Not for publication or presentation

A G E N D A
CIBMTR WORKING COMMITTEE FOR IMMUNOBIOLOGY
Houston, TX
Thursday, February 21, 2019, 12:15 pm– 4:45 pm

Co-Chair:	**Katharina Fleischhauer, MD; Essen University Hospital;**
	Telephone: +49-201-723-4582; E-mail: Katharina.Fleischhauer@uk-essen.de
Co-Chair:	**Katharine Hsu, MD, PhD; Memorial Sloan-Kettering Cancer Center;**
	Telephone: 646-888-2667; E-mail: hsuk@mskcc.org
Co-Chair:	**Sophie Paczesny, MD, PhD; Indiana University Hospital;**
	Telephone: 317-278-5487; E-mail: sophpacz@iu.edu
Co-Scientific Dir:	**Stephanie Lee, MD, MPH, Fred Hutchinson Cancer Research Center**
	Telephone: 206-667-6190; E-mail: sjlee@fredhutch.org
Co-Scientific Dir:	**Stephen Spellman, MBS, CIBMTR Immunobiology Research**
	Telephone: 763-406-8334; E-mail: sspellma@nmdp.org
Statistical Director:	**Tao Wang, PhD, CIBMTR Statistical Center**
	Telephone: 414-955-4339; E-mail: taowang@mcw.edu
Statisticians:	**Michelle Kuxhausen, MS, CIBMTR Statistical Center**
	Telephone: 763-406-8727; E-mail: mformane@nmdp.org

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1. Introduction 12:15pm

a. Minutes and Overview Plan of Immunobiology Working Committee from Tandem 2018 *(Attachment 1)*
b. Newly appointed chair: Steven Marsh, BSc, PhD, ARCS; Anthony Nolan Research Institute; Telephone: +44 20 7284 8321; E-mail: steven.marsh@ucl.ac.uk

2. Published or submitted papers 12:25pm

a. **IB06-05** Patient HLA germline variation and transplant survivorship. Petersdorf EW, Stevenson P, Malkki M, Strong RK, Spellman SR, Haagenson MD, Horowitz MM, Gooley T, Wang T. ***J Clin Oncol*. 2018 Aug 20; 36(24):2524-2531. doi:10.1200/JCO.2017.77.6534. Epub 2018 Jun 14. PMC6097831.**

b. **IB09-06/RT09-04c** Exome chip Analyses Identify Genes affecting mortality after HLA-Matched Unrelated Donor Blood and Marrow Transplantation Qian Liu, Qiang Hu, Leah Preus, Alyssa I. Clay, Ken Onel, Daniel O. Stram, Loreall Pooler, Xin Sheng, Christopher A. Haiman, Xiaochun Zhu, Stephen R. Spellman, Marcelo Pasquini, Philip L. McCarthy , Song Liu, Theresa Hahn, Lara E. Sucheston-Campbell. ***Blood.* 2018 May 31; 131(22):2490-2499. doi:10.1182/blood-2017-11-817973. Epub 2018 Apr 2. PMC5981168.**

c. **IB10-01d** Flow Cytometry using FISH techniques in a Severe Aplastic Anemia population. Gadalla S, Aubert G, Wang T, Haagenson M, Spellman SR, Wang L, Katki HA, Savage S, Lee SJ. ***Mol Genet Genomic Med*. 2016 Jul 1; 4(4):475-479. doi:10.1002/mgg3.220. Epub 2016 Mar 20. PMC4947866.**

d. **IB10-01e** Chromosomal aberrations and survival after unrelated donor hematopoietic stem cell transplant in patients with Fanconi anemia. Wang Y, Zhou W, Alter BP, Wang T, Spellman SR, Haagenson M, Yeager M, Lee SJ, Chanock SJ, Savage SA, Gadalla SM. ***Biol Blood Marrow Transplant.* 2018 Oct 1; 24(10):2003-2008. doi:10.1016/j.bbmt.2018.05.027. Epub 2018 Jun 4. PMC6239962.**

e. **IB10-01g** Telomere length calibration from qPCR measurement: Limitations of current method. Wang Y, Savage SA, Alsaggaf R, Aubert G, Dagnall CL, Spellman SR, Lee SJ, Hicks B, Jones K, Katki HA, Gadalla SM. ***Cells.* 7(11):183. doi:10.3390/cells7110183. Epub 2018 Oct 24. PMC6262465.**

f. **IB11-01a** The effect of NIMA matching in adult unrelated mismatched hematopoietic stem cell transplantation - a joint study of the Acute Leukemia Working Party of the EBMT and the CIBMTR. Pingel J, Wang T, Hagenlocher Y, Hernández-Frederick CJ, Nagler A, Haagenson MD, Fleischhauer K, Hsu KC, Verneris MR, Lee SJ, Mohty M, Polge E, Spellman SR, Schmidt AH, van Rood JJ. ***Bone Marrow Transplant*. doi:10.1038/s41409-018-0345-8. Epub 2018 Oct 2.**

g. **IB12-02c** In silico prediction of nonpermissive HLA-DPB1 mismatches in unrelated HCT by functional distance. Arrieta-Bolaños E, Crivello P, Shaw BE, Ahn KW, Wang H-L, Verneris MR, Hsu KC, Pidala J, Lee SJ, Fleischhauer K, Spellman SR**. *Blood Advances*. 2018 Jul 24; 2(14):1773-1783. doi:10.1182/bloodadvances.2018019620. Epub 2018 Jul 24. PMC6058232.**

h. **IB13-08** Prediction of Acute Graft-Versus-Host Disease Following Hematopoietic Cell Transplantation. Lee C, Haneuse S, Wang H, Rose S, Spellman SR, Verneris M, Hsu K, Fleischhauer K, Lee SJ, Abdi R. ***PLOS1* 13(1):e0190610. doi:10.1371/journal.pone.0190610. Epub 2018 Jan 18. PMC5773230.**

i. **IB14-06** Donor-specific anti-HLA antibodies in unrelated hematopoietic cell transplantation for non-malignant disorders. Woolfrey A, Wang T, Lee SJ, Haagenson MD, Chen G, Fleischhauer K, Horan J, Hsu K, Tyan D, Verneris M, Spellman SR, Fernandez-Vina M. ***Bone Marrow Transplantation.* doi:10.1038/s41409-018-0334-y. Epub 2018 Sep 19.**

j. **IB14-08** Development and validation of a clinical unrelated donor selection score. Shaw BE, Logan BR, Spellman SR, Marsh SGE, Robinson J, Pidala J, Hurley C, Barker J, Maiers M, Dehn J, Wang H, Haagenson M, Porter D, Petersdorf EW, Woolfrey A, Horowitz MM, Verneris M, Hsu KC, Fleischhauer K, Lee SJ. ***Biol Blood Marrow Transplant.* 2018 May 1; 24(5):1049-1056. doi:10.1016/j.bbmt.2018.02.006. Epub 2018 Feb 14. PMC5953795.**

k. **IB15-01** Analysis of single nucleotide polymorphisms in the gamma block of the major histocompatibility complex in association with clinical outcomes of hematopoietic cell transplantation: A CIBMTR study. Askar M, Sayer D, Wang T, Haagenson M, Spellman SR, Lee SJ, Madbouly A, Fleischhauer K, Hsu KC, Verneris MR, Thomas D, Zhang A, Sobecks R,

Majhail NS. ***Biol Blood Marrow Transplant*. doi:10.1016/j.bbmt.2018.12.008. Epub 2018 Dec 18.**

l. **IB15-02** Donor killer-cell immunoglobulin-like receptor (KIR) genotype does not improve graft-versus-leukemia responses in chronic lymphocytic leukemia (CLL) after unrelated donor transplant: a CIBMTR analysis. Bachanova V, Weisdorf DJ, Wang T, Marsh SGE, Cereb N, Haagenson MD, Spellman SR, Lee SJ, Guethlein LA, Parham P, Miller JS, Cooley S. ***Biol Blood Marrow Transplant. doi:10.1016/j.bbmt.2018.12.763. Epub 2018 Dec 27.***

m. **IB15-06b** Evaluation of a Machine Learning-Based Prognostic Model for Unrelated Hematopoietic Cell Transplantation Donor Selection. Buturovic L, Shelton J, Spellman SR, Wang T, Friedman L, Loftus D, Hesterberg L, Woodring T, Fleischhauer K, Hsu KC, Verneris MR, Haagenson M, Lee SJ. ***Biol Blood Marrow Transplant* 2018 Jun 1; 24(6):1299-1306. doi:10.1016/j.bbmt.2018.01.038. Epub 2018 Feb 1. PMC5993610.**

n. **IB10-01c** Telomere length telomerase polymorphism in Severe Aplastic Anemia - Exome Analysis and Mosaicism. Gadalla S, Savage S. **Submitted. Nature Communications**

o. **IB15-04** Clinical outcomes among hematopoietic stem cell transplant recipients as a function of socioeconomic status and related transcriptome differences. Knight J, Rizzo JD, Cole S. **Submitted. JNCI Cancer Spectrum**

p. **IB15-07** Functional genetic variants of the ST2 gene in pairs of recipient and donors for risk stratification of GVHD and TRM outcomes. Paczesny S. **Submitted. Blood**

3. Research repository update and accrual tables *(Attachment 2)* 12:25pm

4. Future/proposed studies and discussion 12:35pm

a. Voting guidelines

NK/KIR

a. **PROP1811-12** Impact of the direction of NK cell alloreactivity predicted by KIR ligand mismatch on engraftment in umbilical cord blood and haploidentical stem cell transplantation (F Otegbeye) – *(Attachment 3)*

b. **PROP1811-97** A Novel KIR-HLA Interaction Scoring System and its Effect on Transplantation Outcomes after HLA Matched Allogeneic Hematopoietic Stem Cell Transplantation (E Krieger/A Toor/R Romee) – *(Attachment 4)*

HLA GENES

c. **PROP1811-03/PROP1811-57/PROP1811-144/PROP1811-186** Effect of Class II HLA mismatching on the outcome of HLA-haploidentical hematopoietic cell transplantation (haploHCT) with high dose, post-transplantation cyclophosphamide (PTCy): a combined CIBMTR/EBMT analysis (S McCurdy/S Solomon/Y Kasamon/A Bashey/E Fuchs) – *(Attachment 5)*

d. **PROP1811-68** Impact of ultra-high resolution HLA matching on the outcome of unrelated donor hematopoietic cell transplantation (N Mayor/S Spellman/S Marsh) – *(Attachment 6)*

e. **PROP1811-95** Evaluation of the impact of HLA Class I and II mismatches potentially non-immunogenic mismatches (A Bertaina/M Fernandez-Vila) – *(Attachment 7)*

f. **PROP1811-115** Effect of HLA-A Expression and HLA-B -21 M/T Dimorphism on Outcomes Following Allogeneic Hematopoietic Cell Transplant (C Camacho-Bydume/J Mytilineos/K Hsu) – *(Attachment 8)*

g. **PROP1811-157** Clinical correlation of DPB1 histocompatibility in BMT clinical outcome (P Cano/J Pidala/C Anasetti) – *(Attachment 9)*

h. **PROP1811-165** Impact of Donor HLA on Transplant Outcomes in NPM1 Mutated AML (R Narayan) – *(Attachment 10)*

i. **PROP1811-185** The impact of single nucleotide gene polymorphisms in the gamma block of the major histocompatibility complex on unrelated donor hematopoietic cell transplants for hematological malignancies Part II: Extension of IB15-01 (M Askar/D Sayer/R Sobecks/N Majhail) – *(Attachment 11)*

OTHER GENES

j. **PROP1812-05** Using whole-exome sequencing to identify novel non-HLA genetic contributors to mortality after blood and marrow transplantation (Q Zhu/L Sucheston-Campbell/T Hahn) – *(Attachment 12)*

Dropped proposals

a. **PROP1801-01** Recipient HLA heterozygosity and the risk of AML/MDS relapse after reduced-intensity HLA-matched unrelated donor allograft – *Overlap with IB18-03*

b. **PROP1811-39** HLA-disparity influence in the setting of matched-unrelated donor and PT-CY based anti-GVHD prophylaxis - *Feasibility*

c. **PROP1811-184** The impact of HLA-A level of expression on clinical outcomes of HCT: extension of IB17-01 - *Feasibility*

BREAK – 20 minutes 2:15pm

5. Studies in progress *(Attachment 13)* 2:35pm

NK/KIR

a. **R02-40/R03-63** Acquisition of natural killer cell receptors in recipients of unrelated transplant (J Miller/E Trachtenberg) **Ongoing**

b. **R04-74d** Functional significance of killer cell immunoglobulin-like receptor genes in HLA-matched and mismatched unrelated HCT (K Hsu) **Manuscript preparation – Update**

c. **IB15-03** Killer Immunoglobulin Receptor (KIR) gene content and pediatric acute leukemia transplant outcomes (MR Verneris/J Miller/S Cooley) **Manuscript preparation**

e. **IB17-02** Donor-recipient NK cell determinants associated with survival in JMML after hematopoietic stem cell transplantation (D Lee/H Rangarahan) **Data file preparation**

f. **IB18-04** Impact of donor KIR genotype on outcome after URD TX in patients with MDS or sAML (J Schetelig/N Kröger/M Robin) **Manuscript preparation – Update** *(Attachment 14)*

g. **IB18-05** Imputation of KIR in GWAS and association of KIR-HLA with outcomes following alloHCT In AML and MDS (C Camacho-Bydume/L Sucheston-Campbell/S Leslie/K Hsu) **Analysis**

HLA GENES – CLASSICAL MATCHING

a. **IB06-05** Use of high-resolution HLA data from the NMDP for the International Histocompatibility Working Group in HCT (E Petersdorf) **Ongoing – Update**
b. **IB14-07** Indirectly recognizable HLA epitopes (PIRCHES): a retrospective validation study on the role of indirect recognition of mismatched HLA in hematopoietic stem cell transplantation outcome (E Spierings) **Manuscript preparation**
c. **IB16-01** The role of HLA-E compatibility in the prognosis of acute leukemia patients undergoing 10/10 HLA matched unrelated HSCT (C Tsamadou/D Fürst/J Mytilineos) **Manuscript preparation** *(Attachment 15)*
d. **IB16-02** Use of HLA structure and function parameters to understand the relationship between HLA disparity and transplant outcomes (LA Baxter-Lowe) **Analysis**
e. **IB18-01** Effect of HLA phenotypes on long term GVHD risk (C Story/M Riches/P Armisted) **Protocol development**
f. **IB18-02** Impact of HLA class I risk alleles associated with AA Immune pathogenesis on allo TX outcomes in patients with SAA (D Babushok/T Olson) **Protocol development**
g. **IB18-03** Effect of HLA Class I Heterozygosity and HLA Supertypes on Outcomes Following Allogeneic HCT for Myeloid and Lymphoid Malignancies (C Camacho-Bydume/K Hsu) **Analysis – Update** *(Attachment 16)*

CYTOKINE/CHEMOKINE

a. **IB14-03a:** The prognostic impact of somatic mutations and levels of CXC chemokine ligands on post hematopoietic cell transplantation (HCT) outcomes in patients with myelodysplastic syndromes (MDS) (W Saber/B Dhakal) **Manuscript preparation**
b. **IB14-03c** Effect of telomere length in MDS patients without TP53/RASTK/JAK2 mutations (RC Lindsley/W Saber) **Manuscript preparation** *(Attachment 17)*

OTHER GENES

a. **IB09-06/RT09-04b** Genetic susceptibility to transplant-related mortality after unrelated donor stem cell transplant (T Hahn/L Sucheston-Campbell) **Ongoing**
b. **IB10-01f** Epigenetic clock: Can this guide donor selection in HCT (S Gadalla/S Savage) **Sample typing**
c. **IB14-04** Assessing the similarity of the T cell receptor repertoire in allogeneic hematopoietic stem cell recipients with the same single human leukocyte mismatches (EH Meyer) **Manuscript preparation – Update**
d. **IB14-05** mtDNA haplotypes and unrelated donor transplant outcomes (M Verneris/J Ross) **Analysis**
e. **IB16-03** Role of recipient and donor genetic polymorphisms in interferon lambda 4 (INFL4) on outcomes after unrelated allogeneic cell transplant (S Gadalla) **Manuscript preparation – Update** *(Attachment 18)*
f. **IB17-03** Identification of genomic markers of post hematopoietic cell transplantation (HCT) outcomes in patients with myelofibrosis: A pilot study (W Saber/S Gadalla) **Sample typing**
g. **IB17-04** Epigenetic profiling of unrelated donor-recipient pairs to improve donor selection during HCT transplants (S Beck/K Peggs/V Rakyan/A Webster) **Analysis**

h. **IB18-06** Clonal mosaicism and HCT outcomes in patients with acute leukemia and myelodysplastic syndromes (S Gadalla/T Hahn/L Sucheston-Campbell) **Protocol development**
i. **IB18-07** Donor and recipient genomic associations with acute GVHD (V Afshar-Khargan) **Protocol pending**

7. Deferred studies pending accrual/funding

a. **IB17-01** The impact of HLA-DPB1 level of expression on clinical outcomes of transplantation (M Askar/M Fernandez-Vina) **Pending funding**

8. Dropped studies

a. **IB09-04** D/R gene polymorphisms of drug metabolisms and innate immune response post allele matched MUD HSCT (V Rocha) – *Lack of progress*
b. **IB11-01b** IPA effect on outcome in URD PBSC/BM HCT (G Ehninger) – *Lack of progress*
c. **IB13-09** Machine learning classifiers to define the alloreactivity of HLA mismatches in URD HCT (Y Louzoun) – *Lack of progress*
d. **IB15-05** Secondary Findings in Exome Sequencing Data (S Savage) – *Lack of progress*

9. Closing remarks

MINUTES
CIBMTR WORKING COMMITTEE FOR IMMUNOBIOLOGY
Salt Lake City, UT
Saturday, February 24, 2018, 12:15 pm– 4:45 pm

Co-Chair:	**Michael Verneris, MD, University of Colorado – Denver;**
	Telephone: 303-724-4006; E-mail: michael.verneris@ucdenver.edu
Co-Chair:	**Katharina Fleischhauer, MD; Essen University Hospital;**
	Telephone: +49-201-723-4582; E-mail: Katharina.Fleischhauer@uk-essen.de
Co-Chair:	**Katharine Hsu, MD, PhD; Memorial Sloan-Kettering Cancer Center;**
	Telephone: 646-888-2667; E-mail: hsuk@mskcc.org
Co-Scientific Dir:	**Stephanie Lee, MD, MPH, Fred Hutchinson Cancer Research Center**
	Telephone: 206-667-6190; E-mail: sjlee@fredhutch.org
Co-Scientific Dir:	**Stephen Spellman, MBS, CIBMTR Immunobiology Research**
	Telephone: 763-406-8334; E-mail: sspellma@nmdp.org
Statistical Director:	**Tao Wang, PhD, CIBMTR Statistical Center**
	Telephone: 414-955-4339; E-mail: taowang@mcw.edu
Statistician:	**Michael Haagenson, MS, CIBMTR Statistical Center**
	Telephone: 763-406-8609; E-mail: mhaagens@nmdp.org

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1. Introduction (M Verneris)

Dr. Michael Verneris opened the Immunobiology Working Committee (IBWC) session at 12:15 PM. The leaders of the IBWC were introduced, including the incoming chair Dr. Sophie Paczesny. Membership to a working committee (WC) is automatically assigned when your badge is scanned at the Tandem IBWC meetings. It is also open to any individual willing to take an active role in the WC studies. Young investigators are encouraged to engage in and perform research. The WC goal is to publish high impact studies in a timely manner. Expectations of members are to provide the current status of ongoing studies and timelines, and to assess and select proposals that will have a high impact on the BMT field. Limitations of studies include statistical time and the availability of appropriate sample types.

Voting for proposals is assessed on a scale from 1 as highest score and 9 as lowest score. Presentations for proposals are limited to 5 minutes with 5-10 additional minutes for discussion. IBWC will only accept up to 6 proposals this year. The decisions of acceptance or declination will be out within a month from the Tandem IBWC meeting. Prioritization will be influenced by voting to quantify the opinion in the room, by what is the current WC portfolio and by the high impact on the BMT field.

Authorship on manuscripts is dependent on the substantial contributions to all parts of the study.

2. Published or submitted papers

Further discussion mentioned 16 published studies and 3 submitted studies within the last year, in journals such as New England Journal of Medicine, Journal of Clinical Oncology, Lancet

Haematology, Public Library of Science One (PLOS1), Blood, Blood Advances, Biology of Blood and Marrow Transplantation and Bone Marrow Transplantation.

3. Research repository update and accrual tables (S Spellman)

Mr. Steve Spellman discussed the Research Sample Repository. There are unrelated donors, cord blood, and transplant recipient pre-transplant/pre-conditioning samples from NMDP facilitated transplants in the Repository. For the unrelated donor repository, there are more than 200 centers participating with more than 38000 adult recipient/donor pairs and more than 4300 recipient/cord pairs. For the related donor repository, there are 52 centers participating with more than 6300 adult recipient/donor pairs. There are more than 2.6 million aliquots stored. More than 8000 samples were distributed in 2017.

Currently, the Repository receives 20 ml of whole blood as frozen aliquots. It also receives 2 mls of ACD-A plasma, 2.5 mls of serum and 2.5 ml EDTA plasma. Sample typing results must be submitted to the CIBMTR database. Samples are being HLA high-resolution and KIR typed retrospectively. There are more than 28000 pairs with HLA data and more than 14000 pairs with KIR presence/absence data.

The BMT CTN Repository began in 2007. Biospecimen collections have clinical samples on more than 6480 subjects, from 21 clinical studies. BMT CTN has supported 55 ancillary and correlative studies. 35 used cryopreserved biospecimens. One GvHD study has 9 ancillary study publications.

The Chronic GVHD Consortium also has a biorepository, largely housed in the Research Sample Repository. Controls are also available. Sample types include PBMC, plasma, serum and urine. Contact Stephanie Lee (sjlee@fredhutch.org) for more information.

4. Future/proposed studies and discussion

a. Voting guidelines

Dr. Katharine Hsu went through the voting guidelines and led the section through the proposals.

b. PROP1710-09 Clonal Mosaicism and HCT Outcomes in Patients with Acute Leukemia and Myelodysplastic Syndromes (Lara Sucheston-Campbell / Theresa Hahn / Shahinaz Gadalla) –

Dr. Shahinaz Gadalla presented this proposal. This proposal would use the cases from the DISCOVeRY-BMT cohort, which has 2107 AML, 777 ALL and 648 MDS cases of first-allo HCT from 2000-2011. A question was raised about the aberrations being considered. Is it disease specific or germline? Dr. Gadalla answered that they may be somatic mutations or germline. Are these aberrations found in normal donors? NCI has a publication in Nature Genetics showing that a normal population has very low level of aberrations. However, they do increase with age. The detection level is typically as low as 5%. Even if this study shows no association with outcome, the descriptive analysis would be beneficial to the community. A question was then raised about whether late effects could be analyzed as well, such as subsequent neoplasms. Another suggestion was to use a more homogeneous disease/disease status cohort. The investigator plans to look first at all disease types and then within disease types.

c. **PROP1711-97** Imputation of KIR in genome-wide association study and the association of KIR-HLA with outcomes following alloHCT In AML and MDS (Christine Camacho-Bydume / Lara Sucheston-Campbell / Stephen Leslie / Katharine Hsu) –
Dr. Brian Shaffer presented this proposal in Dr. Camacho-Bydume's absence. The initial cohort consists of 1771 AML, 577 ALL and 540 MDS first-allo transplants from 2000-2011 in the DISCOVeRY-BMT cohort. They are able to impute KIR gene and allele-level information using their KIR*IMP techniques. There is a 36% overlap between the DISCOVeRY-BMT cohort and the cases they have available. They would analyze the non-overlapping set of cases and do not require biological samples. What is KIR*IMP confidence level cutoff point for looking at outcomes? Imputation technique is quite successful using NGS to impute KIR alleles. 97-99% sensitivity and specificity. The final imputation plan is still in development. Are you going to focus on patients based on race/ethnicity? The investigators agreed that the same races should be used for both training and validation sets. Cohort is highly European Caucasian; probably best to focus on that.

d. **PROP1711-03** Effect of HLA phenotypes on long term GVHD risk (Charlotte Story / Marcie Riches / Paul Armistead) –
Dr. Charlotte Story presented this proposal. Of the 8/8 matched donors available, there are 558 verified matched sibling donors and 7120 8/8 matched URD donors. Suggestion to stick with MUD cohort. Also suggested to drop ATG/CAMPATH use. Data in presentation is from single center results. Did the investigators consider how broad the spectrum of bound peptides is? The informative alleles are combined to form a cumulative score. How do the investigators intend to score alleles not on their list of predetermined alleles? A suggestion was to infer them from sequence similarity. The 16 alleles from the Abelin/Wu immunity study could be considered. Other questions: 1) Does high score associate with outcome of GvHD?; and 2) Can each allele be looked at individually? Even with the large initial set of cases, this study may have a small number of particular alleles. Suggestions were to restrict on age and to develop a global score since the investigators expect a full range of scores.

e. **PROP1711-71** The impact of HLA class I risk alleles associated with AA Immune pathogenesis on allogeneic transplant outcomes in patients with severe acquired aplastic anemia (Daria Babushok / Timothy Olson) –
Dr. Daria Babushok presented this proposals. There are 3015 MSD and 840 8/8 MUD first allo transplants for Severe Aplastic Anemia from 2005 to 2016. In the initial testing set, the population was not a transplant population, just patients on immunosuppression. Will use of 8/8 matching bias results? This will select for patients with common alleles. Dr. Gadalla mentioned that the Loss of Heterozygosity (LOH) in MUD SAA cases from the CIBMTR is about 9%. Dr. Babushok said this proposal will do deeper sequencing to get the mechanism for the loss and not just the presence of the change, and she is open to collaboration on the project. Why use age greater than 50? They want patients with longer exposure to frontline therapy to develop the chromosomal loss/changes and patients over age 50 usually are given immunosuppression before proceeding to a transplant.

f. **PROP1711-06** Role of HLA allotypes in determining CMV and leukemia specific outcomes in patients undergoing unrelated donor alloHCT (Brian Shaffer / Rosa Sottile / Richard O'Reilly / Katharine Hsu) –
Dr. Shaffer presented this proposal. There are 3560 patients with AML, ALL, CML or MDS using first allo, 8/8 MUDs from 2007-2016. HLA allotypes will be divided into strong vs. weak (or avid vs. weak). Question was raised about the CMV unknowns and would they confound the analysis. Primary endpoint is CMV reactivation, not seropositivity. The study will require data collection and extensive data preparation. The CMV Reactivation indicator started being collected in late 2007 to 2008. Question: What is the primary detection method? Methodology is not captured so this is a limitation. Restrict to CMV serostatus positive recipients and plan to control for lymphodepletion.

g. **PROP1711-106** The Impact Of MHC Class I Chain-Related Gene A (MICA) 129 Polymorphism On CMV infection in Unrelated Donor Hematopoietic Cell Transplants (HCT) For Hematological Malignancies – Extension of Study IB13-05 (Medhat Askar / Ronald Sobecks) –
Dr. Medhat Askar presented this proposal. The study population would be the same as IB13-05, which is 163 9/10's with HLA-B mismatches and 555 10/10's where patients have AML, ALL or MDS from 2000-2011. Preliminary data from a single center study shows MICA correlates with CMV reactivation and disease. Incorporating CMV reactivation data is similar for this proposal as the previous proposal. CMV Reactivation was collected starting in late 2007, which cuts off several years of this population. There is linkage with HLA, so could this proposal overlap with the previous proposal? Previous IB13-05 paper looked at other outcomes, not CMV disease.

h. **PROP1711-79** Evaluation of the impact of donor KIR genotype on outcome after unrelated donor transplantation in patients with myelodysplastic syndromes or secondary acute myeloid leukemia – Joint study with EBMT Chronic Leukemia Working Party (Johannes Schetelig / Nicolas Kröger / Marie Robin) –
Dr. Johannes Schetelig presented this proposal. Study population consists of MDS and sAML cases. Goal is to confirm previously observed results. Will need 1400-1700 patients to confirm. Currently have data on 1800 patients, where 585 are directly linked to donor center. Statistical support will be from EBMT with goal of 2019 EBMT presentation. The proponents will start by validating previous results. Questions were raised about HLA-A Bw4 KIR ligand impact since previous analysis only focused on HLA-B and –C. EBMT will only need US patient data from the CIBMTR to complete the study. All KIR testing will be performed at the DKMS Life Sciences Laboratory using stored samples from the DKMS collaborative repository.

i. **PROP1711-128** Chromosomal aberrations and transplant outcomes in patients with inherited bone marrow failure syndromes (Youjin Wang / Shahinaz Gadalla) –
Dr. Youjin Wang presented this proposal. There are 250 URD and 26 related donor transplants where the patients have inborn abnormalities of erythrocyte differentiation and/or function and also have recipient samples available from 2005-2017. Patients with active disease should be excluded. Question: Aren't the investigators looking mostly at Fanconi Anemia (FA) in this project? This diagnosis differs substantially from the other inborn rbc errors. Dr. Gadalla stated they would like to be more descriptive and will look at other IBMFS for comparison.

j. **PROP1711-169** The Effect of HLA Class I Heterozygosity and HLA Supertypes on Outcomes Following Allogeneic Hematopoietic Cell Transplant For Myeloid and Lymphoid Malignancies (Christine Camacho-Bydume / Katharine Hsu) –
Dr. Katharine Hsu presented this proposal in Dr. Camacho-Bydume's absence. There are a total of 8420 adult patients (5342 AML, 1637 ALL and 1423 Lymphoma) that are 8/8 matched URD transplants from 2000-2016. Homozygosity breakdowns include 6383 heterozygous at HLA-A, -B and –C; 1341 homozygous at one HLA class I locus; and 241 homozygous at all 3 class I HLA loci (162 AML alone). No samples will be required nor any outside data collection besides CIBMTR current data forms. This may end up being two separate publications since it contains so much information potentially. Goal is to replicate the published observations first and then expand into peptide binding homozygosity and other potential applications. Will also consider Cw*03:03/Cw*03:04 heterozygosity.

Dropped proposal (pending funding)
The following proposal was dropped due to lack of funding.

a. **PROP1710-14** Whole exome sequencing to simulate alloreactive t cell growth following stem cell transplantation

Dropped proposal (due to limited populations/lack of samples/lack of feasibility)
The following proposals were dropped due to limited populations/lack of samples/lack of feasibility.

a. **PROP1710-04** Human leukocyte antigen B allele polymorphism and its association with leukemia in north indian population
b. **PROP1711-22** Impact of HLA-DRB1 matching on survival following HIDT with PTCy for adults with hematologic malignancies
c. **PROP1711-24** Impact of KIR ligand mismatches on clinical outcomes following haploidentical stem cell transplantation
d. **PROP1711-25** Do hypomethylating agents following HLA C1/C2 mismatched or HLA/KIR mismatched transplantation improve clinical outcome in acute myeloid leukemia?
e. **PROP1711-38** The relationship between HLA epitope mismatch and clinical outcomes in haploHCT with post-transplant cyclophosphamide

BREAK – A 20 minute break was taken. Meeting resumed at 2:35 PM

5. Studies in progress

A study summary was provided in the online materials to give updates on all studies in progress during the year as attachment 12 in the materials. Some additional updates of studies with recent progress were given by the investigator after the break.

NK/KIR
Dr. Katharine Hsu stated that there were no updates in the NK/KIR section.

a. **R02-40/R03-63** Acquisition of natural killer cell receptors in recipients of unrelated transplant (J Miller/E Trachtenberg) **Ongoing**
b. **R04-74d** Functional significance of killer cell immunoglobulin-like receptor genes in HLA-

matched and mismatched unrelated HCT (K Hsu) **Ongoing**

c. **IB15-02** Natural killer cell genomics and outcomes after allogeneic transplantation for chronic lymphocytic leukemia (V Bachanova, JS Miller, D Weisdorf, S Cooley) (Attachment 13) **Manuscript preparation**

d. **IB15-03** Killer Immunoglobulin Receptor (KIR) gene content and pediatric acute leukemia transplant outcomes (MR Verneris, J Miller, S Cooley) **Protocol development** – No update

e. **IB17-02** Donor-recipient NK cell determinants associated with survival in JMML after hematopoietic stem cell transplantation (D Lee, H Rangarahan) **Protocol development**

HLA GENES – CLASSICAL MATCHING

Dr. Katharina Fleischhauer introduced the HLA genes section. She started with her own study IB12-02C.

a. **IB12-02C** Prospective assignment of HLA-DPB1 T cell epitope (TCE) group mismatches by functional distance scores compared to the functional TCE assignment algorithm (K Fleischhauer) **Manuscript preparation** –
Dr. Katharina Fleischhauer presented this study update. TCE groups are based on HLA-DPB1 groups of alleles. There are 3 different TCE assignments. Crivello is a post-doc in Dr. Fleischhauer's lab and led development of the functional distance scoring algorithm (TCE-FD). HLA-DPB1*06:01 and HLA-DPB1*19:01 are in different classifications between the three TCE algorithms. A correlation has been found between the high expression and low expression HLA-DPB1 loci and the TCE groups.

b. **IB13-09** The development of machine learning based classifiers to define the alloreactivity of HLA mismatches in unrelated donor hematopoietic stem cell transplantation (Y Louzoun) **Manuscript preparation** – No update

c. **IB14-08** Development and validation of a clinical unrelated donor selection score. BE Shaw/SJ Lee) (Attachment 14) **Submitted** – No update

d. **IB15-01** The impact of single nucleotide gene polymorphisms (SNP) in the gamma block of the major histocompatibility complex (MHC) on unrelated donor hematopoietic cell transplants (HCT) for hematological malignancies (M Askar/R Sobecks) (Attachment 15) **Manuscript preparation** –
Dr. Medhat Askar presented this study update. This study looks at Gamma Block SNPs located in the central MHC region. Dr. Askar was asked if he had a chance to type using Next Generation Sequencing (NGS). He had, but only on HLA-B since that was the only mismatch allowed in the data set. A suggestion was to look at recipient SNP variation. This data set was powered to detect for the MICA question, but not for the Gamma Block data. Dr. Lara Sucheston-Campbell volunteered their SNP data from the DISCOVeRY-BMT data set.

e. **IB16-01** The role of HLA-E compatibility in the prognosis of acute leukemia patients undergoing 10/10 HLA matched unrelated HSCT (C Tsamadou/D Fürst/J Mytilineos) **Sample typing** – No update

f. **IB16-02** Use of HLA structure and function parameters to understand the relationship between HLA disparity and transplant outcomes (LA Baxter-Lowe) **Data file preparation** – No update

CYTOKINE/CHEMOKINE

a. **IB14-03a:** The prognostic impact of somatic mutations and levels of CXC chemokine

ligands on post hematopoietic cell transplantation (HCT) outcomes in patients with myelodysplastic syndromes (MDS) (W Saber/B Dhakal) **Manuscript preparation** – No update

OTHER GENES
(Chair: K Fleischhauer)

a. **IB06-05** Use of high-resolution HLA data from the NMDP for the International Histocompatibility Working Group in HCT (E Petersdorf) **Ongoing** – No update

b. **IB09-04** D/R gene polymorphisms of drug metabolisms and innate immune response post allele matched unrelated donor HCT (V Rocha) **Manuscript preparation** – No update

c. **IB09-06/RT09-04b** Genetic susceptibility to transplant-related mortality after unrelated donor stem cell transplant (T Hahn/L Sucheston-Campbell) (Attachment 16) **Ongoing** –
Dr. Lara Sucheston-Campbell presented this study update. There are 3 Tandem 2018 abstracts being presented this year, two oral and one poster. There have also been 3 publications from 2017 and 4 potential collaborations started in 2017 by using the same data set. There are two cohorts: 2000-2008 and 2009-2011.

d. **IB10-01c** Chromosome 6 Loss-of-heterozygosity in Pre-transplant Blood Samples of Patients with Severe Aplastic Anemia is Associated with Lower Risk of Acute Graft-versus-Host Disease (S Gadalla) (Attachment 17) **Manuscript preparation** – No update

e. **IB10-01d** Flow Cytometry using FISH techniques in a Severe Aplastic Anemia population. (S Gadalla) (Attachment 18) **Submitted**

f. **IB14-04** Assessing the similarity of the T cell receptor repertoire in allogeneic hematopoietic stem cell recipients with the same single human leukocyte mismatches (EH Meyer) **Manuscript preparation** – No update

g. **IB14-05** mtDNA haplotypes and unrelated donor transplant outcomes (M Verneris/J Ross) **Analysis** – No update

h. **IB15-04** Clinical outcomes among hematopoietic stem cell transplant recipients as a function of socioeconomic status and related transcriptome differences (J Knight, JD Rizzo/S Cole) (Attachment 19) **Manuscript preparation** – No update

i. **IB15-05** Secondary findings in exome sequencing data (S Savage/S Gadalla) **Manuscript preparation** – No update

j. **IB15-07** Functional genetic variants of the ST2 gene in pairs of recipient and donors for risk stratification of GVHD and TRM outcomes (S Paczesny) (Attachment 20) **Manuscript preparation** –
Dr. Lara Sucheston-Campbell presented this study update. GWAS typing determined 390 genome wide SNPs. Associations with GvHD and GvHD death were found. The lower score makes it more interesting. 51 SNPs were replicated. Eight variants have significant sST2 concentrations. Question: How does this correlate with other risk models of GvHD that use other biomarkers? This has not been evaluated yet.

k. **IB16-03** Role of recipient and donor genetic polymorphisms in interferon lambda 4 (INFL4) on outcomes after unrelated allogeneic cell transplant (S Gadalla) **Manuscript preparation** –
Dr. Shahinaz Gadalla presented this study update. Completed genotyping of recipient and donor samples are as follows: For SAA, 500 recipients and 756 donors; For Acute Leukemia (AML/ALL), 531 recipients and 610 donors. Distribution of the interferon lambda 4 genotypes were given. Next steps are to analyze the effects of INFL4 genotypes on outcomes and causes of death.

l. **IB17-03** Identification of genomic markers of post hematopoietic cell transplantation (HCT) outcomes in patients with myelofibrosis: A pilot study (W Saber / S Gadalla) **Sample typing** – No update
m. **IB17-04** Epigenetic profiling of unrelated donor-recipient pairs to improve donor selection during HCT transplants (S Beck/K Peggs/V Rakyan/A Webster) **Sample typing** – No update

SENSITIZATION/TOLERANCE

Dr. Katharina Fleischhauer stated there are no more study updates, so she referred the podium to Dr. Michael Verneris to give his closing remarks.

a. **IB11-01a** Analysis of the NIMA effect on the outcome of unrelated PBSC/BM transplantation (G Ehninger/JJ van Rood/A Schmidt) **Manuscript preparation** – No update
b. **IB11-01b** Analysis of the IPA effect on the outcome of unrelated PBSC/BM transplantation (G Ehninger) **Data Collection/Data File Preparation** – No update
c. **IB14-07** Indirectly recognizable HLA epitopes (PIRCHES): a retrospective validation study on the role of indirect recognition of mismatched HLA in hematopoietic stem cell transplantation outcome (E Spierings) **Manuscript preparation** – No update

6. Deferred studies pending accrual/funding

a. **IB13-06** Role of the complement system in graft-versus-host disease (V Afshar-Kharghan/J Belmont/C Amos) **Pending funding**
b. **IB13-07** Impact of donor signal-regulatory protein alpha (SIRPα) polymorphism on outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT) (A Gassas/J Danska/S Rajakumar) **Pending funding**
c. **IB17-01** The impact of HLA-DPB1 level of expression on clinical outcomes of transplantation (M Askar/M Fernandez-Vina) **Pending funding**

7. Closing remarks

Dr. Michael Verneris closed the meeting by thanking the full committee for the opportunity to spend the last 5 years as a co-chair of the IBWC committee. The meeting was adjourned at 3:05 PM.

Working Committee Overview Plan for 2018-2019

a. **IB09-04:** Donor/recipient gene polymorphisms of drug metabolism and in innate immune response post allele-matched unrelated donor hematopoietic stem cell transplantation (HCT) - The manuscript is in progress and will be submitted by November 2018 The study will be published by June 2019. (Total hours: 70; Allocated for the fiscal year: 70.)

b. **IB10-01e:** Chromosomal aberrations and survival after URD HSCT with Fanconi Anemia – The manuscript is in process and will be submitted by June 2018 (Total hour: 70; Allocated for the fiscal year: 70.)

c. **IB11-01a:** Analysis of the NIMA effect on the outcome of unrelated PBSC/BM transplantation – The manuscript is in progress and will be submitted by June 2018. The study will be published by December 2018. (Total hour: 50; Allocated for the fiscal year: 50.)

d. **IB11-01b:** Analysis of the IPA effect on the outcome of unrelated PBSC/BM transplantation – Preliminary analysis is completed. The study is currently in data collection and will be until June 2019. (Total hour: 250; Allocated for the fiscal year: 0.)

e. **IB12-02c:** Prospective assignment of HLA-DPB1 T cell epitope (TCE) group mismatches by functional distance scores compared to the functional TCE assignment algorithm – The manuscript is in progress and will be submitted by June 2018. The December 2018 goal is to have the manuscript published. (Total hour: 30; Allocated for the fiscal year: 30.)

f. **IB13-09:** The development of machine learning based classifiers to define the alloreactivity of HLA mismatches in unrelated donor hematopoietic stem cell transplantation - The manuscript is in progress. The June 2018 goal is to submit the manuscript. The December 2018 goal is to have it published. (Total hour: 30; Allocated for the fiscal year: 30.)

g. **IB14-03a:** The prognostic impact of levels of CXC chemokine ligands on post hematopoietic cell transplantation outcomes in patients with myelodysplastic syndromes. – The manuscript is in progress and will be submitted by March 2019. (Total hour:70; Allocated for this fiscal year: 70.)

h. **IB14-03c:** Impact of telomere length and telomerase gene mutations on allogeneic stem cell transplantation outcomes in myelodysplastic syndrome – The analysis is underway and will be completed by April 2019, and the manuscript will be in progress by June 2019. (Total hour: 170; Allocated for this fiscal year: 120.)

i. **IB14-04:** Assessing the similarity of the T cell receptor repertoire in allogeneic hematopoietic stem cell recipients with the same single human leukocyte mismatches – The manuscript is in progress. The June 2018 goal is to submit the manuscript. (Total hour: 70; Allocated for this fiscal year: 70.)

j. **IB14-05:** mtDNA haplotypes and unrelated donor transplant outcomes – Analysis is in progress and should be completed by June 2018. The manuscript will be submitted by June 2019. (Total hour: 110; Allocated for this fiscal year: 110.)

k. **IB15-01:** The impact of single nucleotide gene polymorphisms (SNP) in the gamma block of the major histocompatibility complex (MHC) on unrelated donor hematopoietic cell transplants

(HCT) for hematological malignancies – The manuscript is in progress and will be submitted by May 2018. The manuscript will be published by December 2018. (Total hour: 70; Allocated for this fiscal year: 70.)

l. **IB15-02** Natural killer cell genomics and outcomes after allogeneic transplantation for chronic lymphocytic leukemia - The manuscript is in progress. The June 2018 goal is to submit the manuscript. The December 2018 goal is to be published. (Total hour: 70; Allocated for this fiscal year: 70.)

m. **IB15-03** Effect of Killer immunoglobulin like receptors on allogeneic HCT for pediatric acute leukemia – Data file preparation is in process and will be completed by April 2018. Analysis will be completed by June 2018. The June 2019 goal is to submit the manuscript. (Total hour: 70; Allocated for this fiscal year: 70.)

n. **IB15-04** Clinical outcomes among hematopoietic stem cell transplant recipients as a function of socioeconomic status and related transcriptome differences - The manuscript is in progress. The goal is to submit by June 2018. (Total hour: 20; Allocated for this fiscal year: 20.)

o. **IB15-05** Secondary Findings in Exome Sequencing Data - The study is currently in typing The goal is to have the manuscript submitted by June 2019. (Total hour: 50; Allocated for this fiscal year: 50.)

p. **IB15-07:** Functional genetic variants of the ST2 gene in pairs of recipient and donors for risk stratification of GVHD and TRM outcomes – Manuscript is in progress. The June 2018 goal is to submit the manuscript. The manuscript will be published by November 2018. (Total hour: 10; Allocated for this fiscal year: 10.)

q. **IB16-01:** The role of HLA-E compatibility in the prognosis of acute leukemia patients undergoing 10/10 HLA matched unrelated HSCT – The data file preparation is underway. The April 2018 goal is to finalize the data set. Analysis will be completed by June 2018. The February 2019 goal is to have the manuscript submitted. (Total hour: 70; Allocated for this fiscal year: 70.)

r. **IB16-02:** Use of HLA structure and function parameters to understand the relationship between HLA disparity and transplant outcomes - Analysis will be completed by June 2018. The goal is to submit the manuscript by June 2019. (Total hour: 70; Allocated for this fiscal year: 70.)

s. **IB16-03:** Role of recipient and donor genetic polymorphisms in interferon lambda 4 (INFL4) on outcomes after unrelated allogeneic cell transplant – Data file preparation is completed. The June 2018 goal is to finish the analysis. The June 2019 goal is to submit the manuscript. (Total hour: 70; Allocated for this fiscal year: 70.)

t. **IB17-02:** Donor-recipient NK cell determinants associated with survival in JMML after hematopoietic stem cell transplantation – The protocol is being developed and will be finalized by April 2018. Samples will be selected and shipped for typing by June 2018. The final testing

results will be completed and merged with the data file by June 2019. (Total hour: 310; Allocated for this fiscal year: 60.)

u. **IB17-03:** Identification of genomic markers of post hematopoietic cell transplantation (HCT) outcomes in patients with myelofibrosis (MF): A pilot study – Samples are being typed. The final testing results will be completed and merged with the data file by June 2018. The June 2019 goal is to be in analysis. (Total hour: 260; Allocated for this fiscal year: 110.)

v. IB18-01: Effect of HLA phenotypes on long term GVHD risk – The protocol will be finalized by December 2018. The data file preparation will be completed by June 2019. (Total hour: 310; Allocated for this fiscal year: 160.)

w. IB18-02: The impact of HLA class I risk alleles associated with AA Immune pathogenesis on allogeneic transplant outcomes in patients with severe acquired aplastic anemia – The protocol will be finalized by January 2019. The study will still be in data file preparation by June 2019. (Total hour: 320; Allocated for this fiscal year: 70.)

x. IB18-03: The Effect of HLA Class I Heterozygosity and HLA Supertypes on Outcomes Following Allogeneic Hematopoietic Cell Transplant for Myeloid and Lymphoid Malignancies - The protocol will be finalized by November 2018. The data file preparation will be completed by June 2019. (Total hour: 310; Allocated for this fiscal year: 160.)

y. IB18-04: Evaluation of the impact of donor KIR genotype on outcome after unrelated donor transplantation in patients with myelodysplastic syndromes or secondary acute myeloid leukemia - The protocol will be finalized by October 2018. The data file preparation will be completed by June 2019. (Total hour: 360; Allocated for this fiscal year: 210.)

z. IB18-05: Imputation of KIR in genome-wide association study and the association of KIR-HLA with outcomes following alloHCT In AML and MDS - The protocol will be finalized by September 2018. The data file preparation will be completed by December 2018. The analysis will be completed by June 2019. (Total hour: 200; Allocated for this fiscal year: 130.)

OVERSIGHT ASSIGNMENTS FOR WORKING COMMITTEE LEADERSHIP

Sophie Paczesny	**IB11-01a** Analysis of the NIMA effect on the outcome of unrelated PBSC/BM transplantation
	IB11-01b Analysis of the IPA effect on the outcome of unrelated PBSC/BM transplantation
	IB14-05 mtDNA haplotypes and unrelated donor transplant outcomes
	IB15-04 Clinical outcomes among hematopoietic stem cell transplant recipients as a function of socioeconomic status and related transcriptome differences
	IB15-05 Secondary findings in exome sequencing data
	IB15-07 Functional genetic variants of the ST2 gene in pairs of recipient and donors for risk stratification of GVHD and TRM outcomes
	IB16-03: Role of recipient and donor genetic polymorphisms in interferon lambda 4 (INFL4) on outcomes after unrelated allogeneic cell transplant
	IB17-03 Identification of genomic markers of post hematopoietic cell transplantation (HCT) outcomes in patients with myelofibrosis (MF): A pilot study
	IB18-04: Evaluation of the impact of donor KIR genotype on outcome after unrelated donor transplantation in patients with myelodysplastic syndromes or secondary acute myeloid leukemia
Katharina Fleischhauer	**IB12-02C** Prospective assignment of HLA-DPB1 T cell epitope (TCE) group mismatches by functional distance scores compared to the functional TCE assignment algorithm
	IB13-09 The development of machine learning based classifiers to define the alloreactivity of HLA mismatches in unrelated donor hematopoietic stem cell transplantation
	IB15-01 The impact of single nucleotide gene polymorphisms (SNP) in the gamma block of the major histocompatibility complex (MHC) on unrelated donor hematopoietic cell transplants (HCT) for hematological malignancies
	IB16-02: Use of HLA structure and function parameters to understand the relationship between HLA disparity and transplant outcomes
	IB17-01 The impact of HLA-DPB1 levels of expression on clinical

	outcomes of HCT
	IB18-01: Effect of HLA phenotypes on long term GVHD risk
	IB18-02: The impact of HLA class I risk alleles associated with AA Immune pathogenesis on allogeneic transplant outcomes in patients with severe acquired aplastic anemia
	IB18-03: The Effect of HLA Class I Heterozygosity and HLA Supertypes on Outcomes Following Allogeneic Hematopoietic Cell Transplant for Myeloid and Lymphoid Malignancies
Katharine Hsu	**IB15-02** Natural killer cell genomics and outcomes after allogeneic transplantation for chronic lymphocytic leukemia
	IB15-03 Killer Immunoglobulin Receptor (KIR) gene content and pediatric acute leukemia transplant outcomes
	IB09-04 D/R gene polymorphisms of drug metabolisms and innate immune response post allele matched matched unrelated donor HCT
	IB13-04 Discrepancy analysis of microsatellite loci as a proxy measure for ancestral differentiation between donors and recipients: correlation between high scores and poorer overall survival in high resolution matched unrelated donor transplantation
	IB14-03a The levels of CXC chemokine ligands on post hematopoietic cell transplantation outcomes in patients with myelodysplastic syndromes
	IB14-04 Assessing the similarity of the T cell receptor repertoire in allogeneic hematopoietic stem cell recipients with the same single human leukocyte mismatches
	IB16-01: The role of HLA-E compatibility in the prognosis of acute leukemia patients undergoing 10/10 HLA matched unrelated HSCT
	IB17-02 Donor-recipient NK cell determinants associated with survival in JMML after hematopoietic stem cell transplantation
	IB18-05: Imputation of KIR in genome-wide association study and the association of KIR-HLA with outcomes following alloHCT In AML and MDS

Accrual Summary for Immunobiology Working Committee

Comprehensive Report Form (CRF) data

Variable	CIBMTR HLA-identical sibling N (%)	CIBMTR Alternative related N (%)	CIBMTR Unrelated (non-US) N (%)	CIBMTR Unrelated (US) N (%)
Number of patients	47528	9863	9641	45141
Number of centers	519	450	229	212
Recipient age at transplant				
0-9 years	6452 (14)	2364 (24)	2220 (23)	7218 (16)
10-19 years	7745 (16)	1540 (16)	1572 (16)	5157 (11)
20-29 years	8057 (17)	1367 (14)	1439 (15)	5013 (11)
30-39 years	8709 (18)	1194 (12)	1591 (17)	5580 (12)
40-49 years	8145 (17)	1190 (12)	1389 (14)	6838 (15)
50-59 years	8412 (18)	2205 (22)	1429 (15)	15334 (34)
Unknown	8 (N/A)	3 (N/A)	1 (N/A)	1 (N/A)
Median (Range)	32 (0-82)	27 (0-88)	27 (0-76)	39 (0-83)
Recipient race/ethnicity				
Caucasian, non-Hispanic	36161 (79)	6707 (73)	7288 (79)	34887 (79)
African-American, non-Hispanic	2157 (5)	866 (9)	80 (1)	3344 (8)
Asian, non-Hispanic	4410 (10)	871 (9)	1390 (15)	1537 (3)
Pacific islander, non-Hispanic	72 (<1)	23 (<1)	45 (<1)	82 (<1)
Native American, non-Hispanic	87 (<1)	42 (<1)	41 (<1)	166 (<1)
Hispanic, Caucasian	1110 (2)	433 (5)	297 (3)	2959 (7)
Hispanic, African-American	66 (<1)	21 (<1)	15 (<1)	120 (<1)
Hispanic, Asian	12 (<1)	3 (<1)	3 (<1)	18 (<1)
Hispanic, Pacific islander	4 (<1)	0	0	12 (<1)
Hispanic, Native American	21 (<1)	4 (<1)	3 (<1)	38 (<1)
Hispanic, race unknown	144 (<1)	27 (<1)	21 (<1)	741 (2)
Other	1424 (3)	224 (2)	83 (1)	107 (<1)
Unknown	1860 (N/A)	642 (N/A)	375 (N/A)	1130 (N/A)
Recipient sex				
Male	27811 (59)	5965 (60)	5726 (59)	26425 (59)
Female	19717 (41)	3898 (40)	3915 (41)	18716 (41)
Karnofsky score				
10-80	12914 (27)	3232 (33)	2569 (27)	13471 (30)
90-100	32982 (69)	6062 (61)	6697 (69)	29252 (65)
Missing	1632 (3)	569 (6)	375 (4)	2418 (5)
HLA-A B DRB1 groups - low resolution				
<=3/6	0	1463 (61)	21 (1)	256 (1)
4/6	0	515 (21)	219 (9)	4172 (10)
5/6	0	209 (9)	637 (26)	9008 (22)
6/6	47528 (100)	225 (9)	1607 (65)	28139 (68)

Variable	CIBMTR HLA-identical sibling N (%)	CIBMTR Alternative related N (%)	CIBMTR Unrelated (non-US) N (%)	CIBMTR Unrelated (US) N (%)
Unknown	0 (N/A)	7451 (N/A)	7157 (N/A)	3566 (N/A)
High-resolution HLA matches available out of 8				
<=5/8	29 (2)	1602 (80)	239 (15)	5256 (15)
6/8	8 (<1)	114 (6)	186 (12)	3339 (10)
7/8	20 (1)	130 (7)	433 (27)	6957 (20)
8/8	1843 (97)	153 (8)	741 (46)	19327 (55)
Unknown	45628 (N/A)	7864 (N/A)	8042 (N/A)	10262 (N/A)
High-resolution HLA typed and audited				
N	1 (<1)	2 (<1)	24 (4)	991 (4)
Y	1184 (>99)	538 (>99)	531 (96)	22434 (96)
Unknown	46343 (N/A)	9323 (N/A)	9086 (N/A)	21716 (N/A)
Graft type				
Marrow	31820 (67)	6003 (61)	5363 (56)	17225 (38)
PBSC	15129 (32)	3689 (37)	2444 (25)	17199 (38)
UCB	189 (<1)	39 (<1)	1793 (19)	10360 (23)
BM+PBSC	234 (<1)	73 (1)	5 (<1)	9 (<1)
BM+UCB	103 (<1)	12 (<1)	2 (<1)	1 (<1)
PBSC+UCB	4 (<1)	8 (<1)	7 (<1)	250 (1)
Others	49 (<1)	39 (<1)	27 (<1)	97 (<1)
Conditioning regimen				
Myeloablative	38840 (82)	7053 (72)	7169 (74)	29007 (64)
RIC	3456 (7)	824 (8)	1185 (12)	8501 (19)
Nonmyeloablative	3130 (7)	1349 (14)	682 (7)	4888 (11)
Other	2102 (4)	637 (6)	605 (6)	2745 (6)
Donor age at donation				
To Be Determined/NA	1520 (3)	393 (4)	1368 (14)	1703 (4)
0-9 years	5620 (12)	522 (5)	1407 (15)	9481 (21)
10-19 years	7651 (16)	1024 (10)	152 (2)	1096 (2)
20-29 years	8304 (17)	1900 (19)	1984 (21)	12455 (28)
30-39 years	8608 (18)	2453 (25)	2540 (26)	11007 (24)
40-49 years	7858 (17)	1881 (19)	1747 (18)	7350 (16)
50+ years	7967 (17)	1690 (17)	443 (5)	2049 (5)
Median (Range)	31 (0-85)	35 (0-81)	32 (0-80)	29 (0-69)
Disease at transplant				
AML	12394 (26)	2605 (26)	2379 (25)	14157 (31)
ALL	7257 (15)	1645 (17)	1995 (21)	7075 (16)
Other leukemia	866 (2)	130 (1)	166 (2)	1312 (3)
CML	7890 (17)	1039 (11)	1797 (19)	4485 (10)
MDS	4199 (9)	994 (10)	1010 (10)	7465 (17)
Other acute leukemia	369 (1)	118 (1)	126 (1)	450 (1)
NHL	3229 (7)	665 (7)	348 (4)	3387 (8)

Variable	CIBMTR HLA-identical sibling N (%)	CIBMTR Alternative related N (%)	CIBMTR Unrelated (non-US) N (%)	CIBMTR Unrelated (US) N (%)
Hodgkins Lymphoma	448 (1)	172 (2)	61 (1)	834 (2)
Plasma Cell Disorders, MM	1503 (3)	243 (2)	92 (1)	650 (1)
Other malignancies	348 (1)	73 (1)	33 (<1)	100 (<1)
Breast cancer	82 (<1)	26 (<1)	2 (<1)	10 (<1)
SAA	4507 (9)	635 (6)	517 (5)	1470 (3)
Inherited abnormalities erythrocyte diff fxn	3299 (7)	479 (5)	336 (3)	961 (2)
SCIDs	683 (1)	781 (8)	373 (4)	1202 (3)
Inherited abnormalities of platelets	26 (<1)	10 (<1)	14 (<1)	67 (<1)
Inherited disorders of metabolism	271 (1)	168 (2)	255 (3)	974 (2)
Histiocytic disorders	120 (<1)	67 (1)	122 (1)	455 (1)
Autoimmune disorders	19 (<1)	5 (<1)	4 (<1)	23 (<1)
Other	18 (<1)	8 (<1)	11 (<1)	63 (<1)
Unknown	0 (N/A)	0 (N/A)	0 (N/A)	1 (N/A)
Disease status at transplant				
Early	11948 (25)	1982 (20)	1962 (20)	11435 (25)
Intermediate	11862 (25)	2104 (21)	2922 (30)	7880 (17)
Advanced	5896 (12)	1607 (16)	1312 (14)	8240 (18)
Other	17822 (37)	4170 (42)	3445 (36)	17586 (39)
Donor/Recipient CMV serostatus				
Negative/Negative	10617 (22)	2070 (21)	2135 (22)	9196 (20)
Negative/Positive	7297 (15)	1504 (15)	1929 (20)	9812 (22)
Positive/Negative	4359 (9)	1134 (11)	1086 (11)	3798 (8)
Positive/Positive	17913 (38)	3477 (35)	2206 (23)	6598 (15)
Unknown	7342 (15)	1678 (17)	2285 (24)	15737 (35)
GvHD Prophylaxis				
Ex vivo T-cell depletion	3417 (7)	1975 (20)	688 (7)	3464 (8)
CD34 selection	513 (1)	387 (4)	93 (1)	973 (2)
Tacrolimus + MMF +- others	1216 (3)	506 (5)	156 (2)	6327 (14)
Tacrolimus + MTX +- others (except MMF)	4539 (10)	403 (4)	580 (6)	11948 (26)
Tacrolimus + others (except MTX, MMF)	664 (1)	51 (1)	67 (1)	1872 (4)
Tacrolimus alone	336 (1)	70 (1)	30 (<1)	913 (2)
CSA + MMF +- others (except Tacrolimus)	1619 (3)	170 (2)	998 (10)	5878 (13)
CSA + MTX +- others (except Tacrolimus, MMF)	21343 (45)	2134 (22)	4971 (52)	7858 (17)
CSA + others (except Tacrolimus, MTX, MMF)	3693 (8)	290 (3)	1036 (11)	2045 (5)
CSA alone	5091 (11)	459 (5)	444 (5)	417 (1)
Other GVHD prophylaxis	3119 (7)	325 (3)	70 (1)	579 (1)
Missing	1978 (4)	3093 (31)	508 (5)	2867 (6)
Donor/Recipient sex match				
Male/Male	8478 (33)	2204 (37)	2782 (38)	13133 (38)
Male/Female	5636 (22)	1077 (18)	1644 (23)	8185 (23)
Female/Male	6787 (26)	1447 (24)	1548 (21)	7241 (21)

Variable	CIBMTR HLA-identical sibling N (%)	CIBMTR Alternative related N (%)	CIBMTR Unrelated (non-US) N (%)	CIBMTR Unrelated (US) N (%)
Female/Female	5130 (20)	1281 (21)	1325 (18)	6322 (18)
Unknown	21497 (N/A)	3854 (N/A)	2342 (N/A)	10260 (N/A)
Year of transplant				
1964-1985	4814 (10)	889 (9)	42 (<1)	12 (<1)
1986	1375 (3)	260 (3)	14 (<1)	18 (<1)
1987	1466 (3)	249 (3)	32 (<1)	34 (<1)
1988	1622 (3)	246 (2)	55 (1)	96 (<1)
1989	1852 (4)	258 (3)	101 (1)	187 (<1)
1990	1953 (4)	321 (3)	142 (1)	303 (1)
1991	1900 (4)	255 (3)	179 (2)	430 (1)
1992	1995 (4)	281 (3)	237 (2)	502 (1)
1993	2006 (4)	288 (3)	242 (3)	607 (1)
1994	1862 (4)	274 (3)	260 (3)	753 (2)
1995	1938 (4)	344 (3)	347 (4)	906 (2)
1996	1995 (4)	340 (3)	436 (5)	1050 (2)
1997	1688 (4)	312 (3)	415 (4)	1137 (3)
1998	1548 (3)	229 (2)	477 (5)	1172 (3)
1999	1393 (3)	218 (2)	471 (5)	1225 (3)
2000	1511 (3)	217 (2)	523 (5)	1295 (3)
2001	1497 (3)	241 (2)	523 (5)	1392 (3)
2002	1445 (3)	203 (2)	485 (5)	1591 (4)
2003	1232 (3)	175 (2)	516 (5)	1768 (4)
2004	1471 (3)	151 (2)	628 (7)	1983 (4)
2005	1502 (3)	184 (2)	602 (6)	2172 (5)
2006	1261 (3)	151 (2)	503 (5)	2512 (6)
2007	745 (2)	94 (1)	359 (4)	2853 (6)
2008	1074 (2)	242 (2)	326 (3)	2485 (6)
2009	888 (2)	163 (2)	274 (3)	2628 (6)
2010	508 (1)	61 (1)	155 (2)	1903 (4)
2011	318 (1)	69 (1)	114 (1)	1501 (3)
2012	339 (1)	87 (1)	181 (2)	1435 (3)
2013	685 (1)	351 (4)	223 (2)	2196 (5)
2014	1026 (2)	457 (5)	242 (3)	2512 (6)
2015	911 (2)	563 (6)	209 (2)	2368 (5)
2016	844 (2)	705 (7)	189 (2)	2031 (4)
2017	687 (1)	772 (8)	113 (1)	1718 (4)
2018	177 (<1)	213 (2)	26 (<1)	366 (1)
Follow-up among survivors, Months				
N Eval	22849	4331	4306	17027
Median (Range)	94 (0-513)	38 (0-547)	61 (0-336)	71 (0-394)

Unrelated Donor HCT Research Sample Inventory - Summary for First Allogeneic Transplants in CRF and TED with biospecimens available through the CIBMTR Repository stratified by availability of paired samples, recipient only samples and donor only samples. Biospecimens include: whole blood, serum/plasma and limited quantities of viable cells and cell lines (collected prior to 2006). Specific inventory queries available upon request through the CIBMTR Immunobiology Research Program

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
Number of patients	37744	10623	6882
Source of data			
CRF	21889 (58)	5634 (53)	4225 (61)
TED	15855 (42)	4989 (47)	2657 (39)
Number of centers	249	218	316
Disease at transplant			
AML	12782 (34)	3782 (36)	2223 (32)
ALL	5581 (15)	1464 (14)	1153 (17)
Other leukemia	1312 (3)	328 (3)	227 (3)
CML	3217 (9)	856 (8)	715 (10)
MDS	6063 (16)	1873 (18)	915 (13)
Other acute leukemia	388 (1)	119 (1)	72 (1)
NHL	3579 (9)	951 (9)	559 (8)
Hodgkins Lymphoma	800 (2)	162 (2)	120 (2)
Plasma Cell Disorders, MM	766 (2)	228 (2)	108 (2)
Other malignancies	54 (<1)	13 (<1)	17 (<1)
Breast cancer	7 (<1)	3 (<1)	1 (<1)
SAA	1191 (3)	297 (3)	280 (4)
Inherited abnormalities erythrocyte diff fxn	665 (2)	184 (2)	127 (2)
SCIDs	648 (2)	186 (2)	182 (3)
Inherited abnormalities of platelets	38 (<1)	9 (<1)	9 (<1)
Inherited disorders of metabolism	261 (1)	63 (1)	80 (1)
Histiocytic disorders	332 (1)	83 (1)	71 (1)
Autoimmune disorders	16 (<1)	7 (<1)	4 (<1)
Other	44 (<1)	15 (<1)	19 (<1)
AML Disease status at transplant			
CR1	6446 (50)	1924 (51)	970 (44)
CR2	2591 (20)	762 (20)	469 (21)
CR3+	257 (2)	70 (2)	50 (2)
Advanced or active disease	3341 (26)	989 (26)	687 (31)
Missing	143 (1)	37 (1)	43 (2)
ALL Disease status at transplant			
CR1	2643 (47)	730 (50)	464 (40)
CR2	1641 (29)	402 (27)	344 (30)
CR3+	466 (8)	120 (8)	111 (10)

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
Advanced or active disease	787 (14)	198 (14)	202 (18)
Missing	44 (1)	14 (1)	31 (3)
MDS Disease status at transplant			
Early	1233 (20)	327 (18)	212 (23)
Advanced	4332 (72)	1419 (76)	568 (63)
Missing	457 (8)	114 (6)	124 (14)
NHL Disease status at transplant			
CR1	446 (13)	158 (17)	58 (10)
CR2	664 (19)	166 (18)	93 (17)
CR3+	302 (9)	82 (9)	47 (8)
PR	431 (12)	108 (11)	78 (14)
Advanced	1655 (47)	419 (44)	271 (49)
Missing	50 (1)	9 (1)	9 (2)
Recipient age at transplant			
0-9 years	3381 (9)	833 (8)	898 (13)
10-19 years	3499 (9)	871 (8)	820 (12)
20-29 years	4018 (11)	1092 (10)	855 (12)
30-39 years	4481 (12)	1152 (11)	887 (13)
40-49 years	5945 (16)	1647 (16)	1104 (16)
50-59 years	7884 (21)	2177 (20)	1205 (18)
60-69 years	7285 (19)	2369 (22)	975 (14)
70+ years	1251 (3)	482 (5)	138 (2)
Median (Range)	46 (0-84)	49 (0-79)	40 (0-79)
Recipient race/ethnicity			
Caucasian, non-Hispanic	31488 (85)	8851 (85)	5200 (83)
African-American, non-Hispanic	1728 (5)	446 (4)	296 (5)
Asian, non-Hispanic	809 (2)	351 (3)	233 (4)
Pacific islander, non-Hispanic	49 (<1)	17 (<1)	12 (<1)
Native American, non-Hispanic	143 (<1)	47 (<1)	23 (<1)
Hispanic	2619 (7)	655 (6)	452 (7)
Other	44 (<1)	25 (<1)	21 (<1)
Unknown	864 (N/A)	231 (N/A)	645 (N/A)
Recipient sex			
Male	22065 (58)	6269 (59)	4075 (59)
Female	15679 (42)	4354 (41)	2807 (41)
Karnofsky score			
10-80	12440 (33)	3698 (35)	2071 (30)
90-100	23812 (63)	6374 (60)	4287 (62)
Missing	1492 (4)	551 (5)	524 (8)
HLA-A B DRB1 groups - low resolution			
<=3/6	21 (<1)	25 (<1)	1 (<1)

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
4/6	204 (1)	75 (1)	29 (<1)
5/6	5329 (14)	1325 (14)	977 (15)
6/6	31770 (85)	7921 (85)	5383 (84)
Unknown	420 (N/A)	1277 (N/A)	492 (N/A)
High-resolution HLA matches available out of 8			
<=5/8	834 (2)	66 (1)	27 (1)
6/8	1639 (4)	105 (2)	117 (3)
7/8	7450 (20)	1297 (19)	934 (23)
8/8	26608 (73)	5482 (79)	3001 (74)
Unknown	1213 (N/A)	3673 (N/A)	2803 (N/A)
HLA-DPB1 Match			
Double allele mismatch	8931 (30)	536 (25)	298 (28)
Single allele mismatch	16049 (54)	1101 (51)	549 (52)
Full allele matched	4646 (16)	522 (24)	206 (20)
Unknown	8118 (N/A)	8464 (N/A)	5829 (N/A)
High resolution release score			
No	397 (1)	156 (43)	362 (67)
Yes	28516 (99)	207 (57)	178 (33)
Unknown	8831 (N/A)	10260 (N/A)	6342 (N/A)
KIR typing available			
No	24082 (64)	10499 (99)	6842 (99)
Yes	13662 (36)	124 (1)	40 (1)
Graft type			
Marrow	14336 (38)	3792 (36)	3234 (47)
PBSC	23388 (62)	6730 (63)	3645 (53)
BM+PBSC	8 (<1)	6 (<1)	2 (<1)
BM+UCB	0	1 (<1)	0
PBSC+UCB	12 (<1)	94 (1)	1 (<1)
Number of cord units			
1	5 (100)	0	1 (100)
Conditioning regimen			
Myeloablative	24422 (65)	6581 (62)	4710 (68)
RIC/Nonmyeloablative	13151 (35)	4000 (38)	2093 (30)
TBD	171 (<1)	42 (<1)	79 (1)
Donor age at donation			
To Be Determined/NA	180 (<1)	1269 (12)	57 (1)
0-9 years	11 (<1)	15 (<1)	0
10-19 years	1043 (3)	307 (3)	145 (2)
20-29 years	16285 (43)	4188 (39)	2531 (37)
30-39 years	10995 (29)	2720 (26)	2168 (32)
40-49 years	7072 (19)	1608 (15)	1499 (22)

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
50+ years	2158 (6)	516 (5)	482 (7)
Median (Range)	31 (0-69)	30 (0-109)	33 (18-67)
Donor/Recipient CMV serostatus			
+/+	9249 (25)	2887 (28)	1677 (26)
+/-	4528 (12)	1376 (13)	860 (13)
-/+	12323 (33)	3133 (31)	2128 (33)
-/-	11116 (30)	2822 (28)	1874 (29)
CB - recipient +	0	4 (<1)	0
CB - recipient -	0	2 (<1)	0
CB - recipient CMV unknown	0	1 (<1)	0
Unknown	528 (N/A)	398 (N/A)	343 (N/A)
GvHD Prophylaxis			
Ex vivo T-cell depletion	1089 (3)	271 (3)	300 (4)
CD34 selection	665 (2)	282 (3)	105 (2)
Tacrolimus + MMF +- others	4514 (12)	1101 (10)	572 (8)
Tacrolimus + MTX +- others (except MMF)	16190 (43)	4697 (44)	1910 (28)
Tacrolimus + others (except MTX, MMF)	1971 (5)	682 (6)	273 (4)
Tacrolimus alone	919 (2)	285 (3)	117 (2)
CSA + MMF +- others (except Tacrolimus)	2583 (7)	581 (5)	566 (8)
CSA + MTX +- others (except Tacrolimus, MMF)	6473 (17)	1656 (16)	2128 (31)
CSA + others (except Tacrolimus, MTX, MMF)	985 (3)	297 (3)	283 (4)
CSA alone	462 (1)	115 (1)	267 (4)
Other GVHD prophylaxis	687 (2)	202 (2)	116 (2)
Missing	1206 (3)	454 (4)	245 (4)
Donor/Recipient sex match			
Male-Male	15602 (42)	4222 (40)	2727 (40)
Male-Female	9473 (25)	2543 (24)	1586 (23)
Female-Male	6359 (17)	1927 (18)	1323 (19)
Female-Female	6134 (16)	1702 (16)	1201 (18)
CB - recipient M	5 (<1)	53 (1)	0
CB - recipient F	7 (<1)	42 (<1)	1 (<1)
Unknown	164 (N/A)	134 (N/A)	44 (N/A)
Year of transplant			
1986-1990	349 (1)	45 (<1)	85 (1)
1991-1995	1795 (5)	448 (4)	610 (9)
1996-2000	3148 (8)	1113 (10)	894 (13)
2001-2005	5002 (13)	987 (9)	1433 (21)
2006-2010	9213 (24)	1853 (17)	1391 (20)
2011-2015	12850 (34)	3618 (34)	1714 (25)
2016-2019	5387 (14)	2559 (24)	755 (11)
Follow-up among survivors, Months			

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
N Eval	16177	4874	2672
Median (Range)	53 (0-344)	37 (0-325)	51 (0-337)

Unrelated Cord Blood Transplant Research Sample Inventory - Summary for First Allogeneic Transplants in CRF and TED with biospecimens available through the CIBMTR Repository stratified by availability of paired, recipient only and cord blood only samples. Biospecimens include: whole blood, serum/plasma and limited quantities of viable cells and cell lines (collected prior to 2006-recipient only). Specific inventory queries available upon request through the CIBMTR Immunobiology Research Program

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
Number of patients	5199	1233	1179
Source of data			
CRF	3988 (77)	952 (77)	795 (67)
TED	1211 (23)	281 (23)	384 (33)
Number of centers	144	127	188
Disease at transplant			
AML	1937 (37)	411 (33)	381 (32)
ALL	1060 (20)	259 (21)	268 (23)
Other leukemia	91 (2)	25 (2)	23 (2)
CML	112 (2)	29 (2)	28 (2)
MDS	502 (10)	123 (10)	100 (8)
Other acute leukemia	80 (2)	19 (2)	21 (2)
NHL	367 (7)	81 (7)	80 (7)
Hodgkins Lymphoma	93 (2)	25 (2)	21 (2)
Plasma Cell Disorders, MM	34 (1)	10 (1)	5 (<1)
Other malignancies	10 (<1)	0	0
SAA	89 (2)	28 (2)	21 (2)
Inherited abnormalities erythrocyte diff fxn	149 (3)	46 (4)	30 (3)
SCIDs	235 (5)	67 (5)	83 (7)
Inherited abnormalities of platelets	15 (<1)	3 (<1)	4 (<1)
Inherited disorders of metabolism	308 (6)	80 (6)	76 (6)
Histiocytic disorders	97 (2)	25 (2)	31 (3)
Autoimmune disorders	9 (<1)	0	1 (<1)
Other	11 (<1)	2 (<1)	6 (1)
AML Disease status at transplant			
CR1	966 (50)	219 (53)	192 (50)
CR2	548 (28)	104 (25)	104 (27)
CR3+	51 (3)	6 (1)	11 (3)
Advanced or active disease	364 (19)	80 (20)	72 (19)
Missing	8 (<1)	1 (<1)	2 (1)
ALL Disease status at transplant			
CR1	477 (45)	108 (42)	122 (46)
CR2	397 (37)	100 (39)	95 (35)
CR3+	118 (11)	35 (14)	28 (10)

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
Advanced or active disease	68 (6)	16 (6)	23 (9)
MDS Disease status at transplant			
Early	161 (32)	30 (25)	46 (46)
Advanced	306 (61)	86 (71)	43 (43)
Missing	34 (7)	5 (4)	10 (10)
NHL Disease status at transplant			
CR1	56 (15)	5 (6)	17 (22)
CR2	68 (19)	17 (21)	20 (25)
CR3+	42 (12)	10 (12)	9 (11)
PR	65 (18)	12 (15)	10 (13)
Advanced	133 (37)	36 (44)	22 (28)
Missing	0	1 (1)	1 (1)
Recipient age at transplant			
0-9 years	1562 (30)	456 (37)	440 (37)
10-19 years	681 (13)	143 (12)	160 (14)
20-29 years	490 (9)	86 (7)	91 (8)
30-39 years	507 (10)	104 (8)	113 (10)
40-49 years	546 (11)	123 (10)	108 (9)
50-59 years	731 (14)	149 (12)	141 (12)
60-69 years	599 (12)	151 (12)	117 (10)
70+ years	83 (2)	21 (2)	9 (1)
Median (Range)	27 (0-81)	22 (0-75)	19 (0-78)
Recipient race/ethnicity			
Caucasian, non-Hispanic	2908 (59)	739 (63)	650 (62)
African-American, non-Hispanic	743 (15)	167 (14)	136 (13)
Asian, non-Hispanic	300 (6)	70 (6)	74 (7)
Pacific islander, non-Hispanic	23 (<1)	2 (<1)	12 (1)
Native American, non-Hispanic	34 (1)	6 (1)	13 (1)
Hispanic	945 (19)	189 (16)	169 (16)
Other	0	1 (<1)	1 (<1)
Unknown	246 (N/A)	59 (N/A)	124 (N/A)
Recipient sex			
Male	2858 (55)	712 (58)	675 (57)
Female	2341 (45)	521 (42)	504 (43)
Karnofsky score			
10-80	1330 (26)	297 (24)	281 (24)
90-100	3728 (72)	851 (69)	829 (70)
Missing	141 (3)	85 (7)	69 (6)
HLA-A B DRB1 groups - low resolution			
<=3/6	70 (1)	27 (3)	5 (<1)
4/6	2051 (41)	393 (41)	411 (38)

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
5/6	2231 (45)	401 (42)	522 (48)
6/6	630 (13)	136 (14)	158 (14)
Unknown	217 (N/A)	276 (N/A)	83 (N/A)
High-resolution HLA matches available out of 8			
<=5/8	2478 (56)	405 (58)	474 (54)
6/8	1066 (24)	160 (23)	212 (24)
7/8	594 (13)	89 (13)	127 (14)
8/8	294 (7)	49 (7)	66 (8)
Unknown	767 (N/A)	530 (N/A)	300 (N/A)
HLA-DPB1 Match			
Double allele mismatch	682 (40)	42 (45)	39 (38)
Single allele mismatch	858 (50)	41 (44)	51 (50)
Full allele matched	160 (9)	10 (11)	12 (12)
Unknown	3499 (N/A)	1140 (N/A)	1077 (N/A)
High resolution release score			
No	156 (9)	32 (39)	31 (79)
Yes	1499 (91)	50 (61)	8 (21)
Unknown	3544 (N/A)	1151 (N/A)	1140 (N/A)
KIR typing available			
No	3935 (76)	1227 (>99)	1171 (99)
Yes	1264 (24)	6 (<1)	8 (1)
Cord blood number of units			
1	3609 (69)	0	913 (77)
2	1588 (31)	0	266 (23)
3	2 (<1)	0	0
Unknown	0 (N/A)	1233 (N/A)	0 (N/A)
Graft type			
UCB	4951 (95)	1138 (92)	1127 (96)
BM+UCB	1 (<1)	1 (<1)	0
PBSC+UCB	247 (5)	94 (8)	52 (4)
Conditioning regimen			
Myeloablative	3433 (66)	805 (65)	773 (66)
RIC/Nonmyeloablative	1756 (34)	426 (35)	403 (34)
TBD	10 (<1)	2 (<1)	3 (<1)
Donor age at donation			
To Be Determined/NA	137 (3)	76 (6)	65 (6)
0-9 years	4642 (89)	976 (79)	1029 (87)
10-19 years	273 (5)	104 (8)	52 (4)
20-29 years	44 (1)	21 (2)	7 (1)
30-39 years	43 (1)	27 (2)	12 (1)
40-49 years	24 (<1)	11 (1)	4 (<1)

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
50+ years	36 (1)	18 (1)	10 (1)
Median (Range)	4 (0-72)	4 (0-73)	3 (0-67)
Donor/Recipient CMV serostatus			
+/+	1204 (23)	251 (20)	245 (21)
+/-	532 (10)	119 (10)	113 (10)
-/+	966 (19)	223 (18)	217 (18)
-/-	655 (13)	153 (12)	165 (14)
CB - recipient +	1045 (20)	252 (20)	218 (18)
CB - recipient -	714 (14)	187 (15)	178 (15)
CB - recipient CMV unknown	83 (2)	48 (4)	43 (4)
GvHD Prophylaxis			
Ex vivo T-cell depletion	23 (<1)	9 (1)	4 (<1)
CD34 selection	182 (4)	70 (6)	39 (3)
Tacrolimus + MMF +- others	1379 (27)	309 (25)	182 (15)
Tacrolimus + MTX +- others (except MMF)	200 (4)	55 (4)	54 (5)
Tacrolimus + others (except MTX, MMF)	214 (4)	54 (4)	47 (4)
Tacrolimus alone	131 (3)	43 (3)	23 (2)
CSA + MMF +- others (except Tacrolimus)	2453 (47)	518 (42)	586 (50)
CSA + MTX +- others (except Tacrolimus, MMF)	92 (2)	27 (2)	39 (3)
CSA + others (except Tacrolimus, MTX, MMF)	311 (6)	104 (8)	134 (11)
CSA alone	57 (1)	15 (1)	44 (4)
Other GVHD prophylaxis	125 (2)	17 (1)	15 (1)
Missing	32 (1)	12 (1)	12 (1)
Donor/Recipient sex match			
CB - recipient M	2858 (55)	712 (58)	674 (57)
CB - recipient F	2341 (45)	521 (42)	504 (43)
CB - recipient sex unknown	0	0	1 (<1)
Year of transplant			
1996-2000	0	2 (<1)	4 (<1)
2001-2005	113 (2)	82 (7)	22 (2)
2006-2010	1783 (34)	406 (33)	425 (36)
2011-2015	2567 (49)	491 (40)	569 (48)
2016-2019	736 (14)	252 (20)	159 (13)
Follow-up among survivors, Months			
N Eval	2515	664	606
Median (Range)	52 (1-176)	37 (2-191)	49 (1-217)

Related Donor HCT Research Sample Inventory - Summary for First Allogeneic Transplants in CRF and TED with biospecimens available through the CIBMTR Repository stratified by availability of paired, recipient only and donor only samples. Biospecimens include: whole blood, serum/plasma and limited quantities of viable cells and cell lines (collected prior to 2006). Specific inventory queries available upon request through the CIBMTR Immunobiology Research Program

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
Number of patients	6033	890	403
Source of data			
CRF	2317 (38)	276 (31)	161 (40)
TED	3716 (62)	614 (69)	242 (60)
Number of centers	82	63	51
Disease at transplant			
AML	1980 (33)	297 (33)	118 (29)
ALL	946 (16)	170 (19)	60 (15)
Other leukemia	141 (2)	26 (3)	19 (5)
CML	206 (3)	20 (2)	14 (3)
MDS	994 (16)	137 (15)	59 (15)
Other acute leukemia	81 (1)	14 (2)	3 (1)
NHL	631 (10)	84 (9)	58 (14)
Hodgkins Lymphoma	134 (2)	18 (2)	17 (4)
Plasma Cell Disorders, MM	182 (3)	30 (3)	15 (4)
Other malignancies	16 (<1)	0	0
Breast cancer	1 (<1)	0	0
SAA	261 (4)	31 (3)	12 (3)
Inherited abnormalities erythrocyte diff fxn	289 (5)	41 (5)	16 (4)
SCIDs	109 (2)	18 (2)	8 (2)
Inherited abnormalities of platelets	9 (<1)	0	0
Inherited disorders of metabolism	8 (<1)	0	0
Histiocytic disorders	31 (1)	2 (<1)	2 (<1)
Autoimmune disorders	5 (<1)	0	0
Other	9 (<1)	2 (<1)	2 (<1)
AML Disease status at transplant			
CR1	1215 (61)	189 (64)	72 (61)
CR2	312 (16)	33 (11)	12 (10)
CR3+	23 (1)	4 (1)	0
Advanced or active disease	423 (21)	69 (23)	32 (27)
Missing	7 (<1)	2 (1)	2 (2)
ALL Disease status at transplant			
CR1	597 (63)	112 (66)	43 (72)
CR2	257 (27)	35 (21)	10 (17)
CR3+	39 (4)	5 (3)	2 (3)

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
Advanced or active disease	53 (6)	18 (11)	5 (8)
MDS Disease status at transplant			
Early	175 (18)	19 (14)	6 (10)
Advanced	789 (79)	114 (83)	51 (86)
Missing	30 (3)	4 (3)	2 (3)
NHL Disease status at transplant			
CR1	103 (16)	19 (23)	8 (14)
CR2	124 (20)	14 (17)	11 (19)
CR3+	70 (11)	6 (7)	2 (3)
PR	58 (9)	11 (13)	6 (10)
Advanced	270 (43)	32 (39)	31 (53)
Missing	2 (<1)	1 (1)	0
Recipient age at transplant			
0-9 years	556 (9)	70 (8)	29 (7)
10-19 years	629 (10)	65 (7)	25 (6)
20-29 years	484 (8)	93 (10)	37 (9)
30-39 years	470 (8)	79 (9)	33 (8)
40-49 years	844 (14)	121 (14)	57 (14)
50-59 years	1450 (24)	208 (23)	102 (25)
60-69 years	1402 (23)	225 (25)	109 (27)
70+ years	198 (3)	29 (3)	11 (3)
Median (Range)	50 (0-78)	51 (0-76)	52 (0-74)
Recipient race/ethnicity			
Caucasian, non-Hispanic	3899 (67)	485 (58)	264 (68)
African-American, non-Hispanic	690 (12)	89 (11)	43 (11)
Asian, non-Hispanic	269 (5)	73 (9)	22 (6)
Pacific islander, non-Hispanic	22 (<1)	3 (<1)	0
Native American, non-Hispanic	23 (<1)	1 (<1)	0
Hispanic	909 (16)	184 (22)	60 (15)
Unknown	221 (N/A)	55 (N/A)	14 (N/A)
Recipient sex			
Male	3540 (59)	533 (60)	235 (58)
Female	2493 (41)	357 (40)	168 (42)
Karnofsky score			
10-80	2091 (35)	367 (41)	164 (41)
90-100	3801 (63)	502 (56)	221 (55)
Missing	141 (2)	21 (2)	18 (4)
Graft type			
Marrow	1660 (28)	209 (23)	115 (29)
PBSC	4348 (72)	673 (76)	284 (70)
BM+PBSC	6 (<1)	3 (<1)	0

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
BM+UCB	19 (<1)	5 (1)	1 (<1)
PBSC+UCB	0	0	3 (1)
Conditioning regimen			
Myeloablative	3530 (59)	518 (58)	217 (54)
RIC/Nonmyeloablative	2469 (41)	367 (41)	179 (44)
TBD	34 (1)	5 (1)	7 (2)
Donor age at donation			
To Be Determined/NA	34 (1)	4 (<1)	2 (<1)
0-9 years	406 (7)	43 (5)	19 (5)
10-19 years	571 (9)	75 (8)	28 (7)
20-29 years	731 (12)	116 (13)	46 (11)
30-39 years	738 (12)	130 (15)	66 (16)
40-49 years	1004 (17)	148 (17)	57 (14)
50+ years	2549 (42)	374 (42)	185 (46)
Median (Range)	46 (0-81)	45 (0-79)	47 (0-76)
Donor/Recipient CMV serostatus			
+/+	2457 (41)	431 (49)	177 (45)
+/-	650 (11)	62 (7)	49 (13)
-/+	1490 (25)	202 (23)	87 (22)
-/-	1347 (23)	178 (20)	77 (20)
Unknown	89 (N/A)	17 (N/A)	13 (N/A)
GvHD Prophylaxis			
Ex-vivo T-cell depletion	65 (1)	19 (2)	3 (1)
CD34 selection	80 (1)	26 (3)	7 (2)
Post-CY + other(s)	1057 (18)	148 (17)	70 (17)
Post-CY alone	32 (1)	8 (1)	3 (1)
TAC + MMF +- other(s) (except post-CY)	698 (12)	59 (7)	25 (6)
TAC + MTX +- other(s) (except MMF, post-CY)	2520 (42)	331 (37)	196 (49)
TAC + other(s) (except MMF, MTX, post-CY)	557 (9)	195 (22)	45 (11)
TAC alone	51 (1)	7 (1)	2 (<1)
CSA + MMF +- other(s) (except post-CY)	157 (3)	11 (1)	4 (1)
CSA + MTX +- other(s) (except MMF, post-CY)	497 (8)	52 (6)	26 (6)
CSA + other(s) (except MMF, MTX, post-CY)	57 (1)	9 (1)	2 (<1)
CSA alone	53 (1)	8 (1)	2 (<1)
Other(s)	90 (1)	7 (1)	6 (1)
Missing	119 (2)	10 (1)	12 (3)
Donor/Recipient sex match			
Male-Male	1954 (32)	329 (37)	140 (35)
Male-Female	1305 (22)	168 (19)	83 (21)
Female-Male	1569 (26)	199 (22)	94 (23)
Female-Female	1184 (20)	188 (21)	82 (20)

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
CB - recipient M	15 (<1)	4 (<1)	1 (<1)
CB - recipient F	4 (<1)	1 (<1)	3 (1)
Unknown	2 (N/A)	1 (N/A)	0 (N/A)
Year of transplant			
2006-2010	511 (8)	48 (5)	38 (9)
2011-2015	3239 (54)	455 (51)	206 (51)
2016-2019	2283 (38)	387 (43)	159 (39)
Follow-up among survivors, Months			
N Eval	3825	575	258
Median (Range)	25 (1-126)	23 (2-121)	25 (2-109)

Proposal: 1811-12

Title:
Impact of the direction of NK cell alloreactivity predicted by KIR ligand mismatch on engraftment in umbilical cord blood and haploidentical stem cell transplantation

Folashade Otegbeye, MBChB, MPH, folashade.otegbeye@uhhospitals.org, University Hospitals Cleveland Medical Center
Marcelo Fernandez-Vina, PhD, D, marcelof@stanford.edu, Stanford University School of Medicine
Marcos de Lima, MD, marcos.delima@uhhospitals.org, University Hospitals Cleveland Medical Center

Hypothesis:
The direction of mismatches in HLA ligands for killer cell immunoglobulin like receptors (KIRs) on natural killer cells may predict risk of engraftment failure in cord blood and HLA-haploidentical transplantation, as well as the dominant graft when two cord units are transplanted. KIR ligand mismatch with alloreactivity in the host-versus-graft direction is expected to correlate with increased risk of graft failure and or delayed engraftment.

Specific aims:
- To analyze the impact of host-versus-graft directed KIR ligand incompatibility on engraftment(primary graft failure, time to neutrophil/platelet engraftment and mixed chimerism distribution) in umbilical cord blood transplantation (CBT).
- To study if graft-versus-graft directed KIR ligand incompatibility predicts the dominant cord unit indouble cord blood unit transplantation.
- To study the relationship between direction of KIR ligand mismatch and engraftment (primary graftfailure, time to neutrophil/platelet engraftment and mixed chimerism distribution) in haploidentical stem cell transplantation.

Scientific impact:
Alternative donor transplantation using umbilical cord blood (CBT) or HLA-haploidentical donors (HaploSCT) has significantly increased access to allogeneic stem cell transplantation for a variety of diseases. Where multiple such donors are available for a patient, it would be important to guide donor selection using readily available criteria, such as routinely done HLA typing, that can predict preferred outcomes.

Scientific Justification:
Donor-derived natural killer (NK) cell alloreactivity has been shown to improve clinical outcomes following allogeneic stem cell transplant (HSCT).1-6 The reverse, host-versus-graft (HvG) directed NK cell alloreactivity, could significantly impact engraftment, resulting in primary graft failure (PGF) or delayed engraftment, particularly in the setting of reduced intensity conditioning. In an EBMT/CIBMTR study of single cord blood transplants (CBT) for acute myeloid leukemia, host-versus-graft directed KIR-ligand mismatch was associated with increased non-relapse and overall mortality.2 The incidence of graft failure or its associations was not reported in this study.
Several epitopes of HLA-B and HLA-C are ligands for inhibitory killer immunoglobulin-like receptors (KIRs) that regulate NK cell cytotoxicity. These KIR ligands (KIR-L) are considered surrogates of NK cell alloreactivity in the HLA-mismatched setting.1,3,5 In a preliminary, unpublished analysis of cord blood transplants (CBT) at our institution, we detected increased likelihood of graft failure in HvG-directed KIR ligand mismatch (KIR-L-MM). In double CBT, there was also a trend in favor of engrafting the cord unit

with a simultaneous KIR-L-MM in the graft-versus-host (GvH) and graft-versus-other graft (GvG) direction. Interestingly, HvG directed KIR ligand mismatch had no impact on engraftment in our haploidentical cohort, albeit a much smaller population.
For this analysis, we computed KIR-L and direction of KIR-L-MM for recipient/ donor pairs using a KIR ligand calculator available online (https://www.ebi.ac.uk/ipd/kir/ligand.html). In the CBT cohort (N=70), there was HvG- and GvH-directed KIR-L-MM in 32 and 31 recipient-cord blood unit pairs respectively. There were no KIR-L-MM in 26 transplants while seven had KIR-L-MM in both GvH and HvG directions. Overall, 16 CBT failed to engraft (23%). There was an increased risk of graft failure in recipients of HvG KIR-L-MM transplants (PGF rate 39.1%, R.R 2.15; p=0.063) with this trend most significant when the mismatch was in C1/C2 ligand (PGF rate 42.8%, R.R 2.46; p=0.027). In 8 double CBTs where 1 cord unit had HvG-directed KIR-L-MM, the non-mismatched unit fully engrafted in 7 cases (87.5%). In 6 double CBTs where there was KIR-L-MM from one 1 cord unit in both GvH and GvG (graft versus other graft) direction, 4 (67%) of such units became the dominant graft. Of 8 double CBTs in which both cord units had KIR-L-MM in the HvG direction there was graft failure in 3 (37.5%). Time to neutrophil engraftment and lymphocyte recovery when engraftment occurred was not significantly impacted by KIR-L-MM.
In the Haplo cohort (N=26), all five graft failures (19%) were KIR-L mismatched only in the GvH direction (PGF rate 29.4%, R.R 9.53; p=0.114). There were nine HvG KIR-L-MM transplants and they all engrafted. Fourteen transplant pairs in this cohort had KIR-L-MM in the GvH direction; 2 pairs had both HvG and GvH KIR-L-MM while 3 pairs did not have any KIR-L-MM. Time to neutrophil engraftment was not impacted by GvH or HvG KIR-L-MM.
The rationale for this study proposal is to use the large CIBMTR database in exploring these associations with a sample size large enough for engraftment analysis stratified by multiple confounding variables: including degree of HLA mismatch, intensity of conditioning regimen and use of lymphocyte depleting therapies.7 The proposed study will examine three distinct groups: single unit CBT, double unit CBT and haploidentical transplants for any indication. Study end points to address the specific aims are the incidence of primary graft failure and the time to neutrophil and platelet engraftment. Secondary end points will include the incidence of mixed donor chimerism at 30 and 100 days post-transplant.
Based on the preliminary findings discussed above, the minimum sample sizes required to detect a PGF difference with 99% confidence and 80%, 90% or 100% power would be 104, 132 and 652 respectively for CBT; and 29, 36 and 177 for Haplo HSCT. 3

Patient Eligibility Population:
Inclusion Criteria:

- All patients who have received allogeneic HSCT from umbilical cord blood or HLA-haploidentical donor sources for any indication, malignant or benign.
- Transplants up to December 2017 for which allele-level HLA typing is available for both donor and recipient (to allow adequate computation of KIR ligand).

Data Requirements:
Patient data:

- Age at transplant
- Gender
- Race /Ethnicity
- HLA Typing and antigen assessment (Form 2005): HLA-B and HLA-C allele-level typing will be used by study team to calculate KIR ligand (Bw4, Bw6, C1 and C2) and predict direction of NK cell alloreactivity (Host versus Graft and/or Graft versus Host, Graft-versus-Graft [in cord blood transplant]).

Disease-related data:

- Disease sub-classification or histology
- Disease status/stage at transplant
- Pre-HCT disease treatment

Treatment related data:

- Date of allogeneic stem cell transplant
- Donor demographics (Haploidentical): age, gender, relationship with patient (parent, sibling, child)
- Umbilical cord blood graft: number of unit(s), cell dose per unit
- Haploidentical donor: graft source (mobilized PBSC, marrow harvest); cell dose; other graft manipulation
- Donor HLA Typing and antigen assessment: HLA-B and HLA-C allele-level typing will be used by study team to calculate KIR ligand (Bw4, Bw6, C1 and C2) and predict direction of NK cell alloreactivity (Host versus Graft and/or Graft versus Host, Graft-versus-Graft [in cord blood transplant]).
- Preparative regimen and preparative regimen intensity
- Use of Antithymocyte globulin (ATG)
- GVHD prophylaxis

Post-Transplant Assessment Data:

- Time to engraftment: neutrophil, platelets
- Chimerism data: post-transplant day 30 and day 100
- Acute GVHD: Date, Grade, Treatment
- Therapy given for graft failure: stem cell boost (Haplo), second transplant
- Death: Date and Cause

Study Design:

This is a retrospective analysis using data generated from the CIBMTR registry and computed KIR ligands from patient-donor allele-level HLA typing. Single CBT, Double CBT and HLA-Haploidentical (Haplo) transplants will be analyzed as three distinct cohorts. Summary statistics on the incidence of primary graft failure, time to engraftment (neutrophil and platelets), intensity of conditioning regimen, use of ATG and number of donor-recipient HLA mismatches will be analyzed for the entire cohort and in the various categories of KIR-ligand mismatch directions. Primary graft failure will be compared between groups using t tests and analyses of variance. Mixed chimerism will be summarized as quantiles and compared by presence/nature of KIR-ligand mismatch. Two-way ANOVA will be used to compare median time to engraftment between patients transplanted with or without host-versus-graft directed KIR ligand mismatch, in the CBT and Haplo cohorts. Similar analysis will be done for any, graft-versus-graft (double CBT) and graft-versus-host directed mismatches. The effect of host-versus-graft directed KIR-ligand mismatch on time-to-engraftment (neutrophil and platelets) will be analyzed by Cox proportional hazards model accounting for confounding variables such as graft source, cell dose, donor age, number of HLA mismatches, use of ATG and intensity of conditioning regimen.

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Conflicts of Interest:

None

Prop 1811-12- Table 1. First allogeneic unrelated cord blood HCT, 2008-2017

Variable	N	(%)
Number of recipients	8298	
Disease		
AML	3146	(38)
ALL	1735	(21)
Other leukemia	149	(2)
CML	217	(3)
MDS	828	(10)
Other acute leukemia	142	(2)
NHL	649	(8)
Hodgkins Lymphoma	164	(2)
Plasma Cell Disorders, MM	52	(1)
Other malignancies	6	(<1)
SAA	104	(1)
Inherited abnormalities erythrocyte diff function	205	(2)
SCIDs	317	(4)
Inherited abnormalities of platelets	21	(<1)
Inherited disorders of metabolism	409	(5)
Histiocytic disorders	124	(1)
Autoimmune disorders	13	(<1)
Other	17	(<1)
Race/Ethnicity		
Caucasian, non-Hispanic	4592	(59)
African-American, non-Hispanic	1139	(15)
Asian, non-Hispanic	585	(8)
Pacific islander, non-Hispanic	41	(1)
Native American, non-Hispanic	53	(1)
Hispanic, Caucasian	1280	(16)
Hispanic, African-American	47	(1)
Hispanic, Asian	10	(<1)
Hispanic, Pacific islander	5	(<1)
Hispanic, Native American	19	(<1)
Unknown	527	N/A
Recipient age at transplant		
0-10 years	1938	(23)
10-17 years	1064	(13)

Variable	N	(%)
18-29 years	842	(10)
30-39 years	959	(12)
40-49 years	956	(12)
50-59 years	1317	(16)
60 years and older	1222	(15)
Median (Range)	33	(0-81)
Sex		
Male	4658	(56)
Female	3640	(44)
Graft type		
Cord	8298	(100)
Conditioning regimen intensity		
Myeloablative	5172	(63)
Non-myeloablative/RIC	3060	(37)
Unknown	66	N/A
Recipient CMV Serostatus		
Negative	2866	(35)
Positive	5326	(65)
Not tested	63	(1)
Unknown	43	N/A
Karnofsky performance score		
10-80	2142	(27)
90-100	5905	(73)
Unknown	251	N/A
Retrospective high resolution typing		
No	166	(10)
Yes	1535	(90)
Unknown	6597	N/A
High resolution matches out of 8		
1/8	10	(<1)
2/8	116	(1)
3/8	543	(7)
4/8	1491	(18)
5/8	2682	(32)
6/8	1944	(23)
7/8	1026	(12)
8/8	486	(6)
Year of transplant		

Variable	N	(%)
2008	544	(7)
2009	894	(11)
2010	947	(11)
2011	1056	(13)
2012	1033	(12)
2013	951	(11)
2014	886	(11)
2015	820	(10)
2016	691	(8)
2017	476	(6)

Prop 1811-12- Table 2 - Recipients of mismatched related first allogeneic HCT with retrospective high resolution typing available, 2008-2017

Variable	N	(%)
Number of recipients	853	
Disease		
AML	347	(41)
ALL	153	(18)
Other leukemia	11	(1)
CML	38	(4)
MDS	134	(16)
Other acute leukemia	11	(1)
NHL	59	(7)
Hodgkins Lymphoma	19	(2)
Plasma Cell Disorders, MM	11	(1)
Other malignancies	6	(1)
SAA	20	(2)
Inherited abnormalities erythrocyte diff function	17	(2)
SCIDs	22	(3)
Inherited abnormalities of platelets	1	(<1)
Histiocytic disorders	3	(<1)
Other	1	(<1)
Race/Ethnicity		
Caucasian, non-Hispanic	455	(56)
African-American, non-Hispanic	186	(23)
Asian, non-Hispanic	45	(6)
Pacific islander, non-Hispanic	1	(<1)
Native American, non-Hispanic	3	(<1)
Hispanic, Caucasian	116	(14)
Hispanic, African-American	7	(1)
Hispanic, Native American	3	(<1)
Unknown	37	N/A
Recipient age at transplant		
0-10 years	56	(7)
10-17 years	81	(9)
18-29 years	103	(12)
30-39 years	78	(9)
40-49 years	108	(13)

Variable	N	(%)
50-59 years	198	(23)
60 years and older	229	(27)
Median (Range)	50	(0-78)
Sex		
Male	516	(60)
Female	337	(40)
Graft type		
Marrow	384	(45)
PBSC	456	(53)
BM+PBSC	1	(<1)
PBSC+Cord	11	(1)
PBSC+Other	1	(<1)
Conditioning regimen intensity		
Myeloablative	428	(51)
Non-myeloablative/RIC	416	(49)
Unknown	9	N/A
Recipient CMV Serostatus		
Negative	238	(28)
Positive	609	(71)
Not tested	6	(1)
Karnofsky performance score		
10-80	365	(44)
90-100	473	(56)
Unknown	15	N/A
Retrospective high resolution typing		
Yes	853	(100)
Number of high resolution matches out of 10		
5/10	543	(64)
6/10	168	(20)
7/10	64	(8)
8/10	37	(4)
9/10	34	(4)
10/10	7	(1)
Year of transplant		
2008	6	(1)
2009	6	(1)
2010	13	(2)
2011	15	(2)

Variable	N	(%)
2012	32	(4)
2013	57	(7)
2014	132	(15)
2015	194	(23)
2016	254	(30)
2017	144	(17)

Proposal: 1811-97

Title:
A Novel KIR-HLA Interaction Scoring System and It's Effect on Transplantation Outcomes After HLA Matched Allogeneic Hematopoietic Stem Cell Transplantation

Elizabeth Oaks Krieger, MD, Elizabeth.Krieger@VCUHealth.org, Virginia Commonwealth University
Amir Toor, MD, Amir.Toor@VCUHealth.org, Virginia Commonwealth University
Rizwan Romee, MD, Rizwan_Romee@DFCI.Harvard.edu, Harvard Medical School
Steven Spellman, MBS, sspellma@NMDP.ORG, CIBMTR and University of Minnesota
Christina Wiedl, DO, christina.wiedl@vcuhealth.org, Virginia Commonwealth University
Roy T. Sabo, PhD, roy.sabo@vcuhealth.org, Virginia Commonwealth University

Hypothesis:
The cumulative effect of donor natural killer (NK) cell killer immunoglobulin like receptors (KIR) and recipient human leukocyte antigen (HLA), or KIR ligand (KIRL) interactions influence clinical outcomes following hematopoietic stem cell transplantation (HSCT).

Specific aims:
To determine the summative property of NK cell KIR interactions with their HLA-KIRL and estimate the effect of this KIR/KIRL scores on HSCT outcomes.

Scientific impact:
Given this novel scoring system's ease of determination, it may be implemented in the pre-transplant setting to aid in identifying donors who may improve HSCT outcomes by optimizing GVL, which will ultimately reduce relapse and GVHD risk as well as transplant related mortality (TRM).

Scientific justification:
Natural Killer (NK) cells are an integral part of our innate immune system and the first cell to reconstitute after allogeneic HSCT. They have the ability to lyse malignant cells in an antibody independent fashion. This innate alloreactive graft versus leukemia (GVL) effect makes HSCT a potential cure for high risk hematopoietic malignancies[1]. Mechanisms for improving transplant outcomes via T lymphocyte mediated GVL have been proposed and studied at length. T lymphocytes induce GVL by disparities in major and minor histocompatibility antigens. These disparities are not restricted to malignant cells and leave the recipient at risk for Graft versus host disease (GVHD) which can cause major morbidity and mortality. HLA matched HSCT have become the standard of care when matched donors are available. NK cells are known to cause GVL in an HLA matched environment through KIR mediated effects, however this effect is poorly understood[2]. We now strive to develop a quantitative model to help predict SCT outcomes via NK cell mediated alloreactivity.
NK cells effector function is regulated by an array of inhibitory and activating signals transduced by cell surface receptors including KIR which interact with HLA and HLA like molecules. KIR genes vary in number and context from person to person since they segregate independently of HLA class 1 antigens as they are located on different chromosomes (19 and 6 respectively). The overall outcome of the NK cells' receptor interaction with a target cell's HLA molecules is summative. It is either inhibitory, leaving the NK cell inactive as the NK cell has recognized the target cell as self, or activating and leads to the target cell's destruction[3]. Both activating and inhibitory NK cell KIR recognize HLA class 1 receptors on target cells[4]. KIR and HLA interactions were first shown to influence the outcomes of haploidentical transplants in AML patients[4]. Patients lacking the respective HLA antigen for their donor's KIR ligand

("missing self" or "missing ligand") were found to have less relapse and better overall survival. However, no clear consensus has been reached regarding KIR-based selection in HLA matched setting[5]. Conflicting clinical associations have also been drawn with respect to KIR haplotype A vs B and signal activating or inhibitory KIRs[1,6–9]. Herein, we propose a novel scoring system for quantifying the cumulative KIR/HLA interaction where "missing ligand", inhibitory and activating KIR/HLA ligand interactions are summative and the score may be useful in predicting transplant outcomes and thus donor selection.

Previously, we have examined NK cell reconstitution in the 8/8 HLA allele level matched unrelated donor HCT (N=60), and found that while there was no difference in NK cell reconstitution and clinical outcomes between KIR donor haplotype A and B, survival was improved with more robust NK cell reconstitution at day 60 post HCT[10]. Given this effect of NK cell recovery on post-SCT survival, we went forward with a retrospective pilot study to determine the effect of cumulative KIR/KIRL interactions on transplant outcomes. The same 60 adult HCT unrelated donor-recipient pairs who are HLA matched at 8/8 loci (allele level) had known donor KIR typing. The study population has a median age of 52 years; 55% had lymphoid and 45% had myeloid disease; 57% received a reduced intensity conditioning regimen; 82% underwent T cell depletion with ATG; 88% received GCSF mobilized PBSCT.

Donor recipient pairs' KIR/HLA interaction scores were calculated as detailed below in methods. The entire cohort's scores ranged between -5 to +3, with a Median of 0 (Figure 1). The entire cohort was then divided into groups with either low (scores -5 to -1, n=25) or high scorers (0-3, n=35). NK cell recovery analysis revealed that at 30 days, the average CD56+ cell counts were no different in those with low scores, mean 358 μL^{-1} and high scores, mean 274 μL^{-1} (p=0.2). This may be due to the large cytokine effect at day 30 and the relative immaturity of the NK cells. However, at day 60 and 100, the average CD56+ cell counts were significantly different in patients with lower scores, such that they had a higher average CD56+ cell count; day 60, high-scoring patients, 183μL^{-1}, & low-scoring 295 μL^{-1} (p=0.017) and at day 100, low scoring 235 $\mu L^{-1}$and high scoring 161 μL^{-1} (p=0.032). This indicates that the cumulative KIR/HLA score may be associated with NK cell reconstitution after SCT. Since NK cell reconstitution is associated with survival in our cohort, we would like to investigate the impact of KIR/HLA scores on survival and other clinical outcomes. Intriguingly, we did also observe a trend among higher scoring patients for improved survival (p=0.38) (Figure 2).

When differentiated by myeloid and lymphoid disease, there were no difference in high and low scoring patients with lymphoid disease, however, amongst myeloid disease patients (n=33) those with low scores (n=11) had a significantly higher average CD56+ cell count at day 60, 286 μL^{-1} than those with higher scores (n=22) 174 μL^{-1} (p=0.046).

The study cohort was also examined with respect to KIR Haplotype A and B. Donor KIR haplotype B was assigned when *KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5 or KIR3DS1* were present in the donor, and haplotype A in their absence. Haplotype A (n=20) scores ranged between -4 to 3, with a median of 0. Haplotype B donor pair (n=40) scores ranged between -5 to 3 with a median of 0 (T-test p=0.22). Our subset of haplotype A patient's average CD56+ cell counts were not different at day 60 (p=0.37) this may be due to our small sample size in this sub group analysis or due to the importance of activating KIR effect on NK cell proliferation. Haplotype B donor recipient pairs with low scores had a higher CD56 cell count at day 60, 344 μL^{-1} compared to those with high scores 184 μL^{-1} (p=0.018).

Future goals include incorporating further details of the basic biological principles that govern NK cell responses in this model to optimize its ability to predict clinical outcomes. To accomplish this, we are further developing our current scoring system to include the variability associated with NK cell licensing and recipient heterozygosity.

Figure 1

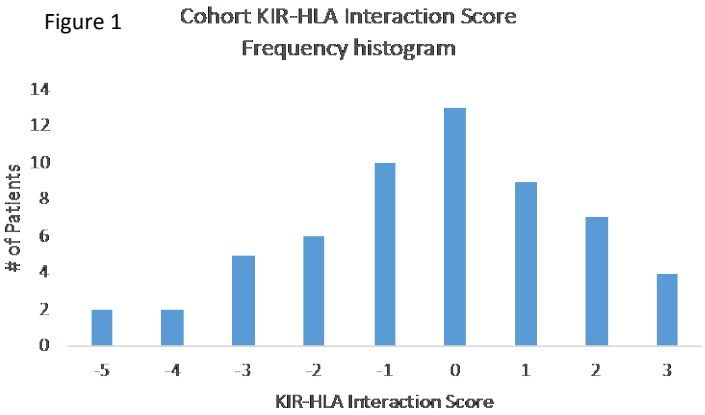
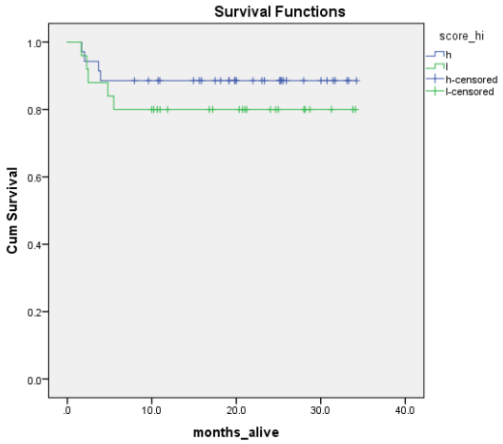


Figure 2



Patient eligibility population:

We will examine the Scoring system in the pediatric and adult setting including patients ages 1-70 who were transplanted for malignancy with known donor KIR typing. We will apply our score to unrelated donor recipient pairs, HLA matched 8/8, with myeloid and lymphoid hematologic malignancy. Our cohort may include both RIT and myeloablative regimens with or without TIB. We will include recipients of both T replete as well as T cell depleted transplants, including those who receive pre-transplant ATG and T cell depletions. Stem cell products may include bone marrow or peripheral blood stem cell (PBSC). Donor recipient pairs must have known donor KIR typing and Recipient HLA typing.

Data requirements:

KIR typing of the Donor

Pre-transplant essential data form 2400; Donor and recipient age, sex, pre-transplant diagnosis and disease state, donor type, stem cell product, conditioning regimen, T cell depletion (yes/no)

Conformation of HLA typing Form 2005; Recipient and donor HLA type i

Hematopoietic Stem Cell Transplant Infusion Form 2006; stem cell product, T cell depletion, CD 34 cell dose when/if available

Post-Transplant essential information form 2450; CD56 and CD 3 cell counts as well as T cell engraftment, when available, Acute and Chronic GVHD (yes and no) with data grading and staging,

GVHD prophylaxis, Days to death, cause of death, Days to relapse, date of last follow up, CMV reactivation yes or no, EBV reactivation yes or no
Infectious disease markers form 2004; CMV serostatus (pos/neg)

Study design:
We will calculate scores by examining donor recipient pairs for the presence or absence of 5 known inhibitory KIR genes 2DL1, 2DL2, 2DL3, 3DL1 and 3DL3, and 4 known activating KIR genes 2DS1, 2DS2, 2DS4 and 2DS5 in the donor. HLA class 1 epitopes, C1, C2, Bw4, will be assigned using the European Bioinformatics Institute's KIR ligand calculator for the recipient[11]. Known KIR ligands include for KIR 2DL1, 2DS1 and 2DS5 HLA C2; 2DL2 and 2DL 3, HLA C1; 2DS2, 2DS4 and 3DL2, HLA A11; and 3DL2 HLA A3[3]. A -1 value was assigned when the donor possessed an inhibitory KIR and the recipient also possessed the corresponding HLA. A value of 0 was assigned when the donor did not possess the inhibitory or activating KIR. A +1 was assigned when the donor possessed the inhibitory KIR and the recipient did not possess the corresponding HLA ("missing ligand") or the donor possessed an activating KIR ligand and the recipient possessed the corresponding HLA receptor. The scores for each donor-recipient pair were then tabulated and could range between -5 and +9. These scores will first be scrutinized using univariate analysis for impact on clinical outcomes including acute. and chronic GVHD, viral reactivation, relapse and survival. A larger cohort will not only allow us to examine these scores using threshold values (such as medians) but also as a continuous variable. Further secondary analysis based on donor type, preparatory regimen, malignancy, T cell depletion, ATG and TBI will be analyzed to determine their effect on transplant out. Finally, multivariate analysis will be utilized to validate the utility of this score amongst all the clinically measurable variables.

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receptor (KIR) genotype matching has a protective effect on chronic graft versus host disease and relapse incidence following HLA-identical sibling hematopoietic stem cell transplantation. *Ann Hematol*. 2018;97(6):1027-1039. doi:10.1007/s00277-018-3274-0

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Conflicts of Interest:
None

Prop 1811-97 – Table 1. First allogeneic unrelated 8/8 matched HCT with KIR typing available 2008-2018, CRF only

Variable	N	(%)
Number of recipients	3614	
Disease		
AML	1521	(42)
ALL	419	(12)
Other leukemia	130	(4)
CML	109	(3)
MDS	1123	(31)
Other acute leukemia	29	(1)
NHL	221	(6)
Hodgkins Lymphoma	33	(1)
Plasma Cell Disorders, MM	29	(1)
Race/Ethnicity		
Caucasian, non-Hispanic	3194	(90)
African-American, non-Hispanic	105	(3)
Asian, non-Hispanic	83	(2)
Pacific islander, non-Hispanic	9	(<1)
Native American, non-Hispanic	10	(<1)
Hispanic, Caucasian	146	(4)
Hispanic, African-American	2	(<1)
Hispanic, Pacific islander	1	(<1)
Hispanic, Native American	2	(<1)
Unknown	62	N/A
Recipient age at transplant		
0-10 years	173	(5)
10-17 years	156	(4)
18-29 years	250	(7)
30-39 years	306	(8)
40-49 years	506	(14)
50-59 years	863	(24)
60 years and older	1360	(38)
Median (Range)	56	(0-82)
Sex		
Male	2141	(59)
Female	1473	(41)
GvHD prophylaxis		
No GVHD prophylaxis	34	(1)
Ex-vivo T-cell depletion	14	(<1)
CD34 selection	33	(1)

Variable	N	(%)
Post-CY + other(s)	91	(3)
TAC + MMF +- other(s) (except post-CY)	635	(18)
TAC + MTX +- other(s) (except MMF, post-CY)	1909	(54)
TAC + other(s) (except MMF, MTX, post-CY)	210	(6)
TAC alone	97	(3)
CSA + MMF +- other(s) (except post-CY)	251	(7)
CSA + MTX +- other(s) (except MMF, post-CY)	163	(5)
CSA + other(s) (except MMF, MTX, post-CY)	33	(1)
CSA alone	24	(1)
Other(s)	43	(1)
Unknown	77	N/A
Graft type		
Marrow	727	(20)
PBSC	2811	(78)
Cord	76	(2)
Conditioning regimen intensity		
Myeloablative	1493	(71)
Non-myeloablative/RIC	596	(29)
Unknown	1525	N/A
Retrospective high resolution typing		
Yes	3614	(100)
High resolution matches out of 10		
8/10	4	(<1)
9/10	208	(6)
10/10	3402	(94)
High resolution matches out of 12		
8/12	2	(<1)
9/12	67	(2)
10/12	1054	(30)
11/12	1827	(52)
12/12	559	(16)
Unknown	105	N/A
Recipient CMV Serostatus		
Negative	1473	(41)
Positive	2116	(59)
Inconclusive	16	(<1)
Not tested	9	(<1)
Year of transplant		
2008	616	(17)
2009	540	(15)
2010	292	(8)

Variable	N	(%)
2011	234	(6)
2012	189	(5)
2013	272	(8)
2014	665	(18)
2015	637	(18)
2016	169	(5)

Combined proposal: 1811-03, 1811-57, 1811-144, 1811-186

Title:
Effect of Class II HLA mismatching on the outcome of HLA-haploidentical hematopoietic cell transplantation (haploHCT) with high dose, post-transplantation cyclophosphamide (PTCy): a combined CIBMTR/EBMT analysis.

Shannon, Rose, McCurdy, MD, shannon.mccurdy@uphs.upenn.edu, The Hospital of the University of Pennsylvania
Scott, R, Solomon, MD, ssolomon@bmtga.com, Northside Hospital
Yvette, Leslie, Kasamon, MD, ykasamon@jhmi.edu, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
Bashey, Asad, MD, PhD, abashey@bmtga.com, Northside Hospital
Ephraim, Joseph, Fuchs, MD, fuchsep@jhmi.edu, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

Hypotheses:
Recipients of grafts from donors mismatched for one HLA-DRB1 antigen in the graft-versus-host direction and/or one non-permissive mismatch in HLA-DPB1 will have improved progression-free survival and overall survival when compared to recipients of grafts from donors without a mismatch in these loci. In contrast, we hypothesize that mismatches in class I loci (HLA-A, -B, and –C) will not influence outcomes after HLA-mismatched transplantation with post-transplantation cyclophosphamide.

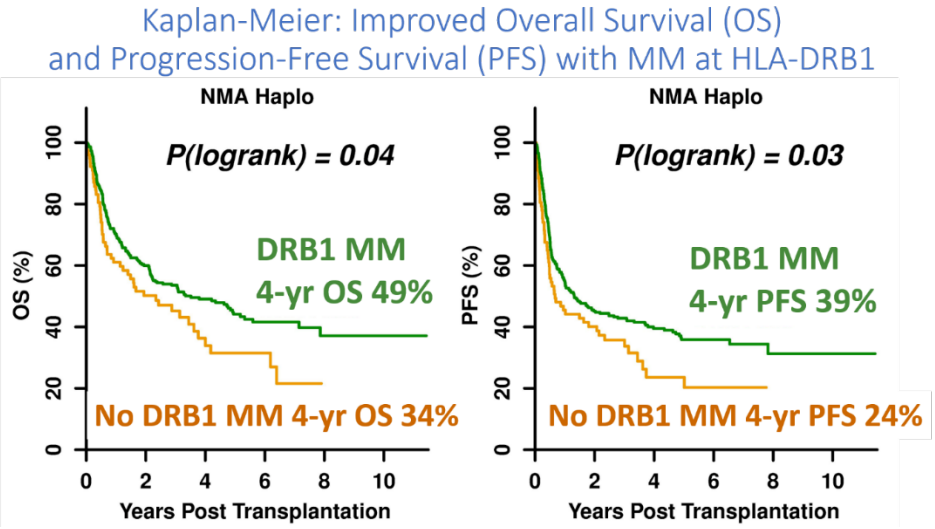
Specific aims:
- To determine whether mismatches in individual loci at HLA-A, -B, -C, -DRB1, -DQB1, and –DPB1 antigens are associated with graft-versus-host disease, nonrelapse mortality, relapse, progression-free survival, and overall survival after HLA-haploidentical blood or marrow transplantation utilizing post-transplantation cyclophosphamide.
- To determine whether combined class II mismatching in both HLA-DRB1 and HLA-DPB1, but not HLA-DQB1, is associated with graft-versus-host disease, nonrelapse mortality, relapse, progression-free survival, and overall survival after HLA-haploidentical blood or marrow transplantation utilizing post-transplantation cyclophosphamide.

Scientific impact:
HLA-haploidentical transplantation with post-transplantation cyclophosphamide (PTCy) is now the most widely used HLA-haploidentical platform.[1] Recipients of HLA-haploidentical transplantation often have multiple potential HLA-haploidentical donors (siblings, children, grandchildren, nieces, nephews) from whom to choose.[2] Currently, donor prioritization strategies rely on the absence of donor-specific antibodies, cytomegalovirus serostatus matching, and ABO match.[3] However, few donor factors have been associated with outcomes after HLA-haploidentical transplantation.[4] Therefore, donor factors that influence outcomes, particularly relapse, have the potential to widely influence donor selection.

Scientific justification:
Given the association of graft-versus-host disease (GVHD) with less relapse after HLA-haploidentical transplantation with PTCy,[5] graft-versus-tumor effects are likely influenced by minor histocompatibility and HLA-mismatches between the recipient and donor, rather than relying solely on the donor immune

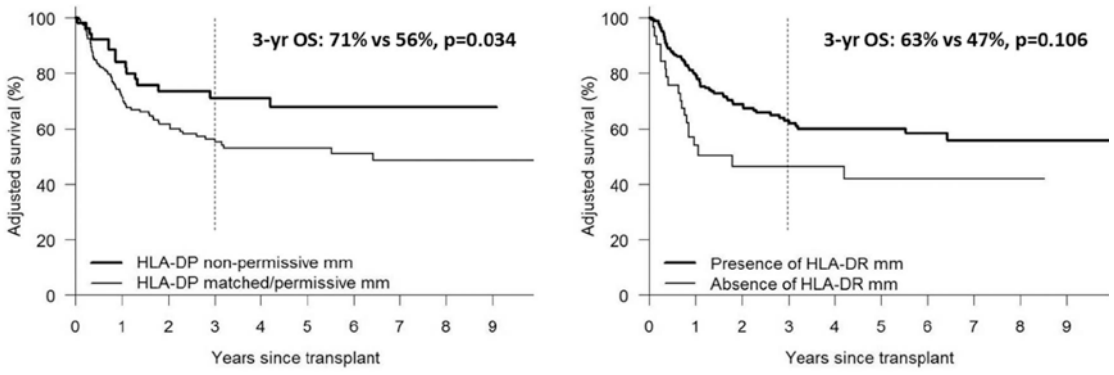
system's identification of tumor antigens. Importantly the influence of donor factors including HLA-mismatching at HLA-DRB1 was associated with grade ≥2 acute GVHD in haploidentical transplantation with PTCy, but not with anti-thymocyte globulin.[6] This suggests that the GVHD prophylaxis employed determines the influence of various alloreactive factors on outcomes and such analyses should be performed in cohorts with uniform GVHD prophylaxis. In an earlier study, we evaluated the effects of HLA-mismatching on outcomes after HLA-haploidentical bone marrow transplantation with PTCy and found that they were not detrimental, in fact, event-free survival (EFS) was improved in patients transplanted from donors with HLA-DRB1 mismatches (HR 0.62, p=.009).[7] In light of this earlier finding, we hypothesize that particular loci may be associated with stronger graft-versus-tumor effects. In particular, HLA-DRB1 has the highest cell surface expression and the greatest polymorphism among HLA Class II molecules, suggesting that it may play the most immunogenic role. We theorize that donor $CD4^+$ T-cell recognition of allogeneic HLA class II molecules, and in particular HLA-DRB1, would result in the delivery of "help" to anti-tumor effector cells, leading to less relapse. This hypothesis is also supported by a recent study in which class II MHC down-regulation was associated with acute myeloid leukemia (AML) relapse.[8] In addition, work from our group and others, showed that HLA loss was associated with immune escape specifically after haploidentical transplantation.[9,10] This shows the importance of these Class II MHC in both the immune system's identification of AML cells in general and in the immune pressure associated with mismatched MHC molecules after HLA-mismatched transplantation, respectively. In an initial study of haploidentical transplantation with PTCy, presented at 2017 Tandem (Abstract ID #9501), but not yet published we found that mismatch at HLA-DRB1 was associated with a higher rate of grade II aGVHD (p=.008) and improved overall survival (OS) (p=.04) and EFS (p=.03) in Kaplan Meier curves (Figure 1). No other loci were associated with this outcome. HLA-DRB1 was not associated with grade III-IV acute GVHD, chronic GVHD, or nonrelapse mortality. In multivariable analysis that adjusted for patient age, cytomegalovirus serostatus, disease risk index (Armand 2012), year of BMT, and nucleated cell graft dose, antigen mismatch at HLA-DRB1 was associated with improved OS (HR 0.73: 95% CI 0.53-1.01, p=.06) and EFS (HR 0.73: 95% CI 0.53-0.98, p=.03). However, given the limited number of patients in that analysis (n=373) and that a number of patients were overlapping from our prior study by Kasamon et al.,[7] we propose to validate these initial findings in a separate and larger cohort. In a similar analysis in a separate cohort of patients receiving haploidentical transplantation with PTCy, non-permissive HLA-mismatch at DPB1 was associated with significantly less relapse and improved overall survival (Figure 2).[11] Finally, in Rimando et al. class II mismatching was associated with significantly reduced relapse and improved relapse-free survival.[12] An American Society of Hematology Meetings 2018 abstract (Lorentino et al. Abstract #482 ASH 2018) compared different immunogenic models of HLA-DPB1 and showed that the most predictive of permissive vs. nonpermissive mismatches was TCE4.[13,14] As such, we proposed to use this model to examine the effect of HLA-DPB1 mismatch on outcomes as well. We believe that HLA-DRB1 and HLA-DPB1 mismatch will be associated with better immune surveillance, prevention of relapse, and improvement in OS after haploidentical transplantation with PTCy.

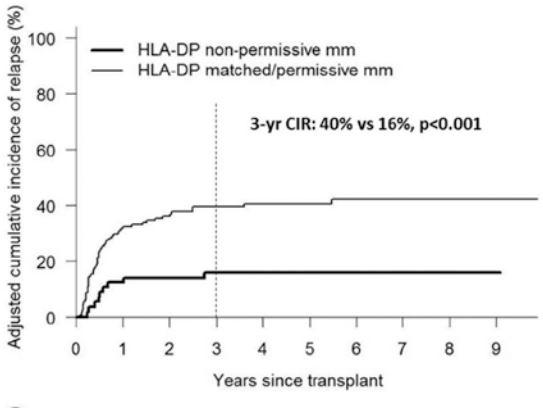
Figure 1: Overall and Progression-Free Survival after HLA-haploidentical bone marrow transplantation with post-transplantation cyclophosphamide by HLA-DRB1 mismatch status

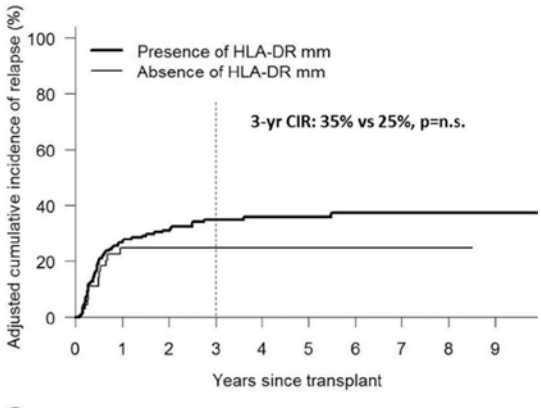


Abbreviations: NMA, nonmyeloablative; Haplo, HLA-haploidentical; DRB1 MM, recipient in whom HLA-DRB1 was mismatched in the graft-versus-host direction; OS, overall survival; No DRB1 MM, patients without a mismatch in HLA-DRB1 in the graft-versus-host direction; PSF, progression-free survival.

Figure 2: Overall Survival and Cumulative Incidences of Relapse by presence of mismatches in HLA-DPB1 and HLA-DRB1.




Adjusted cumulative incidence of relapse (%)
100
80
60
40
20
0
HLA-DP non-permissive mm
HLA-DP matched/permissive mm
3-yr CIR: 40% vs 16%, p<0.001
0 1 2 3 4 5 6 7 8 9
Years since transplant


Adjusted cumulative incidence of relapse (%)
100
80
60
40
20
0
Presence of HLA-DR mm
Absence of HLA-DR mm
3-yr CIR: 35% vs 25%, p=n.s.
0 1 2 3 4 5 6 7 8 9
Years since transplant

Patient eligibility population:
All patients in the United States or Europe receiving HLA-haploidentical transplantation with PTCy for acute leukemia, myelodysplastic syndrome, or lymphoma up through two years prior to the analysis and for whom at least low resolution typing of HLA-A, -B, -Cw, -DRB1, and –DQB1 of both donor and recipient are available. The effect of HLA-DPB1 mismatching will be analyzed when –DPB1 typing is available.

Data requirements:
Forms:

- 2000: Recipient baseline data
- 2005: Confirmation of HLA typing (for both donor and recipient)
- 2450: Post-transplant essential data (for engraftment, chimerism, GVHD, relapse, non-relapse mortality, survival)

We believe the data available through the CIBMTR forms will be adequate to answer our question. If HLA-locus information is unavailable in earlier years, we could request that this data be collected retrospectively if typing information is still available at individual sites.

Study design:
The primary endpoint is progression-free survival according to the presence or absence of HLA-DRB1 mismatch at the antigen level. Power calculations will be based on this primary endpoint. If patient numbers allow, additional calculations will be performed as follows including analysis at HLA-DPB1.
Additional objectives are to compare the incidences of acute and chronic graft-versus-host disease, relapse, non-relapse mortality, and the Kaplan-Meier overall and progression-free survivals between two groups of donor/recipient pairs: 1) pairs in which donor and recipient are **not** mismatched at antigen level for the HLA-DRB1 locus in the graft-versus-host direction; with 2) pairs in which donor and recipient are mismatched for one HLA-DRB1 antigen in the graft-versus-host direction.
The secondary objective is to compare the incidences of acute and chronic graft-versus-host disease, relapse, non-relapse mortality, and the Kaplan-Meier overall and progression-free survivals between two groups of donor/recipient pairs: 1) pairs in which donor and recipient are matched or have permissive mismatches for the HLA-DPB1 locus in the graft-versus-host direction; with 2) pairs in which donor and recipient have nonpermissive mismatches for HLA-DPB1 in the graft-versus-host direction.
Outcomes shall be analyzed for the entire population or according to the following planned subgroups: 1) Diagnosis (lymphoma versus acute leukemia/MDS); 2) stem cell source (peripheral blood vs. bone marrow); 3) Disease risk index; and 4) in patients with high-resolution HLA typing to examine the effect of allele level mismatching.

Endpoints:

			Analyze outcome by antigen mismatch at:						
Outcome	**-A**	**-B**	**-C**	**-DR**	**-DQ**	**-DP**	**Total Class I**	**Total Class II**	**Total mismatch**
GF (1° or 2°)	(H)*	(H)	(H)	(H)	(H)	(H)	H	H	H
aGVHD II-IV	(G)	(G)	(G)	G	G	G	G	G	G
aGVHD III-IV	(G)	(G)	(G)	G	G	G	G	G	G
cGVHD	(G)	(G)	(G)	G	G	G	G	G	G
NRM	(G/H)	(G/H)	(G/H)	G/(H)	G/(H)	G/(H)	G/H	G/H	G/H
Relapse	(G/H)	(G/H)	(G/H)	G/(H)	G/(H)	G/(H)	G/H	G/H	G/H
OS	(G/H)	(G/H)	(G/H)	G/(H)	G/(H)	G/(H)	G/H	G/H	G/H
PFS	(G/H)	(G/H)	(G/H)	G/(H)	G/(H)	G/(H)	G/H	G/H	G/H

Abbreviations: GF, graft failure (primary or secondary); aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; NRM, non-relapse mortality; OS, overall survival; PFS, progression-free survival; G=graft-versus-host direction; H=host-versus-graft direction; G/H= graft-versus-host and/or host-versus-graft directions

*Perform the analysis in the direction(s) indicated in parentheses only if there is a significant effect of the corresponding total class I or class II mismatching.

Variables to be analyzed for inclusion in the multivariable analysis:

Patient-related:

- Age at HCT, years: <55 vs. ≥55 and continuous
- Sex: male vs female
- Karnofsky performance score: ≥90% vs. <90%
- HCT comorbidity index at transplant 0, 1, 2, and ≥ 3
- Race: White vs. Black vs. Asian/pacific islander vs. others
- CMV status: seropositive vs. seronegative.

Disease-related:

- Disease diagnosis
- Disease-Risk Index (low/intermediate vs. high/very high)

Transplant-related:

- Bone marrow vs. peripheral blood as a graft source
- Conditioning regimen: MA vs. RIC vs. NMA (using standard CIBMTR definitions); MA vs. RIC/NMA.

- Year of HCT
- Donor/Recipient gender (F-to-M vs. other)
- Donor/Recipient CMV status (CMV- D/CMV+ R vs. other)
- HLA match
- Donor age - continuous
- Donor relationship – child vs. sibling vs. parent

Non-CIBMTR data source:
Potential for collaboration with the EBMT including Arnon Nagler if it is determined that additional patient numbers are needed for statistical power.

References:
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Conflicts of interest:
None

Table 1a. Recipients with malignant disease receiving PT-Cy for mismatched related first allogeneic HCT, 2008-2018

Variable	N	(%)
Number of recipients	2855	
Disease		
AML	1162	(41)
ALL	482	(17)
Other leukemia	57	(2)
CML	113	(4)
MDS	489	(17)
Other acute leukemia	43	(2)
NHL	321	(11)
Hodgkins Lymphoma	110	(4)
Plasma Cell Disorders, MM	55	(2)
Other malignancies	23	(1)
Race/Ethnicity		
Caucasian, non-Hispanic	1566	(60)
African-American, non-Hispanic	487	(19)
Asian, non-Hispanic	165	(6)
Pacific islander, non-Hispanic	9	(<1)
Native American, non-Hispanic	11	(<1)
Hispanic, Caucasian	369	(14)
Hispanic, African-American	12	(<1)
Hispanic, Asian	1	(<1)
Hispanic, Native American	2	(<1)
Unknown	233	N/A
Recipient age at transplant		
0-10 years	91	(3)
10-17 years	150	(5)
18-29 years	325	(11)
30-39 years	294	(10)
40-49 years	395	(14)
50-59 years	625	(22)
60 years and older	975	(34)
Median (Range)	53	(1-88)
Sex		
Male	1706	(60)
Female	1149	(40)
Graft type		

Variable	N	(%)
Marrow	1109	(39)
PBSC	1739	(61)
BM+PBSC	6	(<1)
PBSC+Other	1	(<1)
Conditioning regimen intensity		
Myeloablative	1123	(39)
Non-myeloablative/RIC	1731	(61)
Unknown	1	N/A
Recipient CMV Serostatus		
Negative	875	(31)
Positive	1965	(69)
Not tested	12	(<1)
Unknown	3	N/A
Karnofsky performance score		
10-80	1100	(40)
90-100	1676	(60)
Unknown	79	N/A
Number of high resolution matches out of 10		
4/10	22	(1)
5/10	1856	(65)
6/10	603	(21)
7/10	260	(9)
8/10	73	(3)
9/10	33	(1)
10/10	8	(<1)
DPB1 Match		
Double allele mismatch	4	(1)
Single allele mismatch	544	(80)
Full allele match	133	(20)
Unknown	2174	N/A
Retrospective high resolution typing		
Yes	669	(100)
Unknown	2186	N/A
Year of transplant		
2009	2	(<1)
2010	13	(<1)
2011	12	(<1)
2012	22	(1)
2013	81	(3)

Variable	N	(%)
2014	347	(12)
2015	590	(21)
2016	722	(25)
2017	817	(29)
2018	249	(9)

Table 1b. Recipients with malignant disease receiving PT-Cy for mismatched related first allogeneic HCT with retrospective high resolution typing available, 2008-2017

Variable	N	(%)
Number of recipients	669	
Disease		
AML	288	(43)
ALL	131	(20)
Other leukemia	9	(1)
CML	30	(4)
MDS	116	(17)
Other acute leukemia	9	(1)
NHL	53	(8)
Hodgkins Lymphoma	19	(3)
Plasma Cell Disorders, MM	10	(1)
Other malignancies	4	(1)
Race/Ethnicity		
Caucasian, non-Hispanic	365	(57)
African-American, non-Hispanic	140	(22)
Asian, non-Hispanic	34	(5)
Pacific islander, non-Hispanic	1	(<1)
Native American, non-Hispanic	2	(<1)
Hispanic, Caucasian	93	(15)
Hispanic, African-American	4	(1)
Hispanic, Native American	1	(<1)
Unknown	29	N/A
Recipient age at transplant		
0-10 years	20	(3)
10-17 years	46	(7)
18-29 years	83	(12)
30-39 years	61	(9)
40-49 years	94	(14)
50-59 years	164	(25)
60 years and older	201	(30)
Median (Range)	52	(1-78)
Sex		
Male	405	(61)
Female	264	(39)
Graft type		
Marrow	318	(48)

Variable	N	(%)
PBSC	350	(52)
BM+PBSC	1	(<1)
Conditioning regimen intensity		
Myeloablative	341	(51)
Non-myeloablative/RIC	328	(49)
Recipient CMV Serostatus		
Negative	189	(28)
Positive	477	(71)
Not tested	3	(<1)
Karnofsky performance score		
10-80	293	(45)
90-100	362	(55)
Unknown	14	N/A
Number of high resolution matches out of 10		
5/10	451	(67)
6/10	141	(21)
7/10	53	(8)
8/10	16	(2)
9/10	6	(1)
10/10	2	(<1)
DPB1 Match		
Double allele mismatch	4	(1)
Single allele mismatch	534	(80)
Full allele match	131	(20)
Retrospective high resolution typing		
Yes	669	(100)
Year of transplant		
2009	1	(<1)
2010	9	(1)
2011	10	(1)
2012	19	(3)
2013	38	(6)
2014	100	(15)
2015	146	(22)
2016	216	(32)
2017	130	(19)

Proposal: 1811-68

Title:
Impact of ultra-high resolution HLA matching on the outcome of unrelated donor hematopoietic cell transplantation

Neema Phillippa Mayor, BSc, PhD, neema.mayor@anthonynolan.org, Anthony Nolan Research Institute
Stephen Rayomond Spellman, BA, MBS, sspellma@NMDP.ORG, (CIBMTR)
Steven Geroge Edward Marsh, BSc, PhD, ARCS, steven.marsh@ucl.ac.uk, Anthony Nolan Research Institute

Hypothesis:
Any degree of genetic variation at the classical HLA loci included when matching patients with hematological disease and their unrelated donor selected for hematopoietic cell transplantation (HLA-A, -B, -C, DRB1, -DQB1 and -DPB1) will result in increased risks of post-transplant complications and mortality.

Specific aims:
- To validate the findings of a UK study that demonstrated that HLA matching at an Ultra-High Resolution (UHR) as achieved with long-read, Next Generation Sequencing (NGS) technology for the six classical HLA loci (HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1), resulted in significant survival advantages for patients undergoing predominantly T-cell depleted (with *in vivo* Alemtuzumab) Unrelated Donor (UD) Hematopoietic Cell Transplantation (HCT) for a hematological malignancy[1].
- To evaluate the impact of UHR HLA matching on a cohort of predominantly T-cell replete UD-HCT pairs from the CIBMTR
- In the event of inconsistencies in study findings, to determine reasons for the difference and propose alternative matching strategies that benefit patient outcomes in this study cohort.
- To make specific clinical recommendations on the resolution of HLA typing required for the best possible prognoses after UD-HCT for hematological malignant disease.

Scientific impact:
Understanding the degree of previously unidentified HLA mismatches that exist for the six classical HLA loci used for matching patients and donors for UD-HCT will help to determine if this polymorphism is having a detrimental effect on patient outcomes. Additionally, it will help elucidate whether non-coding variation (specifically) has a role in UD-HCT prognoses. The impact of these data will help refine donor selection guidelines world-wide and have the potential to become the new standard of care for patients.

Scientific justification:
HCT is a well-established treatment for hematological diseases, including malignant disease. While the use of a related donor remains the primary choice, the probability of identifying an HLA compatible family member is only 25-30%, a factor that is compounded by a trend for smaller family sizes and an aging transplant population. Unrelated donors offer a viable alternative source of cells with the probability of successful outcome improving, in part, by identifying 'well-matched' donors. Current HCT activity reports suggest UD usage is superseding related donors[3,4]. The current optimally matched UD is defined as either an 8/8 or 10/10 HLA match[5-7]. Matching for HLA-DPB1 has repeatedly been shown to improve transplant prognoses and is generally recommended in UD selection[8,9], with models for

permissive mismatching based on the T-cell Epitope models, providing viable alternative options for donor selection[2,10,11].

In recent years, HLA typing for UD-HCT has predominantly focused on regions of the classical HLA genes that encode the Antigen Recognition Domain (ARD), that is exons 2 and 3 for the HLA class I genes, and exon 2 only for the HLA class II genes. These regions were targeted as it was assumed they had more functional relevance, thus introns and untranslated regions have been largely ignored. Due to the hyperpolymorphic nature of the HLA genes and limitations in the technologies available for HLA typing historically, results were often ambiguous, and it was impossible to resolve all possible allelic pairings using the methods available. The introduction of Next- and Third- Generation Sequencing (NGS and TGS respectively) have revolutionized the field of HLA typing, with the ability to sequence through much more of the HLA genes, even the entire gene in some cases, resulting in little or no typing ambiguity. Today, NGS and TGS methods are used widely in clinical HLA typing laboratories, but little is known of the impact of having better HLA typed and matched pairs on patient long-term outcome. Studies using NGS to recharacterize cohorts of previously 10/10 HLA matched UD-HCT pairs have suggested that there is little variation outside of the ARD[12,13]. However, these studies were limited to those pairs that were initially 'well-matched' and excluded the effect of HLA-DPB1 matching at an UHR.

A recent study on the Anthony Nolan UK cohort of predominantly T-cell deplete (*in vivo* Alemtuzumab) UD-HCT pairs has used the TGS method Single Molecule Real-Time (SMRT) DNA sequencing to re-type 891 UD-HCT pairs to determine the impact on outcome[1]. Patients were being treated for a hematological malignancy at a UK transplant center between 1996-2011. Pairs had original HLA typing and matching data available for the six classical HLA loci. For this study, typing and matching was determined at an UHR level, that is whole gene sequence level for HLA class I and an extended HLA class II typing strategy at the coding DNA sequence (CDS) level, where all exons encoding the extracellular portions of the mature protein were included.

In this study, 29% of pairs were found to have mismatches that were not previously identified. Individuals that were a 12/12 UHR match had significantly better survival probability than those previously thought to be 12/12 matched, but were found to have mismatches (5 years: 54.8% vs. 30.1%; p=0.022) or those with any other degree of mismatch (5 years: 55.1% vs. 40.1%; p=0.005). The impact of non-coding variation alone could not be determined in this analysis due to the low number of pairs with only these mismatches (n=13).

Pairs that were a 10/10 UHR match in this study were subdivided into those that were HLA-DPB1 matched (i.e. 12/12 UHR matched), those that were permissively mismatched using the T-cell Epitope model of permissive HLA-DPB1 mismatching[2], and those that were non-permissively mismatched. When compared to an UHR 12/12 matched patient, those that were 10/10 UHR HLA matched but non-permissively HLA-DPB1 mismatched had significantly increased mortality (HR 1.98; p=0.001). The reasons for inferior survival were thought to be an increase in acute Graft-versus-Host Disease (aGvHD; HR 2.37; p=0.018) and a trend for increased non-relapse mortality (HR 1.75, p=0.091). There were no significant differences in disease relapse between the three groups.

Previous data from this cohort indicated that HLA and CMV matching status could be used collectively to predict survival probability[7]. The three groups (12/12 UHR, 10/10 DPB1 permissively mismatched and 10/10 non-permissively mismatched) were further stratified into whether the patient was CMV matched or mismatched with their respective donor. After multivariate analysis, significant differences in outcome were observed between 12/12 UHR, CMV matched patients (best outcome) and either 10/10, non-permissively mismatched, CMV matched patients (RR 2.03, P=0.004) or with 10/10, non-permissively mismatched, CMV mismatched patients (RR 3.25, P<0.0001).

This study would attempt to validate the findings from the UK cohort in a cohort of in vivo T-cell depleted UD-HCT pairs from the CIBMTR. In addition, the study will evaluate the impact of UHR matching in cohort of T-cell replete UD-HCT pairs.

Patient eligibility population:

- Patients with a hematological malignancy (ALL, AML, CML and MDS)
- Adult and pediatric patients
- First allogeneic transplant
- Received stem cells from an unrelated donor either bone marrow or peripheral blood stem cells
- Adequate quantities of good quality genomic DNA available for retrospective UHR HLA typing for both the patient and the unrelated donor
- Previous HLA typing used to match donor and recipient for the six classical HLA loci HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1
- Available UHR typing generated through the CIBMTR Donor-Recipient Pair Project
- Complete and accurate post-transplant follow-up data

Data requirements:

Main effect:

- UHR HLA match per UK study

Patient-related:

- Age: 0-18 vs. 18-29 vs. 30-39 vs. 40-49 vs. 50-59 vs. ≥ 60
- Gender: male vs. female
- Karnofsky score: <90 vs. 90-100%

Disease-related:

- Diagnosis: AML vs. MDS vs. ALL vs. NHL
- Disease status at transplant: early vs. intermediate vs. advanced

Transplant-related:

- Donor and recipient HLA typing and degree match
- Donor age
- Year of transplant
- Condition regimen intensity
- Donor-recipient gender match: M/M vs. M/F vs. F/M vs. F/F
- Source of stem cells: bone marrow vs. peripheral blood
- GVHD prophylaxis
- HLA-DPB1 TCE matching (Crivello et al 2015)

Primary outcome:

- Overall survival

Secondary outcomes:

- Neutrophil engraftment
- Platelet engraftment
- Acute Graft-versus-Host Disease (time to and grade)

- Chronic Graft-versus-Host Disease (time to and extent)
- Relapse
- Non-Relapse Mortality
- Event-Free Survival
- Overall Survival

Study design:
The study team will assign UHR matching per UK study definitions. UHR matching levels will be described at individual locus level and x/10 with comparison to the original ARS defined match.
Analyze impact of UHR matching in in vivo TCD cohort – $p<0.05$ for validation analysis
Analyze impact of UHR matching in T cell replete cohort – $p<0.01$ for analysis
To validate the findings of a UK study that demonstrated that HLA matching at an UHR level in patients undergoing in vivo T-cell depleted (ATG or Alemtuzumab), we will compare cases 10/10 UHR matched vs. mismatched. This validation analysis will use $p<0.05$ for significance for the primary outcome of overall survival.
To evaluate the impact of UHR HLA matching on a cohort of T-cell replete UD-HCT pairs, we will compare cases 10/10 UHR matched vs. mismatched. This validation analysis will use $p<0.01$ for significance for the primary outcome of overall survival.
The contribution of DPB1 TCE match status will be evaluated in both the T replete and T depleted cohorts.
Multivariate analyses will be performed using the Cox proportional hazards model. All variables will be tested for the affirmation of the proportional hazards assumption. Factors violating the proportional hazards assumption will be adjusted via stratification. Then a stepwise model building approach will be used in developing multivariable models for the primary and secondary outcomes. Next, based on the multivariable models, we will assess the association of UHR matching with the HCT outcomes.

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Conflicts of interest:
None

Prop 1811-68 – Table 1. Demographics for 10/10 matched donor-recipient pairs identified for the Whole Gene Cohort

Variable	N	(%)
Number of patients	5928	
Source of data		
CRF	1638	(28)
TED	4290	(72)
Disease at transplant		
AML	2970	(50)
ALL	934	(16)
MDS	2024	(34)
GvHD prophylaxis		
Tacrolimus + MMF +- others	844	(14)
Tacrolimus + MTX +- others (except MMF)	3525	(60)
Tacrolimus + others (except MTX, MMF)	440	(8)
Tacrolimus alone	163	(3)
CSA + MMF +- others (except Tacrolimus)	377	(6)
CSA + MTX +- others (except Tacrolimus, MMF)	367	(6)
CSA + others (except Tacrolimus, MTX, MMF)	24	(<1)
CSA alone	22	(<1)
Other GVHD prophylaxis	86	(1)
Unknown	80	N/A
Use of ATG and Campath		
ATG + CAMPATH	2	(<1)
ATG alone	2179	(37)
CAMPATH alone	203	(3)
Neither ATG nor CAMPATH	3516	(60)
Unknown	28	N/A
Graft type		
Marrow	1099	(19)
PBSC	4824	(81)
BM+PBSC	2	(<1)
PBSC+Cord	1	(<1)
PBSC+Other	2	(<1)
DPB1 match level		
Double allele mismatch	1631	(28)
Single allele mismatch	3205	(54)
Full allele matched	1083	(18)
Unknown	9	N/A

Variable	N	(%)
Recipient age at transplant		
0-9 years	195	(3)
10-19 years	222	(4)
20-29 years	448	(8)
30-39 years	503	(8)
40-49 years	806	(14)
50-59 years	1372	(23)
60-69 years	1922	(32)
70+ years	460	(8)
Median (Range)	56	(1-84)
Recipient race/ethnicity		
Caucasian, non-Hispanic	5234	(90)
African-American, non-Hispanic	118	(2)
Asian, non-Hispanic	114	(2)
Pacific islander, non-Hispanic	9	(<1)
Native American, non-Hispanic	25	(<1)
Hispanic	299	(5)
Unknown	129	N/A
Recipient sex		
Male	3367	(57)
Female	2561	(43)
Conditioning regimen		
Myeloablative	3414	(58)
RIC	2494	(42)
TBD	20	(<1)
Donor age at donation		
To Be Determined/NA	9	(<1)
0-9 years	1	(<1)
10-19 years	247	(4)
20-29 years	3376	(57)
30-39 years	1360	(23)
40-49 years	708	(12)
50+ years	227	(4)
Median (Range)	28	(3-61)
Year of transplant		
2000	1	(<1)
2001	1	(<1)
2003	2	(<1)
2004	10	(<1)

Variable	N	(%)
2005	6	(<1)
2006	6	(<1)
2007	9	(<1)
2008	92	(2)
2009	228	(4)
2010	510	(9)
2011	549	(9)
2012	658	(11)
2013	636	(11)
2014	827	(14)
2015	1315	(22)
2016	883	(15)
2017	195	(3)

Proposal: 1811-95

Title:
Evaluation of the impact of HLA Class I and II mismatches potentially non-immunogenic mismatches.

Alice Bertaina, MD, PhD, aliceb1@stanford.edu, Stanford University/LPCH
Marcelo Fernandez-Viña, PhD, marcelof@stanford.edu, Stanford University

Hypothesis:
HLA mismatching has been established as an important variable affecting the outcomes of hematopoietic stem cell transplants (HSCT). This proposal tests the hypothesis that some HLA Class I and II mismatches are potentially non-immunogenic mismatches. In particular: 1) a single mismatch in alleles presenting only amino acid differences in residues that do not determine peptide binding does not negatively impact on the outcome of 7/8 unrelated donor (UD) transplants when compared to 8/8 UD HSCT and 2) in case of a single mismatch in DRB alleles, permissible and directionally permissible DRB1 mismatches associate significantly with better outcomes than other mismatches.
We propose, thus, to examine the outcomes of transplants including this single amino acid/single allele mismatch and compare with outcomes of transplants with other single amino acid mismatches excluding the mismatches listed below as putative permissible. Because of differential linkage disequilibrium with alleles at other loci of the class II region, we propose to evaluate mismatches at the low expression loci (LE) and DP permissive/non-permissive mismatches as a co-variate and if possible perform sub-analyses stratified according to the number of LEL mismatches and/or non-permissive DP mismatches.

Specific aims:
- To determine the effect of a putative non-immunogenic HLA mismatches on the outcome of UD-HSCT.
- To compare the impact of the single mismatch in alleles presenting only amino acid differences in residues that do not determine peptide binding with other single antigen and/or allele level mismatches on the outcome of UD-HSCT.
- To compare the impact of the single mismatch in DRB alleles in that differ only by one amino acid substitution at residue 86 in which the patient's DRB1 allele carries Valine and the donor carries Glycine with transplants in which the patient's DRB1 mismatched allele carries single mismatch in DRB alleles carries Glycine and the donor carries Valine.
- To compare the impact of the single mismatch in DRB alleles that differ only by one amino acid substitution at residue 86 in which the patient's DRB1 allele carries Valine and the donor carries Glycine with other single antigen and/or allele level mismatches on the outcome of UD-HSCT.
- To compare the impact of the single mismatch in alleles presenting only amino acid differences in residues that do not determine peptide binding with the outcome of transplants in which the patient and the UD are fully matched in HLA-A, B, C or DRB1 loci.
- To compare the impact of the single mismatch in DRB alleles in that differ only by one amino acid substitution at residue 86 in which the patient's DRB1 allele carries Valine and the donor carries Glycine with the outcome of transplants in which the patient and the unrelated donors are fully matched in HLA-A, B, C or DRB1 loci.

Scientific impact:
The identification of Permissible mismatches will lead to expand the pool of suitable donors and will allow for further optimization of donor selection criteria.

Scientific justification:
A study evaluating the cytotoxic T-lymphocyte precursor (CTLp) frequencies directed against incompatibilities at the HLA-A, -B, and -C locus in donor-recipient pairs (1) showed a significant correlation between HLA class I incompatibilities ($p < 0.001$) and high CTLp frequencies. The analysis of HLA amino acid sequences in the HLA-C allele mismatched group showed that mismatches involving alleles with amino acid differences at five polymorphic positions 97, 99, 113, 114, and 116 situated at the peptide binding groove always resulted in the high CTLp frequency group, while the low/absent CTLp frequency group included mainly pairs with the mismatch in the alleles C*03:03/C*03:04 that differ only by one amino acid replacement at residue 91. This residue is not a contact site with the T-cell receptor is not part of any of the pockets that accommodate the peptide side chains in the antigen recognition site. Two CIBMTR outcome studies (2-3) and one study from German centers (4) including unrelated donors have shown that this isolated allele level mismatch in HLA-C does no not associate with detrimental effects in any outcome.

HLA mismatches that do not implicate changes in peptide binding
On the basis of the observations described above, we propose to examine the impact of other allele level mismatches in which the patient and donor alleles differ only by amino acid substitutions in the peptide binding domains that do not affect peptide binding. These substitutions can be identified by compare the amino acid differences in the mismatched alleles and identify the location of the distinguishing residues; if the distinguishing residues do not form part of residues mapped to determine the structure of peptide binding pockets then the mismatches including these alleles can be categorized as putative non-Immunogenic or Permissible mismatches.
The initial study evaluating the mismatch between the alleles C*03:03 and C*03:04 identified frequently this mismatch in patients with European ancestry because these alleles associate frequently with B*15:01. The aim of the present proposal is to expand our knowledge and identify additional permissible mismatches that may be found in other populations. The alleles C*08:01/C*08:03 differ at residue 175 is located at a position that does not affect peptide binding; these alleles are common in Asian and some Native American populations and both associate with B*48:01.
The hypothesis of the evaluating structural location of amino acid differences between mismatched alleles allows also for a generalization that will allow for the systematic assessment of mismatches. For example, the structural analysis of the allele A*02:30 that is not common- however if often found in patients with Jewish ancestry associated with B*38:01- presents only one amino acid difference the common allele A*02:01 at residue 3 that is not part of any peptide binding pocket. Therefore, the mismatch A*02:01/A*02:30 could turn being considered as Permissible. In turn the identification of Permissible mismatches will lead to expand the pool of suitable donors and will allow for further optimization of donor selection criteria.

DRB mismatches that may define directional immunogenic mismatches
The alleles DRB1*11:01 DRB1*11:04 differ only at amino acid position 86 having either Glycine (Gly) or Valine at (Gly) this residue. The valine/glycine dimorphism is highly conserved, it present in almost most HLA-DR alleles and influences peptide-binding.
The dimorphism is likely to influence antigen presentation and forms the molecular basis for differences in stability of Val 86 and Gly 86 DR dimers (5). Studies in the early and mid 1990's by Demotz and coworkers (6) suggested that the natural polymorphism at residue 86 might be a molecular switch that finely tunes the complexity of the peptide collection presented on DR molecules. These studies showed

that self-peptides displayed on DRB1*11:04 molecules represented a fraction of those displayed on DRB1*11:01 molecules; these studies indicated that all naturally processed allo-determinants bound to DRB1*11:04 molecules are a subset of those presented by DRB1*1101. The reciprocal was not true as a fraction of T-cell alloreactive clones raised against DRB1*11:01 did not react with DRB1*11:04. This was probably due to the differential ability of naturally processed self-peptides to bind DRB1*11:01 vs DRB1*11:04 molecules. Analyses of T-cell clones suggest that the substitutions at residue 86 of DRB alone may determine in vivo responses to antigens. These results show that the DRB1*11:04 repertoire is wholly included in the DRB1*11:01 repertoire and strongly support the notion that residue 86 can expand (Gly 86) or reduce (Val 86) the size of the peptide collection presented by DR molecules. The peptide binging groove of the HLA class II molecules is open ended being residue located at the end of the groove; the nature of the substitutions at this residue may have an effect in tuning the set of self-peptides presented by DRB molecules.
In the allogeneic setting in which DRB1*11:01/DRB1*11:04 is the single mismatch, it appears that the substitutions at residue 86 may define a directional mismatch in which cells from an individual carrying DRB1*11:04 (Val 86) may not be stimulatory or immunogenic to T-lymphocytes from an individual carrying DRB1*11:01 (Gly 86). In the opposite direction cells from an individual carrying DRB1*11:01 (Gly 86) may most likely be stimulatory and immunogenic to T-lymphocytes from an individual carrying DRB1*11:04 (Val 86). If this hypothesis is true then patients having DRB1*11:04 transplanted with a donor carrying DRB1*11:01 may be at lower risk for GvHD than patients having DRB1*11:01 with donor carrying DRB1*11:04. This concept could be generalized to transplant with a single amino acid difference in a single DRB1 allele mismatch in which the patient's mismatched allele carries Val at residue 86 and the donor carries Gly at this residue.

Patient eligibility population:
The study population consists of patients receiving their first marrow or peripheral blood stem cell unrelated donor transplantation for the treatment of AML, ALL, CML or MDS. Transplant pairs must be high resolution typed for HLA-A, B, C, DRB1, DQB1 and DPB1 through the NMDP retrospective high resolution typing program.

Data requirements:
Recipient Baseline Data (2000), Confirmation of HLA Typing (2005), Acute Myelogenous Leukemia Pre-HCT Data (2010), Acute Lymphoblastic Leukemia Pre-HCT Data (2011), Chronic Myelogenous Leukemia Pre-HSCT Data (2012), Myelodysplasia / Myeloproliferative Disorders Pre-HCT Data (2014), Post-HSCT Data (2100), Acute Myelogenous Leukemia Post-HCT Data (2110), Acute Lymphoblastic Leukemia Post-HCT Data (2110), Chronic Myelogenous Leukemia Post-HSCT Data (2112), Myelodysplasia / Myeloproliferative Disorders Post-HCT Data (2114), Post-Transplant Essential Data (2450), Recipient Death Data (2900).

Study design:
To compare the impact of the single mismatch in alleles presenting only amino acid differences in residues that do not determine peptide binding with:

- other single antigen and/or allele level mismatches in HLA-C or HLA-A, B or DRB1;
- transplants fully matched in HLA-A, B, C or DRB1 loci;
- transplants in which the patient and the unrelated donors present two mismatches in alleles or antigens of HLA-A, B, C or DRB1 loci excluding mismatches in alleles presenting only amino acid differences in residues that do not determine peptide binding;

- transplants in which the patient and the donor present a single antigen or allele level mismatches HLA-A, B, C or DRB1 other than only amino acid differences in residues that do not determine peptide binding.

To summarize the characteristics of the dataset, descriptive tables of patient-, disease and transplant-related factors will be reported. For discrete factors, the number of cases and their respective percentages will be calculated. Chi-Square tests will be used to compare discrete factors between the HLA matched vs. mismatched groups. For continuous factors, the median and ranges will be calculated. The Kruskal-Wallis test will be used to compare the continuous factors between the HLA matched vs. mismatched groups. Probabilities for overall survival and disease-free survival will be calculated using the Kaplan-Meier estimator with variance estimated by Greenwood's formula. Comparison of survival curves will be done using the log-rank test. Values for other outcomes listed in section 5 will be calculated according to cumulative incidence using a Taylor series linear approximation to estimate the variance.
Multivariate analyses will be performed using the proportional hazards model to compare the homozygous locus mismatched vs. heterozygous mismatched groups. Models will be fit to determine which risk factors may be related to a given outcome. All variables will be tested for the affirmation of the proportional hazards assumption. Factors violating the proportional hazards assumption will be adjusted for first before the stepwise model building approach will be used in developing models for the primary and secondary outcomes.

Outcomes to be studied:

- Overall survival (OS)
- Acute GVHD (grade II-IV and grade III-IV)
- Chronic GVHD
- Relapse (REL)
- Disease-free Survival (DFS)
- Transplant-related mortality (TRM)

Variables to be analyzed:

Main effect to be tested:

- the impact of the single mismatch in alleles presenting only amino acid differences in residues that do not determine peptide binding with other single antigen and/or allele level mismatches;
- the impact of the single mismatch in DRB alleles that differ only by one amino acid substitution at residue 86 in which the patient's DRB1 allele carries Valine and the donor carries Glycine vs transplants in which the patient's DRB1 mismatched allele carries single mismatch in DRB alleles carries Glycine and the donor carries Valine.

Patient-related (at time of transplant):

- Age: in decades (0-9, 10-19, 20-29, 30-39, 40-49, 50 and older).
- Gender: female vs. male
- Lansky/Karnofsky score at transplant: < 90 vs. 90-100

Disease-related:

- Disease at transplant
  - Subanalysis by each disease: ALL, AML, CML and MDS
- Disease status prior to transplant: early (CR1) vs. intermediate (CR2) vs. advanced (≥CR3) vs. others

- Subanalysis by disease stage: early (CR1), intermediate (CR2) and advanced (≥CR3)

Transplant-related:

- Source of stem cells: marrow (BM) vs. peripheral blood stem cells (PB)
- Donor age: in decades (18-29, 30-39, 40-49, 50 and older)
- Year of transplant: (1988-2015)
- Gender match: M-M vs. M-F vs. F-M vs. F-F
- Donor/recipient CMV status: -/- vs. -/+ vs. +/- vs. +/+ vs. Unknown
- Conditioning regimen: Traditional Myeloablative vs. reduced intensity
- GvHD prophylaxis: Tacrolimus +/-others vs. CSA +/-others vs. others

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Conflicts of interest:

None

Prop 1811-95 – Table 1. First allogeneic unrelated myeloablative transplants for AML/ALL/CML/MDS with high resolution typing available, 1999-2011

Variable	N	(%)
Number of recipients	3276	
Disease		
AML	1572	(48)
ALL	874	(27)
CML	502	(15)
MDS	328	(10)
Race/Ethnicity		
Caucasian, non-Hispanic	2593	(90)
African-American, non-Hispanic	75	(3)
Asian, non-Hispanic	43	(1)
Pacific islander, non-Hispanic	2	(<1)
Native American, non-Hispanic	10	(<1)
Hispanic, Caucasian	126	(4)
Hispanic, African-American	2	(<1)
Hispanic, Asian	1	(<1)
Hispanic, race unknown	31	(1)
Other	6	(<1)
Unknown	387	N/A
Recipient age at transplant		
0 <10	249	(8)
1 10-19	370	(11)
2 20-29	561	(17)
3 30-39	570	(17)
4 40-49	706	(22)
5 50-59	638	(19)
6 >60	182	(6)
Median (Range)	38	(0-74)
Sex		
Male	1823	(56)
Female	1453	(44)
Graft type		
Bone marrow	1601	(49)
Peripheral blood	1675	(51)
GvHD prophylaxis		
FK506 + (MTX or MMF or Steroids) +- other	1872	(57)
FK506 +- other	175	(5)
CsA + MTX +- other	893	(27)

Variable	N	(%)
CsA +- other (No MTX)	87	(3)
MMF +- other	5	(<1)
MTX +- other (No CsA)	12	(<1)
T-cell depletion	174	(5)
Other	58	(2)
Number of HLA matches out of 8		
8/8 high-resolution matched	3276	(100)
Karnofsky performance score		
<90	791	(27)
90-100	2172	(73)
Unknown	313	N/A
Year of transplant		
1999	186	(6)
2000	240	(7)
2001	208	(6)
2002	196	(6)
2003	197	(6)
2004	302	(9)
2005	350	(11)
2006	398	(12)
2007	405	(12)
2008	325	(10)
2009	283	(9)
2010	73	(2)
2011	113	(3)

Proposal: 1811-115

Title:
Effect of HLA-A Expression and HLA-B -21 M/T Dimorphism on Outcomes Following Allogeneic Hematopoietic Cell Transplant

Christine Camacho-Bydume, MD, camachc1@mskcc.org, Memorial Sloan Kettering Cancer Center
Joannis Mytilineos, MD, PhD, j.mytilineos@blutspende.de, Institute for Clinical Transfusion Medicine and Immunogenetics, University Hospital Ulm and Red Cross Blood Transfusion Service Baden-Wuerttemberg-Hessia, Germany
Katharine Hsu, MD, PhD, hsuk@mskcc.org, Memorial Sloan Kettering Cancer Center

Hypothesis:
Differential expression levels of human leukocyte antigen (HLA) alleles have been shown to impact viral control of human immunodeficiency virus (HIV). Increased expression of HLA-A was recently associated with impaired control of HIV and more rapid progression to AIDS. Higher levels of HLA-A correlated with increased expression of HLA-E, a ligand for the inhibitory receptor NKG2A of natural killer (NK) cells, which led to enhanced inhibition of NK cell degranulation of HIV infected T-cells[1]. A dimorphism of methionine (M) or threonine (T) located at -21 residue of HLA-B corresponds to position 2 of the anchor residue on peptides binding to HLA-E and impacts overall binding and expression of HLA-E[1,2]. In the same study, -21M variant of HLA-B was associated with more stable binding and enhanced expression of HLA-E and inhibition of NKG2A-expressing NK cells in a similar manner[1].
The effects of HLA-A expression and HLA-B -21 dimorphism on clinical outcomes following allogeneic hematopoietic cell transplant (HCT) remain unknown, but the contribution of NK and T cells in graft-versus-leukemia (GVL) and graft-versus-host disease (GVHD) illustrate their potential impact post-transplant. Given their effect of increased HLA-E expression, increased HLA-A expression and/or the presence of -21M dimorphism in HLA-B could potentially be associated with higher rates of disease relapse by promoting inhibition of early reconstituting NKG2A+ NK cells leading to poor tumor surveillance. In addition, higher HLA-A expression could promote higher rates of GVHD due to greater antigen presentation; the impact of HLA-B -21 dimorphism on GVHD is unknown, but could tilt in favor of GVHD if NKG2A+ NK cells are inhibited due to higher HLA-E expression.

Specific aims:
This study will evaluate how polymorphism controlling HLA-A surface expression and dimorphism of HLA-B at residue -21 impact outcomes following allogeneic 8/8 HLA-matched HCT for treatment of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), and lymphoma. This will be studied in the following four specific aims:

- To investigate the association of high versus low HLA-A expression on post-transplant outcomes of relapse, survival, acute GVHD, and transplant-related mortality (TRM).
- To evaluate the association of dimorphism of -21M versus -21T in HLA-B with outcomes of relapse, survival, acute GVHD, and TRM.
- To analyze the combined effects of HLA-A expression and -21 M/T dimorphism on outcomes of relapse, survival, acute GVHD, and TRM.

- To evaluate high versus low HLA-E expression, as defined by the two alleles E*01:01 and E*01:03, in a subset of patients where typing is available, and effect on outcomes of relapse and survival.

Scientific impact:
As the number of allogeneic transplants continues to increase, there remains a crucial need to further investigate methods to maximize GVL effect and minimize serious post-transplant complications. Despite multiple advances in high-resolution HLA typing, conditioning regimens, and supportive care measures, disease relapse and acute GVHD account for 22% and 10%, respectively, of all mortality during first 100 days post-HCT from unrelated donors[3]. Although much of the attention on HLA polymorphism has focused on improved allele matching in HCT, recent findings illustrate that allele-related expression levels of HLA may also play a role in the pathogenesis of certain diseases and subsequent immune responses.
With the observed variations in HLA class I expression during viral infection and cancer, differential expression of HLA alleles may expose a mechanism by which the immune response can be calibrated post-transplant[4]. Differential expression of HLA-C was previously shown to impact the risk of acute GVHD, non-relapse mortality, and overall mortality in the setting of 9/10 mismatched HCT[5]. Recent findings have demonstrated that differential expression of HLA-A alleles and the -21 dimorphism in HLA-B impact viral control of HIV, potentially due to their effect on NKG2A-mediated NK cell inhibition via differential expression of HLA-E[1]. Given the central role of donor NK and T cells in GVL and GVHD, differential HLA-A expression and -21 dimorphism of HLA-B may contribute to the calibration of NK and T cell alloreactivity post-transplant and impact clinical outcomes. Recognition of an association of differential HLA-A expression and HLA-B -21 dimorphism with post-transplant outcomes may provide further prognostic information to minimize relapse and morbidity post-transplant.

Scientific justification:
NK cells are the first donor-derived immune cells to reconstitute within the first month following HCT. NKG2A-expressing NK cells are known to be the predominant phenotype of early reconstituted NK cells[4]. NKG2A is an inhibitory receptor mainly expressed on NK cells and contributes to NK cell education and responsiveness. As part of the innate immune response, these donor NK cells are crucial in eliciting GVL effect. Although donor T-cells are the major effector cells responsible for mediating GVHD, NK cells have been implicated in reducing acute GVHD by targeting host antigen-presenting cells (APC) and donor alloreactive T-cells[6]. For example, NKG2A-expressing NK cells have been shown to inhibit activated T-cells. Determining ways to maximize this dual role of NK cells following HCT remains paramount to improving post-transplant outcomes.
Differential levels of HLA expression have been evaluated for their role in impacting the pathogenesis of certain diseases and infections and subsequent immune responses[4]. For example, HLA-C expression was previously shown to be associated with HIV control and impact the strength and likelihood of cytotoxic T-cell responses to HIV[7]. A recent study demonstrated that differential levels of HLA-A expression also influence viral control of HIV. By measuring mRNA levels of 22 different HLA-A antigens and calculating z-scores to represent degree of variation from the mean expression level, HLA-A allotypes were categorized as low versus high expressing and compared for their effects on outcomes[1]. Increased HLA-A expression was shown to impair HIV control and be associated with more rapid progression to AIDS. Through increased production of a HLA-A derived signal peptide, higher levels of HLA-A correlated with increased expression of HLA-E, which acts as a ligand for the inhibitory receptor NKG2A on NK and T-cells and leads to enhanced inhibition of NK cell degranulation of virally infected T-cells[1]. In the same study, HLA-B dimorphism of -21M or -21T was also shown to influence HLA-E expression and thus

impact NK cell education and inhibition[1,2]. Greater inhibition of NK cell degranulation of infected HIV target cells was observed when HLA-A expression increased in individuals homozygous for the -21M dimorphism, illustrating their complementary effects of HLA-E expression and NK cell inhibition[1].
As a non-classical HLA class I molecule with limited genetic variation, HLA-E is expressed primarily on lymphoid and endothelial cells and has been shown to regulate innate and adaptive immune responses[8]. Two common HLA-E alleles exist (E*01:01 and E*01:03) in relatively equal frequencies in most populations, but show variations in expression levels and peptide binding affinities[9]. Patients with acute leukemia with the higher expressing allele of HLA-E*01:03 were shown to be associated with worse overall survival and disease-free survival when compared to patients with E*01:01 in multivariate analysis[10].
The effect of differential HLA-A and HLA-E expression and the -21 dimorphism of HLA-B can be applied to allogeneic HCT, where NKG2A+ NK cells are known to be the predominant phenotype of early reconstituted NK cells[6]. Although preventative measures are employed to minimize the risk of GVHD, acute GVHD remains a common complication due to differences in minor MHC antigens. Following HCT, enhanced inhibition of NKG2A+ NK cells could lead to decreased suppression of activated donor-derived T-cells causing GVHD. Recent findings illustrated that patients who developed acute GVHD had lower numbers of NKG2A+ NK cells when compared to patients without acute GVHD following HCT[6].
Therefore, we hypothesize that increased HLA-A expression and presence of alleles harboring the -21M dimorphism in HLA-B may be associated with higher rates of disease relapse and GVHD following allogeneic HCT due to higher HLA-E expression and subsequent NK inhibition.

Patient eligibility population:
The study population will include patients greater than 18 years old diagnosed with AML, MDS, ALL, CML, and lymphoma, who receive 8/8 HLA-matched allogeneic HCT from an unrelated or related donor.

Data requirements: The proposed study does not require collection of any supplemental data outside of current data collection forms.

Patient-related

- Age: 18-29 vs. 30-39 vs. 40-49 vs. 50-59 vs. ≥ 60
- Gender: male vs. female
- Race/ethnicity
- Karnofsky score: <90 vs. 90-100%

Disease-related

- Diagnosis: AML vs. MDS vs. ALL vs. CML vs. Lymphoma (NHL, HL)
- Disease status at transplant

Transplant-related

- Donor and recipient HLA typing
- Donor age
- Degree of HLA typing: 8/8 matched (HLA-A, -B, -C, -DR)
- Year of transplant: 2000-2017
- Condition regimen intensity: myeloablative
- Will include non-myeloablative or reduced intensity conditioning for lymphoma patients only
- Donor-recipient gender match: M/M vs. M/F vs. F/M vs. F/F

- Source of stem cells: bone marrow vs. peripheral blood
- Donor type: related vs. unrelated donor
- Type of graft: T-cell replete

Study design:
In the proposed study, we will initially examine the differential expression of HLA-A alleles and its association with outcomes of relapse, survival, GVHD, and TRM following allogeneic HCT. We will divide recipients into disease-specific cohorts for AML, MDS, ALL, CML, and lymphoma (NHL, HD). We will categorize recipients by their HLA-A alleles into high versus low subtypes according to the model of HLA-A expression described in Ramsuran et al, *Science* 2018. For example, we will calculate the sum of z-scores corresponding to the two HLA-A alleles for each recipient. We will categorize high expression as combined z-scores greater than zero and low expression as less than zero. We will then analyze recipients with high HLA-A expression compared to recipients with low HLA-A expression and their association with outcomes of survival and relapse in disease-specific cohorts. We will evaluate the association of HLA-A expression on the outcomes of acute GVHD and TRM in the collective cohort by combining all disease groups.
We will also investigate whether the -21M or -21T dimorphism of HLA-B influences post-transplant outcomes. We will classify recipients based on their HLA-B alleles into -21M or -21T variants, according to Horowitiz et al. *Sci Immunol* 2016. Those recipients homozygous for -21T/T will be compared to those recipients heterozygous for -21M/T or homozygous for -21M/M to determine their association with outcomes of relapse and survival in disease-specific cohorts. The outcomes of acute GVHD and TRM will be analyzed in the overall cohort with all disease groups combined.
If similar associations are observed in high HLA-A expression and presence of -21M dimorphism, HLA-E may be responsible for mediating such effects on outcomes and thus evaluating their combined effect is beneficial. By scoring recipients based on their level of HLA-A expression and -21 dimorphism of HLA-B (i.e. low HLA-A expression and -21T/T, low HLA-A expression and -21M/T or -21M/M, high HLA-A expression and -21T/T, high HLA-A expression and -21M/T or -21 M/M), we will analyze their association with relapse and survival in disease specific cohorts and with acute GVHD and TRM in the overall cohort.
In a subset of leukemia patients where HLA-E typing is available, we will adjust the analyses to include recipient HLA-E polymorphism, hypothesizing that recipients with the high expressing allele HLA-E*01:03 versus recipients with only the low expressing allele HLA-E*01:01 (HLA-E*01:01/01:01) will exhibit higher rates of relapse post-HCT.

Data source: This study will use the CIBMTR Research Database.

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Conflicts of interest:
None

Prop 1811-115 – Table 1. First allogeneic 8/8 matched HCT for malignant disease with retrospective high resolution typing available, 2000-2018

Variable	N	(%)
Number of recipients	15635	
Disease		
AML	6837	(44)
ALL	2100	(13)
CML	838	(5)
MDS	4078	(26)
NHL	1514	(10)
Hodgkins Lymphoma	268	(2)
Race/Ethnicity		
Caucasian, non-Hispanic	13800	(90)
African-American, non-Hispanic	399	(3)
Asian, non-Hispanic	311	(2)
Pacific islander, non-Hispanic	16	(<1)
Native American, non-Hispanic	40	(<1)
Hispanic, Caucasian	709	(5)
Hispanic, African-American	9	(<1)
Hispanic, Asian	2	(<1)
Hispanic, Pacific islander	12	(<1)
Hispanic, Native American	3	(<1)
Hispanic, race unknown	37	(<1)
Other	8	(<1)
Unknown	289	N/A
Recipient age at transplant		
10-17 years	264	(2)
18-29 years	1654	(11)
30-39 years	1826	(12)
40-49 years	2710	(17)
50-59 years	4213	(27)
60 years and older	4968	(32)
Median (Range)	54	(18-84)
Sex		
Male	8962	(57)
Female	6673	(43)
Graft type		
Marrow	3075	(20)
PBSC	12520	(80)
Cord	34	(<1)

Variable	N	(%)
BM+PBSC	3	(<1)
PBSC + Cord	1	(<1)
PB + Other	2	(<1)
Donor type		
HLA-identical sibling (may include non-monozygotic twin)	1524	(10)
Sysgeneic (monozygotic twin)	17	(<1)
Unrelated donor	10588	(68)
HLA-matched other relative	35	(<1)
HLA-mismatched relative	6	(<1)
HLA-matched unrelated	3149	(20)
HLA-mismatched unrelated	210	(1)
Unknown	106	N/A
Conditioning regimen intensity		
Myeloablative	8910	(61)
Non-myeloablative/RIC	5726	(39)
Unknown	999	N/A
Recipient CMV Serostatus		
Negative	6004	(39)
Positive	9412	(61)
Inconclusive	27	(<1)
Not tested	54	(<1)
Unknown	138	N/A
Karnofsky performance score		
10-80	5645	(38)
90-100	9267	(62)
Unknown	723	N/A
Number of high resolution matches out of 10		
8/10	18	(<1)
9/10	837	(5)
10/10	14779	(95)
Unknown	1	N/A
HLA-E typing available		
Yes	1796	(11)
No	13839	(89)
Retrospective high resolution typing		
Yes	15635	(100)
Year of transplant		
2000	316	(2)
2001	331	(2)
2002	337	(2)

Variable	N	(%)
2003	397	(3)
2004	532	(3)
2005	678	(4)
2006	772	(5)
2007	934	(6)
2008	910	(6)
2009	986	(6)
2010	1024	(7)
2011	1037	(7)
2012	1282	(8)
2013	1600	(10)
2014	1698	(11)
2015	1536	(10)
2016	1031	(7)
2017	234	(1)

Proposal: 1811-157

Title
Clinical correlation of DPB1 histocompatibility in BMT clinical outcome

Pedro Cano, M.D., Moffitt Cancer Center.
Joseph Pidala, M.D., Moffitt Cancer Center
Claudio Anasetti, M.D., Moffitt Cancer Center

Hypothesis:
This is a proposal to test the following hypothesis:
Matching for the main functional DP groups (DP1, DP2, DP3 and DP4) improves outcome after bone-marrow transplantation.

Specific aims:
- Study the correlation between DPB1 histocompatibility in the graft-versus-host direction based on DP1, DP2, DP3 and DP4 functional epitopes and acute graft-versus-host disease.
- Assess the effect on outcome of functional-epitope (DP1 through DP4) histocompatibility, after adjusting for 3'UTR-SNP histocompatibility.
- Assess the effect on outcome of functional-epitope (DP1 through DP4) histocompatibility, after adjusting for T cell epitope histocompatibility, as defined by Fleischhauer.
- Evaluate the role of other functional epitopes on acute graft-versus-host disease.

Definition of matching:
Matching for the DP functional epitopes is defined as two alleles being in the same functional group. For instance:
- DPB1*04:01:01:01 and DPB1*02:02 are a match (both in DP4)
- DPB1*04:02:01:01 and DPB1*02:01:02 are a match (both DP2)
- DPB1*04:01:01:01 and DPB1*04:02:01:01 are *not* a match (DP4 versus DP2)
- DPB1*02:02 and DPB1*02:01:02 are *not* a match (DP4 versus DP2)

Scientific Impact
If the proposed study shows that DPB1 histocompatibility using functional epitopes significantly affects BMT outcomes, donor selection criteria will be amended and patient outcomes will improve.
It is possible that the observed effect of HLA-DPB1 3'-UTR SNP histocompatibility on BMT outcomes is caused by functional-epitope histocompatibility due to the very high correlation between the two sets of variables. This study would through light on this distinction.

Scientific justification
HLA allele nomenclature is not reliable to establish functional and clinical correlations. Using allele full names (4 fields), or even 2-field names, hide similarities among alleles. Abbreviated names (1-field names or serologic-equivalent names) hide gross differences among alleles. The amino acid sequence of alleles must always be taken into account. Although the full amino acid sequence would make any data analysis in functional and clinical correlation studies too complex, there are obvious methods to extract the most relevant information.

Structural crystallographic studies reveal which positions in the HLA molecule make up the peptide binding groove being in contact with the bound peptide and which positions are in contact with the T-cell receptor. These positons are of primary relevance.
Anti-HLA antibody specificity studies allow the molecular mapping of serologic epitopes to specific amino acids at specific positions.
Outside the coding region, HLA genes carry additional information that may affect their expression, like the binding site of microRNAs to the 3'UTR region.
HLA-DP molecules are particular difficult to evaluate in clinical studies. Allele-level studies may attenuate a real clinical effect to the point that it appears insignificant or even non-existent. When correlations are established with specific molecular properties of HLA-DP molecules, then these effects might become obvious.
The following facts must be considered:

- There are two dimorphic epitopes (DPB1:{56}:(A)/(E) and DPB1:{85-86-87}:(EAV)/(GPM)) that classify DPB1 alleles in four functional groups (DP1, DP2, DP3 and DP4). These 2 functional epitopes are immunodominant and account for the specificity of T-cell clones and the majority of anti-DP antibodies. [P Cano and M Fernández Viña 2009 Hum Immunol 70:836]

		85-86-87	
		EAV	GPM
56	A	**DP1**	**DP4**
	E	**DP3**	**DP2**

  The gene frequency is p(DP1) = 0.14, p(DP2) = 0.26, p(DP3) = 0.15, and p(DP4) = 0.45.
- These basic DPB1 allele groups identified by serologic methods had long ago also been identified by T-cell detection methods ('primed lymphocyte typing' or PLT) showing how these functional epitopes are recognized by both antibodies and T cells. [A Urlacher et al. 'T cell clones show polymorphism within DPw1, DPw2 and DPw3 specificities' in the 1987 10th International Histocompatibility Workshop.]
  The two polymorphisms (DPB1:{56}:(A)/(E) and DPB1:{85-86-87}:(EAV)/(GPM)) should be matched one at a time in the graft-versus-host direction, and scored according to the following histocompatibility tables:

	{56}			
		recipient		
		A+A	E+E	A+E
donor	A+A	0	1	1
	E+E	1	0	1
	A+E	0	0	0

	{85-86-87}			
		recipient		
		EAV+EAV	GPM+GPM	EAV+GPM
donor	EAV+EAV	0	1	1
	GPM+GPM	1	0	1
	EAV+GPM	0	0	0

  Where '0' means compatible or match and '1' means incompatible or mismatch.
- A recipient-donor pair can be matched or mismatched at each of these two functional-epitope loci. There are 4 logical possibilities and 3 serologic matching scores:

{56}	{85-86-87}	serologic matching score
0	0	0
0	1	1
1	0	1
1	1	2

- This functional matching score (in the GvH direction) can then be used to study correlation with acute graft-versus-host disease
- DPB1 and DPA1 gene products form the DP heterodimer, a functional unit. Although products of these two genes are strongly associated, this association is not perfect and the combined effect of the two genes forming one heterodimer may have to be taken into account.

- Antibody studies show that although DPA1 codes protein regions that are certainly the target of antibodies, the role of this alpha gene is not as prominent as in the case of DQA1 in the case of anti-DQ antibodies.
- There have been several independent observations of the effect of 3'UTR-SNP variation on BMT outcome. [EW Petersdorf et al. 2015 N Eng J Med 373:599] This SNP variation, however, is strongly correlated with the DP1 through DP4 functional epitope variants and this effect must be carefully reevaluated. Based on 4,587 DPB1 allele assignments, 3'UTR SNPs (P Cano unpublished work) can be strongly correlated with serologic groups:

3'UTR SNP	functional group	χ^2
AA	DP2 or DP4	4046
GA	DP1	4192
GG	DP3	4137

- The reported risk of GVHD associated with HLA-DPB1 mismatching influenced by the DPB1 rs9277534 expression marker in the 3'UTR region can actually be linked to disparity for the functional-DP-epitopes DP1 thorugh DP4.
  Although this link between 3'UTR SNPs and functional epitopes does not seem to have been widely recognised yet, the link with the coding region has. [B Schöne et al. 2018 Hum Immunol 79:20; Morishima et al. 2018 Blood 131:808]
- T-cell receptor epitopes (TCE) have been identified in some DPB1 alleles. [K Fleischhauer et al. 2001 Blood 98:1122; E Zino et al. 2004 Blood 103:1417] TCE do not correlate with the proposed DP1 through DP4 functional epitopes, as defined by Cano (unpublished work).

Patient eligibility population
Post-hematopoietic-stem-cell-transplantation patients with full matches in all loci except DPB1 and DPA1.

Data requirements
High-resolution typing for all HLA loci, including A, B, C, DRB1, DRB3/4/5, DQA1, DQB1, DPA1 and DPB1.

Study design
This is a retrospective study of cases with known clinical outcome after bone marrow transplantation and different degrees of DPB1 histocompatibility.
Covariates:

- DPB1 {56} + {85-86-87} functional matching score in the graft-versus-host direction (as described above). These two variables can be assessed independently.
- Alleles where their functional epitopes do not correlate with the 3'UTR SNP variant alleged to have an effect on outcome. These alleles include:
  - DPB1*31:01:01:01
  - DPB1*106:01
  - DPB1*55:01:01:01
  - DPB1*30:01:01:01
  - DPB1*34:01:01:01
  - DPB1*15:01:01:01
  - DPB1*17:01:01:01
  - DPB1*124:01:01:01

  - DPB1*463:01:01:01,and
  - DPB1*18:01:01:01.

All of these alleles together have a gene frequency of about 3%.

- DPB1 3'UTR SNP
- DPB1 alleles classified by the T cell defined epitopes, according to Fleischauer.
- Secondary DPB1 functional epitopes including: DPB1:{8-9-11}, {33-35-36}, {55-56-57}, {84} and {11-65-76}. (Cano, unpublished observation.)

Primary clinical endpoints:

- Acute GVHD grades II- IV (during first 100 days post-transplantation)

Secondary clinical endpoints:

- Grade III-IV acute GVHD
- Overall survival
- Disease-free survival
- Treatment-related mortality
- Relapse
- Chronic GVHD

Statistical considertation:

Among 8/8-matched unrelated donors, in 37% cases both DPB1 alleles are matched, in 46% cases one DPB1 is matched and the other is not, and in 17% of cases both alleles are mismatched. The most informative comparisons will be between the 0-allele-matched group and the 1-allele matched group since these comparisons will reveal the effect of a single allele mismatch.

Alleles like DPB1*17:01:01:01 (with a gene frequency of 2%) are particularly interesting because their functional epitopes do not correlate with the 3'UTR SNP variant alleged to have an effect on outcome. So about 3% of the samples will be critical in the analysis, and since 46% of cases show 1-allele mismatches, in order to get n informative cases we will need x total cases: x * 0.03 * 0.46 = n. For n = 100, x = 7,246 and for n= 200, x = 14,462. The number x refers to the lowest number of cases required to be able to extract confident conclusions in regard to distinguishing whether it is the functional epitopes proposed in this study or the effect of the altered expression by alleles carrying the 3'UTR SNP variant that is responsible for the increase rate of GVHD. The minimal value of n depends on the magnitude of the difference on outcome found between the compatible and the incompatible.

Power calculations are expected from the CIBMTR statistical team.

Supplementary documents

- Definition of DP functional epitopes and correlation between the primary functional epitopes and 3'UTR SNPs (See attached document: DPB1_FunctionalGroups.xlsx)

Prop 1811-157 – Table 1. First allogeneic 10/10 matched HCT with retrospective high resolution typing available, 2000-2018

Variable	N	(%)
Number of recipients	14088	
Disease		
AML	5997	(43)
ALL	2199	(16)
Other leukemia	334	(2)
CML	767	(5)
MDS	3320	(24)
Other acute leukemia	108	(1)
NHL	984	(7)
Hodgkins Lymphoma	195	(1)
Plasma Cell Disorders, MM	169	(1)
Other malignancies	15	(<1)
Race/Ethnicity		
Caucasian, non-Hispanic	12564	(91)
African-American, non-Hispanic	299	(2)
Asian, non-Hispanic	234	(2)
Pacific islander, non-Hispanic	12	(<1)
Native American, non-Hispanic	38	(<1)
Hispanic, Caucasian	594	(4)
Hispanic, African-American	11	(<1)
Hispanic, Asian	2	(<1)
Hispanic, Pacific islander	7	(<1)
Hispanic, Native American	4	(<1)
Hispanic, race unknown	41	(<1)
Other	7	(<1)
Unknown	275	N/A
Recipient age at transplant		
0-10 years	619	(4)
10-17 years	814	(6)
18-29 years	1366	(10)
30-39 years	1483	(11)
40-49 years	2267	(16)
50-59 years	3381	(24)
60 years and older	4158	(30)
Median (Range)	52	(0-84)
Sex		
Male	8116	(58)
Female	5972	(42)

Variable	N	(%)
Graft type		
Marrow	3548	(25)
PBSC	10448	(74)
Cord	88	(1)
BM+PBSC	1	(<1)
PBSC+Cord	1	(<1)
PBSC + Other	2	(<1)
Donor type		
Unrelated donor	11120	(79)
HLA-matched unrelated	2865	(20)
HLA-mismatched unrelated	103	(1)
Conditioning regimen intensity		
Myeloablative	8231	(62)
Non-myeloablative/RIC	4954	(38)
Unknown	903	N/A
Recipient CMV Serostatus		
Negative	5653	(40)
Positive	8266	(59)
Inconclusive	24	(<1)
Not tested	32	(<1)
Unknown	113	N/A
Karnofsky performance score		
10-80	4810	(36)
90-100	8658	(64)
Unknown	620	N/A
Number of high resolution matches out of 10		
10/10	14088	(100)
DPB1 match		
Double allele mismatch	4047	(29)
Single allele mismatch	7699	(55)
Full allele match	2342	(17)
Retrospective high resolution typing		
Yes	14088	(100)
Year of transplant		
2000	341	(2)
2001	343	(2)
2002	351	(2)
2003	280	(2)
2004	494	(4)
2005	577	(4)

Variable	N	(%)
2006	660	(5)
2007	723	(5)
2008	805	(6)
2009	984	(7)
2010	970	(7)
2011	909	(6)
2012	1161	(8)
2013	1445	(10)
2014	1477	(10)
2015	1416	(10)
2016	960	(7)
2017	192	(1)

Proposal: 1811-165

Title:
Impact of Donor HLA on Transplant Outcomes in NPM1 Mutated AML

Narayan, Rupa, MD, rnarayan3@mgh.harvard.edu, Massachusetts General Hospital
Meyer, Everett, MD, PhD, evmeyer@stanford.edu, Stanford University
Chen, Yi-Bin, MD, YChen6@partners.org, Massachusetts General Hospital

Hypothesis:
We hypothesize that donor HLA haplotype impacts the outcome of patients with NPM1 mutated AML undergoing allogeneic transplantation.

Specific aims:
We will evaluate the impact of donor HLA haplotype on disease outcomes including incidence of relapse, progression free survival and overall survival in patients with NPM1 mutated AML undergoing matched related or matched unrelated allogeneic transplantation.

Scientific justification:
Somatic mutations in cancer have been found to be a potential source of cancer specific neoantigens.[1–3] Acute myeloid leukemia (AML) has common recurrent mutations shared between patients in addition to private mutations specific to individuals. In our recent work, we hypothesized that recurrent mutations in AML may result in shared neoantigens. We searched for shared neoantigens by predicting *in silico* HLA Class I binding affinities of peptide sequences from common recurrent AML mutations compared to their corresponding wildtype sequences using NetMHC (manuscript under review). The results for mutated *NPM1* were of particular interest due to the high frequency of this mutation amongst AML patients and the predicted binding affinity pattern of mutation bearing peptide sequences to common HLA alleles (Figure 1).
NPM1 is recurrently mutated in approximately 30% of adult AML,[4] and generally arises from base pair insertions which result in a frameshift, and consequently novel C terminus sequences[5] which are markedly different from the wildtype sequence. The resultant mutation-bearing C-terminal protein sequences contain ligands with predicted binding affinity for common HLA alleles such as A2, A3, and A11. For instance, peptides AIQDLCLAV and CLAVEEVSL are predicted to bind HLA-A*02:01; peptide AVEEVSLRK is predicted to bind A*03:01 and A*11:01, with weak predicted binding to A*30:01, A*66:01, and A*68:01. HLA-A*02:01 and A*03:01 are among the most common HLA alleles in the US.[6,7] We subsequently surveyed peripheral blood mononuclear samples (PBMCs) from HLA-A2 and A3 healthy donors using A*02:01 and A*03:01 tetramers loaded with the respective mutant NPM1 neoantigen peptides, and found a small but consistent percentile of CD8+ T cells that were able to bind the mutant-NPM1-tetramer complex (manuscript under review). The significance of allogeneic neoantigen recognizing T cells is not well understood but has potential impacts in the transplant setting for graft versus leukemia effect.
Further, mutant NPM1 tends to be localized to the cytoplasm in contrast to the nuclear localization of wildtype NPM1.[5] This change in localization may affect protein processing of mutant NPM1 and has been hypothesized to result in more efficient HLA presentation of both unmutated and mutated peptides from mutant NPM1 protein.[8]
Prior work in field:
Several studies have supported the immunogenicity of mutated NPM1. Kuzelova et al.,[8] compared HLA Class I frequencies in patients with AML compared to normal individuals. Interestingly they found that

several HLA allele groups were less frequently found in NPM1 mutated patients (including B*07, B*18, B*40 and a trend for A*03, A*11, B*39, C*03 and C*07) compared to the general population. Intriguingly, amongst patients with mutated NPM1, those with at least one of these types of alleles had overall survival advantage which was not found amongst those with wildtype NPM1 in this study. While several of these alleles are predicted binders to mutation-bearing NPM1 peptides, others are predicted to bind non-mutated peptides from NPM1. Their work suggests that the cytoplasmic localization itself of mutant NPM1 results in HLA immune interactions, possibly from more efficient processing of both mutated and unmutated peptides from cytoplasmic mutant NPM1 protein. In another study evaluating allogeneic transplant recipients,[9] sera from patients who developed extensive graft versus host disease were collected and found to stain the nucleolar region of target cells; target antigens were found to include B23 (NPM1) and C23.[9] Greiner et al.,[10] found that synthetic peptides from mutated NPM1, which were predicted HLA-A2 binders, elicited *in vitro* CD8+ T cell responses in both healthy donors and AML patients. They also found a statistically significant increase in PD-L1 expression in the leukemic stem cell fraction of NPM1 mutated AML compared to NPM1 wildtype AML.[11]

Based on these specific features, we hypothesize that donor HLA haplotype will impact the outcome of patients with NPM1 mutated AML through immunological interactions with tumor HLA presentation of NPM1 peptides. Data from the CIBMTR will be critical for this type of analysis as it will allow us to evaluate the impact of varied HLA alleles which may not otherwise be apparent with small cohort numbers.

Prior evaluation of outcome of NPM1 mutated AML post-allogeneic transplant: An EBMT study of 156 patients with normal karyotype AML with mutated NPM1 and FLT3-ITD negative status transplanted between 2006-2012 using matched donors[12,13] reported the following results: 2-year cumulative incidence of relapse (27%), non-relapse mortality (13%), chronic GVHD (37%), and overall survival (70%). Disease remission status (CR1 vs CR2 vs beyond/other), use of an unrelated donor, and age impacted outcomes in univariate analysis, with disease remission status impacting survival in multivariate analyses. In another study of isolated mutated NPM1 AML patients undergoing transplantation in CR1,[14] including 72 patients with matched related donors and 59 with matched unrelated donors, 2 year overall survival was 83%, with donor type and age affecting outcomes. In a donor versus no donor analysis among 304 patients on the SAL-AML-2003 study with intermediate cytogenetic risk NPM1 mutated AML,[15] 3-year relapse-free rates were 47% and 71% in the no-donor versus donor group with 3-year overall survival being similar amongst the two groups (60% versus 70% respectively). Age and FLT3-ITD status were found to impact relapse free survival rates, with LDH, FLT3-ITD, and age being found to impact OS in a multivariate analysis. In summary, while factors such as disease remission status (CR1 vs CR2 vs beyond/other), age, FLT3-ITD status, and donor type (related vs unrelated) have been found to influence outcomes post-allogeneic transplantation in NPM1 mutated AML, the impact of donor haplotype in the context of this mutation has not been well studied.

Scientific impact:

There are several potential impacts of this work:

- NPM1 is mutated in approximately 30% of adult AML.[4] While isolated NPM1 mutated AML is generally not transplanted in first CR, approximately 30-70% of patients with NPM1 mutated AML have disease relapse within five years, depending on factors such as age and the presence of concurrent mutations including FLT3-ITD.[16–18] If HLA haplotype is found to impact outcome, this may affect decisions regarding post-transplant consolidation for haplotypes with higher risk of poor disease outcome.
- The clinical relevance of neoantigen recognizing T cells in the context of allogeneic hematopoietic cell transplant (HCT) has not been well characterized. We anticipate that this

work will lead to future novel studies evaluating the role of allogeneic neoantigen-specific T cells on graft versus leukemia and graft versus host biology.
- We anticipate the proposal of future studies evaluating donor samples for the presence of neoantigen specific T cells and whether such findings can be therapeutically manipulated to improve patient outcomes, such as with post-transplant neoantigen vaccination with checkpoint blockade or through novel neoantigen specific cellular therapy.
- The study may provide context for future studies evaluating impact of HLA haplotype on other potential neoantigens.

Patient eligibility population:
- Adults age 18 and older
- Disease: De novo AML in CR1/CRi1 or CR2/CRi2
- Mutated NPM1 status from diagnosis (Form 2402, Q22-33)
- Graft source: bone marrow and peripheral blood
- Donor: HLA matched related (8/8), HLA matched unrelated (8/8)
- Transplant period: tentatively 2008-2016 (to allow minimum two-year follow-up)
- Conditioning: any
- GVHD prophylaxis: any
- Therapies prior to transplant: any

Exclusions:
- Ex-vivo T cell depletion protocols
- Exclude haploidentical and umbilical cord blood donor-based transplants

Data requirements:
Data collection forms:
- Form 2000, Recipient Baseline Data
- Form 2004, Infectious Disease markers
- Form 2150, Viral infection Diagnosis and Treatment
- Form 2005, Confirmation of HLA typing
- Form 2006, Hematopoietic Stem Cell Transplant infusion
- Form 2010, AML Pre-HCT Data
- Form 2100, Post-HSCT Data
- Form 2110, AML post-HCT Data
- Form 2400, Pre-transplant essential data
- Form 2402, Pre-transplant essential data, disease classification Q3-89, AML
- Form 2450, Post-transplant essential data
- Form 2500, Recipient eligibility form
- Form 2900, Recipient death data

Variables for description and analysis:
Please see attached appendix

Data sources:
- CIBMTR Research Database
- Study team will provide HLA predicted binding affinity data for both mutated and unmutated peptides from mutated NPM1 protein and predicted HLA stratification

- Study team includes a computational immunologist (Dr. Niroula) who will be able to help with creation of coding script to help group HLA types to evaluate outcomes.

Study design:

The goal of this study is to examine whether donor HLA haplotype impacts outcomes in NPM1 mutated AML. Patients with de novo AML with mutated *NPM1* (from the time of diagnosis) in CR1 or CR2 will be included. De novo AML is selected (with exclusion of secondary and therapy related disease) to reduce disease type as confounder. Matched related and matched unrelated with exclusion of mismatch unrelated, haploidentical, and cord blood based transplantation is selected to reduce donor cell type as a confounder as much as possible. Disease status of CR1/CRi1 and CR2/CRi2 is selected as we anticipate that CR1/CRi1 may include patients with clinically relevant FLT3 mutated/NPM1 mutations and that CR2/CRi2 may include additional patients with either isolated NPM1 mutations or with co-occurring relevant mutations. A research period of 2008-2016 is selected as NPM1 mutation testing became routinely assessed around 2006-2008 in most centers and using 2016 as cutoff to allow for a minimum of 2-year follow-up. A limitation of this study is that the CIBMTR collection forms do not report the type of *NPM1* mutation. However, the majority of *NPM1* mutations are due to mutations A, B, and D, with mutation A accounting for approximately 70-80% of all *NPM1* mutations.[19–21] Further, in terms of peptide sequence, the C-terminal sequence AVEEVSLRK is shared by the vast majority of NPM1 mutations.[19]

We will initially obtain the number of patients with NPM1 mutated AML along with donor HLA haplotype. We will evaluate donor HLA in two ways. First, we will group HLA haplotypes that are predicted binders versus predicted non-binders to peptides from the mutated region of NPM1. In the second analysis evaluating whether the cytoplasmic localization of NPM1 causes both mutated and unmutated peptides to be presented, we will group HLA by predicted binders versus predicted non-binders to peptides from both mutated and unmutated peptides from mutated NPM1 protein. Another consideration is for an unbiased approach with setting an outcome cutoff and evaluating which HLA haplotypes group into favorable outcomes versus non-favorable outcomes.

For comparison between the predicted binder versus predicted non-binder groups, chi-square testing will be used for categorical variables and mann-whitney testing for continuous variables. Primary endpoints will be evaluation of progression-free survival (PFS) and overall survival (OS). Secondary endpoints will include cumulative incidences of relapse (CIR), non-relapse mortality (NRM) and acute and chronic graft versus host disease (AGVHD and CGVHD respectively). PFS and OS will be analyzed by Kaplan Meier estimator. Events for PFS analysis will be defined as relapse or death from any cause. Events for OS analysis will be defined by death from any cause. Cumulative incidences will be analyzed via competing risk method.

Potential confounders on outcome based on prior studies include age, cytogenetic status, disease remission status (CR1 vs CR2 vs CRi1 vs CRi2), donor type (matched related versus matched unrelated) and FLT3 status (FLT3-ITD mutated versus not).[13,15–18] NPM1 measurable residual disease (MRD) status at the time of transplant has also been shown to impact outcome,[22] however a limitation of this study will be the unavailability of this data. We would propose that these potential confounders be fitted in a multivariate Cox model.

Outcomes:

- Overall survival (OS): defined as time from day of transplant until date of death from any cause, with live patients being censored at the time of last known follow-up.
- Progression free survival (PFS): defined as time from day of transplant until date of morphologic relapse or death from any cause. [based on CIBMTR hematologic remission criteria, cytogenetic or molecular relapse alone will not be classified as relapse events]

- Cumulative incidence of relapse will be calculated using graft failure or death as competing risks
- Cumulative incidence of NRM will be calculated using relapse as a competing risk.
- AGVHD will be described using grade II-IV skin, liver, or gastrointestinal GVHD. The cumulative incidence of AGVHD will be calculated using graft failure or death as competing risks.
- CGVHD affecting any organ will be described. The cumulative incidence of CGVHD will be calculated using graft failure or death as competing risks.
- Cumulative incidence of graft failure will be calculated using relapse or death as competing risks. Graft failure is defined as having less than 5% donor type CD3+ peripheral cells without concurrent relapse.

Conflicts of interest:
No conflicts of interest

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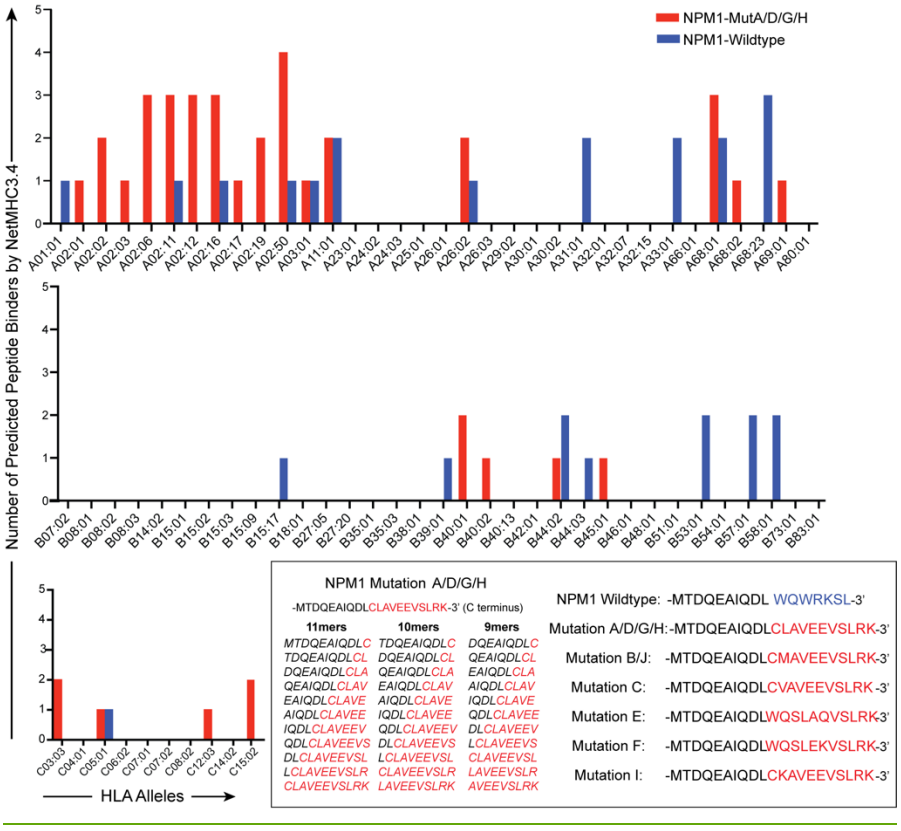
Figure 1. Evaluation of the number of peptides from the C-terminal mutation bearing sequence of mutated NPM1 (mutation A/D/G/H) that have predicted binding affinity to Class I HLA alleles using NetMHC3.4, with comparison to binding affinity from peptides of the corresponding wildtype sequence. (Predicted binding affinity of less than 500 nM [half-maximum inhibitory concentration, IC50] were counted as predicted binders; does not count predicted binders from non-C terminal nonmutated NPM1).



Appendix:

Sample HLA-predicted binder calling table:
(Study PI will provide predicted HLA affinity table; computational immunologist from study team will help create script to group)

First Scenario: Group predicted HLA binder versus predicted non-binder using donor's HLA and predicted binding affinity of peptides from NPM1 mutation bearing C terminal sequence based on hypothesis that mutated sequences lead to potentially immunogenic neoantigens that are not tolerized by immune system.

CIBMTR provided	**CIBMTR provided**	**Study team script**
Donor	**HLA haplotype**	**Predicted Binder or not**
Donor 1	HLA----	Predicted Binder
Donor 2	HLA-----	Predicted Binder
Donor 3	HLA-----	Predicted Non-Binder

Second Scenario: Group predicted HLA binder versus predicted non-binder using donor's HLA and predicted binding affinity of peptides from the entire mutated NPM1 protein (which consists of mutated and unmutated sequences) based on hypothesis that mutation causes cytoplasmic localization resulting in whole protein being more prone to HLA processing.

CIBMTR provided	**CIBMTR provided**	**Study team script**
Donor	**HLA haplotype**	**Predicted Binder or not**
Donor 1	HLA----	Predicted Binder
Donor 2	HLA-----	Predicted Binder
Donor 3	HLA-----	Predicted Non-Binder

Third Scenario: Unbiased approach with grouping based on pre-defined outcome (such as survival) and using cutoff to define which HLA groupings may be predictive of outcomes for future models.

Variables of interest:

	NPM1 mutated Predicted HLA Binder	NPM1 mutated Predicted HLA non-binder
Pre-transplant		
Number of patients		
Number of centers		
Year of Transplant 2008-2011 2011-2014		

2014-2016		
Age (median, range) [continuous variable]		
Age Categories 18-40 41-55 56-65 >66		
Gender Male Female		
Race Caucasian Black Asian Other Missing		
Ethnicity Hispanic Non-Hispanic Missing		
KPS 100 90 80 </=70 Missing		
HCT-CI 0 1 2+ Missing		
WBC at diagnosis <10 10-50 51-100 >100 Missing		
ELN 2010 classification at Dx Favorable Intermediate-I Intermediate-II Adverse Missing/Unassigned		
AML Cytogenetic Classification at Dx		

Favorable Intermediate-Abnormal Intermediate-Normal karyotype Poor Unknown/Missing		
Complex Karyotype at Dx Yes No Missing		
Monosomal Karyotype at Dx Yes No Missing		
Molecular: FLT3-ITD at Dx FLT3-ITD mutated FLT3-ITD unmutated Missing/Unknown		
Molecular: FLT3-TKD at Dx FLT3-TKD mutated FLT3-TKD unmutated Missing/Unknown		
Molecular: CEBPA at Dx Mutated Unmutated Missing/Unknown		
Molecular: IDH1 at Dx Mutated Unmutated Missing/Unknown		
Molecular: IDH2 at Dx Mutated Unmutated Missing/Unknown		
Molecular: KIT at Dx Mutated Unmutated Missing/Unknown		
Molecular: Other marker at Dx Mutated Unmutated Missing/Unknown		

Therapy Prior to Transplant Induction Induction+HMA HMA alone Other Missing		
Therapy Prior to Transplant: Number of Lines of Induction therapy prior to CR1 Duration of CR1 Number of consolidation cycles prior to transplant		
Disease Status at transplant Morphological Cri1 Morphological CR1 Morphological Cri2 Morphological CR2 Missing		
Transplant Characteristics		
Donor Age (median, range)		
Donor Type Matched related Matched unrelated		
Gender Donor/Recipient F/F F/M M/M M/F		
CMV Donor/Recipient Reactive/reactive Reactive/nonreactive Nonreactive/nonreactive Nonreactive/reactive		
Stem cell source: BM PB		
Conditioning Regimen MA RIC		
Conditioning Regimen TBI+Cy		

TBI+Cy+Other TBI+other Bu+Flu+TBI Bu+Flu+other Bu+Cy Bu+Cy+Others Flu/Mel Flu+Mel+Other Bu/Flu TBI+Cy+Flu TBI+Bu+Flu		
GVHD prophylaxis Tacrolimus based CsA based Others		
ATG Yes No		
Campath Yes No		
Post-tx Cy Yes No		
Planned DLI or other cell product: Yes/No		

Prop 1811-165 – Table 1. First allogeneic HCT for patients with de novo AML and NPM1 mutation identified, 2008-2018

Variable	N	(%)
Number of recipients	937	
Disease		
AML	937	(100)
Disease status at transplant		
CR1	679	(72)
CR2	258	(28)
Race/Ethnicity		
Caucasian, non-Hispanic	810	(90)
African-American, non-Hispanic	21	(2)
Asian, non-Hispanic	25	(3)
Native American, non-Hispanic	1	(<1)
Hispanic, Caucasian	37	(4)
Hispanic, African-American	1	(<1)
Hispanic, Native American	2	(<1)
Unknown	40	N/A
Recipient age at transplant		
18-29 years	37	(4)
30-39 years	84	(9)
40-49 years	178	(19)
50-59 years	224	(24)
60 years and older	414	(44)
Median (Range)	58	(18-80)
Sex		
Male	403	(43)
Female	534	(57)
Graft type		
Marrow	121	(13)
PBSC	816	(87)
Donor type		
HLA-identical sibling (may include non-monozygotic twin)	81	(9)
Unrelated donor	844	(90)
HLA-matched other relative	7	(1)
HLA-mismatched relative	5	(1)
NPM1		
Positive	937	(100)
FLT3 ITD mutation		
Negative	403	(45)

Variable	N	(%)
Positive	490	(55)
Unknown	44	N/A
FLT3 D835 point mutation		
Negative	591	(84)
Positive	111	(16)
Unknown	235	N/A
Number of high resolution HLA matches out of 8		
8/8	937	(100)
Conditioning regimen intensity		
Myeloablative	522	(56)
Non-myeloablative/RIC	415	(44)
Recipient CMV Serostatus		
Negative	303	(32)
Positive	629	(67)
Not tested	2	(<1)
Unknown	3	N/A
Karnofsky performance score		
10-80	423	(46)
90-100	501	(54)
Unknown	13	N/A
Year of transplant		
2009	1	(<1)
2012	2	(<1)
2013	55	(6)
2014	160	(17)
2015	163	(17)
2016	206	(22)
2017	183	(20)
2018	167	(18)

Proposal: 1811-185

Title:
The Impact of Single Nucleotide Gene Polymorphisms (SNP) in the Gamma Block of the Major Histocompatibility Complex (MHC) on Unrelated Donor Hematopoietic Cell Transplants (HCT) For Hematological Malignancies: Part II (Extension of study IB15-01- Analysis only)

Medhat Askar, MD, PhD, medhat.askar@bswhealth.org, Baylor University Medical Center
David Sayer, PhD, david_sayer@hotmail.com, CareDx, Inc
Ronald Sobecks, MD, sobeckr@ccf.org, Cleveland Clinic
Navneet Majhail, MD, majhain@ccf.org, Cleveland Clinic

Hypothesis:
Major Histocompatibility Complex Gamma block SNP profile (GBSP) matching impacts clinical outcomes of unrelated donor HCT for hematological malignancies.

Specific aim:
To investigate the impact of individual GBSP donor and recipient genotypes on transplant outcomes in 8/8 and 7/8 HLA matched HCT unrelated donor/recipient pairs on the clinical outcomes particularly acute and chronic graft versus host disease (GvHD) of unrelated donor HCT in Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML) or Myelodysplastic Syndrome (MDS).

Scientific justification:
The impact of donor recipient matching at HLA loci A, B, C, DRB1, DQB1, & DPB1 on clinical outcomes of unrelated HSCT has been established by numerous studies [1-3]. It's noteworthy that the MHC genomic region harbors more than 400 genes, some of which may encode unidentified transplantation. Matching for these antigens, or other non-HLA, MHC sequences, through HLA haplotype matching was reported to correlate with the GVHD risk in HLA-identical transplants [4]. Mismatches at non-HLA antigens encoded by MHC such as MHC class I chain-related gene A (MICA) has been also reported in association with significantly higher risk of severe GVHD [5,6]. However, current standard of care relies only on matching at class I (A, B, C) and class II (DRB1) loci. Although recombination is not uncommon, the MHC is typically inherited as a conserved genomic block. Family pedigree analysis of HLA typing has shown that recombination occurs at specific locations within the MHC, leading to a block like structure of 4 major genomic blocks. Dawkins and colleagues referred to these blocks as the alpha, beta, gamma and delta blocks [7]. The alpha block contains HLA-A, the beta block contains HLA-C and HLA-B, the gamma block contains the complement proteins C2, C4, and factor B (Bf) and the delta block contains the HLA DR and DQ genes [8].
The HLA genes are the most polymorphic genes within their blocks and serve as block "markers". Therefore Human Leukocyte Antigen (HLA) typing is the current method used to infer MHC haplotypes when matching unrelated patients and donors. HLA alleles however, are not always haplotype specific, so haplotype matching cannot always be predicted.
The Gamma block, as well as the central MHC region between the Gamma and Beta blocks, contain many inflammatory and immune regulatory genes. However, the gamma block is rarely typed, leaving a large region of the central MHC uncharacterized during donor/recipient matching. The Gamma block is a key locus when predicting haplotype matching. GBSP mismatching indicates the presence of recombinant haplotypes and potential genetic mismatching across the beta – gamma block region. Given that the central MHC contains pro-inflammatory cytokine and immune response genes and that it has been implicated in many MHC disease associations, it can be speculated that this region may also be key to outcome in unrelated HCT.

In a single center study (unpublished data submitted as an abstract to 2015 BMT Tandem Meetings) 236 HCT recipient/unrelated donor pairs transplanted at Cleveland Clinic (2000-2010) were tested for GSBP

using commercially available Gamma-Type™ kits (Conexio Genomics Pty Ltd, Wangara, Australia) to test the hypothesis that mismatches in the GBSP increase the risk of GVHD [9]. In that study SNPs c.2918+98G, c.3316C, and c.4385C (reference sequence C4A NG_011638.1) were associated with severe GVHD and analyzed as a composite variable. Mismatch at ≥1 of the 3 SNPs occurred in 49 pairs (21%) and 187 were matched across all 3 SNPs. SNP mismatch was associated with increased risk of severe GVHD in univariable analysis (HR 2.43, 95% CI 1.32-4.47, P= 0.004, Figure 1).

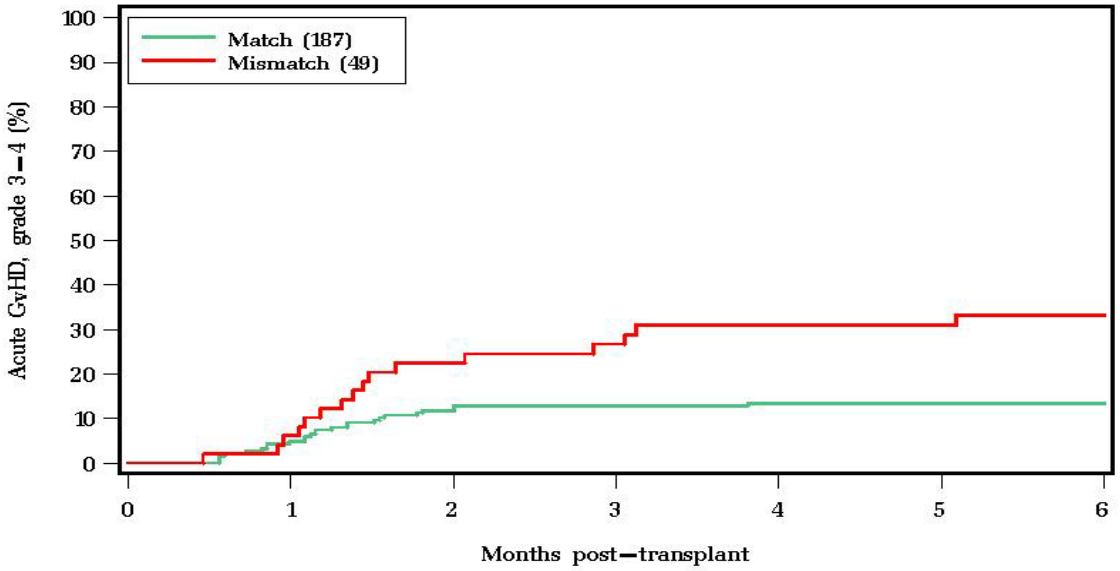


Figure 1: The Association Between c.2918+98G, c.3316C, and c.4385C SNP mismatches and severe acute GVHD (Grades III-IV)

There were no significant differences among SNP match and mismatch pairs regarding HLA match (10/10 vs. <10/10, 8/8 vs. <8/8, DPB1), MICA match, and patient, disease or transplant characteristics. SNP mismatch remained significantly associated with severe GVHD in a multivariable analysis (HR 2.54, P= 0.002) after adjusting for graft source, HLA and MICA mismatch. These results suggest that GBSP mismatches are common in HLA matched unrelated transplants and are associated with a higher risk of severe acute GVHD independent of the HLA and MICA match and other clinical risk factors. These results also questioned whether avoiding GBSP mismatches can potentially reduce the risk of severe acute GVHD and warrant further investigation in larger cohorts.

An attempt to validate these results in a larger CIBMTR cohort of ALL, AML and MDS patients from in study IB15-01. The results were presented in the 2018 BMT Tandem Meetings (Manuscript is in submission) [10]. In the validation study, we investigated the association of GBSP mismatches with outcomes after 10/10 & 9/10 URD HCT (n=714). The primary outcome was aGVHD. OS, DFS, TRM, relapse, cGVHD, and engraftment were also analyzed. DNA samples were GBSP genotyped by identifying 338 SNPs across 20kb using the Illumina NGS platform.

The overall 100 day incidence of aGVHD grades 2-4 & 3-4 were 41% and 17%, respectively. The overall incidence of matching at all GBS tested and at C4 SNP were 23% & 81%, respectively. Neither being matched across all GBSP tested (vs. mismatched) nor having higher number of GBSP mismatches was associated with transplant outcomes. There was no association between C4 SNP mismatches and outcomes except an unexpected significant association between having 2 C4 SNP mismatches and higher hazard ratio for relapse (association seen in 15 patients only, HR: 3.38, 95% CI: 1.75-6.53, p=0.0003). These data did not support the hypothesis that mismatching at GB is associated with outcomes after HCT.

The lack of significant association between GBSP mismatches and HCT outcomes and the unexpected findings in the validation study does not necessarily disprove a potential clinical relevance of GB composition. A CIBMTR genome wide association study identified SNP mismatches within the MHC

region in association with higher risk of GVHD [11]. This study identified 12 SNPs with significant associations with OS, RFS, TRM, aGVHD (II-IV & III-IV) and cGVHD. Only 6 of those SNPs conferred risks by donor/recipient mismatching (rs2242656, rs209130, rs2523957, rs3830076, rs2071479 and rs107822). The remaining SNPs conferred risks by a given recipient genotype (rs429916, rs915654 and rs2075800) or a given donor genotype (rs2244546, rs986522 and rs394657) regardless of donor/recipient matching at these SNPs. In the validation study, individual donor and recipient SNP genotypes were not considered. Investigating donor and recipient genotypes at GBSP is critical for further understanding of a potential role of this genomic region in the clinical outcomes of HCT.

Patient eligibility, samples and data requirements:
No additional sample or data collection is needed. Continuation of the analysis of the data already collected in study IB15-01

Study design (scientific plan):
The proposed extension of the study IB15-01 will consist of data analysis of the association between donor and recipient GSPS genotypes and clinical HCT outcomes. In this analysis already identified donor and recipient GBSP genotypes will be considered the primary explanatory variable and will be correlated with the study outcomes.

Study outcomes:

- Primary outcome: GVHD (III-IV & any)
- Secondary outcomes: OS, GRFS, DFS, relapse, TRM, and cGVHD (extensive & any)

Variables to be analyzed:
Donors and recipient genotypes across each SNP will be correlated with HCT clinical outcomes. Variables will be considered in multivariate analyses includes patient, disease and transplant characteristics. Patient-related variables will include age at time of transplant, gender, race/ethnicity and Karnofsky score. Disease-related variables will include disease (ALL, AML, or MDS) and disease status (early vs. intermediate vs. advanced vs. others). Transplant-related variables will include source of hematopoietic cells (bone marrow vs. peripheral blood stem cells), donor age and race/ethnicity, year of transplant, gender match (M-M vs. M-F vs. F-M vs. F-F), donor/recipient CMV status (-/- vs. -/+ vs. +/- vs. +/+ vs. unknown), conditioning regimen (myeloablative vs. reduced intensity/non-myeloablative) and GvHD prophylaxis (Tacrolimus +/-others vs. CSA +/-others vs. others).

Statistical analysis:
Multivariable models will be constructed for OS, DFS, relapse, TRM, aGVHD (Grades II-IV and III-IV), and cGVHD using the Cox proportional hazards model. All the clinical variables will be tested for the affirmation of the proportional hazards assumption ($P<0.01$). Factors violating the proportional hazards assumption will be adjusted through stratification. All outcome events will be defined based on competing risks, censored as appropriate and included in the cox models. Fine & Gray's models will be used for modeling the sub-distribution hazard. A stepwise variable selection procedure was then used to select adjusted clinical variables for each outcome with a threshold of 0.05 for both entry and retention in the model. The association of GBSP genotypes with clinical outcomes will be tested with adjustments for the selected clinical variables. The 'center' effect will also be adjusted in all the models. Appropriate p value to be considered significant to adjust for multiple testing will be determined by the CIBMTR biostatistics group. SAS version 9.4 (SAS Institute, Cary, NC) was used for all the analyses.

Experience of the investigators in the proposed methods:
The team of co-PIs has strong relevant experience and a demonstrable track record of productive collaboration in the proposed area of research. The 1st and 2nd co-PIs are laboratorians with strong experience with the gamma block genomic regions and prior studies in this area. The 3rd and 4th co-PIs

are accomplished academic investigator physicians with extensive clinical HCT related experience and prior studies in this area. CVs are available on request.

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Table 1. IB15-01 - Summary for SNPs demographics split by All SNPs Matched vs. SNPs Mismatched -

Variable	SNP Matched N (%)	SNP Mismatched N (%)
Number of patients	163	551
Number of centers	59	98
Recipient age at transplant		
10-19 years	4 (2)	16 (3)
20-29 years	31 (19)	90 (16)
30-39 years	17 (10)	67 (12)
40-49 years	37 (23)	109 (20)
50-59 years	74 (45)	269 (49)
Median (range)	47 (18-74)	50 (18-75)
Recipient race / ethnicity		
Caucasian, non-Hispanic	152 (95)	498 (92)
African-American, non-Hispanic	0	10 (2)
Asian, non-Hispanic	2 (1)	5 (1)
Native American, non-Hispanic	0	2 (<1)
Hispanic, Caucasian	5 (3)	24 (4)
Hispanic, African-American	0	1 (<1)
Hispanic, race unknown	1 (1)	1 (<1)
Unknown	3 (N/A)	10 (N/A)
Recipient sex		
Male	88 (54)	290 (53)
Female	75 (46)	261 (47)
Karnofsky score		
10-80	50 (34)	187 (36)
90-100	97 (66)	328 (64)
Unknown	16 (N/A)	36 (N/A)
High-resolution HLA matches available out of 10		
9/10	16 (10)	147 (27)
10/10	147 (90)	404 (73)
Graft type		
Bone marrow	39 (24)	139 (25)
Peripheral blood	124 (76)	412 (75)
Conditioning regimen		
Myeloablative	117 (72)	383 (70)
RIC	36 (22)	116 (21)
Nonmyeloablative	6 (4)	32 (6)
Other	4 (2)	20 (4)
Donor age at donation		
10-19 years	7 (4)	17 (3)
20-29 years	68 (42)	168 (30)
30-39 years	56 (34)	175 (32)

Variable	SNP Matched N (%)	SNP Mismatched N (%)
40-49 years	25 (15)	137 (25)
50+ years	7 (4)	54 (10)
Median (range)	31 (18-56)	35 (18-61)
Disease at transplant		
AML	91 (56)	307 (56)
ALL	27 (17)	84 (15)
MDS	45 (28)	160 (29)
Disease status at transplant		
Early	114 (70)	385 (70)
Intermediate	1 (1)	5 (1)
Advanced	44 (27)	137 (25)
Other	4 (2)	24 (4)
Donor / recipient CMV serostatus		
Negative / negative	51 (31)	150 (27)
Negative / positive	55 (34)	188 (34)
Positive / negative	17 (10)	59 (11)
Positive / positive	36 (22)	143 (26)
Unknown	4 (2)	11 (2)
GvHD prophylaxis		
Tacrolimus + MMF ± others	33 (20)	89 (16)
Tacrolimus + MTX ± others (except MMF)	92 (56)	329 (60)
CSA + MMF ± others (except Tacrolimus)	12 (7)	44 (8)
CSA + MTX ± others (except Tacrolimus, MMF)	26 (16)	89 (16)
Donor / recipient sex match		
Male / male	62 (38)	200 (36)
Male / female	54 (33)	149 (27)
Female / male	26 (16)	90 (16)
Female / female	21 (13)	112 (20)
Year of transplant		
2000	4 (2)	8 (1)
2001	2 (1)	12 (2)
2002	7 (4)	15 (3)
2003	7 (4)	35 (6)
2004	8 (5)	45 (8)
2005	18 (11)	80 (15)
2006	21 (13)	76 (14)
2007	31 (19)	75 (14)
2008	26 (16)	66 (12)
2009	21 (13)	63 (11)
2010	16 (10)	43 (8)
2011	2 (1)	33 (6)

Proposal: 1812-05

Title:
Using whole-exome sequencing to identify novel non-HLA genetic contributors to mortality after blood and marrow transplantation

Qianqian Zhu, PhD, Qianqian.Zhu@RoswellPark.org, Roswell Park Comprehensive Cancer Center
Lara E. Sucheston-Campbell, PhD, sucheston-campbell.1@osu.edu, The Ohio State University
Theresa Hahn, PhD, Theresa.Hahn@RoswellPark.org, Roswell Park Comprehensive Cancer Center

Hypothesis:
We hypothesize that functional coding genetic variants in non-HLA loci significantly affect patient survival after HLA-matched unrelated donor (URD) blood and marrow transplant (BMT). We will use whole-exome sequencing (WES) technology to systematically survey coding variants in both recipients and donors and then test the association between BMT outcomes and variant genotypes in recipients and donors respectively as well as variant genotype mismatches between recipients and donors. The BMT outcomes we will investigate include overall survival (OS), transplant-related mortality (TRM), and disease-related mortality (DRM).

Specific aims:
- Aim1: To whole-exome sequence 3,000 HLA-matched unrelated recipient-donor pairs, and carry out both variant-level and gene-level association tests to identify new non-HLA loci affecting mortality after BMT.
- Aim2: To perform meta-analysis of BMT outcomes on ~5,500 HLA-matched unrelated recipient-donor pairs by integrating the new WES data and our existing genotype data from ~2,500 recipient-donor pairs.

Scientific impact:
This study is the first to use the most cutting edge next-generation sequencing (NGS) technology to analyze the contribution of non-HLA coding variants on survival outcomes after BMT. Involving ~5,500 HLA-matched unrelated recipient-donor pairs, our study is also the largest one of its kind. Our findings may help provide individualized risk prediction and prognosis, as well as alternative donor selection strategies for patients undergoing transplant.

Scientific justification:
We have previously carried out and published the first exome-wide association study (EXWAS) based on Illumina exome array [1]. Our study included ~2,500 patients from the DISCOVeRY-BMT (Determining the Influence of Susceptibility COnveying Variants Related to one-Year mortality after BMT) cohorts [2, 3] who were treated with URD-BMT for Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML) and Myelodysplastic syndrome (MDS) and their 10/10 HLA matched (high resolution HLA-A, -B, -C, -DRB1 loci) unrelated donors. The patients were treated from 2000 to 2011 and reported to the Center for International Blood and Marrow Transplant Research (CIBMTR). In this study, we discovered that genotype mismatches between recipients and donors in a

Table 1. The variant in *TEX38* whose allele mismatches contribute to TRM in recipients.

Variant	Chr	Position	Alleles (Ref/Alt)	AA change†	MAF*	p-value
rs200092801	1	47139039	C/T	P178S	0.31%	3.51×10^{-7}

†: the amino acid changes are based on transcript NM_001145474.
*: minor allele frequency

rare nonsynonymous variant of *TEX38* gene significantly increased patients' risk of TRM (Table 1), and that rare variants of a few additional genes in recipients or donors significantly affected survival outcomes (Table 2). Our work started to uncover the biological relevance of these new and unexpected genes and shed light on new areas that were not considered before. For example, we found rs200092801 can induce strong binding of mutant TEX38 antigen peptides with MHC-I molecules, which possibly trigger downstream immune response to the mutant antigens and lead to TRM. We also found the rare *NT5E* mutations present in donors can impact various aspects of the enzyme activity and lower its efficiency of generating adenosine. Therefore the recipients whose donors carried these mutations experience NT5E inhibition, which had been shown to effectively inhibit tumor growth [4-13].

Our EXWAS has demonstrated the important roles played by rare coding variants. However, the exome arrays used in our study by design misses a majority of rare variants, particularly the novel ones that may only occur in our patient and donor cohorts. Among rare variants (MAF < 1%) of European populations that were contained in the largest variant catalog to date, Genome Aggregation Database [14] , only 2% were included in Illumina exome array. On the other hand, driven by its ability to un-biasedly interrogate all genetic variants in an individual, NGS technology has been quickly adopted by the field in searching for genetic variants and genes influencing human traits [15-18]. Despite dramatic reduction in DNA sequencing price, it is still cost prohibitive to conduct whole-genome sequencing in large number of samples. The exome, defined as the complete set of coding regions, represents only 1% of the human genome but accounts for ~85% of all variants identified in human disorders. WES, which is targeted sequencing of the protein coding regions in the human genome, provides a good balance between cost and yield for searching novel genetic causal variants. This powerful approach has been successfully applied in discovery of genetic variants responsible for various human diseases and traits [15, 18, 19]. Therefore we plan to systematically survey coding variants in human exomes to identify genes that contribute to patient survival after HLA-matched URD-BMT. Importantly, we already had extensive genotype data for ~2,500 recipient-donor pairs in the DISCOVeRY-BMT from our prior EXWAS and genome-wide association studies (GWAS) [3, 20]. This allows us to integrate the new genotype data from WES with our existing data to conduct a much more powerful meta-analysis of BMT outcomes on exomes of totally ~5,500 recipient-donor pairs.

Table 2. The genes significantly associates with survival outcomes in recipients or donors.

Outcome	Gene	Chr	p-value	# of variants*
Recipient Genotype (Exome-wide significance < 4.05×10^{-6})				
OS	*OR51D1*	11	9.48×10^{-7}	6
TRM	*OR51D1*	11	1.05×10^{-6}	6
Donor Genotype (Exome-wide significance < 4.10×10^{-6})				
OS	*ALPP*	2	1.05×10^{-6}	3
	EMID1	22	1.05×10^{-6}	2
	SLC44A5	1	1.05×10^{-6}	2
	LRP1	12	2.86×10^{-6}	27
TRM	*HHAT*	1	9.34×10^{-7}	6
DRM	*LYZL4*	3	1.05×10^{-6}	3
	NT5E	6	1.05×10^{-6}	6

* the number of single variants included in the meta SKAT-O test.

Patient eligibility population:

This study will include patients who were treated with an 8/8 HLA-matched T-cell replete unrelated donor BMT from 2012-2018 for ALL, AML, and MDS, as well as their 8/8 HLA matched unrelated donors. All eligible recipient-donor pairs who have an available sample for either the recipient or donor are included. All ages, race/ethnicity and conditioning regimens are included. Exclusion criteria are *in vivo* or *ex vivo* T-cell depletion, cord blood transplants, <8/8 HLA matched unrelated donor, related donor BMT, or lack of an available sample.

CRF track patients are preferred in this study in order to adjudicate causes of death and other outcomes. However depending on sample availability TED track patients will also be considered to reach our target sample size.

Data requirements:
The proposed study does not require collection of any supplemental data. Variables needed for our analyses are listed below.

Variables needed for both recipients and donors:

- Age at BMT/donation
- Gender: male/female
- HLA typing and degree match
- Race and ethnicity
- CMV serostatus

Recipient-only variables:

- Height and weight
- Karnofsky or Lansky performance score
- Disease: AML/MDS/ ALL
- Disease status at BMT: early/intermediate/advanced
- Conditioning regimen
- Condition regimen intensity: myeloablative/reduced intensity
- GVHD prophylaxis regimen
- Source of stem cells: bone marrow/peripheral blood
- Cause(s) of death
- Date of last follow-up, survival status
- Disease best response post BMT and date of disease progression

Variables needed for adjudicating causes of death:

- ANC and Platelet recovery and dates
- Acute GVHD overall grade, organs involved and stage, and date of onset
- Chronic GVHD grade, severity, date of onset, treatments
- Clinically significant infections, dates of onset
- Clinically significant organ impairment
- Second malignancy and date of diagnosis
- Graft failure and date
- Date of subsequent DLI, CD34 boost or 2nd BMT

Sample requirements:
DNA or whole blood samples of the eligible recipients and donors.
DNA samples will be sent to Center for Inherited Disease Research (CIDR, http://www.cidr.jhmi.edu) at the Johns Hopkins University for sequencing. Supported by NIH CIDR program, CIDR provides high quality next generation sequencing (NGS) and genotyping services to investigators working to discover genes that contribute to human health and disease. CIDR has completed more than 900 genetic studies encompassing over 200 phenotypes and over 1.2 million samples from over 300 different principal investigators located world-wide, resulting in over 1,190 peer-review publications.

Based on the sequencing data, CIDR will detect genetic variants and provide their genotypes in the sequenced samples. Drs. Zhu, Sucheston-Campbell, and Hahn will work together on the association analysis between variant genotypes and BMT outcomes. We have extensive experience in genetic studies utilizing approaches of GWAS, EXWAS, and NGS, as evidenced by more than 16 peer-review publications (see the brief curriculum vitae accompanying this proposal).

Study design:
We will perform WES on DNA samples from 3,000 new recipient-donor pairs that do not overlap with the DISCOVeRY-BMT cohorts. These recipients were treated with URD-BMT from 2012-2018 and reported to CIBMTR. We will carry out both variant-level and gene-level association tests between genotypes (recipient genotypes, donor genotypes, and recipient-donor genotype mismatches) and survival outcomes (OS, TRM, DRM) one year after BMT as we have done in our prior EXWAS.
To increase our power for evaluating variants' contribution to BMT outcomes, we will integrate genotypes from the new WES data and our existing genotype data of ~2,500 recipient-donor pairs from the DISCOVeRY-BMT cohorts, and then conduct a meta-analysis of BMT outcomes on exonic variants of ~5,500 HLA-matched unrelated recipient-donor pairs. This meta-study will allow us to validate our prior findings from EXWAS and to detect associations with smaller effect sizes. Importantly, it will enable us to evaluate genetic variants and BMT outcomes in minority populations, which are not possible due to limited sample size.

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Conflicts of interest:
None

Prop 1812-05 - Table 1a. 8/8 HLA-matched T-cell Replete Unrelated Donor HCT Research Sample Inventory - Summary for First Allogeneic Transplants in TED with biospecimens available through the CIBMTR Repository stratified by availability of paired samples, recipient only samples and donor only samples, Biospecimens include: whole blood, serum/plasma and limited quantities of viable cells and cell lines (collected prior to 2006)

Variable	**Samples Available for Recipient and Donor** N (%)	**Samples Available for Recipient Only** N (%)	**Samples Available for Donor Only** N (%)
Number of patients	2954	846	313
Source of data			
TED	2954 (100)	846 (100)	313 (100)
Number of centers	136	103	91
Disease at transplant			
AML	1828 (62)	505 (60)	194 (62)
ALL	621 (21)	171 (20)	57 (18)
MDS	505 (17)	170 (20)	62 (20)
AML disease status at transplant			
CR1	1129 (62)	315 (62)	110 (57)
CR2	303 (17)	70 (14)	35 (18)
CR3+	15 (1)	4 (1)	0
Advanced or active disease	377 (21)	112 (22)	49 (25)
Missing	4 (<1)	4 (1)	0
ALL disease status at transplant			
CR1	418 (67)	128 (75)	36 (63)
CR2	132 (21)	24 (14)	10 (18)
CR3+	27 (4)	7 (4)	3 (5)
Advanced or active disease	44 (7)	12 (7)	7 (12)
Missing	0	0	1 (2)
MDS disease status at transplant			
Early	86 (17)	23 (14)	12 (19)
Advanced	398 (79)	144 (85)	48 (77)
Missing	21 (4)	3 (2)	2 (3)
GvHD prophylaxis			
CD34 selection	8 (<1)	5 (1)	1 (<1)
Tacrolimus + MMF +- others	293 (10)	83 (10)	31 (10)
Tacrolimus + MTX +- others (except MMF)	1706 (58)	465 (55)	153 (49)
Tacrolimus + others (except MTX, MMF)	318 (11)	129 (15)	48 (15)
Tacrolimus alone	20 (1)	5 (1)	4 (1)

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
CSA + MMF +- others (except Tacrolimus)	179 (6)	44 (5)	24 (8)
CSA + MTX +- others (except Tacrolimus, MMF)	188 (6)	27 (3)	28 (9)
CSA + others (except Tacrolimus, MTX, MMF)	3 (<1)	1 (<1)	2 (1)
CSA alone	17 (1)	3 (<1)	0
Other GVHD prophylaxis	40 (1)	12 (1)	1 (<1)
Missing	182 (6)	72 (9)	21 (7)
Use of ATG/Campath			
Neither ATG nor CAMPATH	2930 (100)	838 (100)	309 (100)
Unknown	24 (N/A)	8 (N/A)	4 (N/A)
Graft type			
Marrow	555 (19)	127 (15)	60 (19)
PBSC	2399 (81)	718 (85)	253 (81)
BM+PBSC	0	1 (<1)	0
High resolution HLA matches available out of 8			
8/8	2954 (100)	846 (100)	313 (100)
High resolution HLA matches available out of 10			
8/10	2 (<1)	1 (<1)	0
9/10	147 (5)	28 (4)	16 (5)
10/10	2745 (95)	731 (96)	288 (95)
Unknown	60 (N/A)	86 (N/A)	9 (N/A)
Recipient age at transplant			
0-9 years	83 (3)	19 (2)	9 (3)
10-19 years	107 (4)	25 (3)	14 (4)
20-29 years	243 (8)	67 (8)	20 (6)
30-39 years	286 (10)	86 (10)	29 (9)
40-49 years	423 (14)	104 (12)	42 (13)
50-59 years	697 (24)	195 (23)	76 (24)
60-69 years	893 (30)	282 (33)	101 (32)
70+ years	222 (8)	68 (8)	22 (7)
Median (Range)	55 (1-81)	57 (2-78)	56 (2-75)
Recipient race/ethnicity			
Caucasian, non-Hispanic	2954 (100)	846 (100)	313 (100)
Recipient sex			
Male	1627 (55)	458 (54)	169 (54)
Female	1327 (45)	388 (46)	144 (46)
Karnofsky score			

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
10-80	1248 (42)	354 (42)	134 (43)
90-100	1665 (56)	486 (57)	177 (57)
Missing	41 (1)	6 (1)	2 (1)
Conditioning regimen			
Myeloablative	1863 (63)	490 (58)	168 (54)
RIC/Nonmyeloablative	1082 (37)	355 (42)	143 (46)
TBD	9 (<1)	1 (<1)	2 (1)
Donor age at donation			
To Be Determined/NA	8 (<1)	32 (4)	0
10-19 years	144 (5)	38 (4)	16 (5)
20-29 years	1794 (61)	460 (54)	183 (58)
30-39 years	597 (20)	174 (21)	71 (23)
40-49 years	300 (10)	113 (13)	29 (9)
50+ years	111 (4)	29 (3)	14 (4)
Median (Range)	27 (18-61)	28 (18-63)	28 (18-61)
Year of transplant			
2012	577 (20)	73 (9)	47 (15)
2013	518 (18)	114 (13)	40 (13)
2014	432 (15)	137 (16)	49 (16)
2015	471 (16)	125 (15)	46 (15)
2016	434 (15)	173 (20)	73 (23)
2017	522 (18)	224 (26)	58 (19)
Follow-up among survivors, Months			
N Eval	1677	529	186
Median (Range)	25 (3-76)	13 (3-74)	24 (3-73)

Prop 1812-05 - Table 1b. 8/8 HLA-matched T-cell Replete Unrelated Donor HCT Research Sample Inventory - Summary for First Allogeneic Transplants in CRF with biospecimens available through the CIBMTR Repository stratified by availability of paired samples, recipient only samples and donor only samples, Biospecimens include: whole blood, serum/plasma and limited quantities of viable cells and cell lines (collected prior to 2006)

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
Number of patients	1767	508	151
Source of data			
CRF	1767 (100)	508 (100)	151 (100)
Number of centers	112	80	48
Disease at transplant			
AML	733 (41)	183 (36)	61 (40)
ALL	162 (9)	43 (8)	14 (9)
MDS	872 (49)	282 (56)	76 (50)
AML disease status at transplant			
CR1	479 (65)	107 (58)	38 (62)
CR2	100 (14)	28 (15)	11 (18)
CR3+	3 (<1)	2 (1)	1 (2)
Advanced or active disease	148 (20)	46 (25)	11 (18)
Missing	3 (<1)	0	0
ALL disease status at transplant			
CR1	107 (66)	31 (72)	9 (64)
CR2	39 (24)	7 (16)	3 (21)
CR3+	5 (3)	0	1 (7)
Advanced or active disease	11 (7)	4 (9)	1 (7)
Missing	0	1 (2)	0
MDS disease status at transplant			
Early	108 (12)	34 (12)	7 (9)
Advanced	740 (85)	240 (85)	67 (88)
Missing	24 (3)	8 (3)	2 (3)
GvHD prophylaxis			
CD34 selection	2 (<1)	0	0
Tacrolimus + MMF +- others	177 (10)	42 (8)	15 (10)
Tacrolimus + MTX +- others (except MMF)	1106 (63)	322 (63)	87 (58)

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
Tacrolimus + others (except MTX, MMF)	159 (9)	67 (13)	18 (12)
Tacrolimus alone	5 (<1)	2 (<1)	0
CSA + MMF +- others (except Tacrolimus)	86 (5)	24 (5)	11 (7)
CSA + MTX +- others (except Tacrolimus, MMF)	66 (4)	10 (2)	7 (5)
CSA + others (except Tacrolimus, MTX, MMF)	1 (<1)	0	0
CSA alone	5 (<1)	0	0
Other GVHD prophylaxis	21 (1)	6 (1)	0
Missing	139 (8)	35 (7)	13 (9)
Use of ATG/Campath			
Neither ATG nor CAMPATH	1752 (100)	508 (100)	149 (100)
Unknown	15 (N/A)	0 (N/A)	2 (N/A)
Graft type			
Marrow	316 (18)	81 (16)	41 (27)
PBSC	1450 (82)	427 (84)	110 (73)
BM+PBSC	1 (<1)	0	0
High resolution HLA matches available out of 8			
8/8	1767 (100)	508 (100)	151 (100)
High resolution HLA matches available out of 10			
9/10	73 (4)	25 (5)	5 (3)
10/10	1683 (96)	443 (95)	139 (97)
Unknown	11 (N/A)	40 (N/A)	7 (N/A)
Recipient age at transplant			
0-9 years	28 (2)	9 (2)	2 (1)
10-19 years	28 (2)	6 (1)	3 (2)
20-29 years	65 (4)	22 (4)	6 (4)
30-39 years	82 (5)	27 (5)	7 (5)
40-49 years	154 (9)	37 (7)	13 (9)
50-59 years	381 (22)	93 (18)	31 (21)
60-69 years	768 (43)	233 (46)	62 (41)
70+ years	261 (15)	81 (16)	27 (18)
Median (Range)	62 (1-82)	64 (1-76)	62 (4-77)
Recipient race/ethnicity			
Caucasian, non-Hispanic	1767 (100)	508 (100)	151 (100)
Recipient sex			

Variable	Samples Available for Recipient and Donor N (%)	Samples Available for Recipient Only N (%)	Samples Available for Donor Only N (%)
Male	1065 (60)	319 (63)	86 (57)
Female	702 (40)	189 (37)	65 (43)
Karnofsky score			
10-80	769 (44)	250 (49)	67 (44)
90-100	981 (56)	255 (50)	83 (55)
Missing	17 (1)	3 (1)	1 (1)
Conditioning regimen			
Myeloablative	857 (49)	239 (47)	72 (48)
RIC/Nonmyeloablative	910 (51)	269 (53)	79 (52)
Donor age at donation			
To Be Determined/NA	5 (<1)	8 (2)	0
10-19 years	71 (4)	28 (6)	8 (5)
20-29 years	1055 (60)	267 (53)	90 (60)
30-39 years	390 (22)	128 (25)	29 (19)
40-49 years	175 (10)	49 (10)	18 (12)
50+ years	71 (4)	28 (6)	6 (4)
Median (Range)	27 (18-62)	28 (18-61)	26 (18-56)
Year of transplant			
2012	149 (8)	31 (6)	26 (17)
2013	322 (18)	67 (13)	18 (12)
2014	389 (22)	106 (21)	26 (17)
2015	393 (22)	82 (16)	22 (15)
2016	282 (16)	129 (25)	34 (23)
2017	232 (13)	93 (18)	25 (17)
Follow-up among survivors, Months			
N Eval	961	291	70
Median (Range)	25 (3-76)	24 (3-71)	24 (3-73)

TO: Immunobiology Working Committee Members

FROM: Stephanie Lee, MD, MPH; Co-Scientific Director for the Immunobiology WC
Stephen Spellman, MBS; Co-Scientific Director for the Immunobiology WC

RE: Studies in Progress and Publication Summary

Studies in Progress Summary

NK/KIR
R02-40/R03-63: Choosing donors with favorable KIR B genotypes for unrelated hematopoietic cell transplantation (HCT) results in superior relapse protection and better relapse-free survival for patients with acute myeloid leukemia (AML) (J Miller) This is an ongoing study in support of Dr. Miller's NK Biology program project grant. Ongoing.

R04-74d Functional significance of killer cell immunoglobulin-like receptor genes in HLA-matched and mismatched unrelated HCT (K Hsu) This is an ongoing study in support of the IHWG KIR component led by Dr. Hsu. Ongoing.

IB15-03: Effect of Killer immunoglobulin like receptors on allogeneic HCT for pediatric acute leukemia (M Verneris/J Miller/S Cooley) The primary aim of the study is to examine the association of donor KIR genotype (A/A vs B/x) on relapse and disease free survival (DFS) in children undergoing allogeneic transplantation (allo-HCT) with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). The analysis did not show any association between KIR and relapse, DFS or the secondary endpoints in this population. Manuscript preparation is underway.

IB17-02 Donor-recipient NK cell determinants associated with survival in JMML after hematopoietic stem cell transplantation (D Lee/H Rangarajan) This study will test whether determinants of NK cell function are associated with relapse and survival in patients transplanted for JMML. Data file preparation is in progress.

IB18-04 Impact of donor KIR genotype on outcome after URD TX in patients with MDS or sAML (J Schetelig/N Kröger/M Robin) This study is evaluating the role of donor KIR genotype on transplant outcome in a cohort of European patients. Donor samples were collected by the DKMS biorepository and KIR typing performed at the DKMS Life Sciences Laboratory. This is a joint analysis with the EBMT Acute Leukemia Working Party, CIBMTR and DKMS. Manuscript preparation is underway.

IB18-05 Imputation of KIR in GWAS and association of KIR-HLA with outcomes following alloHCT In AML and MDS (C Camacho-Bydume/L Sucheston-Campbell/S Leslie/K Hsu) This study is evaluating the

potential to impute KIR genotypes from GWAS data generated through the DISCOVeRY-BMT project and correlate with transplant outcome. Data analysis is in progress.

HLA GENES

IB06-05 Use of high-resolution HLA data from the NMDP for the International Histocompatibility Working Group in HCT (E Petersdorf) This study proposes to identify novel major histocompatibility complex resident SNPs of clinical importance. This is a collaborative study with the International Histocompatibility Working Group – HCT component (IHWG). Ongoing.

IB14-07 Indirectly recognizable HLA epitopes (PIRCHES): a retrospective validation study on the role of indirect recognition of mismatched HLA in hematopoietic stem cell transplantation outcome (E Spierings) This study was a validation analysis of the PIRCHES algorithm for identification of less immunogenic HLA mismatches in unrelated donor HCT. Manuscript preparation is underway.

IB16-01 The role of HLA-E compatibility in the prognosis of acute leukemia patients undergoing 10/10 HLA matched unrelated HSCT (C Tsamadou/ D Furst/ J Mytilineos) The primary objective of the study is to investigate whether HLA-E donor-recipient incompatibility is associated with better HCT outcomes in a cohort of acute leukemia patients transplanted with HLA-A, B, C, DRB1 and DQB1 matched unrelated donors. HLA-E matching was not associated with outcomes. However, donor HLA-E 01:03 homozygosity was associated with worse DFS. Manuscript preparation is underway.

IB16-02: Use of HLA structure and function parameters to understand the relationship between HLA disparity and transplant outcomes (LA Baxter-Lowe) The main objective of the study is to determine the relationship between HLA disparities ranked by their impact on T cell receptor docking, peptide binding and the combination of docking and binding. The docking and binding assignment algorithm has been locked and the analysis is in progress.

IB18-01 Effect of HLA phenotypes on long term GVHD risk (C Story/M Riches/P Armisted) The goal of this study is to test whether the frequency of peptide binding by class I and II alleles is associated with acute GVHD or relapse. This analysis will be conducted in HLA-matched related and unrelated donor pairs. Protocol development is in progress.

IB18-02 Impact of HLA class I risk alleles associated with AA Immune pathogenesis on allo TX outcomes in patients with SAA (D Babushok/T Olson) Protocol development is in progress.

IB18-03 Effect of HLA Class I Heterozygosity and HLA Supertypes on Outcomes Following Allogeneic HCT for Myeloid and Lymphoid Malignancies (C Camacho-Bydume/K Hsu) The goal of this study is to examine the impact of HLA heterozygosity on survival and other clinical outcomes in patients with myeloid and lymphoid malignancies. Data analysis is in progress. Results thus far show no association with heterozygosity or expression of Bw4/HLA-C. Two associations of unclear clinical significance were seen with HLA-B supertype B62 and B27.

CYTOKINE/CHEMOKINE

IB14-03a The prognostic impact of somatic mutations and levels of CXC chemokine ligands on post hematopoietic cell transplantation (HCT) outcomes in patients with myelodysplastic syndromes (MDS) (W Saber/B Dhakal) The primary objective of the study is to evaluate the plasma CXCL4 and CXCL7 chemokine levels in patients undergoing transplantation for MDS in comparison to those with other

conditions. Although levels of CXCL4 and CXCL7 were lower in MDS compared to normal donors, results did not show an association of CXC chemokine-related variables and transplant outcomes. Manuscript preparation is underway.

IB14-03c Effect of telomere length in MDS patients without TP53/RASTK/JAK2 mutations (RC Lindsley/W Saber) The goal of this study is to evaluate the impact of recipient telomere length on clinical outcomes based on treatment intensity in patients with MDS receiving HCT. Manuscript preparation is underway.

OTHER GENES

IB09-05 Identification of functional single nucleotide polymorphisms (SNPs) in umbilical cord blood transplant (E Petersdorf) The primary hypothesis of the study is that umbilical cord blood units and recipients differ for genome-wide single nucleotide polymorphism and gene copy number variation and that these differences may define putative transplant outcome determinants. This is a collaborative study with IHWG. Ongoing.

IB09-06/RT09-04: Genetic susceptibility to transplant-related mortality after matched unrelated stem cell transplant (T Hahn) This is a joint study with the Regimen Related Toxicity working committee and is supported by an R01 grant to Drs. Hahn and Sucheston-Campbell. This study will test for a genetic association with transplant-related and overall mortality in recipients of myeloablative and reduced intensity conditioning matched unrelated donor HCT. Multiple manuscripts are in progress. Ongoing.

IB09-07: Clinical significance of genome-wide variation in unrelated donor hematopoietic stem cell transplantation (HCT) (E Petersdorf) This study is designed to assess the impact of genome-wide variation between donors and recepients in HLA matched unrelated donor HCT. This is a collaborative study with the IHWG. Ongoing.

IB10-01c Telomere length telomerase polymorphism in Severe Aplastic Anemia - Exome Analysis and Mosaicism (S Gadalla/S Savage) Two SNPs in the HMC region were associated with increased incidence of SAA. This manuscript was submitted to Nature Communications and is under review.

IB10-01f Epigenetic clock: Can this guide donor selection in HCT (S Gadalla/S Savage) The goal of this study is to evaluate the association between pre-HCT donor methylation age and post-transplant outcomes in patients receiving unrelated donor HCT for SAA. Sample typing is underway.

IB14-04: Assessing the similarity of the T cell receptor repertoire in allogeneic hematopoietic stem cell recipients with the same single human leukocyte mismatches (EH Meyer) The goal of this study is to measure T cell alloreactivity following hematopoietic stem cell transplantation by examining cases where transplant recipients have the same HLA single, double or multiple HLA mismatch, to see if they develop similar alloreactivity. Control transplant recipients with the same HLA type who did not receive an HLA-mismatched transplant will be also analyzed. The number of samples was very limited and results were not conclusive. Manuscript preparation is underway.

IB14-05: mtDNA haplotypes and unrelated donor transplant outcomes (M Verneris/L Spector) The goal of this study is to test whether patient or donor mitochondrial haplotypes predict outcomes of unrelated donor transplantation, particularly GVHD, relapse and survival. The study is supported by an R01 grant to Drs. Verneris and Spector. Data analysis is in progress.

IB15-04: Clinical outcomes among hematopoietic stem cell transplant recipients as a function of socioeconomic status and related transcriptome differences (J Knight/JD Rizzo/S Cole) The primary hypothesis of the study is that increased expression of the conserved transcriptional response to adversity (CTRA) gene profile will be associated with lower socioeconomic status (SES) and worse clinical outcomes among a group of unrelated donor (URD) myeloablative (MA) acute myelogenous leukemia (AML) recipients in CR1. Results showed that very high or very low CTRA inflammatory gene profiles were associated with relapse and disease-free survival. The manuscript was submitted to JNCI Cancer Spectrum and is under review.

IB15-07: Functional genetic variants of the ST2 gene in pairs of recipient and donors for risk stratification of GVHD and TRM outcomes (S Paczesny) The serum biomarker sST2 is associated with an increased risk for therapy-resistant GVHD and death. The primary hypothesis is that similar to the heritability of sST2 in the Framingham Offspring Cohort explaining sST2 as a predictor of cardiovascular risk, the 16 SNPs most associated with sST2 will determine which donor /recipient pair is at risk of developing acute graft-versus-host disease (aGVHD) and transplant-related mortality (TRM) following allogeneic hematopoietic cell transplantation in a well-controlled cohort. The study will use the GWAS typing results and dataset from the DISCOVERY-BMT (IB09-06/RT09-04) cohort to evaluate the association of 16 sST2 SNPs. Results showed that ST2 SNPs correlated with sST2 levels but ST2 SNPs did not strongly correlate with GVHD. The manuscript was submitted to Blood and is under review.

IB16-03: Role of recipient and donor genetic polymorphisms in interferon lambda 4 (INFL4) on outcomes after unrelated allogeneic cell transplant (S Gadalla/L Prokunina-Olsson) The primary goal of the study is to evaluate the effect of recipient and donor genetic polymorphisms in the type-III interferon, interferon lambda 4 (INFL4) on outcomes following unrelated donor HCT for SAA and acute leukemia. The dG homozygous and heterozygous patients had higher TRM than the homozygous TT patients. Grade II-IV and III-IV acute GVHD were not different. Results are being compared with a cohort from Fred Hutchinson and DISCOVeRY-BMT. Manuscript preparation is underway.

IB17-03 Identification of genomic markers of post hematopoietic cell transplantation (HCT) outcomes in patients with myelofibrosis: A pilot study (W Saber/ S Gadalla) The goal of this study is to describe mutations associated with MF, and to correlate these abnormalities with clinical outcomes. Samples are being analyzed.

IB17-04 Epigenetic profiling of unrelated donor-recipient pairs to improve donor selection during HCT transplants (S Beck/K Peggs/V Rakyan/A Webster) The goal of this study is to determine whether donor specific epigenetic patterns associate with risk of acute GVHD III-IV and, if so, develop an epigenetic profile based donor selection algorithm. Sample analysis is underway. A preliminary signature has been identified and a replication cohort is being identified.

IB18-06 Clonal mosaicism and HCT outcomes in patients with acute leukemia and myelodysplastic syndromes (S Gadalla/T Hahn/L Sucheston-Campbell) Protocol development is in progress.

IB18-07 Donor and recipient genomic associations with acute GVHD (V Afshar-Khargan) Protocol is pending.

Publication Summary – Published manuscripts

IB06-05 Patient HLA germline variation and transplant survivorship. Petersdorf EW, Stevenson P, Malkki M, Strong RK, Spellman SR, Haagenson MD, Horowitz MM, Gooley T, Wang T. ***J Clin Oncol*. 2018 Aug 20; 36(24):2524-2531. doi:10.1200/JCO.2017.77.6534. Epub 2018 Jun 14. PMC6097831.**

Patient germline HLA-DRB1 alleles that encode amino acid substitutions that influence the peptide repertoire of HLA-DRβ predispose to increased death after transplantation. Patient germline variation informs transplantation outcomes across US populations and may provide a means to reduce risks for high-risk patients through pretransplantation screening and evaluation.

IB09-06/RT09-04c Exome chip Analyses Identify Genes affecting mortality after HLA-Matched Unrelated Donor Blood and Marrow Transplantation Qian Liu, Qiang Hu, Leah Preus, Alyssa I. Clay, Ken Onel, Daniel O. Stram, Loreall Pooler, Xin Sheng, Christopher A. Haiman, Xiaochun Zhu, Stephen R. Spellman, Marcelo Pasquini, Philip L. McCarthy , Song Liu, Theresa Hahn, Lara E. Sucheston-Campbell. ***Blood.* 2018 May 31; 131(22):2490-2499. doi:10.1182/blood-2017-11-817973. Epub 2018 Apr 2. PMC5981168.**

To identify non-HLA genetic contributors to mortality after BMT, we performed the first exome-wide association study in the DISCOVeRY-BMT cohorts using the Illumina HumanExome BeadChip. Genotype mismatches between recipients and donors in a rare nonsynonymous variant of testis-expressed gene *TEX38*significantly increased risk of TRM, which was more dramatic when either the recipient or donor was female. Using the SKAT-O test to evaluate gene-level effects, variant genotypes of *OR51D1* in recipients were significantly associated with OS and TRM. In donors, 4 (*ALPP*, *EMID1*, *SLC44A5*, *LRP1*), 1 (*HHAT*), and 2 genes (*LYZL4*, *NT5E*) were significantly associated with OS, TRM, and DRM, respectively. Inspection of NT5E crystal structures showed 4 of the associated variants affected the enzyme structure and likely decreased the catalytic efficiency of the enzyme.

IB10-01d Flow Cytometry using FISH techniques in a Severe Aplastic Anemia population. Gadalla S, Aubert G, Wang T, Haagenson M, Spellman SR, Wang L, Katki HA, Savage S, Lee SJ. ***Mol Genet Genomic Med*. 2016 Jul 1; 4(4):475-479. doi:10.1002/mgg3.220. Epub 2016 Mar 20. PMC4947866.**

We identified 197 marrow failure patients who received unrelated donor HCT between 1988-2004, and for whom donor cryopreserved peripheral blood mononuclear cells were available. We used flow cytometry and fluorescence in situ hybridization (Flow FISH) analysis to measure telomere length in 4 lymphocyte cell subtypes: naïve enriched T-cells (CD45RA+CD20-), memory enriched T-cells (CD45RA-CD20-), NK-fully differentiated T cells (CD45RA+CD57+), and B cells (CD45RA+CD20+). Longer donor telomere lengths in B cells (HR=0.63, 95% CI=0.46-0.87, p=0.006), and possibly NK- fully differentiated T cells (HR=0.7, 95% CI=0.51-0.97, p=0.03) were associated with lower risk of infection-related death. No statistically significant associations were seen with telomere length of the naïve or memory T-cells in relation to death due to infection. Donor telomere lengths in any of the tested lymphocyte subsets were not associated with death caused by GvHD or graft failure ($p<0.05$).

IB10-01e Chromosomal aberrations and survival after unrelated donor hematopoietic stem cell transplant in patients with Fanconi anemia. Wang Y, Zhou W, Alter BP, Wang T, Spellman SR, Haagenson M, Yeager M, Lee SJ, Chanock SJ, Savage SA, Gadalla SM. ***Biol Blood Marrow Transplant.* 2018 Oct 1; 24(10):2003-2008. doi:10.1016/j.bbmt.2018.05.027. Epub 2018 Jun 4. PMC6239962.**

We used genome-wide single nucleotide polymorphism arrays to identify chromosomal aberrations in pre-HCT blood samples. Chromosomal aberrations were detected in 16 (22%) patients; most frequent were clonal copy loss in chromosome 7 (9.6%), clonal copy gains in the long arm (q) of chromosome 1 ($chr1q^+$) (8.2%), and clonal or complete copy gains in the q arm of chromosome 3 ($chr3q^+$) (8.2%). Seven

(9.6%) patients had alterations in 3 or more chromosomes. Poor post-HCT overall survival (OS) was noted in patients with chr3q$^+$ (P = .04), or those with abnormalities in ≥3 chromosomes (P = .03). The 1-year OS was 0% versus 45% in patients with either alteration versus its absence. No statistically significant differences in OS were noted in patients carrying deletions in chr7 (1-year OS = 29% versus 42%; log-rank P = .74).

IB10-01g Telomere length calibration from qPCR measurement: Limitations of current method. Wang Y, Savage SA, Alsaggaf R, Aubert G, Dagnall CL, Spellman SR, Lee SJ, Hicks B, Jones K, Katki HA, Gadalla SM. ***Cells.*** **7(11):183. doi:10.3390/cells7110183. Epub 2018 Oct 24. PMC6262465.**

This study aimed to assess the validity of converting the quantitative polymerase chain reaction (qPCR) telomere/single copy gene (T/S) ratio to TL in kilobases (kb). We developed a linear regression equation to predict TL from qPCR T/S using flow cytometry with fluorescence in situ hybridization (flow FISH) TL data. TL measurements by qPCR and flow FISH were modestly correlated (R^2 = 0.56, p < 0.0001). In Bland-Altman analyses, individuals with the shortest (≤10th percentile) or longest (≥90th) flow FISH TL had an over- or under-estimated qPCR TL (bias = 0.89 and -0.77 kb, respectively). Comparisons of calculated TL from the NMDP samples and 1810 age- and sex-matched individuals from the National Health and Nutrition Examination Survey showed significant differences (median = 7.1 *versus* 5.8 kb, respectively, p < 0.0001). Differences in annual TL attrition were also noted (31 *versus* 13 bp/year, respectively, p = 0.02). Our results demonstrate that TL calculated in kb from qPCR T/S may yield biased estimates for individuals with the shortest or longest TL, those often of high clinical interest. We also showed that calculated TL in kb from qPCR data are not comparable across populations and therefore are not necessarily useful.

IB11-01a The effect of NIMA matching in adult unrelated mismatched hematopoietic stem cell transplantation - a joint study of the Acute Leukemia Working Party of the EBMT and the CIBMTR. Pingel J, Wang T, Hagenlocher Y, Hernández-Frederick CJ, Nagler A, Haagenson MD, Fleischhauer K, Hsu KC, Verneris MR, Lee SJ, Mohty M, Polge E, Spellman SR, Schmidt AH, van Rood JJ. ***Bone Marrow Transplant*****. doi:10.1038/s41409-018-0345-8. Epub 2018 Oct 2.**

Fetal exposure to non-inherited maternal antigens (NIMA) may impart lifelong NIMA tolerance modulating the immune response. This retrospective analysis examined if 9/10 matched unrelated donor HSCT benefits from NIMA matching. No significant differences between NIMA-matched and NIMA-mismatched groups were found for any outcomes with similar OS and TRM rates within both groups.

IB12-02c In silico prediction of nonpermissive HLA-DPB1 mismatches in unrelated HCT by functional distance. Arrieta-Bolaños E, Crivello P, Shaw BE, Ahn KW, Wang H-L, Verneris MR, Hsu KC, Pidala J, Lee SJ, Fleischhauer K, Spellman SR**.** ***Blood Advances*****. 2018 Jul 24; 2(14):1773-1783. doi:10.1182/bloodadvances.2018019620. Epub 2018 Jul 24. PMC6058232.**

We compared clinical outcome associations of nonpermissive DPB1 mismatches defined by actual or insilico prediction of nonpermissive HLA-DPB1 mismatches. Concordance between the 2 models was 92.3%, with most differences arising from DPB1*06:01 and DPB1*19:01 being differently assigned. In both models, nonpermissive mismatches were associated with reduced overall survival (hazard ratio [HR], 1.15, P < .006 and HR, 1.12, P < .03), increased transplant-related mortality (HR, 1.31, P < .001 and HR, 1.26, P < .001) as well as acute (HR, 1.16, P < .02 and HR, 1.22, P < .001) and chronic (HR, 1.20, P < .003 and HR, 1.22, P < .001) graft-versus-host disease (GVHD). We show that in silico prediction of nonpermissive DPB1 mismatches significantly associated with major transplant outcomes is feasible for any DPB1 allele with known exon 2 sequence based on experimentally elaborated FD scores.

IB13-08 Prediction of Acute Graft-Versus-Host Disease Following Hematopoietic Cell Transplantation. Lee C, Haneuse S, Wang H, Rose S, Spellman SR, Verneris M, Hsu K, Fleischhauer K, Lee SJ, Abdi R. ***PLOS1*** **13(1):e0190610. doi:10.1371/journal.pone.0190610. Epub 2018 Jan 18. PMC5773230.**

Using data on 9,651 patients who underwent first allogeneic HLA-identical sibling or unrelated donor HCT between 01/1999-12/2011 for treatment of a hematologic malignancy, we developed and evaluated a suite of risk prediction tools for: (i) acute GVHD within 100 days post-transplant and (ii) a composite endpoint of acute GVHD or death within 100 days post-transplant. We considered two sets of inputs: (i) a series of clinical factors that are typically readily-available included as main effects; and, (ii) main effects combined with a selection of a priori specified two-way interactions. Throughout we used the super learner, a recently developed ensemble learning statistical framework that draws on multiple other algorithms/methods to construct the best prediction tool. Across the final super learner prediction tools, the area-under-the curve (AUC) ranged from 0.613-0.640. Improving the performance of risk prediction tools will likely require the inclusion of biological variables as predictive factors, including genetic and proteomic biomarkers, although the measurement of these factors may currently not be practical in standard clinical settings.

IB14-06 Donor-specific anti-HLA antibodies in unrelated hematopoietic cell transplantation for non-malignant disorders. Woolfrey A, Wang T, Lee SJ, Haagenson MD, Chen G, Fleischhauer K, Horan J, Hsu K, Tyan D, Verneris M, Spellman SR, Fernandez-Vina M. ***Bone Marrow Transplantation.*** **doi:10.1038/s41409-018-0334-y. Epub 2018 Sep 19.**

We tested 236 patients with pre-transplant samples for HLA-DSA by solid phase assays utilizing single antigen bead preparations that included detection of IgG antibodies or by complement fixing antibodies based on the C1q binding assay. HLA-DSA was evaluated by analyzing the reactivity against the mismatched donor antigens determined by IgG or C1q assays; mean fluorescence intensity (MFI) >1,000 was considered positive, MFI >500 and <1,000 was considered potentially positive, and MFI< 500 was considered negative. The HLA-DSA-positive (MFI>1000) cohort was similar with respect to age at HCT, race, sex, type of NMD, Karnofsky/Lansky score, and year of HCT, however there was a slightly higher proportion of marrow recipients (95% vs 80%, p=.04) when compared to the HLA-DSA-negative cohort. The C1q positive group did not differ from the C1q negative group for these variables. There was a lack of association of HLA-DSA with graft failure and survival. Results were similar when HLA-DSA IgG positive and C1q positive (11.5%) were combined for analysis. We then used an MFI>5000 as the cutoff value to define a positive HLA-DSA; however, results remained non-significant for an association with graft failure.

IB14-08 Development and validation of a clinical unrelated donor selection score. Shaw BE, Logan BR, Spellman SR, Marsh SGE, Robinson J, Pidala J, Hurley C, Barker J, Maiers M, Dehn J, Wang H, Haagenson M, Porter D, Petersdorf EW, Woolfrey A, Horowitz MM, Verneris M, Hsu KC, Fleischhauer K, Lee SJ. ***Biol Blood Marrow Transplant.*** **2018 May 1; 24(5):1049-1056. doi:10.1016/j.bbmt.2018.02.006. Epub 2018 Feb 14. PMC5953795.**

The goal of this study was to develop and validate a donor selection score that prioritizes donor characteristics associated with better survival in 8/8 HLA-matched URDs. Despite studying over 10,000 URD transplants, we were unable to validate a donor selection score. The only donor characteristic associated with better survival was younger age, with 2-year survival being 3% better when a donor 10 years younger is selected. These results support previous studies suggesting prioritization of a younger 8/8 HLA-matched donor. This large dataset also shows that none of the other donor clinical factors tested were reproducibly associated with survival, and hence flexibility in selecting URDs based on other characteristics is justified.

IB15-01 Analysis of single nucleotide polymorphisms in the gamma block of the major histocompatibility complex in association with clinical outcomes of hematopoietic cell transplantation: A CIBMTR study. Askar M, Sayer D, Wang T, Haagenson M, Spellman SR, Lee SJ, Madbouly A, Fleischhauer K, Hsu KC, Verneris MR, Thomas D, Zhang A, Sobecks R, Majhail NS. ***Biol Blood Marrow Transplant*****. doi:10.1016/j.bbmt.2018.12.008. Epub 2018 Dec 18.**

We investigated the association of GB SNP (GBS) mismatches with outcomes after 10/10 & 9/10 URD HCT (n=714). The primary outcome was aGVHD. OS, DFS, TRM, relapse, cGVHD, and engraftment were also analyzed. DNA samples were GBS genotyped by identifying 330 SNPs across 20kb using the Illumina NGS platform.

The overall 100 day incidence of aGVHD grades 2-4 & 3-4 were 41% and 17%, respectively. The overall incidence of matching at all GBS tested and at C4 SNP were 23% & 81%, respectively. Neither being matched across all GB SNP tested (vs. mismatched) nor having higher number of GBS mismatches was associated with transplant outcomes. There was no association between C4 SNP mismatches and outcomes except an unexpected significant association between having 2 C4 SNP mismatches and higher hazard ratio for relapse (HR: 3.38, 95% CI: 1.75-6.53, p=0.0003). These data did not support the hypothesis that mismatching at GB is associated with outcomes after HCT.

IB15-02 Donor killer-cell immunoglobulin-like receptor (KIR) genotype does not improve graft-versus-leukemia responses in chronic lymphocytic leukemia (CLL) after unrelated donor transplant: a CIBMTR analysis. Bachanova V, Weisdorf DJ, Wang T, Marsh SGE, Cereb N, Haagenson MD, Spellman SR, Lee SJ, Guethlein LA, Parham P, Miller JS, Cooley S. ***Biol Blood Marrow Transplant. doi:10.1016/j.bbmt.2018.12.763. Epub 2018 Dec 27.***

We examined 573 unrelated adult donor (URD)-CLL recipient pairs. KIR genotype (presence/absence) was determined for each donor and comprehensive modeling of interactions with recipient HLA class I loci (KIR ligands) used to predict relapse and survival. Recipients had a median age of 56 years, and most were not in remission (65%). Both 8/8 HLA-matched (81%) or 7/8 antigen HLA-matched grafts (19%) were studied. Cox regression models comparing donors with A/A vs B/x KIR haplotypes and those with KIR gene content scores of 0 vs 1 vs >2 yielded similar rates of non-relapse mortality, relapse, acute graft-versus-host disease (GVHD), and chronic GVHD and the same progression-free survival (PFS) and overall survival (OS). Presence of KIR3DL1 genes in donor grafts transplanted into HLA C1/1 vs C2 recipients did not affect relapse risk. The described models lacked prognostic value for KIR genotype on transplant outcomes. Factors associated with improved OS were reduced intensity conditioning (HR of death 0.76) and good performance status (HR 0.46), while allo-HCT in non-remission (HR 1.96) and mismatched donors (HR 2.01) increased mortality.

IB15-06b Evaluation of a Machine Learning-Based Prognostic Model for Unrelated Hematopoietic Cell Transplantation Donor Selection. Buturovic L, Shelton J, Spellman SR, Wang T, Friedman L, Loftus D, Hesterberg L, Woodring T, Fleischhauer K, Hsu KC, Verneris MR, Haagenson M, Lee SJ. ***Biol Blood Marrow Transplant*** **2018 Jun 1; 24(6):1299-1306. doi:10.1016/j.bbmt.2018.01.038. Epub 2018 Feb 1. PMC5993610.**

In order to improve the donor selection process, we attempted to create an algorithm to quantify the likelihood of survival to five years after unrelated donor HCT for acute leukemia, based on the clinical characteristics of the donor selected. All standard clinical variables were included in the model, which also included average leukocyte telomere length (ATL) of the donor based on its association with recipient survival in severe aplastic anemia, and links to multiple malignancies. We developed a multivariate classifier that assigned a Preferred or NotPreferred label to each prospective donor based on the survival of the recipient. In a prior analysis using a resampling method, recipients whose donors were labeled Preferred experienced clinically compelling better survival compared to donors labeled as

NotPreferred by the test1. However, in a pivotal validation study in an independent cohort of 522 patients, the overall survival of the Preferred and NotPreferred donor groups was not significantly different.

Publication Summary – Submitted manuscripts – see above for description of results

IB10-01c Telomere length telomerase polymorphism in Severe Aplastic Anemia - Exome Analysis and Mosaicism. Gadalla S, Savage S. **Submitted. Nature Communications**

IB15-04 Clinical outcomes among hematopoietic stem cell transplant recipients as a function of socioeconomic status and related transcriptome differences. Knight J, Rizzo JD, Cole S. **Submitted. JNCI Cancer Spectrum**

IB15-07 Functional genetic variants of the ST2 gene in pairs of recipient and donors for risk stratification of GVHD and TRM outcomes. Paczesny S. **Submitted. Blood**

2018 EUROPEAN SOCIETY FOR BLOOD AND MARROW TRANSPLANTATION (EBMT) ABSTRACT

Does Donor KIR-Genotype Impact Outcome After Unrelated Hematopoietic Stell Cell Transplantation for Myelodysplastic Syndromes or Secondary Acute Myeloid Leukemia?

Johannes Schetelig, Henning Baldauf, Linda Koster, Michelle Kuxhausen, Falk Heidenreich, Liesbeth de Wreede, Stephen Spellman, Michel van Gelder, Benedetto Bruno, Francesco Onida, Vinzenz Lange, Carolin Massalski, Victoria Potter, Per Ljungman, Nicolaas Schaap, Patrick Hayden, Stephanie Lee, Nicolaus Kröger, Kathy Hsu, Ibrahim Yakoub-Agha, Marie Robin

Introduction: A series of findings suggest that optimizing natural killer (NK) cell reactivity could further improve outcome after allogeneic hematopoietic cell transplantation (alloHCT). This could be achieved by killer cell immunoglobulin-like receptor (KIR) genotype informed donor selection. An enhanced Receptor-Ligand model which used KIR2DS1 and KIR3DL1 donor genotype information to augment NK cell activation and minimize inhibition demonstrated improved survival in one large study (Boudreau et al, JCO 2017). A second model, built on the classification of centromeric and telomeric KIR haplotype motifs, also predicted mortality after alloHCT (Cooley et al, Blood 2010). This joint EBMT and CIBMTR study aimed at validating the two approaches in an independent cohort of patients with MDS or secondary AML.

Methods: Donor samples were retrieved from the Collaborative Biobank (Dresden, Germany) and mapped to patient outcome data extracted from the EBMT and CIBMTR. Genotyping of all KIR genes by sequencing exons 3, 4, 5, 7, 8, and 9 was performed by high resolution amplicon-based next generation sequencing. The impact of the classifiers on time-to-event outcomes was tested in cause-specific Cox regression models adjusted for patient age, a modified disease risk index, performance status, donor age, HLA-match, sex match, CMV match, conditioning intensity, type of T-cell depletion and graft type.

Results: Clinical data from 1704 patients were analyzed. The median age at alloHCT was 59.4 years (range, 18.1 to 79.6 years). The indication for alloHCT was MDS for 72% and sAML for 28% of patients. Disease risk was low/intermediate and high/very high in 41% and 59%, respectively. Donors were 10/10 matched for 79% of patients. Myeloablative, reduced-intensity and non-myeloablative conditioning regimens were used in 31%, 57%, and 12% of patients, respectively. Peripheral blood stem cells were the predominant graft source (93% of patients). ATG was administered in 56% and alemtuzumab in 9% of patients. In univariable and multivariable analyses of the whole cohort, overall survival and the cumulative incidence of relapse of patients with KIR-advantageous versus disadvantageous donors were not significantly different. As shown in figure 1 we could not replicate the pattern of outcomes predicted by the KIR3DL1/KIR2DS1-Receptor-Ligand model and the B content haplotype model (A/A vs B/x).

Conclusions: Relapse incidence and overall survival after unrelated donor alloHCT could not be predicted using the KIR3DL1/KIR2DS1-Receptor-Ligand model and centromeric/telomeric KIR-motif

model in this cohort of patients with MDS or secondary AML. This points at the possibility of interactions between NK-cell mediated allo-reactivity and procedural variations of alloHCT. Information on KIR-genes which have not been investigated so far and on alternative classification models will be explored in additional analyses.

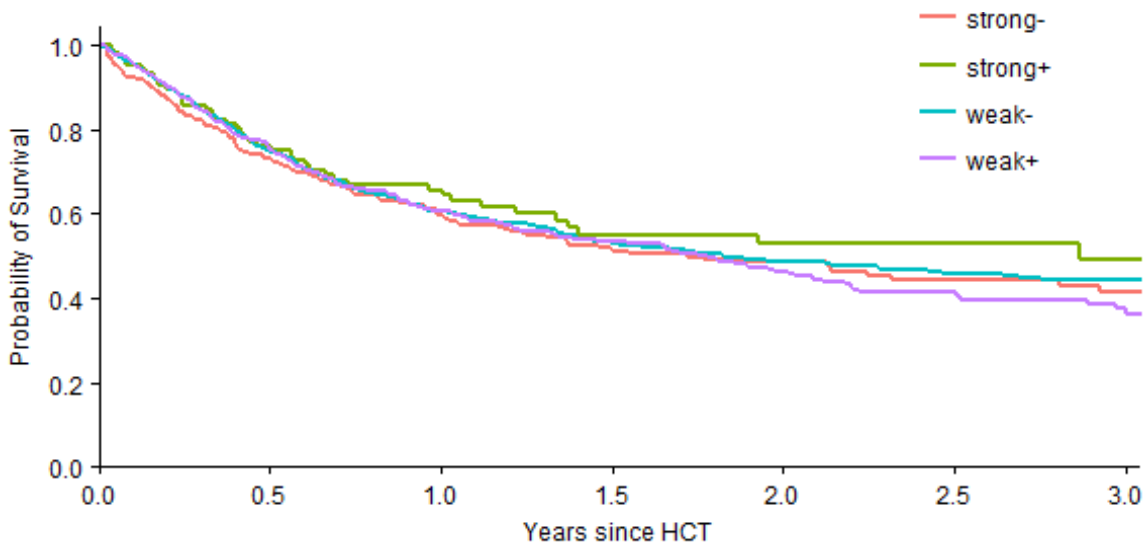

Figure 1. Overall Survival by predicted inhibition/activation of NK cells through KIR2DS1 and KIR3DL1

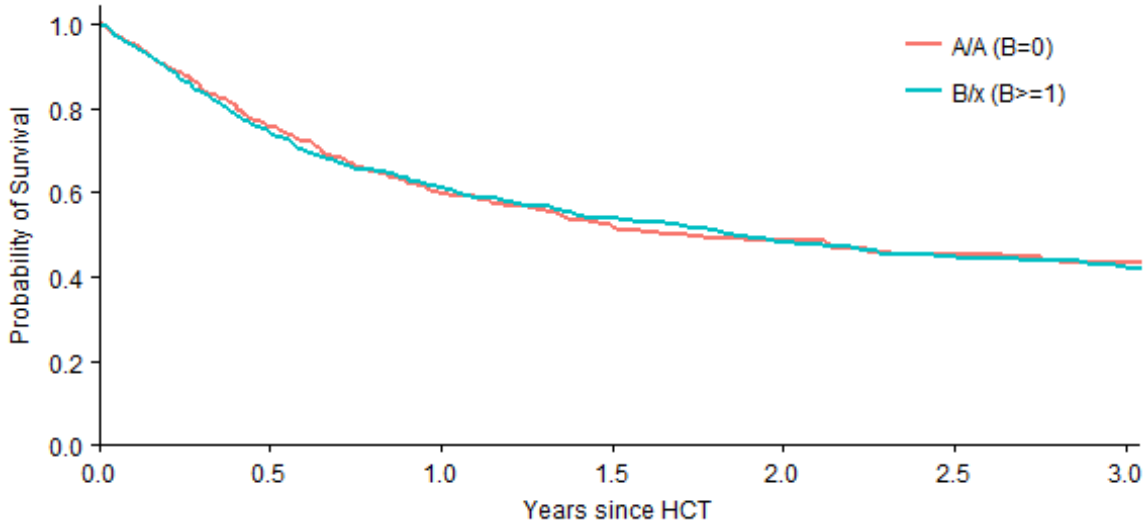

Figure 2. Overall Survival by centromeric or telomeric Haplotype B content

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2018 AMERICAN SOCIETY OF HEMATOLOGY (ASH) MEETINGS ABSTRACT

The Role of HLA-E Polymorphism in Acute Leukemia Patients Receiving a 10/10 HLA Matched Unrelated HSCT

Chrysanthi Tsamadou, MD, Tao Wang, PhD, Daniel Forst, MD, PhD, Stephanie J. Lee, MD, MPH, Stephen R. Spellman, MS, Katharina Fleischhauer, MD, Katharine C. Hsu, MD, PhD, Sophie Paczesny, MD, PhD, Michael R Verneris, MD, Christine Neuchel, PhD, Hubert Schrezenmeier, MD, PhD, Joannis Mytilineos, MD, PhD

Introduction: HLA-E is a class I HLA molecule that is involved in NK and CTL functions. It has limited polymorphism but can present unconventional peptides if it is aberrantly expressed under stress conditions. Donor-recipient (D/R) mismatching may potentiate NK-mediated alloreactivity. The functional role of HLA-E in HSCT is uncertain. We used CIBMTR data and samples to investigate the effect of HLA-E polymorphism on HSCT outcome in a 10/10 HLA matched unrelated acute leukemia setting by addressing the following questions: 1) Does D/R HLA-E match status affect HSCT outcome? 2) Does a specific DIR HLA-E genotype correlate with outcome?

Methods: The study included 1840 adult AML/ALL patients in complete remission who received first T-cell replete, 10/10 high resolution HLA matched transplants from unrelated donors between 2000 to 2015. Cohort characteristics are summarized in Table 1. Both D/R were HLA-E genotyped by NGS using a validated protocol on an Illumina Miseq platform. Overall survival (OS), leukemia free survival (LFS), relapse (RI), transplant related mortality (TRM), acute GvHD (aGvHD) and chronic GvHD (cGvHD) were evaluated; $p< 0.01$ was considered significant. Factors violating the proportional hazards assumption were adjusted via stratification, while a stepwise model building approach was used to select variables related to a given outcome with a threshold of 0.05 for both entry and retention in the model. HLA-E genotype effects in D/R were tested separately by comparing 0, 1 or 2 copies of a particular HLA-E allele (i.e. HLA-E*01 :03). Transplant pairs with HLA-E genotypes including other HLA-E alleles (i.e. other than HLA-E*01:01 or*01:03) were excluded from this analysis. The joint effect of D/R HLA-E genotype was also explored through a 4-level analysis of DIR HLA-E*01:03 homozygous vs other.

Results: HLA-E*01:01 and *01:03 frequencies in both DIR as well as the rate of HLA-E discrepant transplant pairs (32.7%) were in accordance with previously reported data. Distribution of HLA-E matched vs HLA-E mismatched cases was balanced for all significant clinical predictors, while no significant interactions between the HLA-E matching status variable and the adjusted covariates were detected. HLA-E mismatch had no significant association with any of the outcome endpoints in both univariate and multivariate models (data not shown). With respect to the HLA-E genotype effect on outcome, 1827/1840 transplant pairs were considered for analysis, as 13 transplant pairs were excluded due to new or rare HLA-E genotypes. When tested separately, both DIR HLA-E*01 :03/01:03 genotypes were associated with significantly lower LFS (HR=1.28, p=0.0027 and HR=1.31, p=0.0017 respectively). While the impact of HLA-E genotype on other outcome endpoints was not statistically significant,

relapse rates were higher in cases with D/R HLA-E*01:03/01:03 genotype. Joint DIR HLA-E genotype analysis suggest that donor HLA-E*01:03/01:03 is driving this effect. The results of LFS and RI multivariate models are summarized in Table 2. No significant interactions were observed between the HLA-E genotype variable and the adjusted covariates in the multivariate models.

Conclusion: This is the largest study investigating the effect of HLA-E polymorphism on HSCT outcome. We found no apparent effect of HLA-E mismatch on HSCT outcome in AMUALL patients in complete remission, while donor HLA-E*01:03/01 :03 genotype was associated with lower LFS, contrary to some previous reports. Differential efficiency of the two HLA allelic forms in inducing the CD94/NKG2A inhibitory pathway may account for their divergent effect on leukemia control, although this hypothesis is yet to be confirmed by functional assays. Our results suggest that avoidance of HLA-E*01:03/01:03 donors may improve the outcome of HSCT from 10/10 matched unrelated grafts.

Table 1. Cohort characteristics

Variable	**n**	**%**
Number of patients	1840	-
Number of centers	116	-
Patient-related		
Median age (range)	46 (18-77)	-
Gender		
Male	975	53 .0
Female	865	47.0
Karnofsky score prior to Tx		
<90	540	29.3
290	1209	65.7
Missing	91	5.0
Disease-related		
Diagnosis		
AML	1379	75.0
A LL	461	25.0
Disease status		
Early	1265	68 .8
Intermediate	575	31.2
Transplant-related		
Graft type		
Bone Marrow	469	25 .5
PBSC	1371	74.5
Conditioningintensity		
MAC	1414	76.8
RIC	426	23.2
Donor median age	31 (18-61)	-
Donor-recipient sex match		
M-M	724	39.4
M-F	552	30 .0
F-M	251	13 .6
F-F	313	17.0
DPB1 TCE matching		
Fully matched	292	15.9
Per missive	807	43 .8
Non-permissive	644	35.0
Missing	97	5.3

Donor-recipient CMV match		
-/-	508	27.6
-/+	679	36.9
+/-	204	11.1
+/+	414	22.5
Missing	35	1.9
ATG and/or Campath		
Yes	541	29.4
No	1297	70.5
Missing	2	0.1
Median time from diagnosis to Tx	6 months	-
Median follow-up of survivors	90 (9-185)	-

Table 2. D/R HLA-E genotype results for LFS and Relapse

HLA-E genotype	HR	95% CI	p-value	HR	95% CI	p-value
	LFS			Relapse		
Donor overall			0.0039			0.0270
Donor: 01:01/01:01, n=577	1.00			1.00		
Donor: 01:01/01:03, n=905	1.01	0.89-1.16	0.8348	1.09	0.91-1.30	0.3678
Donor: 01:03/01:03, n=333	1.28	1.09-1.51	0.0027	1.35	1.08-1.69	0.0083
Recipient overall			0.0045			0.0131
Recipient: 01:01/01:01, n=584	1.00			1.00		
Recipient: 01:01/01:03, n=908	1.18	1.03-1.34	0.0164	1.24	1.04-1.49	0.0177
Recipient: 01:03/01:03, n=318	1.31	1.11-1.55	0.0017	1.38	1.09-1.73	0.0067
Joint D/R overll			0.0055			0.0545
Donor: 01:01+ Recipient: 01:01+, n=1352	1.00			1.00		
Donor: 01:01+ Recipient: 01:03/01:03, n=123	1.08	0.86-1.36	0.4906	1.08	0.80-1.47	0.6122
Donor: 01:03/01:<J3 Recipient: 01:01+, n=136	1.29	1.04-1.59	0.0184	1.29	0.97-1.71	0.0793
Donor: 01:03/01:03 Recipient: 01:03/01:03, n=195	1.31	1.09-1.56	0.0035	1.33	1.05-1.70	0.0197

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2019 TANDEM BMT MEETINGS (ASBMT/CIBMTR) ABSTRACT

Effect of heterozygosity of human leukocyte antigen on outcomes following allogeneic hematopoietic cell transplant for myeloid and lymphoid malignancies

Christine Camacho-Bydume, MD, Tao Wang, PhD, Jennifer A. Sees, MPH, Katharina Fleischhauer, MD, Sophie Paczesny MD, PhD, Stephen R. Spellman, MS, Stephanie J. Lee, MD, MPH, Katharine C. Hsu, MD, PhD

Background: A recent study demonstrated that heterozygosity at HLA class I loci is associated with improved survival in patients with advanced solid tumors treated with immune checkpoint inhibitors when compared to patients homozygous in at least one HLA class I locus (Chowell et al. Science 2018). HLA heterozygosity allows for greater diversity of peptide presentation to T-cells and activation of a diversified immune response. In HCT, we hypothesize that HLA heterozygosity impacts disease control and survival in patients with myeloid and lymphoid malignancies.

Design: Patients who underwent 8/8 HLA-matched first allogeneic HCT for AML, MDS, ALL, or NHL between 2000 – 2015 were identified from CIBMTR database. Patients who received non-myeloablative and reduced intensity conditioning were excluded from analysis, except for those with NHL. HLA zygosity was characterized to the allele level as either heterozygous at all HLA class I loci or homozygous in at least one HLA class I locus. Primary outcomes of overall survival (OS) and relapse were analyzed. Secondary outcomes included transplant-related mortality (TRM) and acute graft-versus-host disease (aGVHD). Multivariate analysis using Cox proportional hazards model was performed with factors violating the proportional hazards assumptions adjusted through stratification.

Results: A total of 7,474 patients received 8/8 HLA-matched allogeneic HCT according to our selection criteria. 5,775 patients were heterozygous at all HLA class I loci, while 1,699 patients were homozygous in at least one HLA class I locus. OS was not statistically different with HLA homozygosity when compared to heterozygosity (HR=0.98, P=0.6849). When analyzed in disease-specific cohorts, there were no differences in OS with homozygosity when compared to heterozygosity: AML (HR=0.99, P=0.8315), MDS (HR=0.88, P=0.3198), ALL (HR=0.97, P=0.6398), and NHL (HR=0.99, P=0.8862). Relapse was not associated with HLA homozygosity when compared to heterozygosity (HR=0.93, P=0.1028). This remained consistent when evaluated by diseases: AML (HR=0.89, P=0.0891), MDS (HR=0.89, P=0.5106), ALL (HR=0.97, P=0.7669), and NHL (HR=0.91, P=0.3413). There were also no differences in TRM (HR=1.05, P=0.3110) and aGVHD grade 2-4 (HR=1.06, P=0.2213).

Conclusions: Zygosity of HLA class I loci was not associated with outcomes following allogeneic transplants for myeloid and lymphoid malignancies. Alloreactivity of T-cells in conjunction with presence of minor MHC antigens may possibly override the effect of HLA zygosity seen in an autologous setting with immune checkpoint inhibitors. Our findings may be limited by small sample sizes in the disease

subgroups. Future analysis into differential expression and epitope presentation of HLA may help reveal the effect of HLA zygosity on antigen presentation to T-cells and impact on outcomes.

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2018 AMERICAN SOCIETY OF HEMATOLOGY (ASH) MEETINGS ABSTRACT

Telomere Length and Telomerase Complex Mutations Predict Fatal Treatment Toxicity after Stem Cell Transplantation in Patients with Myelodysplastic Syndrome

Mikko Myllymaki, MD, PhD, Robert A. Redd, MS, Corey S. Cutler, MD, MPH, Wael Saber, MD, MS, Zhen-Huan Hu, MPH, Tao Wang, PhD, Stephen R. Spellman, Christopher J. Gibson, MD, Maxine M Chen, Esther Orr, David P. Steensma, MD, Joseph H. Antin, MD, Immaculata De Vivo, Donna S Neuberg, ScD, Suneet Agarwal, MD, PhD and R. Coleman Lindsley, MD, PhD

Introduction: Identifying patients at high risk of fatal treatment toxicity is a central challenge in hematopoietic stem cell transplantation (HSCT). Objective metrics that enable more accurate prediction of non-relapse mortality (NRM) could inform clinical decisions about timing and modality of HSCT. Short telomere length, mediated by inherited or acquired factors, impairs cellular response to genotoxic and replicative stress. We therefore evaluated the impact of recipient telomere length on clinical outcomes based on treatment intensity in patients with myelodysplastic syndrome (MDS) receiving HSCT.

Methods: We used qPCR to measure relative telomere lengths in whole blood DNA samples from 1514 patients who received allogeneic HSCT for MDS and were enrolled in the Center for International Blood and Marrow Transplant Research Repository. Within the cohort, patients age 40 and older were grouped into those with short (<25th), intermediate (25-75th) or long (>75th percentile) telomeres. To evaluate germline determinants of telomere length, we sequenced 7 genes involved in telomere maintenance and mutated in dyskeratosis congenita: TERC, TERT, DKC1, TINF2, NHP2, WRAP53 and CTC1. Putative germline variants were classified as “rare” if the allele frequency was <0.001 in all Genome Aggregation Database (gnomAD) populations.

Results: Among patients age 40 and older (n=1267), those with short (HR 1.52, 1.24-1.85, p<0.001) or intermediate (HR 1.35, 1.13-1.61, p<0.001) telomere length had poor overall survival compared with those having long telomeres (Fig 1A). In a competing risks regression model, the adverse effect of shorter telomeres was driven by a significantly higher risk of NRM among patients with short (HR 1.57, 1.20-2.06, p=0.001) and intermediate (HR 1.32, 1.03-1.69, p=0.03) telomere length. The association between telomere length and NRM was evident in patients receiving myeloablative (MAC, p=0.002) but not reduced-intensity conditioning (RIC, p=0.2) regimens (Fig 1B). We observed no association between telomere length and disease relapse in patients receiving MAC or RIC regimens. In a multivariable regression model, the prognostic significance of telomere length was independent of clinical and genetic factors, including age, Karnofsky performance status, hematologic parameters, IPSS-R risk category, donor mismatch, donor age, and TP53 mutation.

We identified 40 patients (2.6% of the cohort) with rare germline TERT variants. Patients with rare TERT variants had significantly shorter telomeres than patients with common (p=0.001) or no (p<0.0001) TERT variants and were diagnosed with MDS at an earlier age than patients with common (52.2 vs. 58.4 years, p=0.01) or no (52.2 vs. 57.9 years, p=0.01) TERT variants. The domain distribution of rare TERT variants mirrored that of validated pathogenic germline TERT mutations, primarily affecting the reverse transcriptase and C-terminal extension domains. Rare variants in

TERC (0.4%) and DKC1 (0.2%) were also associated with shorter telomeres (p=0.02 and p=0.04, respectively). In total, we identified germline telomerase complex mutations in 49 of 1514 MDS patients (3.2%), even though only 1 patient had a clinical diagnosis of dyskeratosis congenita. Together, patients with telomerase complex mutations had shorter overall survival than those without mutations (unadjusted p=0.008), attributable to a marked increase in the risk of early NRM among those receiving MAC (1 year cumulative incidence of NRM 48% vs. 26%, p=0.03). The impact of shorter telomeres on NRM was similar in patients with and without identified core telomerase complex mutations, suggesting that additional mechanisms of impaired telomere length maintenance may contribute to MDS pathogenesis and outcome.

Conclusion: Recipient telomere length is independently associated with overall survival after allogeneic HSCT for MDS. Patients age 40 or older with shorter blood cell telomere length have a significantly elevated risk of early NRM with myeloablative conditioning regimens. Clinically unrecognized germline mutations in the telomerase genes TERT, TERC, and DKC1 define a distinct subset of adult patients with sporadic MDS and short telomeres who have poor transplant outcomes. Together, these results indicate that short telomere length in MDS patients mediates fatal treatment toxicity that may be attenuated by lower intensity conditioning approaches.

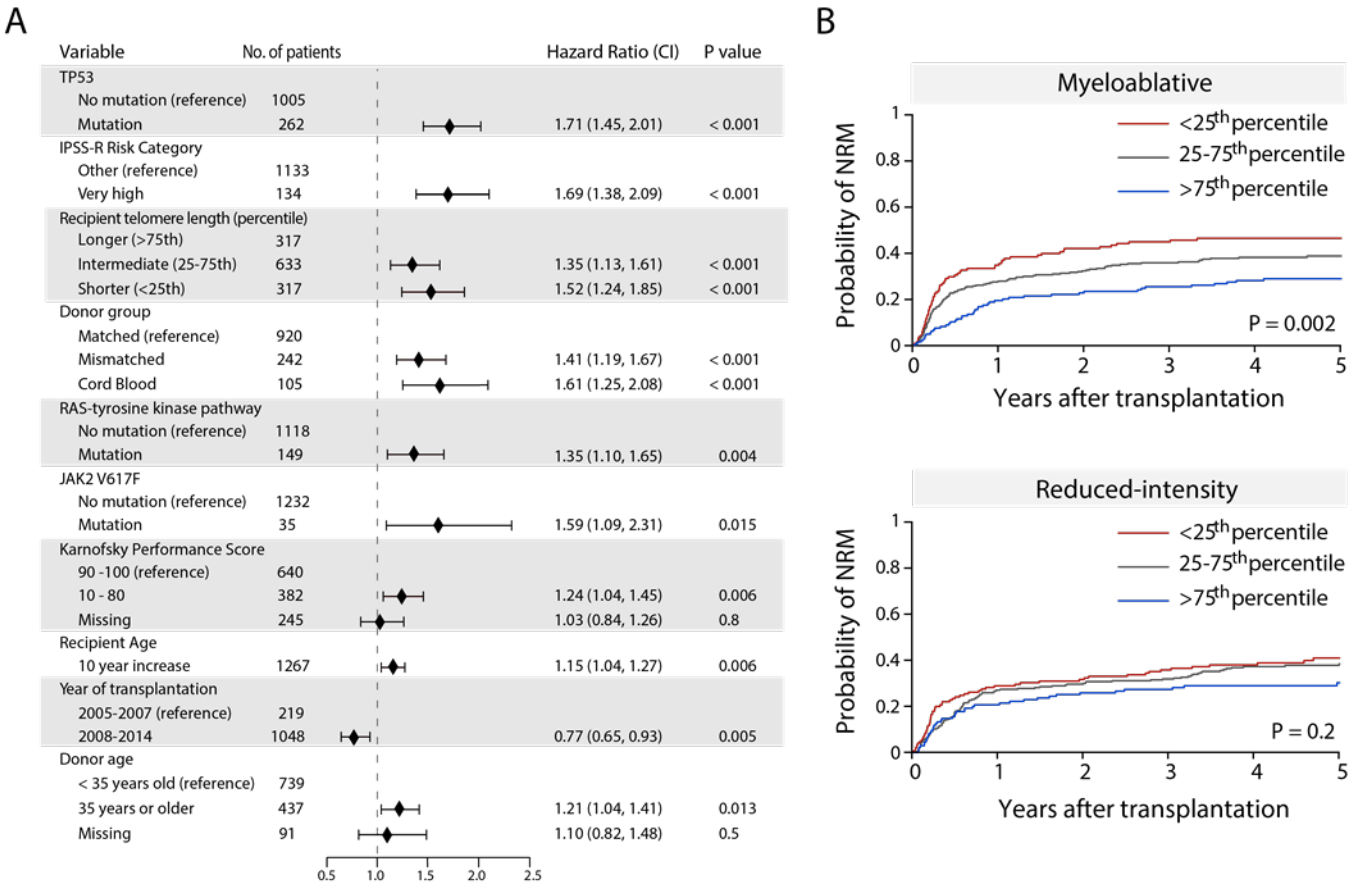

Figure 1. (A) Stepwise multivariable Cox regression model for overall survival in patients age 40 and older. (B) Cumulative incidence of non-relapse mortality (NRM) according to relative telomere length in patients receiving myeloablative (n=582) or reduced-intensity (n=554) conditioning regimens.

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2018 AMERICAN SOCIETY OF HEMATOLOGY (ASH) MEETINGS ABSTRACT

Donor IFNL4 Genotype is Associated with Transplant-related Mortality After Unrelated Donor Myeloablative Hematopoietic Cell Transplantation in Patients with Acute Leukemia

Shahinaz M. Gadalla, Tao Wang, Olusegun Onabajo, Youjin Wang, Michael D. Haagenson, Jennifer A. Sees, Stephen R. Spellman, Hormuzd A. Katki, Stephanie J. Lee, Ludmila Prokunina-Olsson

Introduction: Interferon Lambda 4 gene (IFNL4) encodes IFN-λ4 protein, a new member of the type-III interferon family. IFNL4 genotype (rs368234815-dG allele), defines the genetic ability to produce IFN-λ4 and has been associated with reduced clearance of hepatitis C virus (HCV) infection. Given antiviral activity and immune modulation properties of IFN-λ4, we hypothesized that IFNL4 genotype of recipient and/or donor may modulate post-transplant survival outcomes, possibly through control of viral infections, and/or alloreactivity.

Methods: From the Center for International Blood and Marrow Transplant Research (CIBMTR) database, we randomly selected 627 patients who received unrelated hematopoietic cell transplantation (HCT) for acute myeloid leukemia (AML, N=449) or acute lymphocytic leukemia (ALL, N=178). The patients had to match the following criteria: 1) HCT between 2004 and 2012, 2) available pre-HCT blood sample for the donor and recipient, 3) 8/8 HLA matching, and 3) myeloablative conditioning. IFNL4 genotyping was completed for 619 donors and 522 recipients using a custom-designed TaqMan assay for rs368234815. Multivariable Cox proportional hazard models were used for statistical analyses. Follow-up ended in November 2017.

Results: Median age at HCT was 40 years (range=<1-68). Most patients (66%, N=411) were in first complete remission, had a Karnofsky Performance Score (KPS) between 90-100% (70%, N=436), and received peripheral blood stem cell grafts (70%, N=439). The median post-HCT follow-up was 68 months (range=5-122). Donor IFNL4 genotype was associated with risk of transplant-related mortality (TRM); with 5 years probabilities=19%, 27%, and 30% for donor TT/TT (n=286), TT/dG (n=267), and dG/dG (n=64) genotypes, respectively, p=0.02. The results remained significant in multivariable analysis (p=0.002); compared with patients receiving HCT from donors with TT/TT genotype, with the HR=1.59 (95% CI=1.13-2.23, p=0.007) for TT/dG donors and HR=1.95 (95% CI=1.18-3.23, p=0.009) for dG/dG donors. The data suggested that donor IFNL4 genotype may also predict risk of disease-free survival (DFS; HR=1.43, 95% CI=1.02-2.00, p=0.03), and overall survival (OS; HR=1.40 (95% CI=0.98-1.99, p=0.06) for donor dG/dG genotype (Table1). No association between recipient genotype and any survival outcome was observed (p>0.05 for OS, DFS, and TRM).

Conclusion: Donor IFNL4 genotype is associated with risk of transplant-related mortality in patients with acute leukemia. The data suggest that avoiding donors with dG/dG genotype will improve HCT outcomes without limiting the potential donor pool. A validation study is needed; if confirmed, IFNL4 genotype may provide an added value to donor selection criteria.

Table 1. The association between donor *IFNL4* genotype and post-transplant survival outcomes in patients with AML and ALL in multivariable models

	TT/dG *vs.* TT/TT		dG/dG *vs.* TT/TT	
	N events/total	HR (95% CI) *P-value*	N events/total	HR (95% CI) *P-value*
Overall survival (OS)	143/269	1.08 (0.85-1.36) 0.54	40/64	1.40 (0.98-1.99) 0.06
Disease-free survival (DFS)	153/267	1.01 (0.81-1.27) 0.92	44/64	1.43 (1.02-2.00) 0.03
Transplant-related mortality (TRM)	82/267	1.59 (1.13-2.23) 0.007	21/64	1.95 (1.18-3.23) 0.009

Models adjusted for donor age, GVHD prophylaxis, recipient age, use of total body irradiation, and stratified on graft source; carriers of TT/TT genotype do not produce IFN-λ4 protein; carries of TT/dG and dG/dG genotypes have one or two copies of IFNL4 gene, respectively and can produce IFN-λ4 protein.