**Single patient compassionate use of alpha-beta T Cell and CD19 B Cell depletion of peripheral blood stem cell graft**

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

Age: 5 years old

Gender: female

Weight: 22.6kg

Diagnosis: Relapsed Juvenile Myelomonocytic Leukemia (JMML) after stem cell transplant

* 1. **Clinical history and reasoning for alpha-beta T Cell andCD19 B Cells depletion of peripheral blood stem cell graft**

This patient is a 5 year old with a history of very high risk JMML diagnosed in November 2023. Her JMML was very high risk due to her age, presentation and the multiple genetic mutations present in her leukemia cells (PTPN11, NF1, NRAS and KRAS). She received a stem cell transplant from a matched unrelated donor with a Busulfan, Cyclophosphamide, Melphalan, Anti-thymocyte globulin myeloablative preparative regimen in March 2024. She did well after stem cell transplant without any major toxicities other than mild veno-occlusive disease. She went into remission after stem cell transplant, but unfortunately six months after stem cell transplant her bone marrow revealed relapsed JMML.

She is currently receiving bridging chemotherapy with her oncologist to decrease her leukemia burden prior to stem cell transplant. The only curative therapy for relapsed JMML after allogenic stem cell transplant is a second stem cell transplant. Second allogenic stem cell transplant has resulted in outcomes similar to that of the first stem cell transplants in patients with JMML and is the best chance this patient has at a cure of her leukemia (1,2). It has been shown in prior studies the graft versus host disease, and therefore graft versus leukemia is an important way in which JMML cells are eliminated (3). Therefore, I will not use the same matched unrelated donor for this patient as she did not get any graft versus host disease or enough graft versus leukemia to keep her in remission. Given the need to move to a second transplant as soon as possible and the need for graft versus leukemia, I will use this patients mother as a haploidentical donor.

Haplo 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 post transplant cyclophosphamide or alpha-beta T Cell and CD19 B Cell depletion. This patient is at risk for toxicities post stem cell transplant given the proximity to her first stem cell transplant (8 months). We need to eliminate the post transplant cyclophosphamide infusions to decrease the risk of toxicity and therefore transplant related morbidity and mortality. By depleting the alpha-beta T Cells and B Cells ex vivo and without additional immune suppression to the patient, we can remove the cells responsible for graft versus host disease and post-transplant lymphoproliferative disorder but maintain hematopoietic progenitor and stem cells for engraftment, as well as cells to elicit graft versus leukemia and provide anti-infective activity. Alpha-beta T Cell and CD19 B Cell depletion is commonly used for refractory and relapsed leukemia and there are many publications that describe is use and success (4,5).

**I am requesting compassionate use of alpha-beta T Cell and CD19 B Cell depletion to provide this patient with the best leukemia control and engraftment while decreasing the toxicity by not using high doses of cyclophosphamide.**

This is the most effective treatment available for this patient with the least toxicities.

The alpha-beta T cell and CD19 B Cell depletion is currently available on a clinical trial offered at our institution for a different underlying disease (NCT03619551). 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 alpha-beta T cell and 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 JMML.

The donor for this patient is her mother. 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 early to mid-November. The mother’s stem cells will be peripherally collected and will be alpha-beta T Cell and CD19 BCell depleted using the technique described below. After they have been alpha-beta T Cell + CD19 depleted, they will be frozen until the cell infusion day. Due to the tenuous clinical status of the patient’s leukemia we need to proceed with stem cell transplant urgently. This timeline would put the cell collection on a shortened holiday week. Due to staffing and accessibility, alpha-beta TCell and CD19 Depletion cannot occur during this week. To not compromise the treatment of her leukemia, we will tentatively plan to collect the patient’s mother’s stem cells before the holiday week which is approximately 6 days prior to when they will be thawed and infused.

* 1. **Patient Treatment Plan**

Day -12 to Day -10: rabbit anti-thymocyte globulin

Day -5 to Day -2 Clofarabine

Day -5 to Day -2 Fludarabine

Day -4 Thiotepa

Day -3 to Day -2 Melphalan

Day -1 Rest

Day 0 – infusion of alpha – beta T Cell + CD19 Depletion peripheral blood stem cell infusion

1. **Background and Rationale for alpha-beta T Cell + CD19 Delpeted grafts**

The risk of severe graft versus host disease (GVHD) and other complications is higher with unrelated donors, or partially matched related donors (6). GVHD can be mitigated by the use of post stem cell transplant (SCT) immunosuppression. The use of post SCT immunosuppression, typically with calcineurin inhibitors such as cyclosporine A or tacrolimus, delays immunocompetence by interfering with T cell function for up to a year post-HCT and has significant side effects including nephrotoxicity, hypertension, electrolyte derangement and neurotoxicity. Post transplant cyclophosphamide is used in addition to a calcineurin inhibitor in haploidentical stem cell transplants and further adds to the toxicity and immunosuppression.

There is thus a critical need for more effective techniques that prevent GVHD while minimizing toxicity and facilitating rapid post-SCT T cell immunocompetence. One such promising technique is the use of the CliniMACS® system to deplete only CD3+ T cells expressing αβ T cell receptors (TCRαβ+ T cells) along with depletion of CD19+ B cells to minimize risks of post-HCT EBV-LPD (7). While the vast majority of allogeneic T cells are removed by this technique, T cells expressing γδ T cell receptors are retained, and infusion of TCR γδ+ T cells appears to facilitate engraftment and provide post-HCT adoptive immunity (8,9). In addition, retention of neutrophils, monocytes, macrophages, NK-cells, and other WBC lineages within the graft provide other immune benefits to the recipient.

Pilot studies in haploidentical peripheral blood SCT, including children, have been encouraging. An initial study of 5 children with advanced leukemias and haploidentical mobilized peripheral blood stem cells noted a 4.5 log depletion of alpha-beta T cells, with 72% recovery of CD34+ cells and 80% recovery of γδ T cells (10). No GVHD prophylaxis was given with chemotherapy conditioning only. All patients had rapid hematologic engraftment and rapid immune reconstitution. One patient had grade III skin GVHD, but there was no other acute GVHD noted. Of note, ATG was used during conditioning to achieve additional host immunosuppression. More recent publications have expanded upon this pilot study and trials have been performed internationally (4,11-13) that consistently show good engraftment, rapid immune reconstitution, and low rates of severe GVHD.

1. **Alpha-beta T Cell + CD19 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

 mother 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 x 10^6 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 on day 6 if the target cell number is not reached after the first collection.

Apheresis will typically begin on the 5th day of mobilization. Apheresis may need to be performed over a two-day period, though most donors are likely to be able to provide the stem cell doses required for transplant after one day of apheresis. If required, additional days of apheresis will be done at the transplant or apheresis physician’s discretion.

All donors will be monitored and PBSC products will be collected according to FACT-JACIE international standards for cell therapy, product collections, processing and administration.

All cell processing 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 T- & B-cell depletion. HPC-A products will be T- & 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 Hematopoetic 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 x 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 in order to optimize the CD34+ numbers for infusion.

• Rituximab will be given on Day -1 200mg/m^2 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 TCRαβ 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 T & B-cell depleted HPC-A cell infusions, plus additional CD34+ selected cells if necessary to increase the CD34+ dose.

The final TCRαβ and CD19 depleted cryopreserved product will be infused intravenously according to institutional standards.

This patient will not receive GVHD prophylaxis because <1.0 x 105 αβ+CD3+ cells/kg recipient weight will generally be administered

The rest of the supportive care before, during and after stem cell transplant will follow institutional standards.

* 1. **Cryopreservation Process**

Cryopreservation will be performed per our institutional standards of care. Storage duration will be approximately 6 days.

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 (14).

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.

**3.3 Manufacturing Responsibilities**

The alpha-beta T Cell + CD19 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

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

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

**3.4 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.

**3.5 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.

**3.6 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. 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 alpha beta T Cell + CD19 depletion based on institutional standards for eligibility for stem cell transplant. She will have a work up that includes organ function, infection assessment and disease status.

* 1. **Donor eligibility**

This patient’s donor is her mother and her back up donor is her father in the event that her mother becomes ineligible. Patient’s mother 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. (See DHHQ as a separate appendix)
		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
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**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.**

|  |  |  |  |
| --- | --- | --- | --- |
| 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 |