–1315, 1 fev. 2007.

TING, A. et al. The association of cytotoxic antidonor HLA antibodies and mismatched marrow graft rejection. **Transplantation Proceedings**, v. 18, n. 2, p. 264–266, abr. 1986.

TOLAR, J.; SODANI, P.; SYMONS, H. Alternative donor transplant of benign primary hematologic disorders. **Bone Marrow Transplantation**, v. 50, n. 5, p. 619–627, 1 maio 2015.

TREMBLAY, S. et al. A prospective, iterative, adaptive trial of carfilzomib-based desensitization. **American Journal of Transplantation**, v. 20, n. 2, p. 411–421, fev. 2020.

TYAN, D. B. Application, technical issues, and interpretation of C1q for graft outcome. **Current Opinion in Organ Transplantation**, v. 22, n. 5, p. 505–510, out. 2017.

UMEDA, K. Unresolved issues in allogeneic hematopoietic cell transplantation for non-malignant diseases. **International Journal of Hematology**, v. 116, n. 1, p. 41–47, 1 jul. 2022.

VALENZUELA, N. M. et al. Minimal data reporting standards for serological testing for histocompatibility. **Human Immunology**, v. 79, n. 12, p. 865–868, dez. 2018.

VELLAICHAMY SWAMINATHAN, V. et al. Augmented immunosuppression and PTCY-based haploidentical hematopoietic stem cell transplantation for thalassemia major. **Pediatric Transplantation**, v. 25, n. 2, p. e13893, mar. 2021.

VISENTIN, J. et al. Measuring anti-HLA antibody active concentration and affinity by surface plasmon resonance: Comparison with the luminex single antigen flow beads and T-cell flow cytometry crossmatch results. **Molecular Immunology**, v. 108, p. 34–44, abr. 2019.

VO, A. A. et al. A Phase I/II Trial of the Interleukin-6 Receptor-Specific Humanized Monoclonal (Tocilizumab) + Intravenous Immunoglobulin in Difficult to Desensitize Patients. **Transplantation**, v. 99, n. 11, p. 2356–2363, nov. 2015.

VRIESENDORP, H. M. Aims of conditioning. **Experimental Hematology**, v. 31, n. 10, p. 844–854, 1 out. 2003.

WANG, J. et al. EDTA is superior to DTT treatment for overcoming the prozone effect in HLA antibody testing. **HLA**, v. 89, n. 2, p. 82–89, 1 fev. 2017.

WEHMEIER, C.; HÖNGER, G.; SCHAUB, S. Caveats of HLA antibody detection by solid-phase assays. **Transplant International**, v. 33, n. 1, p. 18–29, 1 jan. 2020.

WEHMEIER, C.; KARAHAN, G. E.; HEIDT, S. HLA-specific memory B-cell detection in kidney transplantation: Insights and future challenges. **International Journal of Immunogenetics**, v. 47, n. 3, p. 227–234, 1 jun. 2020.

WEINSTOCK, C.; SCHNAIDT, M. The complement-mediated prozone effect in the Luminex single-antigen bead assay and its impact on HLA antibody determination in patient sera. **International Journal of Immunogenetics**, v. 40, n. 3, p. 171–177, jun. 2013.

WIEBE, C. et al. Evaluation of C1q Status and Titer of De Novo Donor-Specific Antibodies as Predictors of Allograft Survival. **American Journal of Transplantation**, v. 17, n. 3, p. 703–711, mar. 2017.

WOODARD, P. et al. Etiology and Outcome of Graft Failure in Pediatric Hematopoietic Stem Cell Transplant Recipients. **Journal of Pediatric Hematology/Oncology**, v. 25, n. 12, 2003.

WOOLFREY, A. et al. Donor-specific anti-HLA antibodies in unrelated hematopoietic cell transplantation for non-malignant disorders. **Bone Marrow Transplantation**, v. 54, n. 3, p. 494–496, mar. 2019.

XIE, Y. et al. Donor-Specific Antibodies and Primary Graft Failure in Allogeneic Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. **Transplantation and Cellular Therapy**, v. 27, n. 8, p. 687.e1-687.e7, ago. 2021.

XU, H. et al. Humoral immunity is the dominant barrier for allogeneic bone marrow engraftment in sensitized recipients. **Blood**, v. 108, n. 10, p. 3611–3619, 15 nov. 2006.

YABE, H. et al. Post-transplantation-emerging anti-HLA DQA1/DQB1 antibody possibly responsible for graft rejection after myeloablative-unrelated marrow grafting. **Bone Marrow Transplantation**, v. 51, n. 4, p. 601–603, abr. 2016.

YOSHIHARA, S. et al. Risk and prevention of graft failure in patients with preexisting donor-specific HLA antibodies undergoing unmanipulated haploidentical SCT. **Bone Marrow Transplantation**, v. 47, n. 4, p. 508–515, abr. 2012.

ZACHARY, A. A. et al. Rituximab Prevents an Anamnestic Response in Patients With Cryptic Sensitization to HLA. **Transplantation**, v. 95, n. 5, p. 701–704, 15 mar. 2013.

ZHANG, X.; REINSMOEN, N. L. Comprehensive assessment for serum treatment for single antigen test for detection of HLA antibodies. **Human Immunology**, v. 78, n. 11–12, p. 699–703, nov. 2017.

ZHAO, X. et al. Donor-Specific Anti-Human Leukocyte Antigen Antibodies Predict Prolonged Isolated Thrombocytopenia and Inferior Outcomes of Haploidentical Hematopoietic Stem Cell Transplantation. **Journal of Immunology Research**, v. 2017, p. 1–8, 2017.

ZOU, J. et al. Untreated donor specific antibodies against HLA are associated with poor outcomes in peripheral blood haploidentical hematopoietic cell transplantation. **Bone Marrow Transplantation**, v. 52, n. 6, p. 898–901, jun. 2017.

APPENDIX 1. CHC/UFPR institutional review board approval

HOSPITAL DE CLÍNICAS DA UNIVERSIDADE FEDERAL DO PARANÁ - HCUFPR

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: CARACTERIZAÇÃO DOS ANTICORPOS ANTI-HLA EM PACIENTES COM DOENÇAS HEMATO-ONCOLÓGICAS E INVESTIGAÇÃO DO IMPACTO DE ANTICORPOS ESPECÍFICOS CONTRA O DOADOR NO TRANSPLANTE DE CÉLULAS-TRONCO HEMATOPOIÉTICAS

Pesquisador: ALBERTO CARDOSO MARTINS LIMA

Área Temática:

Versão: 3

CAAE: 96085718.6.0000.0096

Instituição Proponente: Hospital de Clínicas da Universidade Federal do Paraná

Patrocinador Principal: ASSOCIACAO DOS AMIGOS DO HOSPITAL DE CLINICAS

DADOS DO PARECER

Número do Parecer: 4.760.973

Apresentação do Projeto:

Resumo: Este estudo tem como objetivo detectar a presença de anticorpos contra moléculas HLA alogênicas no soro de receptores de células tronco hematopoiéticas. Quando o receptor apresentar anticorpos específicos contra o doador (DSA = Donor Specific Antibodies) será prosseguido à caracterização destes anticorpos mediante a análise epitôpica e outros testes complementares, com o intuito de identificar de modo fidedigno a especificidade dos mesmos. Após a etapa de caracterização será investigado o impacto destes aloanticorpos na evolução dos transplantes de células tronco hematopoiéticas (TCTH). Hipótese: A caracterização de anticorpos anti-HLA fundamentada na análise epitôpica, com utilização de estratégias complementares quando pertinente, superará limitações do método de moléculas HLA individualmente conjugadas às microesferas de poliestireno (Single Antigen Beads - SAB) e evitará resultados falso-positivos e falso-negativos na prova cruzada virtual. Esta abordagem permitirá uma melhor estratificação de risco dos anticorpos anti-HLA doador específicos (DSA), otimizando o processo de seleção de doadores e minimizando o risco de falha de pega/rejeição do enxerto. Objetivo Primário: Caracterizar os anticorpos anti-HLA em pacientes com doenças onco-hematológicas, transplantados no HC-UFPR entre janeiro de 2008 e dezembro de 2017, e avaliar o impacto dos anticorpos específicos contra o doador (DSA) na evolução clínica dos transplantes de células-

tronco hematopoiéticas com incompatibilidades HLA. Objetivo Secundário: Objetivo Específico 1: Caracterização dos anticorpos anti-HLA - Analisar o padrão de reatividade epitôpica dos DSA;Investigar a incidência de DSA da classe IgM; Confirmar a prova cruzada virtual fundamentada na análise epitôpica por meio de adsorção/eluição; Verificar a força de reação dos DSA pelo teste SAB/plataforma Luminex nos soros puros e diluidos 1:8; Investigar a incidência de DSA contra epítopos crípticos em moléculas HLA desnaturadas;Avaliar a capacidade de fixação do complemento dos DSA pelo teste SAB/plataforma Luminex com adição do C1q. Objetivo Específico 2: Investigar a influência dos DSAs na evolução clínica pós -transplante na falha de pega do enxerto, sobrevida global e mortalidade póstransplante.A investigação dos anticorpos incluirá a determinação da patogenicidade dos DSAs pela avaliação da força/título, classe IgG versus IgM, capacidade de fixar complemento, epítopo alvo e nível de expressão da molécula HLA alvo. Metodologia Proposta: 1) Pesquisa de anticorpos anti-HLA pelo método de antígenos HLA isolados conjugados a microesferas de poliestireno em plataforma Luminex (SAB):Este método baseia-se na incubação do soro do paciente com um conjunto de antígenos HLA isolados de origem recombinante, que se encontram imobilizados em microesferas codificadas por coloração fluorescente, e posterior reação com imunoglobulina anti-IgG ou anti-IgM marcada com ficoeritrina (SCHINSTOCK; GANDHI; STEGALL, 2016). A designação da presença de anticorpos anti-HLA é feita com auxílio do programa HLA Fusion (One Lambda Inc., USA). Os testes do soro adsorvido e do eluato, soro diluído 1/8, HLA desnaturado após tratamento ácido e C1q utilizam esta mesma metodologia de antígenos HLA conjugados a microesferas de poliestireno em plataforma Luminex. Os soros que apresentarem background elevado na análise dos resultados pelo HLA Fusion devem ser tratados com reagente tipo “Adsorb Out” para a remoção de interferentes e submetidos novamente ao teste do A falha de pega do enxerto é uma grave complicação após o TCTH e está associada com morbidade e mortalidade elevadas (MATTSSON; RINGDÉN; STORB, 2008). Este processo fisiopatológico pode ser decorrente de alorreatividade celular mediada por células T e ou células natural killer bem como por alorreatividade humoral mediada por anticorpos específicos para moléculas HLA (LOCATELLI; LUCARELLI; MERLI, 2014). A associação da imunidade humoral com a incidência da rejeição do enxerto foi inicialmente descrita em 1989 pela observação de prova cruzada por citotoxicidade dependente do complemento (CDC) positiva entre o soro do receptor e linfócitos do doador (ANASETTI et al., 1989), sendo este fato posteriormente confirmado por Ottinger e colaboradores (OTTINGER et al., 2002). A evolução tecnológica das metodologias para detecção de anticorpos anti-HLA culminou com o desenvolvimento do sistema de moléculas HLA individualmente conjugadas às microesferas de

HOSPITAL DE CLÍNICAS DA UNIVERSIDADE FEDERAL DO PARANÁ - HCUFPR

poliestireno (Single Antigen Beads - SAB) (PEI et al., 2003). Este sistema se tornou amplamente difundido e possibilitou a identificação dos anticorpos anti-HLA com maior acurácia (GEBEL; LIWSKI; BRAY, 2013). A detecção de anticorpos contra moléculas HLA do doador no soro de receptores de células tronco hematopoiéticas, por métodos mais sensíveis como o SAB, tem sido associada à falha primária de pega do enxerto e/ou rejeição em vários estudos mais recentes (BRAND; DOXIADIS; ROELEN, 2013; FOCOSI; ZUCCA; SCATENA, 2011; YOSHIHARA et al., 2012). A presença de aloanticorpos no receptor com especificidade contra moléculas HLA incompatíveis do doador tem sido associada a efeitos adversos nos transplantes haploidênticos (CHANG et al., 2015; CIUREA et al., 2009; GLADSTONE et al., 2013), transplantes com sangue de cordão umbilical (CUTLER et al., 2011; FERNANDEZ-VINA; DE LIMA; CIUREA, 2011; TAKANASHI et al., 2010) e com doadores não consanguíneos (CIUREA et al., 2011; SPELLMAN et al., 2010). Os resultados de identificação de aloanticorpos específicos contra o doador são considerados na seleção de doadores com incompatibilidades HLA, a fim de reduzir o risco de falha de pega de enxerto e demais complicações pós-transplante decorrentes deste evento (KEKRE; ANTIN, 2014). Entretanto, o processo de seleção do doador pode ser prejudicado devido a algumas limitações da tecnologia Single Antigen Beads em plataforma Luminex (SCHINSTOCK; GANDHI; STEGALL, 2016; TAIT et al., 2013). Este sistema, atualmente o padrão ouro, tem algumas limitações tais como a identificação de anticorpos contra epítopos crípticos que são expostos devido à desnaturação de alguns tipos de moléculas HLA durante o processo de conjugação às microesferas (EL-AWAR et al., 2009b; MORALES-BUENROSTRO et al., 2008). Estes anticorpos contra epítopos crípticos não se mostraram clinicamente relevantes nos transplantes de órgãos sólidos (GOMBOS et al., 2013; SICARD et al., 2014), porém o impacto dos mesmos ainda não foi investigado nos TCTH. Uma das estratégias para sobrepujar esta limitação tecnológica é a caracterização destes aloanticorpos anti-HLA sob a luz da análise epitôpica (CAI; TERASAKI, 2008; ELLIS, 2013; TAMBUR; CLAAS, 2015). Outras ferramentas complementares que podem ser necessárias para a identificação fidedigna destes anticorpos são a adsorção seguida de eluição do soro com células que expressem a(s) molécula(s) HLA alvo dos supostos anticorpos doador-específicos, ou ainda a utilização de Single Antigen Beads com moléculas HLA intencionalmente desnaturadas após tratamento ácido (EL-AWAR et al., 2009a, 2009b; VISENTIN et al., 2014).Os estudos prévios que associaram DSA com falha de pega /rejeição do enxerto levaram em consideração somente o valor de cutoff na interpretação dos resultados do SAB (BRAND; DOXIADIS; ROELEN, 2013; YOSHIHARA et al., 2012), sendo este tipo de análise insuficiente para caracterizar com acurácia os anticorpos anti-HLA. A utilização somente do cutoff como critério de análise pode gerar resultados falso-positivos que excluam

HOSPITAL DE CLÍNICAS DA UNIVERSIDADE FEDERAL DO PARANÁ - HCUFPR

desnecessariamente um doador, ou resultados falso-negativos que levem à escolha de um doador que aumente o risco de falha de pega/rejeição do enxerto (ELLIS, 2013). Isto demonstra a relevância do presente estudo que visa conduzir uma identificação mais abrangente destes anticorpos utilizando estratégias fundamentadas na análise epitôpica.

Metodologia Proposta: 1) Pesquisa de anticorpos anti-HLA pelo método de antígenos HLA isolados conjugados a microesferas de poliestireno em plataforma Luminex (SAB):Este método baseia-se na incubação do soro do paciente com um conjunto de antígenos HLA isolados de origem recombinante, que se encontram imobilizados em microesferas codificadas por coloração fluorescente, e posterior reação com imunoglobulina anti-IgG ou anti-IgM marcada com ficoeritrina (SCHINSTOCK; GANDHI; STEGALL, 2016). A designação da presença de anticorpos anti-HLA é feita com auxílio do programa HLA Fusion (One Lambda Inc., USA). Os testes do soro adsorvido e do eluato, soro diluído 1/8, HLA desnaturado após tratamento ácido e C1q utilizam esta mesma metodologia de antígenos HLA conjugados a microesferas de poliestireno em plataforma Luminex. Os soros que apresentarem background elevado na análise dos resultados pelo HLA Fusion devem ser tratados com reagente tipo “Adsorb Out” para a remoção de interferentes e submetidos novamente ao teste do SAB. 2)Programas computacionais de análise e interpretação dos resultados para caracterização dos anticorpos HLA: A análise da reatividade epitôpica dos anticorpos anti-HLA será realizada com auxílio de dois programas computacionais denominados HLA Fusion (One Lambda Inc., USA) e EpVix (Universidade Federal do Piauí, Brasil; http://www.epvix.com.br). O HLA Fusion utiliza os Epítopos Terasaki também denominados “TerEps” (EL-AWAR et al., 2009a), enquanto o EpVix (ANUNCIAÇÃO et al., 2015) utiliza os epítopos definidos por Duquesnoy que são denominados “Eplets” (DUQUESNOY, 2006). As análises dos “TerEps” e dos “Eplets” serão realizadas de modo independente e depois integradas para obtenção do resultado final. Os epítopos HLA caracterizados serão validados com os dados presentes no website HLA Epitope Registry (http://www.epregistry.com.br/) (DUQUESNOY et al., 2013). 3)Prova Cruzada Virtual: será realizada por meio da comparação dos anticorpos antiHLA presentes no soro do paciente com a tipificação HLA do doador de células-tronco hematopoiéticas (GUTMAN et al., 2009; TAMBUR et al., 2009). Para obtenção de uma prova cruzada virtual fidedigna é necessário que os anticorpos anti-HLA no receptor estejam caracterizados em nível epitópico e que a tipificação HLA do doador esteja completa para os locos HLA-A, B, C, DRB1, DRB3,4,5, DQA1, DQB1, DPA1 e DPB1. De modo geral, valores de MFI acima de 5000 condizem com prova cruzada positiva por citometria de fluxo, e valores de MFI maiores que 10.000 com resultado positivo na prova cruzada por CDC (BRAND; DOXIADIS; ROELEN, 2013). Critério de Inclusão: Pacientes que

Endereço: Rua Gal. Carneiro, 181
Bairro: Alto da Glória **CEP:** 80.060-900
UF: PR **Município:** CURITIBA
Telefone: (41)3360-1041 **Fax:** (41)3360-1041 **E-mail:** cep@hc.ufpr.br

realizaram transplante de células-tronco hematopoiéticas no Hospital de Clínicas da UFPR, no período entre 2008 e 2017, com doadores que apresentaram incompatibilidades HLA,e com teste de pesqusia de anticorpos anti-HLA no soro do receptor antes do transplante.

Objetivo da Pesquisa:

Objetivo Primário: Caracterizar os anticorpos anti-HLA em pacientes com doenças onco-hematológicas, transplantados no HC-UFPR entre janeiro de 2008 e dezembro de 2017, e avaliar o impacto dos anticorpos específicos contra o doador (DSA) na evolução clínica dos transplantes de células-tronco hematopoiéticas com incompatibilidades HLA. Objetivo Secundário: Objetivo Específico 1: Caracterização dos anticorpos anti -HLA - Analisar o padrão de reatividade epitôpica dos DSA;Investigar a incidência de DSA da classe IgM; Confirmar a prova cruzada virtual fundamentada na análise epitôpica por meio de adsorção/eluição; Verificar a força de reação dos DSA pelo teste SAB/plataforma Luminex nos soros puros e diluidos 1:8; Investigar a incidência de DSA contra epítopos crípticos em moléculas HLA desnaturadas;Avaliar a capacidade de fixação do complemento dos DSA pelo teste SAB/plataforma Luminex com adição do C1q. Objetivo Específico 2: Investigar a influência dos DSAs na evolução clínica pós-transplante na falha de pega do enxerto, sobrevida global e mortalidade póstransplante.A investigação dos anticorpos incluirá a determinação da patogenicidade dos DSAs pela avaliação da força/título, classe IgG versus IgM, capacidade de fixar complemento, epítopo alvo e nível de expressão da molécula HLA alvo.

Avaliação dos Riscos e Benefícios:

Riscos: Não haverá riscos diretos para os participantes porque este estudo será constituído de avaliação retrospectiva de resultados dos testes laboratoriais, principalmente a pesquisa de anticorpos anti-HLA, realizados somente in vitro. O único risco indireto associado a este estudo seria um eventual extravio de informações dos pacientes participantes. Para minimizar este fato, o acesso à informação será limitado aos pesquisadores integrantes deste estudo. Benefícios: Não ocorrerá beneficio direto algum aos participante, uma vez que o estudo proposto trata-se de análise retrospectiva. No entanto, os achados deste estudo poderão fornecer informações relevantes para transplantes futuros quanto: 1) ao processo de seleção de doadores HLA incompatíveis; 2) as estratégias terapêuticas empregadas para contornar a presença destes anticorpos quando necessário utilizar um doador que apresente moléculas HLA incompatíveis e alvo dos anticorpos HLA; 3) a estratificação de risco dos DSAs.

CEP/HC/UFPR

HOSPITAL DE CLÍNICAS DA UNIVERSIDADE FEDERAL DO PARANÁ - HCUFPR

Comentários e Considerações sobre a Pesquisa:
Emenda encaminhada pelo investigador Nº3 de 30/05/2021

• ALTERAÇÃO DE CRONOGRAMA PARA FINALIZAÇÃO DO PROJETO E INCLUSÃO DE CASUÍSTICA

1-QUAL A JUSTIFICATIVA PARA EMISSÃO DESTE DOCUMENTO:
O cronograma de atividades deste projeto foi severamente comprometido por vários entraves burocráticos e logísticos ao longo de 2019 e 2020. Informamos que a liberação dos recursos provenientes do projeto PRONON ocorreu somente no dia 25/04/2019. Além disso, a aquisição, importação e entrega dos reagentes do projeto foi prejudicada pelo valor elevado da cotação do dólar ao longo de 2019 e 2020 (Cotação de R$ 3,25 na apresentação do projeto em 2016 e R$ 5,2830 no momento da efetivação da importação) e pelas dificuldades logísticas decorrentes da Pandemia de Coronavírus. Desta forma, foi solicitada a extensão do prazo do projeto junto ao Programa Nacional de Apoio à Atenção Oncológica (PRONON). A alteração do novo plano de trabalho, com 06 meses de prorrogação e data de finalização até outubro de 2021, foi aprovada em 22/01/2021 pelo PRONON/Ministério da Saúde (Parecer Técnico Nº 12/2021-COPP/CGFPS/DECIT/SCTIE/MS). Ressaltamos que a primeira remessa de reagentes importados foi entregue somente no dia 10/11/2020, e a última aquisição de reagentes do presente projeto só foi concretizada em 27/04/2021. Desta forma, solicitamos ao CEP a extensão de 6 meses adicionais do referido projeto, em conformidade com a nova proposta de cronograma anexada na Plataforma Brasil

Considerações sobre os Termos de apresentação obrigatória:
Estão adequados

Recomendações:
Não há recomendações

Conclusões ou Pendências e Lista de Inadequações:
Emenda aprovada

Considerações Finais a critério do CEP:
Diante do exposto, o Comitê de Ética em Pesquisa em Seres Humanos do HC-UFPR, de acordo com as atribuições definidas na Resolução CNS 466/2012 e na Norma Operacional Nº 001/2013 do CNS, manifesta -se pela aprovação da Emenda.

Endereço: Rua Gal. Carneiro, 181
Bairro: Alto da Glória **CEP:** 80.060-900
UF: PR **Município:** CURITIBA
Telefone: (41)3360-1041 **Fax:** (41)3360-1041 **E-mail:** cep@hc.ufpr.br

HOSPITAL DE CLÍNICAS DA UNIVERSIDADE FEDERAL DO PARANÁ - HCUFPR

Solicitamos que sejam apresentados a este CEP, relatórios semestrais sobre o andamento da pesquisa, bem como informações relativas às modificações do protocolo, cancelamento, encerramento e destino dos conhecimentos obtidos. Manter os documentos da pesquisa arquivados.
É dever do CEP acompanhar o desenvolvimento dos projetos, por meio de relatórios semestrais dos pesquisadores e de outras estratégias de monitoramento, de acordo com o risco inerente à pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1495489_E1.pdf	30/05/2021 18:24:16		Aceito
Outros	CARTA_PARA_APRESENTACAO_JUSTIFICATIVA_PARA_EMENDA.docx	23/05/2021 14:38:51	ALBERTO CARDOSO	Aceito
Solicitação Assinada pelo Pesquisador Responsável	CARTA_PARA_APRESENTACAO_JUSTIFICATIVA_PARA_EMENDA.pdf	23/05/2021 14:32:08	ALBERTO CARDOSO MARTINS LIMA	Aceito
Cronograma	CRONOGRAMA_EMENDA.pdf	19/05/2021 15:08:34	ALBERTO CARDOSO	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_ANTICORPOS_ANTI_HLA_TCTH_ATUALIZADO_EMENDA.pdf	19/05/2021 15:08:17	ALBERTO CARDOSO MARTINS LIMA	Aceito
Declaração de Pesquisadores	CARTA_ENCAMINHAMENTO_CEP_RESPOSTAS_PARECER_2880419.pdf	26/09/2018 12:30:41	ALBERTO CARDOSO	Aceito
Orçamento	OFICIO_AAHC_ORCAMENTO_PARECER_2880419.pdf	26/09/2018 12:28:48	ALBERTO CARDOSO	Aceito
Orçamento	TERMO_DE_COMPROMISSO_PROJETO_AC_ANTI_HLA.pdf	26/09/2018 12:27:47	ALBERTO CARDOSO	Aceito
Orçamento	DOU_04_JUNHO_2018_32.pdf	26/09/2018 12:27:13	ALBERTO CARDOSO	Aceito
Orçamento	DOU_01_DEZEMBRO_2017_113.pdf	26/09/2018 12:27:05	ALBERTO CARDOSO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TERMO_ASSENTIMENTO_INFORMADO_LIVRE_ESCLARECIDO_12_17_ANOS_ATUALIZADO.pdf	26/09/2018 12:26:33	ALBERTO CARDOSO MARTINS LIMA	Aceito
TCLE / Termos de Assentimento / Justificativa de	TERMO_ASSENTIMENTO_INFORMADO_LIVRE_ESCLARECIDO_7_12_ANOS_ATUALIZADO.pdf	26/09/2018 12:26:23	ALBERTO CARDOSO MARTINS LIMA	Aceito

Endereço: Rua Gal. Carneiro, 181
Bairro: Alto da Glória **CEP:** 80.060-900
UF: PR **Município:** CURITIBA
Telefone: (41)3360-1041 **Fax:** (41)3360-1041 **E-mail:** cep@hc.ufpr.br

HOSPITAL DE CLÍNICAS DA UNIVERSIDADE FEDERAL DO PARANÁ - HCUFPR

Continuação do Parecer: 4.760.973

Ausência	TERMO_ASSENTIMENTO_INFORMADO_LIVRE_ESCLARECIDO_7_12_ANOS_ATUALIZADO.pdf	26/09/2018 12:26:23	ALBERTO CARDOSO MARTINS LIMA	Aceito
Orçamento	ORCAMENTO_AAHC_ATUALIZADO_TERMO_COMPROMISSO.pdf	26/09/2018 12:21:50	ALBERTO CARDOSO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TERMO_CONSENTIMENTO_LIVRE_ESCLARECIDO_CRIANCA.pdf	13/08/2018 14:55:25	ALBERTO CARDOSO MARTINS LIMA	Aceito
Outros	CHECK_LIST.pdf	13/08/2018 14:51:53	ALBERTO CARDOSO	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_ANTICORPOS_ANTI_HLA_TCTH.pdf	13/08/2018 14:50:46	ALBERTO CARDOSO MARTINS LIMA	Aceito
Orçamento	ORCAMENTO_AAHC.pdf	13/08/2018 14:33:07	ALBERTO CARDOSO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	DISPENSA_TERMO_CONSENTIMENTO.pdf	13/08/2018 14:32:50	ALBERTO CARDOSO MARTINS LIMA	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TERMO_ASSENTIMENTO_INFORMADO_LIVRE_ESCLARECIDO.pdf	13/08/2018 14:32:28	ALBERTO CARDOSO MARTINS LIMA	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TERMO_CONSENTIMENTO_LIVRE_ESCLARECIDO_ADULTO.pdf	13/08/2018 14:32:02	ALBERTO CARDOSO MARTINS LIMA	Aceito
Declaração de Pesquisadores	TERMO_RESPONSABILIDADE_PESQUISA.pdf	13/08/2018 14:31:40	ALBERTO CARDOSO	Aceito
Declaração de Pesquisadores	TERMO_COMPROMISSO_UTILIZACAO_DADOS_ARQUIVOS.pdf	13/08/2018 14:31:28	ALBERTO CARDOSO	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	TERMO_GUARDA_MATERIAL_BIOLOGICO.pdf	13/08/2018 14:31:10	ALBERTO CARDOSO MARTINS LIMA	Aceito
Declaração de Pesquisadores	QUALIFICACAO_TODOS_OS_PESQUISADORES.pdf	13/08/2018 14:30:46	ALBERTO CARDOSO	Aceito
Declaração de Pesquisadores	DECLARACAO_COMPROMISSO_PESQUISADORES.pdf	13/08/2018 14:30:04	ALBERTO CARDOSO	Aceito
Declaração de Pesquisadores	DECLARACAO_USO_MATERIAL_DADOS_COLETADOS.pdf	13/08/2018 14:29:50	ALBERTO CARDOSO	Aceito
Declaração de Pesquisadores	DECLARACAO_DE_TORNAR_PUBLICOS_OS_RESULTADOS.pdf	13/08/2018 14:29:29	ALBERTO CARDOSO	Aceito
Declaração de Pesquisadores	TERMO_CONFIDENCIALIDADE.pdf	13/08/2018 14:29:02	ALBERTO CARDOSO	Aceito

HOSPITAL DE CLÍNICAS DA UNIVERSIDADE FEDERAL DO PARANÁ - HCUFPR

Continuação do Parecer: 4.760.973

Declaração de Instituição e Infraestrutura	CONCORDANCIA_STMO.pdf	13/08/2018 14:27:33	ALBERTO CARDOSO MARTINS LIMA	Aceito
Declaração de Instituição e Infraestrutura	CONCORDANCIA_ULAC.pdf	13/08/2018 14:27:19	ALBERTO CARDOSO MARTINS LIMA	Aceito
Declaração de Instituição e Infraestrutura	CONCORDANCIA_IMUNOGENETICA.pdf	13/08/2018 14:27:13	ALBERTO CARDOSO MARTINS LIMA	Aceito
Declaração de Pesquisadores	DECLARACAO_ORIENTADOR_ALUNO.pdf	13/08/2018 14:26:48	ALBERTO CARDOSO	Aceito
Declaração de Pesquisadores	CARTA_ENCAMINHAMENTO_CEP.pdf	13/08/2018 14:25:21	ALBERTO CARDOSO	Aceito
Folha de Rosto	FOLHA_DE_ROSTO_PLATAFORMA_BRASIL.pdf	13/08/2018 14:12:16	ALBERTO CARDOSO	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CURITIBA, 08 de Junho de 2021

Assinado por:
Niazy Ramos Filho
(Coordenador(a))

Endereço: Rua Gal. Carneiro, 181
Bairro: Alto da Glória **CEP:** 80.060-900
UF: PR **Município:** CURITIBA
Telefone: (41)3360-1041 **Fax:** (41)3360-1041 **E-mail:** cep@hc.ufpr.br

APPENDIX 2. List of awards, other publications, scientific presentations, and educational activities during the Ph.D. (2019-2023)

Awards as the first author:

- *“2022 José Roberto Moraes Award - Best Scientific Abstract in Histocompatibility and Immunogenetics”*, 2022 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy.
- *“2021 José Roberto Moraes Award - Best Scientific Abstract in Histocompatibility and Immunogenetics”*, 2021 Annual Meeting of the Brazilian Society of Organ Transplantation.
- *“2020 José Roberto Moraes Award - Best Scientific Abstract in Histocompatibility and Immunogenetics”*, 2020 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation.
- *“2021 Alirio Pfiffer Award - Best Abstract in Bone Marrow Failure”*, 2021 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy.
- *“2020 Alirio Pfiffer Award - Best Abstract in Bone Marrow Failure”*, 2020 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy.

Awards as the co-author heading the statistical analyses:

- *“2021 Mary Flowers Award - Best Scientific Abstract in Clinical Aspects of HSCT”*, 2021 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy.
- *“2021 Carmem Bonfim Award - Best Scientific Abstract in the Pediatric HSCT”*, 2021 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy.

Publications as the co-author heading the statistical analyses:

- Bonfim C, Nichele S, Loth G, Funke VAM, Nabhan SK, Pillonetto DV, **Lima ACM**, Pasquini R. *Transplantation for Fanconi anaemia: lessons learned from Brazil*. Lancet Haematol. 2022 Mar;9(3): e228-e236. doi: 10.1016/S2352-3026(22)00032-1.
- Belinovski AR, Pelegrina PD, **Lima ACM**, Dumke CCK, Rodrigues AM, Loth G, Benini FML, Rodrigues ALM, Motta FA, Prando C, Bonfim C. *Immune reconstitution after*

allogenic stem cell transplantation: An observational study in pediatric patients. Hematol Transfus Cell Ther. 2022 Jul 7: S2531-1379(22)00090-6. doi: 10.1016/j.htct.2022.05.008.

- Pillonetto DV, Piovezan BZ, Nichele S, **Lima ACM**, Pasquini R, Pereira NF, Bonfim C. *Investigation of mutations in Fanconi anemia genes and malignancy predisposition in Brazilian patients*. Int J Lab Hematol. 2022 Nov 5. doi: 10.1111/ijlh.13986.

Publications as the senior author:

- Torres MA, Rampim GF, Cardoso JF, Vergueiro C, Pereira NF, **Lima ACM**. *DONOR SELECTION: IMMUNOGENETICS AND HISTOCOMPATIBILITY*. JBMTCT, 2(1), 22–27. https://doi.org/10.46765/2675-374X.2021v4n1p22-27 (Consensus Guidelines for HSCT from the Brazilian Society for Blood and Marrow Transplantation and Cellular Therapy).

Scientific presentations in International Meetings as first author:

- **Lima, ACM**; Getz, J; do Amaral, GB; Pereira, NF; Bonfim, C; Loth, G; Nabhan, SK; Pasquini, R. *Donor-specific anti-HLA antibodies are associated with poor outcomes following haploidentical transplantation with posttransplant cyclophosphamide in pediatric patients with nonmalignant disorders*. HUMAN IMMUNOLOGY, 2021. P43. Volume 82, Supplement, September 2021, Page 94-95.
- **Lima, ACM**; Getz, J; Dornelles, LN; Amaral, GB; Loth, G; Funke, VA; Nabhan, SK; Pereira, NF; Bonfim, C; Pasquini, R. *Impact of donor-specific anti-HLA antibodies on graft failure after unrelated donor hematopoietic cell transplantation*. HUMAN IMMUNOLOGY, 2020. O186. Volume 81, Supplement, November 2020, Page 30.
- **Lima, ACM**; Feitosa, MK; Dornelles, LN; do Amaral, GB; Loth, G; Nichele, S; Funke, VAM; Nabhan, SK; Pereira, NF; Bonfim, C; Pasquini, R. *Alloreactivity Against HLA-DPB1 in Host-Versus-Graft Direction is Associated with Increased Risk of Graft Failure After Matched Unrelated Donor Transplantation for Nonmalignant Diseases*. BONE MARROW TRANSPLANTATION. 2020. O166, Vol. 55, SUPPL 1: Page 169-169.

Scientific presentations in International Meetings as co-author heading the statistical analyses:

- Bonfim, C; Conti, BFMD; Muratori, R; Nichele, S; Loth, G; Koliski, A; Dumke, CCK; Pelegrina, PRD; Benini, FMD; Mosquer, RATG; Rodrigues, AM; Ribeiro, L; Bach, JLM; Peixoto, CMD; Trennepohl, JP; de Gouvea, LMM; **Lima, ACM**; Pasquini, R. *Excellent*

outcomes after haploidentical bone marrow transplantation using post-transplant cyclophosphamide in 25 patients with Wiskott-Aldrich syndrome. BONE MARROW TRANSPLANTATION. 2022. O133, Vol. 57, SUPPL 1: Page 85.

- Muratori, R; Nichele, S; Loth, G; Koliski, A; Dumke, CCK; Benini, FMD; Pelegrina, PRD; Mosquer, RATG; Rodrigues, AM; Rossoni, AMD; Ribeiro, L; Bach, JLD; Peixoto, CMD; Trennepohl, JP; de Gouvea, LMM; **Lima, ACM**; Pasquini, R; Bonfim, C. *Alternative donor stem cell transplantation with post-transplantation cyclophosphamide in 37 children with severe combined immunodeficiency (SCID)*. BONE MARROW TRANSPLANTATION. 2022. O74, Vol. 57, SUPPL 1: Page 51.
- Funke, VAM.; **Lima, ACM**; Hamerschlak, N; et al. *161 - DIPSS Score Validation as a Tool to Estimate Survival and Non-Relapse Mortality after Hematopoietic Stem Cell Transplant in a Brazilian Multicentric Cohort*. ABSTRACTS FROM THE 2022 TANDEM MEETINGS OF ASTCT AND CIBMTR, v. 28, n. 3, Supplement, p. S131–S133, 2022.
- Bonfim, C; Nichele, S; Ribeiro, L; Loth, G; Kuwahara, C; Rodrigues, ALM; Benini, FML; Koliski, A; Marinho, D; Mousquer, RTG; Renoste, S; Trennepohl, J; Rodrigues, AM; **Lima, ACM**; Pilonetto, D; Pereira, NF; Pasquini, R. *Haploidentical transplantation with post-transplant cyclophosphamide (Haplo-PTCy) for 71 patients with acquired or inherited bone marrow failure syndromes (BMF): The experience from Curitiba*, Brazil. BONE MARROW TRANSPLANTATION. 2019. O26, Vol. 54, SUPPL 1: Pages 28-29.

Lectures on DSA and HSCT in International Meetings:

- *“Donor-specific HLA antibodies in hematopoietic stem cell transplantation: Practical aspects in alternative donor selection”*, XVI Portuguese Transplantation Meeting, Portuguese Transplantation Society (2022).

Lectures on DSA and HSCT in National Meetings:

- *“Primary graft rejection and poor graft function after haploidentical HSCT with PTCy: Impact of donor-specific HLA antibodies (DSA)”,* 2022 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy.
- *“The perspective of HLA laboratory on post-transplant DSA monitoring: a challenging case”*, 2021 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy.
- *“Donor anti-HLA antibodies in allo-HSCT: State of the Art in 2021”*, 2021 Brazilian Transplantation Congress, Brazilian Society of Organ Transplantation.

- *“Graft Failure after Allogeneic Hematopoietic Cell Transplantation: HLA Lab perspective”*, 2020 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy.
- *“Virtual Crossmatch in Bone Marrow Transplantation”*, 2019 Brazilian Transplantation Congress, Brazilian Society of Organ Transplantation.
- *“The Epitope Time - Finding the Target of Anti-HLA Antibodies: The HC/UFPR approach”*, 2019 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation.
- *“Donor anti-HLA antibodies in Haploidentical HCT for Nonmalignant Diseases: The HC/UFPR experience”*, 2019 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation.

Lectures on DSA and HSCT to Professional Societies:

- *“Impact of DSA in allo-HSCT”* lecture for the Pediatric Group of Brazilian Society of Bone Marrow Transplantation and Cellular Therapy, Brazilian Association of Histocompatibility and Immunogenetics, and Department of Immunobiology of the Brazilian Society of Organ Transplantation (2019, 2020, and 2022).

Lectures on DSA and HSCT to Brazilian HLA laboratories and HSCT units:

- "*Impact of DSA in allo-HSCT*" lecture for CHC/UFPR HSCT Unit, Hospital Pequeno Príncipe HSCT Unit, UFMG HSCT Unit, UFJF HSCT Unit, Hospital Santa Marcelina HSCT Unit, Hospital Brigadeiro HSCT Unit, HLA laboratory of Hospital Cajuru, HLA laboratory of Hospital de Clínicas de Porto Alegre, HLA laboratory of the Fundação Centro de Hemoterapia e Hematologia do Pará (2019, 2020, 2021, 2022, and 2023).

Lectures on Donor Selection and Immunological Risk Stratification:

- *“Immunological Risk Stratification: Practical Aspects of Donor Selection”*, Anat Tambur HLA Laboratory, Northwestern University, Chicago, USA (2022).
- *“Immunological Risk Stratification: Practical Aspects of Donor Selection”*, Paraná Regional Meeting of the Brazilian Society of Bone Marrow Transplantation (2022)
- *“Immunological Risk Stratification: Practical Aspects of Donor Selection”,* for the Hospital Alemão Oswaldo Cruz HSCT Unit, Hospital Santa Marcelina HSCT Unit, Hospital Brigadeiro HSCT Unit, and Hospital São Paulo HSCT fellows (2022).

- *“Immunological Risk Stratification: Practical Aspects of Donor Selection”,* for the Multiprofessional Residency and Laboratory Medicine Fellowship Program of the Complexo do Hospital de Clínicas, Federal University of Paraná (2022).
- *“Immunobiology of Relapse: The impact of HLA-DPB1 on Unrelated Donor Selection”*, 2022 I Multiregional Meeting “Relapse after HSCT: prevention and treatment”, Brazilian Society of Bone Marrow Transplantation and Cellular Therapy.
- *“HLA-DPB1 in Unrelated Hematopoietic Cell Transplantation for Nonmalignant Diseases”*, 2020 Annual Meeting of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy.
- *“HLA-DPB1 permissiveness and unrelated donor selection for allo-HSCT”,* for the Pediatric Group of Brazilian Society of Bone Marrow Transplantation and Cellular Therapy (2019, 2020).

Educational Activities on Histocompatibility and Donor Selection:

- *“Histocompatibility in HSCT course”*, for the Histocompatibility and Immunogenetics Group of the Portuguese Transplantation Society and Brazilian Association of Histocompatibility and Immunogenetics (2023).
- *“HLA and Donor Selection module”* in the "*Mais TMO/PROADI*" project of the Beneficência Portuguesa de São Paulo (2022).
- *“Histocompatibility Training course”* (*Chapters: DSA in HSCT and Immunobiology of Rejection and GVHD*), Brazilian Histocompatibility and Immunogenetics Association (2021).

Educational Activities on Statistical Analyses:

- *“Statistical Methods in HSCT: Part I and II”* course for the Pediatric Group of Brazilian Society of Bone Marrow Transplantation and Cellular Therapy (2022).
- *“Statistics in Postgraduate Studies”* course for the Doctoral program in Internal Medicine and Health Sciences, Federal University of Paraná (2020, 2021, and 2022).
- *“Statistics in Postgraduate Studies”* course for the Multiprofessional Residency and Laboratory Medicine Fellowship Program of Complexo do Hospital de Clínicas, Federal University of Paraná (2022)
- *“Survival Analysis and Multivariable Regression in HSCT”,* lecture for the HSCT Unit of the CHC/UFPR (2022).

- *“Advanced Statistics module”*, in the HSCT's Data Managers training course, Brazilian Society of Bone Marrow Transplantation and Cellular Therapy (2022)
- *“Statistics and Use of EZR”* lecture at the HSCT's Data Managers meeting, Brazilian Society of Bone Marrow Transplantation and Cellular Therapy congress (2020).