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Sipuleucel-T

DEFINITION

Sipuleucel-T is an autologous cellular immunotherapy. The active components of sipuleucel-T are autologous antigen presenting cells (APCs) and a recombinant protein, PAP-GM-CSF that consists of human prostatic acid phosphatase (PAP) fused through the C-terminus to the N-terminus of human granulocyte-macrophage colony-stimulating factor (GM-CSF) by a Gly-Ser linker. PAP is an antigen expressed in prostate cancer tissue and GM-CSF is an immune cell activator. Minimal residual levels of the intact PAP-GM-CSF are detectable in the final product.

Sipuleucel-T is prepared using a patient's own peripheral blood mononuclear cells obtained via a 1.5–2.0 blood volume leukapheresis approximately 3 days prior to the infusion date. The cells obtained are further processed and then cultured for 36–44 h in a nutrient-rich medium in the presence of PAP-GM-CSF. The cells are then aseptically harvested, washed, formulated, and packaged for intravenous infusion. Sipuleucel-T is a sterile, live cell suspension for autologous use only. Each dose contains a minimum of 50 million autologous CD54+ cells activated with PAP-GM-CSF, suspended in 250 mL of Lactated Ringer's Injection, *USP*.

IDENTIFICATION

• **A.** Meets the requirements in the *Assay*

ASSAY

• **Procedure**

[Note—To determine potency by flow cytometry, both an in-process sample taken prior to culture with PAP-GM-CSF and a sipuleucel-T final product sample are tested.]

Phosphate buffered saline (PBS): Use Dulbecco's phosphate-buffered saline without calcium or magnesium, pH 7.0–7.2.

Analysis: Stain one 30-µL aliquot of cells (containing 20×10^6) with 40 µL of monoclonal anti-CD54 antibody conjugated 1:1 with phycoerythrin (PE) and a second aliquot with 40 µL of isotype control antibody conjugated 1:1 with PE.[1] Incubate for 15–25 min in the dark, then wash away unbound antibody with *PBS* by centrifugation. Resuspend cells in 400 µL of *PBS* containing 1 µg/mL of laser dye styryl–751 (LDS-751),[2] stain for 5 min, and then add an equal volume of *PBS* to each sample. Reconstitute PE-conjugated flow cytometry bead standards[3] in 300 µL of *PBS* containing 0.5 µg/mL of LDS-751.

Data acquisition: Acquire the flow cytometry bead standards on a multi-color flow cytometer using an acquisition plot with forward scatter (FSC) vs. side scatter (SSC) axes and a gate to encompass approximately 10,000 bead singlet events. Use a second acquisition plot gated on the bead singlet events to graph only the events in the singlet gate by PE fluorescence vs. event count. Use histogram region markers to calculate the geometric mean fluorescence intensity (MFI) of each of the labeled bead populations. The number of PE molecules reported by the manufacturer is used to generate a PE vs. MFI standard curve from which the number of PE molecules on the surface of the test sample population can be calculated.

Acquire approximately 10,000 events for the CD54-PE and isotype control-stained test samples using an acquisition plot with LDS-751 fluorescence vs. FSC and an inclusion gate set on LDS+ leukocytes. This gate allows the user to remove nonspecific signal from debris and/or erythrocytes from the evaluation. Precise gating and therefore removal (from data analysis) of these two smaller product subcomponents is not critical due to the use of a second acquisition plot that focuses solely on the analysis of CD54+ large cell events.

Calculating number of CD54+ cells: LDS fluorescence vs. FSC is evaluated for both the anti-CD54-PE (1:1)-stained and the isotype control-stained samples. The second analysis plot, which includes only the LDS+ leukocyte populations in both the test and control samples, evaluates PE fluorescence vs. FSC, and is used to derive statistics for each sample. A pair of stacked regions allow the user to set a threshold (lower region) based on the isotype control-stained cells, while placing the upper region to encompass the large CD54+ leukocytes. The latter region provides the results for both MFI and the percentage of large CD54+ cells present in each test sample. The percentage of large CD54+ cells is multiplied by both the total leukocyte concentration (determined using an automated hematology analyzer) and the percent of viable cells to obtain the number of CD54+ cells in the product.

Calculating CD54 upregulation: To determine CD54 upregulation, stain and acquire preculture samples and final product samples as described above. For each sample, calculate the MFI for the large CD54+ cells for both the isotype control and the anti-CD54-PE (1:1)-stained sample. Using the corresponding bead standard PE vs. MFI standard curves, convert the test MFI results to the number of PE molecules bound per cell. To correct for nonspecific staining, subtract the isotype control result from the corresponding result for the anti-CD54-PE (1:1)-stained sample to obtain the number of PE molecules specifically bound. Divide the final product number of PE molecules specifically bound by the preculture number to obtain the CD54 upregulation ratio.

Acceptance criteria: The preculture material must contain NLT 165 × 10^6 CD54+ cells to meet in-process acceptance criteria. Sipuleucel-T final product must contain NLT 50 × 10^6 CD54+ cells and must demonstrate NLT a 2.6-fold CD54 upregulation.

SPECIFIC TESTS

• **ELISA Assay**

[Note—To ensure that the cells have been treated with the appropriate antigen, an ELISA test is used to measure residual intact PAP-GM-CSF in the final product.]

Phosphate buffered saline (PBS): Prepare as directed in the *Assay*.

Capture antibody: Prepare a solution containing 5 µg of monoclonal anti-PAP antibody/mL[4] of *PBS*.

Assay diluent: Prepare a solution of 10% blocking solution[5] in Lactated Ringer's Injection, *USP*.

Secondary antibody: Prepare a solution containing anti-GM-CSF antibody[6] conjugated with horseradish peroxidase (HRP) diluted in blocking solution.

2X positive control: Prepare a solution containing 500 pg of PAP-GM-CSF/mL of *Assay diluent*.

Wash buffer: Dilute concentrated wash buffer[7] to the recommended working strength.

Test sample: A 2-mL test sample is removed from the final product formulation. A 1-mL aliquot of this sample is centrifuged to pellet the cells, and then 900 µL of supernatant is combined with 100 µL of blocking solution in preparation for loading on the plate.

Analysis: Prepare assay plates by coating microtiter plate wells with *Capture antibody* for 120 min and then blocking with undiluted blocking solution. Load separate wells with either 50 µL of *Assay diluent* (in triplicate), 50 µL of *Test sample*, or 50 µL of a 1:1 mixture of *Test sample* with *2X positive control*. The triplicate *Assay diluent* wells act as a negative control for the establishment of a background signal. Add 50 µL of *Secondary antibody* to each well, and incubate for 90 min at room temperature. Wash the plate with *Wash buffer,* and add 100 µL of TMB Chromogen.[8] After 30 min, stop the reaction with 100 µL of stop solution[9] and, using a plate reader, determine the absorbance of the well contents with an optical density of 450 nm, with a reference filter set to 570 nm.

Calculations and system suitability: The mean and standard deviation of the negative control sample replicates (*Assay diluent*) are calculated. The mean of the *Test sample* results is also calculated. The threshold value is calculated as the mean absorbance of the negative control plus three times the standard deviation. The mean absorbance of the negative control must be NMT 0.061.

Acceptance criteria: If the *Test sample* mean absorbance is greater than the threshold value, then the *Test sample* is identified as sipuleucel-T that has been exposed to PAP-GM-CSF.

• **Total Nucleated Cell Count Assay**

Test sample: 2 mL of Sipuleucel-T. If required, dilute the sample with *PBS* to obtain a result within the linear range of the method being used.

Analysis: Use a commercially available hematology analyzer or a manual counting procedure to determine the white blood cell concentration on triplicate analyses of neat sample derived from the appropriate process step. Proceed as directed in the instrument operating manual or manual procedure.

Calculation: Multiply the white blood cell concentration by the net volume and the viability of the sample to determine the total nucleated cell count.

Acceptance criteria: NLT 5 × 10^7 cells in the final product. The total nucleated cell result for the final product formulation is used to determine the number of CD54+ cells.

• **Trypan Blue Exclusion (Viability) Assay**

Test sample: 2 mL of Sipuleucel-T

Analysis: Based on the cell concentration (which can be determined manually, or using an automated hematology analyzer), dilute duplicate aliquots of the *Test sample* with *PBS* to yield total counts of 100–400 cells per hemacytometer chamber. Mix equal volumes of each *Test sample* with the same volume of 0.4% isotonic trypan blue. Load the samples in a hemacytometer, and count the cells in the four large corner squares in each chamber.

Calculation: Calculate the percentage of viable cells (unstained cells) relative to the total number of cells (stained and unstained cells).

Acceptance criteria: NLT 80% viability in both the incoming leukapheresis component and in the sipuleucel-T final product.

• **Sterility Tests ⟨71⟩:** Meets the requirements

• **Bacterial Endotoxins Test ⟨85⟩:** NMT 0.5 USP Endotoxin Unit/mL of final product. A 2-mL sample is removed from the final product formulation and 0.45-mL of this sample is mixed with 0.45 mL of LAL Reagent Water, then 0.10-mL duplicate samples are analyzed.

ADDITIONAL REQUIREMENTS

• **Labeling:** The final product is labeled to indicate the identity of the patient, the expiration time and date, the required storage conditions (refrigerated at 2–8°), and the lot number. The label indicates that the product is for autologous use only, and that the product is not routinely tested for transmissible infectious diseases.

• **Packaging and Storage:** Sipuleucel-T is supplied in a sealed infusion bag labeled for the specific recipient. The product is shipped in a cardboard shipping box with a special insulated polyurethane container inside. The insulated container and gel packs within the container maintain the appropriate transportation and storage temperature of sipuleucel-T until infusion. The infusion bag must remain within the insulated polyurethane container and shipping box until the time of administration.

OFFICIAL

[1] Suitable anti-CD54-PE (1:1) and isotype control-PE (1:1) antibodies can be obtained from Becton Dickinson, catalog# 332811.

[2] Suitable laser dye styryl–751 (LDS-751) can be obtained from Molecular Probes, catalog# L-7595.

[3] Suitable flow cytometry standards, QuantiBRITE-PE™ Beads, can be obtained from Becton Dickinson, catalog# 340495.

[4] A suitable antibody can be produced with the hybridoma cell line, ATCC catalog No. HB-8526, or Aragen Bioscience, special order.

[5] A suitable blocking solution can be obtained from Thermo Scientific, catalog# 337516.

[6] A suitable antibody can be obtained from Anogen, catalog# MO-C40090T.

[7] A suitable wash buffer concentrate can be obtained from Invitrogen, catalog# WB01.

[8] A suitable TMB Chromogen can be obtained from Invitrogen, catalog# SB01.

[9] A suitable stop solution can be obtained from Invitrogen, catalog# SS01.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
SIPULEUCEL-T	Rebecca C. Potts Associate Scientific Liaison	BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines

Chromatographic Database Information: Chromatographic Database

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