SOP: D03.05.22 CHARACTERIZATION AND FREEZING OF CYTOTOXIC T-LYMPHOCYTES

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1. Purpose:

1.1. To characterize and cryopreserve ex-vivo expanded Cytotoxic T-Lymphocytes (CTL).

1.2. Prior to freeze, discuss with the principal investigator the number of cells required for one or two patient doses based on patient body surface area and current dose escalation. Process sufficient cells to allow for loss during washing (+20%) and for samples required for characterization and safety testing.

1.3. Samples of CTL and supernatant will be analyzed to test for microbiological safety. In the case of transduced cells, samples will be analyzed or archived for testing of retroviral safety and documenting the efficacy of transduction and the potential for autonomous growth.

2. Scope

2.1. This procedure is to be performed by trained GMP manufacturing staff.

3. Definitions and Abbreviations:

3.1.	CTL(s)	Cytotoxic T-lymphocytes
3.2.	EBV	Epstein Barr Virus
3.3.	DMSO	Dimethyl Sulfoxide
3.4.	HSA	Human Serum Albumin
3.5.	CPF	Cell Processing Facility
3.6.	PCR	Polymerase Chain Reaction
3.7.	TMH	The Methodist Hospital Laboratories
3.8.	TCR	T cell receptor
3.9.	CFR	Code of Federal Regulations
3.10.	HBSS	Hanks' Balanced Salt Solution

4. Materials and Equipment:

NOTE: All materials in contact with cells must be sterile, pyrogen-free and used according to the manufacturer's directions unless stated otherwise. Equivalent materials and equipment may be used but all changes must be recorded in the appropriate worksheets.

4.1. Specimen

4.1.1. Cytotoxic T-lymphocytes (CTLs) derived from bone marrow donor or patient enrolled on immunotherapy protocols.

4.2. Reagents and Supplies

4.2.1. Hank's balanced saline solution – Phenol red-free

4.2.2. Human serum albumin

4.2.3. DMSO

4.2.4. Cryovials

4.2.5. Centrifuge tubes

4.2.6. Serological Pipettes

4.2.7. Fungal and Aerobic and Anaerobic Bacterial Bactec Bottles

4.2.8. Cryomed Controlled-Rate Freezer

5. Procedure:

Note: Set aside samples for QC and proceed with cryopreservation as rapidly as possible to conserve cell viability.

5.1. Prepare wash solution of HBSS containing 10% HSA.

5.2. Harvest CTLs from wells or GRex flasks and pool into appropriately sized sterile container.

5.3. Count CTLs according to SOP and record the percent viability.

5.4. Calculate cell number for QC and set aside.

5.4.1. 5×10^5 cells for mycoplasma (PCR)

5.4.2. 5×10^6 cells for HLA typing

5.4.3. 2×10^6 cells for phenotyping (at this time or within five days of freezing)

5.4.4. 5×10^6 cells for cytotoxicity (at this time or within five days SOP D03.04)

5.4.5. **If cells are transduced**:-

5.4.5.1. 3-5 $\times 10^6$ cells for transduction efficiency PCR

5.4.5.2. 2×10^6 cells for transgene analysis by FLOW

5.4.5.3. 2 x 10^6 cells to test for autonomous growth

5.4.6. Calculate volume of freeze medium (10% DMSO, 50% HSA, 40% HBSS) required.

5.4.6.1. Prepare by adding DMSO to HBSS, thoroughly resuspend, then add HSA

5.4.6.2. Place on ice and prepare the Cryomed.

5.5. Centrifuge remaining cells at 400 x G for 5 minutes.

5.5.1. Pool and set aside the cell culture supernatant in appropriately sized sterile container.

5.6. Wash cells by centrifugation at 400 x G four times in HBSS containing 10% HSA

5.6.1. Save and set aside the final wash.

5.7. Place cell pellet on ice for 10 minutes.

5.8. Resuspend cells in ice cold freezing media in suitable aliquots (as discussed with P.I. prior to procedure) for infusion (up to 10^7 cells/ml).

5.9. 3% of the cells or 15x10^6 (whichever is greater) should be aliquoted for RCR in 3 vials labeled **for RCR**.

5.9.1. Cryovials must be labeled with recipient's name, recipient's CAGT number and hospital number, donor CAGT number (if appropriate), type of component, component number, number of cells and date of freezing and expiration date (5 years from date of freeze).

5.10. Aliquot cells from centrifuge tube into labeled cryovials.

5.11. Transfer cells to controlled rate freezer (Cryomed) and cryopreserve according to SOP C03.34.

5.12. Transfer cells to liquid nitrogen tank.

5.12.1. Place copy of freezer inventory form in patient folder.

5.12.2. Log storage locations in worksheet.

5.13. Disposition of QC samples

5.13.1. Label all storage containers with recipient's name, CAGT number and hospital number, donor CAGT number (if appropriate), type of component, component number, and date,

5.13.2. Complete sample submission form for each different sample.

5.13.3. **Cells**

5.13.3.1. 5 x 10^5 cell suspension for mycoplasma (PCR) in cryovial.

5.13.3.2. 5 x 10^6 cell suspension for HLA typing.

5.13.3.3. 2 x 10^6 cell suspension for phenotyping (at this time or within five days of freezing).

5.13.3.4. 5 x 10^6 cells for cytotoxicity (at this time or within five days SOP D03.04).

5.13.3.4.1. Can be given fresh to PI or cryopreserved in Nalgene container.

5.13.3.5. **If cells are transduced**:

5.13.3.5.1. 3-5 x 10^6 cell pellet for PCR for transduction efficiency testing to GLP Laboratory.

5.13.3.5.2. 2 x 10^6 cell suspension transgene phenotype (to FLOW or PI)

5.13.3.5.3. Culture 2 x 10^6 cells without growth factors for two weeks to test for autonomous growth (compare with non-transduced).

5.13.3.5.4. If the cells are transduced, at least 3 cell aliquots for retrovirus safety testing should be cryopreserved.

5.13.3.5.4.1. Each aliquot should contain 1% of total cells calculated in section 5.3.

5.13.3.5.4.2. No less than 5 x 10^6 cells should be frozen per vial.

5.13.4. **Cell culture supernatant**

5.13.4.1. Freeze 1 ml for Endotoxin testing.

5.13.4.2. Freeze 2 x 1 ml samples for Archive purposes.

5.13.4.3. Inoculate blue-top Bactec bottle with 2 ml for BACTERIAL Screen.

5.13.4.4. Inoculate red-top Bactec bottle with 2 mls for FUNGAL Screen.

5.13.4.5. Freeze 2ml aliquot in 2 ml cryovial for Bacterial CFR (TMH).

5.13.4.6. Freeze 2 ml aliquot in 2 ml cryovial for Fungal CFR (TMH).

5.13.4.7. **If transduced**, archive 5% of the supernatant and snap freeze on a dry ice/ethanol bath. Freeze remaining supernatant in 20 ml aliquots at -80^{o}C. Submit to QC for archiving.

5.13.4.7.1. Supernatant from non-transduced CTLs should be stored at -80ºC for future testing.

5.13.4. **Final Wash**

5.13.4.1. Aliquot 2 mLs into Bactec bottle for sterility

5.13.4.2. Save remaining supernatant at -80^{o}C until final product is released

5.13.5. **Final product**

5.13.5.1. Inoculate aerobic bacterial Bactec bottle with 100ul of final cell suspension that has been brought up in 1 ml of sterile HBSS.

5.14. Release criteria for administering aliquots to patients include:

5.14.1. Viability > 70%.

5.14.2. Negative culture for bacteria and fungi after 7 days.

5.14.3. Endotoxin testing ≤ 5 EU/ml.

5.14.4. Negative result for mycoplasma.

5.14.5. HLA Class I identical to CTL donor.

5.14.6. Less than 10% killing of autologous lymphoblasts at 20:1 ratio for allogeneic products.

5.14.7. Phenotype is protocol specific

6. Notes: N/A

7. References: N/A

8. Attachments:

8.14.1. DW03.05.XA Non-transduced CTL Freezing – Patient Doses

8.14.2. DW03.05.XB Transduced CTL Freezing – Patient Doses

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8. Review and Revisions

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Date issued: 06/08/10 Replaces: SOP # D03.05.21

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Biennial Review:

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