**Compassionate use of αβ+ T Cell/CD19+ B cell Depletion in a single patient with Severe Aplastic Anemia using the CliniMACS Plus®**

**Institution name**: Riley Hospital for Children – Indiana University

**Sponsor:**

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1. **Brief Clinical History and Rationale for Use in this patient**
	1. **Patient information**

Age: 17 years old

Gender: female

Weight: 54.4kg

Diagnosis: Refractory Severe Aplastic Anemia after immunosuppressive therapy (IST)

* 1. **Clinical history and reasoning for αβ+ T Cell/CD19+ B cell Depletion of peripheral blood stem cell graft**

This patient is a 17-year-old with a history of refractory severe aplastic anemia after IST who originally presented in October 2024. Unfortunately, she did not have a matched sibling donor and was started on IST in October 2024. She has had several complications during IST including:

1. multiple admissions for febrile neutropenia
2. A persistent tongue lesion that was concerning for fungal infection but was biopsied and found to be negative
3. Peri-mandibular lymph node swelling thought to be infectious but biopsy negative
4. Acute kidney injury from IST (cyclosporine and/or tacrolimus)
5. Mild iron overload.

She is 5 months from the initiation of IST and has not had a hematological response thus far requiring platelet transfusions 1-2 times a week and blood transfusions 2-3 times a month. At this point in her treatment alternative unrelated transplant or haplo-identical transplant would be strongly considered and is her best chance at long term survival. We had requested >5 unrelated donors from various national bone marrow programs but no donors responded to our request, so we plan to proceed with haplo-identical transplant using her older sister.

Haplo-identical stem cell transplants require profound T cell depletion strategies of the graft to minimize the risk of severe graft versus host disease. This can be performed using in-vivo methods (post-transplant cyclophosphamide) or ex-vivo (x). This patient has had several complications and toxicities thus far during her IST. We need to eliminate the post-transplant cyclophosphamide infusion to decrease the risk of toxicity and therefore transplant related morbidity and mortality. By depleting the αβ+ T Cell/CD19+ B cell with ex vivo depletion we can remove the cells responsible for graft versus host disease (GvHD). Additionally, this method of graft manipulation will also allow for faster engraftment with higher number of CD34+ cells. This most likely will result in decreased periods of neutropenia and transfusion independence for this patient.

I am requesting compassionate use of αβ+ T Cell/CD19+ B cell depletion to provide this patient with the best chance at cure for her refractory severe aplastic anemia while decreasing the toxicity by not using high doses of cyclophosphamide or other immunosuppressants.

The αβ+ T Cell/CD19+ B cell depletion is currently available on a clinical trial offered at our institution for a different underlying disease (NCT03619551). We have also performed one additional treatment under an IND which has been very successful. This protocol is a derivation of this clinical trial that has been approved for other indications and used for this indication at other institutions. The αβ+ T Cell/CD19+ B cell depletion will be completed based on the clinical trial offered at our institution, however the stem cell transplant preparative regimen will be different from the protocol to target her underlying severe aplastic anemia.

The donor for this patient is her older sister. She is determined to be the best donor option available for this patient. She will undergo history, physical exam and blood work to determine suitability. The timeline for donor clearance and then collection will be mid-April. The sister’s stem cells will be peripherally collected and will be αβ+ T Cell/CD19+ B cell depleted using the technique described below. After they have been αβ+ T Cell/CD19+ B cell depleted, they may be frozen until the cell infusion day. Due to the tenuous clinical status of the patient’s severe aplastic anemia we need to proceed with stem cell transplant urgently.

* 1. **Patient Treatment Plan**

The conditioning regimen uses FDA approved agents and is most proper for the patient’s underlying disease and would be considered standard of care (SOC).

Day -9 to Day -7: rabbit anti-thymocyte globulin

Day -6: Total body irradiation (TBI)

Day -6 to Day -2: Fludarabine

Day -5 to Day -4: Cyclophosphamide

Day -3 to Day -2 Thiotepa

Day -1: Rest

Day 0 – infusion of αβ+ T Cell/CD19+ B cell depleted peripheral blood stem cell infusion

1. **Background and Rationale for αβ+ T Cell/CD19+ B cell grafts**

Allogeneic stem cell transplant is the only curative choice for many patients with malignant and non-malignant disorders. Unfortunately, only ~25% of patients who are seeking curative therapy with allogeneic transplantation will have an HLA identical sibling donor. In those instances, the use of a well-matched unrelated donor or mismatched related donor may be the only curative possibility available to these patients. However, when using an alternative donor transplant acute and chronic GvHD can lead to significant morbidities in these patients. Studies have shown that severe acute GvHD (grades III-IV) occurs in up to 50% of patients when using an alternative donor for stem cell transplant. Furthermore, data has shown that extensive chronic GvHD can occur in >20% of patients when using an alternative donor transplant.

Various methods of T cell depletion are used to try an overcome the morbidity of GvHD in unrelated donor and mismatched related donor. These techniques typically involve T cell depletion through either an in-vivo or ex-vivo method. Ex-vivo depletion includes mechanical methods such as CD34+ selection and broad or narrow specificity antibodies (1-2). Using cell sorting techniques to select CD34+ stem cells have allowed the use of mismatch donors with reliable donor engraftment and reduce GvHD (1). New methods have been developed using magnet-activated cell sorting to help keep other cells that may help facilitate engraftment (NK cells). These include CD3+ depletion or more recently αβ+ T cell depletion.

Numerous studies in the U.S.A. and in Europe are currently underway using the CliniMACS Plus® device for αβ+ T cell depletion. A large-scale method for selective depletion of αβ+ T cells from mobilized PSC has been developed and appears effective for reducing the risk of GvHD while preserving CD34+ cell enrichment. The CliniMACS Plus® device is used to deplete αβ+ T cells with the final product keeping NK cells, dendritic cells, and γδ+ T cells. This selective process allows the final product to retain γδ T+ cells and NK cells which can exert pro-engraftment, anti-leukemic, and anti-viral effector function (3). In mice, these grafts resulted in rapid engraftment of myeloid and lymphoid cells. Pilot studies in haploidentical PSCT which have included children have been encouraging thus far. An initial study of the efficacy of αβ+ T cell depletion of 102 mobilized PBSC products conducted in Tubingen Germany noted a 4.7 log depletion of αβ+ T cells, with 73% recovery of CD34+ cells and 83% recovery of γδ+ T cells (4).

Results recently reported from a large Italian cohort of 80 pediatric patients with acute leukemia who underwent haploidentical PSCT with αβ+ T cell depletion from 2011 to 2014 were promising (5). In this study, overall efficiency of αβ+ T cell depletion was excellent with all patients receiving a CD34+ stem cell dose greater than 6 x 106/kg and a αβ+ T cell dose of less than 1 x 105/kg. All but two patients engrafted, and there were no reports of Grade III-IV acute GvHD or chronic extensive GvHD. In a prior study, the same group investigated the use of αβ+ T cell depletion of haploidentical PSCT grafts in 23 children receiving HSCT for non-malignant diseases (6). Results last reported showed that 21 of 23 patients were alive at the time of analysis and no patients developed grade III-IV acute GvHD or chronic extensive GvHD. Median time to neutrophil (ANC) and platelet engraftment was very rapid at 13 and 10 days, respectively. Four patients did experience graft failure (2 primary and two secondary), but all were successfully re-transplanted.

One disadvantage with using T cell depleted grafts is the increased risk of Epstein-Barr Virus (EBV) induced lymphoproliferative disorders. Most patients will have been exposed to EBV prior to transplant. After primary infection with EBV the virus stays dormant in B cells for the lifetime of the B cell. Reactivation of EBV is tightly controlled by the immune system, and particularly the T cells. Studies have shown that the simultaneous removal of donor B cells from the products that are T cell depleted reduces the risk of EBV reactivation during the period before immune recovery (7). Additionally, most centers performing T cell depletion give an added dose of Rituximab to further suppress B cell numbers.

1. **αβ+ T Cell/CD19+ B cell Depletion procedure**
	1. **Procedure overview**

A mobilization regimen with granulocyte colony stimulating factor (G-CSF) will be used to obtain a PBSC product from the patient’s sister after obtaining written informed consent. Apheresis will be performed according to institutional standards aiming for 3-4 total blood volumes as tolerated by the donor. The target infusion goal will be at least 10-15x106 CD34+ cells/kg. The donor will mobilize with G-CSF on Days 1-4 with 10mcg/kg/day subcutaneous. On day 5, the donor will continue with the dosing of G-CSF and apheresis will begin and may continue through day 6 if the target cell number is not reached after the first collection. Based upon experience, sufficient donor cells will be collected in one or two collections. Of note, donor cells may be collected before the timeline above and cryopreserved if necessary. The donor will be monitored and PBSC products will be collected according to FACT-JACIE international standards for cell therapy, product collections, processing, and administration.

Processing of hematologic stem cells from apheresis will take place at the IU Health Cellular Therapy Lab using validated Standard Operating Procedures. If more than 1 apheresis event is performed to collect a sufficient cell dose, HPC-A products may be combined prior to αβ+ T Cell/CD19+ B cell depletion. HPC-A products will be αβ+ T Cell/CD19+ B cell depleted using the CliniMACS® device according to the manufacturer’s instructions.

Briefly, the starting material will be assessed by a cell count, viability and flow cytometry for TCR a/b+ cells and CD19+ cells. Prior to immunomagnetically labeling the Hematopoietic progenitor cell by apheresis (HPC-A) product, the HPC-A product will be washed to remove platelets, and the cell concentration will be adjusted in preparation for antibody labeling. The peripheral blood stem cell (PBSC) product will be labeled using the CliniMACS® TCRαβ Biotin kit and CD19+ Microbeads. After immunomagnetically labeling cells with TCRαβ and CD19 antibodies, the cells will be washed to remove unbound microbeads. The HPC-A product will be loaded onto the CliniMACS® device, and the negative cell fraction will be collected. The cells from the negative fraction will then be centrifuged and reconstituted to obtain the final product. Cell viability, cell counts, sterility, gram stain and endotoxin testing will be performed on the final product prior to infusion. In addition, the final product will be enumerated and assessed for viable stem cell (CD34+) T-cell (TCR αβ+CD3+, TCR γδ+CD3+) and B-cell content using anti-CD20 (due to blockage of the CD19 antigen by the reagent used for depletion) by flow cytometry. The HPC-A graft product will be infused fresh after completion of release testing, stored overnight at 4oC prior to infusion or frozen for future infusion at the discretion of the stem cell transplant physician.

Cell dose parameters for the *primary HSC* infusion donor graft are as follows. The cell doses noted are defined as the total αβ+CD3+, CD34+, and B-cell counts contained in the final product:

• The target HSC dose is at least 10-15x 106 CD34+ cells/kg recipient weight. The minimum cell dose will be 2-5 x 106 CD34+ cells/kg with no maximum dose, but the CD34+ dose is usually limited by the need to not exceed the αβ+CD3+ dose threshold below.

• The target αβ+ CD3+ cell dose content in the primary infusion donor product will be ≤1.0 x 105 αβ+CD3+ cells/kg recipient weight. If projected CD34+ content of a graft will be < 5.0 x 106 /kg, the αβ+CD3+ in the graft may be increased to a maximum of 5.0 x 105 αβ+CD3+ cells/kg to increase CD34+ content in the graft. Alternatively, the Cellular Therapy Lab may perform a CD34+ selection on a portion of the collection or a second collection to optimize the CD34+ numbers for infusion.

• Rituximab will be given on Day +1 375mg/m2 IV x 1 dose to reduce the risk of Epstein-Barr virus (EBV) related post-transplant lymphoproliferative disorder (PTLD).

*Optional CD34+ selection to boost CD34+ dose:*If at the end of the negative depletion procedure, the residual number of αβ T cells would be greater than 1x105/kg recipient weight, a residual part of the selected graft may undergo further CD34+ selection and both allograft products returned to the subject. Alternatively, a saved portion of the first collection or a second leukapheresis collection may be selected for CD34+ after the target residual αβ-T cells has been reached. CD34+ selection will be performed following the standardized protocol in the User’s Manual for the CliniMACS (Miltenyi Biotech), operating under the Cellular Therapy Lab’s standard operating laboratory procedures. The total graft will consist of one or two sequential αβ+ T Cell/CD19+ B cell depleted HPC-A cell infusions, plus additional CD34+ selected cells if necessary to increase the CD34+ dose.

The final αβ+ T Cell/CD19+ B cell depleted fresh product (or cryopreserved) will be infused intravenously according to institutional standards. Due to αβ+ T Cell/CD19+ B cell depletion of donor stem cell product pharmacologic GvHD prophylaxis will not be needed following stem cell infusion in the absence of prior GvHD symptoms. Prevention of graft failure will include agents such as Tacrolimus or Sirolimus to prevent graft rejection and will be left to the attending transplant physician’s discretion based on individual patient risks/factors and will not be a violation of the protocol. All transplant-related care of the recipient including pre-transplant evaluations, conditioning, supportive care, and post-transplant evaluations will be performed as per our institutional standard of care. The stem cell processing and infusion of the donor cells are considered the study-related procedure and experimental.

* 1. **Cryopreservation Process (if needed)**

Cryopreservation will be performed per our institutional standards of care. HCP products are cryopreserved using 10% DMSO plus 5% HSA.  After addition of the cryopreservation solution the bags are labeled per ISBT standards and placed in a controlled rate freezer.  The controlled rate freezer reduces the temperature of the cells slowly to minimize any damage to the cells. The cell bags reach -90 degrees C at which they are held for 15 minutes, they are then transferred to liquid nitrogen vapor phase storage freezers.  The LN2 storage freezers are kept <=-150 degrees C. Please reference CMC for more details on cryopreservation process.

Cryopreservation of hematopoietic stem cells is a common procedure in our cellular therapy lab. Stability testing is performed on HPC products every year and has consistently shown great recovery from the cryopreserved products. Appendix B shows our institution’s 2022 data with great viability recovery. The length of time of cryopreservation for this patient is quite short and should not cause a significant decrease in viability or decrease engraftment. At our institution, there has been successful engraftment of clinically collected and thawed PBSC following cryopreservation up to 18 years in mice, which signifies likely successful clinical transplantation following long-term cryopreservation (8).

Our institution has recently performed cryopreservation on CD34+ selected grafts with great success, see Appendix C for more details. On the one with a post thaw viability available, there was a post thaw viability was 72%. Our CD34+ collection dose will be robust and should still be well within the accepted CD34+ dose for the patient if a 20-30% decrease in viability is seen.

* 1. **Manufacturing Responsibilities**

The αβ+ T Cell/CD19+ B cell depletion will be manufactured using the Miltenyi Biotec CliniMACS device.

Apheresis PBMC collections are performed at the following FACT accredited facility:

Transfusion Medicine - Apheresis

Indiana University Hospital

550 University Blvd

Indianapolis, IN 46202

**Transfusion Medicine Director:** Elaine Skipworth, MBA, MT(ASCP)HP

Depletion of the product is performed in the IU Health Cell Therapy Laboratory, a FACT accredited facility:

Transfusion Medicine – Cell Therapy Laboratory

Indiana University Hospital

550 University Blvd

Indianapolis, IN 46202

**CTL Manager:** Dave Schwering, MT(AMT), CABP

Product testing for CD19/CD20 and CD3 TCRa/b will be completed in the Cell Immunotherapy and Transduction Facility (CIT) at the Indiana University School of Medicine:

Cell Immunotherapy and Transduction Facility

Cell and Gene Therapy Manufacturing

Indiana University School of Medicine

Indiana University Hospital

550 University Blvd
Indianapolis, IN 46202

**CIT Manager:** Christina Vaughan, MS, CABP

**CIT/CGTM Director:** Emily Hopewell, PhD, CABP(H)

Clinical products are distributed for infusion by the following FACT accredited facility:

Transfusion Medicine – Cell Therapy Laboratory

Indiana University Hospital

550 University Blvd

Indianapolis, IN 46202

Patients are infused at the following facility:

Riley Hospital for Children - Indiana University

705 Riley Hospital Dr

Indianapolis, IN 46202

* 1. **Assessment and release criteria for final product**

The final selected product will be assessed for residual CD3+, TCRa/b+ and CD20+ cell content as described above. In addition, release criteria will include general institutional product standards such as CD34+ cell count, total cell count, viability, and sterility testing of the final product per institution SOPs.

* 1. **Packaging, labeling and storage**

The final product will be packaged and labelled according to institutional SOPs which incorporate FDA, FACT, and other accreditation standards. If the final product is frozen prior to infusion, cell freezing and storage will be carried out per institutional SOPs as well.

* 1. **Institutional experience with CliniMACS Reagent System**

CTL has experience with CD34 selections including with NK cell add-back, and has performed alpha-beta T Cell + CD19 depletion validation(s) for the C-SIDE study and one IND. CIT Lab manufactures CAR T cells using the CliniMACS Prodigy device and performs flow on CAR T products.

1. **Patient and donor eligibility**
	1. **Patient eligibility**

This patient is deemed eligible for αβ+ T Cell/CD19+ B cell depletion based on institutional standards for eligibility for stem cell transplant. She will have a workup that includes organ function, infection assessment and disease status.

* 1. **Donor eligibility**

This patient’s donor is her sister and her back up donor is her mother if her sister becomes ineligible. Patient’s sister will undergo history, physical exam and blood work to determine suitability per institutional standards according to 21 CFR 1271(15) and FACT standards.

* + 1. History: The donor will undergo a consultation with a transplant donor physician. This will include review of medical records that includes risk factors for and clinical evidence of, relevant communicable disease agents and diseases, as well as any conditions that that ay increase risk during mobilization or peripheral blood stem cell collection. A list of current medications will be reviewed. Part of the history taking will involve reviewing and discussing the donor health history questionnaire (DHHQ) that is completed by the donor. This screening tool to determine if the donor is eligible or ineligible to donate and will indicate if there is a risk of communicable disease transmission to the recipient, including questions about travel and/or living in Europe and history of or exposure to CJD.
		2. Physical Exam: A physical exam, including vital signs, will be performed by the transplant donor physician to ensure the donor is suitable to donate stem cells.
		3. Laboratory Testing: All lab tests performed are drawn prior to the collection of the peripheral blood stem cells. The CBC and infectious disease marker (IDM) testing must be performed within 30 days of the collection.
			1. List of Labs: CBC with differential, Complete metabolic profile, sickle cell screen, ABO-rh, chimerism testing, IDM testing performed by Versiti (Appendix A). Infectious disease testing includes: Hepatitis B Virus (HBV); Anti-HBs (Hepatitis B surface antibody) blood automatically sent by IBC if Anti-HBc is reactive, HBsAG (Hepatitis B surface antigen), Anti-HBc (Hepatitis B core antibody), HBV NAT, Hepatitis C Virus (HCV); Anti-HCV (Hepatitis C antibody), HCV NAT, Anti-HTLV I/II (Human T-Lymphotrophic Virus I/II), HIV Testing; Anti-HIV 1+2, HIV-1 NAT, STS (syphilis), WNV-NAT (West Nile Virus), Chagas, CMV IgG, Epstein-Barr Virus (EBV) VCA IgG and IgM, Herpes Simplex Virus (HSV) IgG, Varicella Zoster Virus (VZV) IgG, Toxoplasmosis IgG and IgM. If any concerning signs or symptoms noted on history and physical exam, testing for Chlamydia and Gonorrhea will be completed.
		4. Imaging: EKG, Chest Xray and other diagnostic test or imaging will be obtained if clinically indicated
1. **References**
2. Bunjes, D., et al., *T-cell depleted allogeneic peripheral blood stem cell transplants in 30 patients with haematological malignancies: Benefits and risks.* Blood, 1997. **90**: p. 103a.
3. Watts, M., et al., *Variable product purity and functional capacity after CD34 selection: a direct comparison of the CliniMACS (v2.1) and Isolex 300i (v2.5) clinical scale devices.* Br J Haematol, 2002. **118**: p. 117-123.
4. Maniar, A., et al., *Human gammadelta T lymphocytes induce robust NK cell-mediated antitumor cytotoxicity through CD137 engagement.* Blood, 2010. **116**(10): p. 1726-33.
5. Schumm M, Lang P, Bethge W, et al. Depletion of T-cell receptor alpha/beta and CD19 positive cells from apheresis products with the CliniMACS device. Cytotherapy 2013;**15**:1253-8.
6. Locatelli F, Merli P, Pagliara D, et al. Outcome of children with acute leukemia given HLA-haploidentical HSCT after alphabeta T-cell and B-cell depletion. Blood 2017;130:677-85.
7. Bertaina A, Merli P, Rutella S, et al. HLA-haploidentical stem cell transplantation after removal of alphabeta+ T and B cells in children with nonmalignant disorders. Blood 2014;**124**:822-6.
8. Bethge, W.A., et al., *Haploidentical allogeneic hematopoietic cell transplantation in adults with reduced-intensity conditioning and CD3/CD19 depletion: fast engraftment and low toxicity.* Exp Hematol, 2006. **34**(12): p.1746-52.
9. Underwood J, Rahim M, West C, Britton R, Skipworth E, Graves V, Sexton S, Harris H, Schwering D, Sinn A, Pollok KE, Robertson KA, Goebel WS, Hege KM. How old is too old? *In vivo* engraftment of human peripheral blood stem cells cryopreserved for up to 18 years - implications for clinical transplantation and stability programs. World J Stem Cells. 2020 May 26;12(5):359-367. doi: 10.4252/wjsc.v12.i5.359. PMID: 32547684; PMCID: PMC7280863.
10. <https://www.ecfr.gov/current/title-21/chapter-I/subchapter-L/part-1271/subpart-C>

**Appendix A.**

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| **Versiti Indiana Blood Center CLIA# 15D0664398** |
| **HBsAg** | **Abbott Alinity HBsAG Reagent Kit** |
| **Anti-HBc** | **Abbott Alinity Anti-HBc Reagent Kit** |
| **Anti-HCV** | **Abbott Alinity Anti-HCV II Reagent Kit** |
| **Anti-HTLV I/II** | **Abbott Alinity HTLV I/II Reagent Kit** |
| **HIV-1 NAT** | **Cobas MPX Multiplex HIV, HCV, & HBV nucleic acid test** |
| **HCV NAT** | **Cobas MPX Multiplex HIV, HCV, & HBV nucleic acid test** |
| **Anti-HIV 1,2** | **Abbott Alinity HIV Ag/Ab Combo Reagent Kit** |
| **STS** | **Beckman Coulter PK7400 TP HA Reagent** |
| **WNV NAT** | **Cobas WNV test** |
| **Chagas** | **Abbott Alinity Chagas Reagent Kit** |
| **HBV NAT** | **Cobas MPX Multiplex HIV, HCV, & HBV nucleic acid test** |
| **DPLM at IU Health Pathology CLIA #15D0902829** |
| **Anti-CMV** | **Biomerieux Vidas** |

**Appendix B.**

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| **2022 Stability Testing Summary** |  |
| **Auto or Allo** | **Thaw Date** | **Storage Time (yrs)** | **Viability** | **Viable CD34+ Recovery (%)** | **Mean # Colonies CFU** | **TNC Recovery (%)** | **Sterility** | **Data Entry ID** | **Condition of: (Visual Inspection)** | **Freezer Location During Storage Period** |
| **Container** | **Label** | **Product** |
| Auto | 08.21.2019 | 16 | 75 | 100 | 56 | 93 | ng/nf | JD | OK | OK | OK | 7 |   |
| Auto | 08.21.2019 | 10 | 88 | 98 | 206 | 86 | ng/nf | JD | OK | OK | OK | 12 |   |
| Auto | 08.21.2019 | 10 | 83 | 100 | 18 | 87 | ng/nf | JD | OK | OK | OK | 11 |   |
| Auto | 08.21.2019 | 13 | 83 | 94 | 99 | 94 | ng/nf | JD | OK | OK | OK | 21 |   |
| Allo | 08.21.2019 | 7 | 77 | 100 | 19 | 92 | ng/nf | JD | OK | OK | OK | 16 |   |
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Allo | 01.07.2020 | 9 | 77 | 100 | 29 | 73 | ng/nf | JD | OK | OK | OK | Q |   |
| Auto | 01.08.2020 | 8 | 75 | 100 | 12 | 77 | ng/nf | JD | OK | OK | OK | 16 |   |
| Auto | 01.07.2020 | 11 | 76 | 100 | 107 | 66 | ng/nf | JD | OK | OK | OK | 14 |   |
| Auto | 01.08.2020 | 10 | 64 | 100 | 43 | 52 | ng/nf | JD | OK | OK | OK | 14 |   |
| Auto | 01.07.2020 | 8 | 70 | 77 | 14 | 56 | ng/nf | JD | OK | OK | OK | 14 |   |
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Allo | 4/9/2021 | 10 | 68 | 100 | 8 | 104 | ng/nf | DS | OK | OK | OK | 15 |   |
| Auto | 4/9/2021 | 15 | 86 | 100 | 14 | 98 | ng/nf | DS | OK | OK | OK | 9 |   |
| Auto | 4/9/2021 | 15 | 83 | 100 | 306 | 88 | ng/nf | DS | OK | OK | OK | 4 and 9 |   |
| Auto | 10/26/2021 | 15 | 80 | 100 | 59 | 75 | ng/nf | DS | OK | OK | OK | 9 |   |
| Auto | 10/26/2021 | 18 | 84 | 100 | 62 | 83 | ng/nf | DS | OK | OK | OK | 9 |   |
|   |  |  |  |  |  |  |  |  |  |  |  |   |  |
| Auto | 3/10/2022 | 12 | 92 | 100 | 152 | 81 | ng/nf | DS | OK | OK | OK | 14 |  |
| Auto | 3/10/2022 | 20 | 87 | 100 | 205 | 86 | ng/nf | DS | OK | OK | OK | 17 |  |
| Allo | 3/10/2022 | 9 | 88 | 100 | 18 | 98 | ng/nf | DS | OK | OK | OK | 16 |  |
| Auto | 9/13/2022 | 13 | 71 | 100 | 2 | 101 | ng/nf | DS | OK | OK | OK | 14 |  |
| Auto | 9/13/2022 | 7 | 88 | 100 | 85 | 80 | ng/nf | DS | OK | OK | OK | 18 |  |

**Appendix C.**

|  |  |  |  |
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| Date Performed | CD34 Purity | Cell Viability | Post Thaw Viability |
| 2023-SEP-23 | 92.1% | 99% | N/A |
| 2023-OCT-23 | 93.8% | 99% | N/A |
| 2023-DEC-07 | 97.4% | 99% | 72% |
| 2024-JAN-03 | 99.2% | 99% | N/A |
| 2024-JUL-25 | 99.5% | 99% | N/A |