Supplementary Appendix

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List of Investigators

John Magenau[1], M.D.

Daniel Peltier[1], M.D. Ph.D.

Mary Riwes[1], D.O.

Attaphol Pawarode[1], M.D.

Brian Parkin[1], M.D

Thomas Braun[2], Ph.D.

Sarah Anand[1], M.D.,

Monalisa Ghosh[1], M.D.

John Maciejewski[1], M.D. Ph.D.

Gregory Yanik[1], M.D.

Sung Choi[1], M.D.

Moshe Talpaz[3], M.D.

Pavan Reddy[1], M.D.

(1) Blood and Marrow Transplantation Program, University of Michigan Rogel Cancer Center, Ann Arbor, MI

(2) Department of Biostatistics, University of Michigan, Ann Arbor, MI

(3) Division of Hematology-Oncology, University of Michigan Comprehensive Cancer Center, Ann Arbor, MI

SUPPLEMENTARY METHODS

Plasma and peripheral blood mononuclear cells (PBMC) from recipients and donors were collected and cryopreserved prior to conditioning and on days 28, 56, 100 and 180 post-HCT. For cellular immune assays, PBMCs were isolated using Ficoll-Premium (GE Healthcare), frozen in heat-inactivated fetal bovine serum containing 10% dimethyl sulfoxide and stored in liquid nitrogen. For surface staining, PBMCs were washed with FACS buffer, incubated with Human TrueStain FcX Blocking Buffer (BioLegend) on ice for 10 minutes, stained with surface markers on ice for 30 minutes, and then rinsed twice with FACS buffer. For pSTAT1 staining, PBMCs were fixed at 37^0C for 15 minutes (BioLegend Fixation Buffer), permeablized with TruePhos Perm Buffer (BioLegend) for one hour at -20^0C, rinsed with FACS buffer, stained at room temperature, and rinsed twice with FACS buffer. PBMCs from healthy donors were used as positive staining, gating and compensation controls. To identify cross-presenting DCs, PBMCs were stained with Live/Dead Fixable Near-IR (ThermoFisher) to exclude dead cells as well as fluorescently-labeled monoclonal antibodies to human CLEC9A-PE (AF9, BioLegend), CD45-PE-Cy7 (2D1, BioLegend), and CD141-APC (M80, BioLegend). To identify WT1-specific T-cells, PBMCs were stained with CD4-FITC (RPA-T4, BioLegend), CD8a-Pacific Blue (SK1, BioLegend), WT1 HLA-A*0201 (SLGEQQYSV)-APC (Immundex), and WT1 HLA-A*0201 (VLDFAPPGA) (Immundex). WT1 dextramer gates were set using healthy donor PBMCs stained with a negative control HLA-A*0201-APC dextramer in place of WT1 dextramers. Dextramer stained cells were analyzed using a MoFlo Astrios Cell

Sorter (Beckman Coulter) operated by the University of Michigan Flow Cytometry Core. All other samples were analyzed using an Attune NxT flow cytometer (Fisher). Data was analyzed using GraphPad Prism 8.0.0 (San Diego, CA).

Table S1: Infections and severity[1] through day 180 after HCT

Bacterial Species	Site	Grade 1	Grade 2	Grade 3	Total
Staphylococcus epidermidis	Bloodstream	8	0	0	8
Staphylococcus epidermidis	Joint	1	0	0	1
Staphylococcus aureus	Conjuntiva	1	0	0	1
Enteroccus species	Bloodstream	0	4	0	4
Serratia marcescens	Bloodstream	0	1	0	1
Klebsiella pneumoniae	Bloodstream	0	1	0	1
Pantoea species	Bloodstream	0	1	0	1
Clostridium innocuum	Bloodstream	0	1	0	1
Clostridium difficile	Stool	1	0	0	1
Paenibacillus species	peritoneal fluid	1	0	0	1
Total		12	8	0	20*

* 15 of 36 patients experienced ≥ 1 bacterial infections; 5 of 36 patients experience ≥ 2 infections

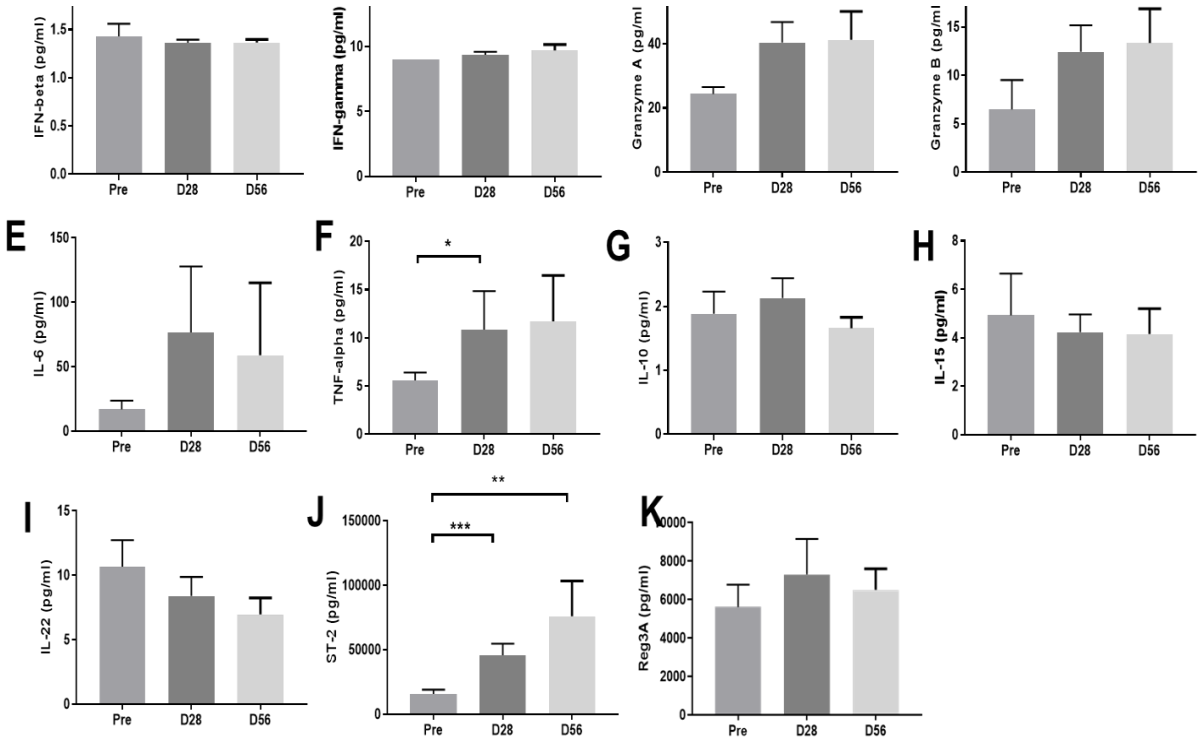
Virus	Site (s)	Grade 1	Grade 2	Grade 3	Total
Cytomegalovirus	Bloodstream	19	0	0	19
Cytomegalovirus	Bloodstream / Enteritis	0	0	1	1
HHV-6	Bloodstream	0	1	0	1
HHV-6	Bloodstream / CSF	0	0	1	1
Adenovirus	Bloodstream	1	0	0	1
Respiratory syncytial virus (R	nasopharynx	0	1	0	1
Rhinovirus - enterovirus	nasopharynx	0	1	0	1
Total		20	3	2	25

* 20 of 36 patients experienced ≥ 1 viral infections

Fungal	Site (s)	Grade 1	Grade 2	Grade 3	Total
Candida parapsilosis	Bloodstream	0	0	1	1
Rhizopus species	skin	0	0	1	1
Total		0	0	2	2

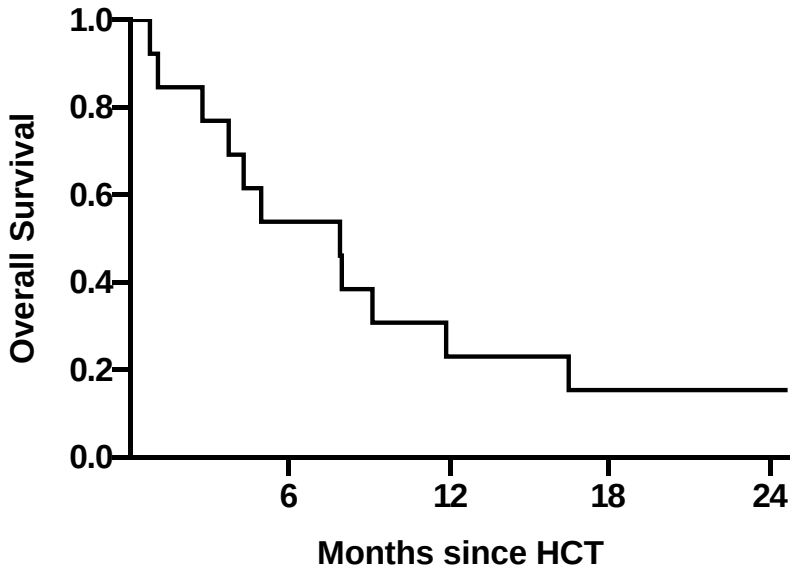
[1] Severity grade assigned per BMT Clinical Trial Network Technical MOP v3.0

FIGURE S1: cytokines and GVHD biomarkers



Supplemental Figure 1. Plasma Cytokines and GVHD Biomarkers. Paired plasma levels are shown at baseline (pre-conditioning), day 28 and day 56 (A) IFN-β (B) IFN-gamma (C) Granzyme A (D) Granzyme B (E) IL-6 (F) TNF-alpha (G) IL-10 (H) IL-15 (I) IL-22 (J) ST-2 and (K) Reg3α

FIGURE S1: OS of contemporary AML cohort not in remission at time of HCT



Supplemental Figure 2. OS for 13 AML patients at University of Michigan from 2014-2017 who underwent HCT not in remission. All patients received myeloablative conditioning (Clofarabine/Busulfan; n=7 or Fludarabine/Busulfan; n=6) without pegIFNα. Characteristics included median age of 61 years (24-72 years), HCT-CI of ≥ 3 (n=4) or < 3 (n=9) and % of blasts at HCT of ≥5% (n=7) and <5% (n=6). Reasons for not proceeding on the clinical trial include: trial not open (n=5) and lack of informed consent (n=8).