Video Article

Automated Cell Enrichment of Cytomegalovirus-specific T cells for Clinical Applications using the Cytokine-capture System

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Abstract

The adoptive transfer of pathogen-specific T cells can be used to prevent and treat opportunistic infections such as cytomegalovirus (CMV) infection occurring after allogeneic hematopoietic stem-cell transplantation. Viral-specific T cells from allogeneic donors, including third party donors, can be propagated *ex vivo* in compliance with current good manufacturing practice (cGMP), employing repeated rounds of antigen-driven stimulation to selectively propagate desired T cells. The identification and isolation of antigen-specific T cells can also be undertaken based upon the cytokine capture system of T cells that have been activated to secrete gamma-interferon (IFN-γ). However, widespread human application of the cytokine capture system (CCS) to help restore immunity has been limited as the production process is time-consuming and requires a skilled operator. The development of a second-generation cell enrichment device such as CliniMACS Prodigy now enables investigators to generate viral-specific T cells using an automated, less labor-intensive system. This device separates magnetically labeled cells from unlabeled cells using magnetic activated cell sorting technology to generate clinical-grade products, is engineered as a closed system and can be accessed and operated on the benchtop. We demonstrate the operation of this new automated cell enrichment device to manufacture CMV pp65-specific T cells obtained from a steady-state apheresis product obtained from a CMV seropositive donor. These isolated T cells can then be directly infused into a patient under institutional and federal regulatory supervision. All the bio-processing steps including removal of red blood cells, stimulation of T cells, separation of antigen-specific T cells, purification, and washing are fully automated. Devices such as this raise the possibility that T cells for human application can be manufactured outside of dedicated good manufacturing practice (GMP) facilities and instead be produced in blood banking facilities where staff can supervise automated protocols to produce multiple products.

Video Link

The video component of this article can be found at https://www.jove.com/video/52808/

Introduction

Hematopoietic stem-cell transplantation (HSCT) [1] can be combined with adoptive T-cell therapy to improve graft-versus-tumor effect and to provide immunity to opportunistic infections[2]. Generation of antigen-specific donor-derived T cells for infusion has historically required skilled personnel and use of specialized facilities that are GMP-compliant. The delivery of such T cells has resulted in resolution of opportunistic infections[3] as well as treating the underlying malignancy[4]. Recently, investigators have demonstrated that the adoptive transfer of only few thousand virus-specific T cells (~ 1×10^4 – 2.5×10^5 cells/kg recipient body weight) can successfully treat opportunistic CMV infections after allogeneic HSCT[5-9]. A limited number of GMP facilities with associated skilled manufacturing requirements and the high cost associated with cell production has, however, restricted patient access to promising T-cell therapies[10]. One approach to isolating antigen-specific T cells is based on the CCS using a bi-specific reagent to recognize CD45 and IFN-γ. As is shown, this methodology can be used to generate clinical-grade CMV-specific T cells employing an automated cell enrichment CCS device (**Figure 1B**).

CMV-specific T cells are generated by incubating overlapping peptides from CMV pp65 antigen with leukapheresis total nuclear cells (TNC) from CMV-seropositive donors. These peptides, displayed in the context of human leukocyte antigen (HLA), activate the CMV pp65-specific T cells within the TNC to secrete IFN-γ. These T cells can then be “captured” and magnetically separated. The operation of the first-generation cell enrichment device (**Figure 1A**) required personnel skilled in cell culture under GMP conditions, and coordination of staff to undertake the multiple steps necessary to generate a “captured” product.

The procedure typically required 10 to 12 hr of continuous operation, and therefore personnel likely need to work over two shifts in the GMP facility. These constraints are now obviated by the implementation of a second-generation device (shown in **Figure 1B**). This device undertakes magnetic enrichment, similar to the first generation device, but automates other aspects of the CCS in an unbreached approach.

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This significantly reduces the burden on the GMP team as most of the steps can be accomplished unattended by staff. Furthermore, since the device operates as a closed system, the antigen-specific T cells can be captured and processed on the benchtop except the steps involved in leukapheresis isolation and preparation of materials before starting the instrument. Details of the complete instrumentation and functionality of this second-generation cell enrichment device have been published[11].

Here, we describe the steps to enrich CMV pp65-specific T cells from a steady-state apheresis product using the automated cell enrichment CCS system. Once isolated, these CMV-specific T cells may be immediately infused into a patient.

Protocol

1. Preparation of Materials under Sterile Conditions (See Materials and Equipment Table)

1. Prepare 3 L of PBS/EDTA buffer supplemented with human serum albumin (HSA) to a final concentration of 0.5% (w/v).
2. Prepare 1 L bag of clinical grade 0.9 % sodium chloride (NaCl) solution and 2 L of GMP grade cell culture medium.
3. Prepare 60 nmol of CMV-specific peptide antigen cocktail by reconstituting one vial of CMV pp65 with 8 ml of sterile water.
4. Transfer CMV pp65 peptide cocktail into a 50 ml volume freezing bag using a Luer/Spike interconnector and clamp with locking forceps to avoid subsequent distribution of cocktail into the tubing set. Open cell enrichment tubing set (TS 500) under sterile conditions.
5. Using sterile tubing welder, connect peptide cocktail freezing bag into tube connection for valve 2 of tubing set TS 500. Do not open the peptide cocktail bag clamp at this time.
6. Remove 1 x 10^9 TNC from starting cellular product and suspend in PBS/EDTA buffer containing 2.5% HSA to a total volume of 50 ml. Inject the cellular product into a 150 ml transfer bag.

2. Preparation and Use of Automated Cell Enrichment System (See Materials and Equipment Table)

1. Switch on the cell enrichment system (**Figure 1B**) and select the program " *CCS_IFN-γ Enrichment*". Observe a user interface showing screens with instructions and pictures guiding the operator through the procedure.
2. Enter the parameter "*Operator*" and "*Tubing Set P/N No*". Next, install the Tubing Set 500 to automated cell enrichment device as per instructions displayed on the interactive monitor screen.
3. Follow the step by step instructions displayed on the screen to connect the medium and buffers to the device. Record catalogue number and lot number of the reagents before connecting to the instrument.
4. After the final check of the tubing set, open the clamp of the peptide cocktail bag. Open the medium bag and initiate automatic priming of the tubing set.
5. After the priming step is completed, supplement HSA (2.5%) into NaCl buffer in the reservoir bag (200 ml) with the help of the sterile tubing welder. Transfer the starting cellular product into the "Application bag" using the sterile tubing welder.
6. Connect CCS (IFNγ) reagents into respective tubing via adapters. Enter preferred time to collect a fraction of cellular material before enrichment process. Review and verify the accuracy of all data/parameters entered. Start the process.
7. Before the start of automated cell enrichment process, remove the Quality Control Bag (QCB, original fraction (ori) contains approximately 1.3 ml out of 100 ml chamber content diluted with PBS/EDTA buffer). Seal the QCB, weigh, and store at 4 °C.
8. Start the enrichment process. At the end of the process, target cells will be eluted with an approximate volume of elution buffer from the reservoir bag.
9. Seal the Non Target Cell Bag (NTCB, negative fraction = neg) and Target Cell Bag (TCB, positive fraction = pos) and weigh each bag. The weights will be used later for calculation of the cell numbers.
10. Immediately after the enrichment procedure collect two aliquots per fraction for flow cytometry analysis, and store the rest of the samples at 4 °C. Use one sample aliquot for cell count determination and the other sample aliquot for the enrichment performance analysis (**Table 1**).
11. Remove the tubing set from the cell enrichment instrument. Transfer the log file to a USB drive for future use.
    NOTE: All reagents should be prepared under sterile conditions. The use of a Biosafety type II hood is highly recommended. Use steady-state apheresis cellular product (non-mobilized) isolated from a healthy CMV-seropositive donor to enrich CMV antigen specific T cells. Only FDA licensed HSA should be used. The buffer for cell preparation should be kept at +19 °C to +25 °C as lower or higher ambient temperatures will result in reduced purity and a reduced yield of the target cells.

3. Cell Count Determination

1. Take the aliquots of QCB , NTCB and TCB for cell counts as shown in **Table 1**. Add CD45-VoBlue to each aliquot (titer 1:11) and incubate in the dark for 10 min at 4 °C.
2. Add 1.5 ml freshly prepared red blood cell lysis solution (1x) to the original fraction and negative fraction, 450 µl freshly prepared red blood cell lysis solution to the positive fraction, and incubate all fractions for 15 min at RT.
3. Just prior to analysis, add propidium iodide to a final concentration of 1 µg/ml (1:100 dilution of 100 µg/ml). Use automatic cell counter to determine cell count and viability. Use cell counter device recommended software for flow cytometry analysis. Determine the absolute counts of leukocytes for original, negative and positive fractions.
   NOTE: The cell count of viable leukocytes per ml of the samples taken for cell count analysis is determined using the cell analyzer recommended software.
4. Set the region as shown in **Figure 2** (region 5, viable leukocytes). Use the following gating strategy to determine cell count. A viable leukocyte in original fraction is shown in **Figure 2**.
5. The indicated regions (**Figure 2, 1-6**) are hierarchically as follows:
   1: Time gate → 2: Single cells → 3: $CD45^+$ cells → 4: Leukocytes (debris excluded) → 5: Viable leukocytes → 6: Viable lymphocytes

6. Repeat the same steps to determine cell counts for negative and positive fractions. Calculate the cell count of the whole fraction by considering the diluent factor of the sample and total volume of the fraction (**Table 2**).

4. Examination of the Separation Performance

1. Wash the aliquots of QCB, NTCB and TCB fraction cells with pre-chilled PBS/EDTA buffer/0.5% AB serum. Centrifuge the cells at 300 x g for 5 min at 4 °C and aspirate the supernatant.
2. Resuspend cells in 100 µl antibody-fluorochrome staining mixture containing: CD3-FITC, CD4-APC, CD8-APC-Vio770, CD14-PerCP, CD20-PerCP, CD45-VioBlue and anti-IFNγ-PE (titer 1:11) and incubate in the dark for 10 min at 4 °C.
3. Add 1 ml freshly prepared red blood cell lysis solution (1x) and incubate for 15 min at RT. Centrifuge at 300 x g for 5 min at 4 °C and aspirate the supernatant. Resuspend the cells in an adequate volume of PBS/EDTA Buffer/ 0.5% AB Serum.
4. Add propidium iodide to a final concentration of 1 µg/ml just prior to analysis (1:100 dilution of 100 µg/ml). Perform flow cytometry analysis to evaluate the purity of the sample.
5. Use the following gating strategy to calculate the $CD3^+$ T cells in viable leukocyte Gating strategy for determining the $CD3^+$ T cells is shown in positive fraction after CCS enrichment process. The indicated regions (**Figure 3A and 3B, 1-6**) are hierarchically linked as follows:
   1: Time gate → 2: Single cells → 3: $CD45^+$ cells → 4: Cells (debris excluded) → 5: Viable leukocytes → 6: Viable $CD3^+$ cells population
6. Determine the frequencies of CD4+, CD8+, CD4+ IFN-γ^+ and CD8+ IFN-γ^+ T cells after CCS enrichment process (**Table 2**).
7. Use the gating strategy to determine the frequencies of $CD4^+$, $CD8^+$, $CD4^+$ IFN-γ^+ and $CD8^+$ IFN-γ^+ T cells shown below for an original and enriched (captured) positive fraction after CCS process. The indicated regions are hierarchically linked and named as follows:
   1: Time gate → 2: Single cells → 3: $CD45^+$ cells → 4: Cells (debris excluded) → 5: Viable Leukocytes → 6: Viable $CD3^+$ cells → 7: $CD4^+$ cells → 7a: $CD4^+$ IFN-γ^+ cells (box) → 8: $CD8^+$ cells → 8a: $CD8^+$ IFN-γ^+ cells (box)

NOTE: The first 6 indicated regions of the hierarchy links are the same as **Figure 3, (1-6)** and last 2 regions are shown in **Figure 4 (6-8a)**.

Representative Results

In this study, an automated cell enrichment CCS System was used for automated production of CMV pp65-specific T cells. CMV-specific T cells were enriched from three apheresis cell products. The steady-state apheresis product was harvested over 2 hr from a CMV-seropositive donor and generated 10^{10} total nuclear cells (TNC). 10^9 TNC were then activated with CMV pp65-derived peptides (60 nmol) for 4 hr and the IFN-γ secreting T cells were isolated using the CCS on the automated cell enrichment device. An operator was needed at the beginning of the experiment to load all the reagents and tubing sets. Setup of the system from the initial unpacking to starting the device took approximately 60 to 120 min. Note, the machine can then be programmed to start after the reagents and tubing sets are loaded thereby enabling the machine to operate unattended (such as overnight). The operator was needed again after 15 hr to perform characterization of the final products for cell purity and viability. After enrichment the cell supernatant was screened for mycoplasma and endotoxin presence. After validation, the processed cells can be infused directly into patients or cryopreserved for later applications.

Cell counts were determined following cell counting standard practices using the formulas given in **Table 2**. Each type of cell count was repeated three times and the results were expressed as mean total cell counts with standard deviation (SD). Reports were then analyzed using cell analyzer recommended software. Gating to determine viable leukocytes and lymphocytes is shown in **Figure 2**. The data for the cell counts are presented in **Table 3**. The gating strategy used to determine IFN-γ^+ T cells is shown in **Figure 3** and **Figure 4**. Before enrichment, the viability of cells was routinely > 95%. After enrichment, the viability of cells was < 50%. The absolute count of IFN-γ^+ T cells was assessed before and after the enrichment process. The total number of IFN-γ^+ T cells before enrichment was $1.14 \times 10^6 \pm 0.35 \times 10^6$ as derived from 10^9 starting TNC, and after enrichment was $3.09 \times 10^5 \pm 1.70 \times 10^5$ IFN-γ^+ T cells. There were 0.16 ± 0.18% IFN-γ^+ $CD4^+$ T cells present prior to processing and this increased to 47.5 ± 34.7% after enrichment. The purity percentage of $CD8^+$ IFN-γ^+ T cells prior to capture was 0.47 ± 0.1%, and increased to 90.3 ± 1.7% after enrichment (**Table 3 and 4**). Sample recovery in the captured (positive) fraction was 32.9 ± 15.7% for $CD4^+$ T cells and 31.8 ± 13.2% for $CD8^+$ T cells based on measurement of IFN-γ^+ T cells in the starting population (**Table 4**). These data indicate that both $CD4^+$ and $CD8^+$ CMV pp65-specific T cells can be harvested automatically in a manner suitable for their human application.

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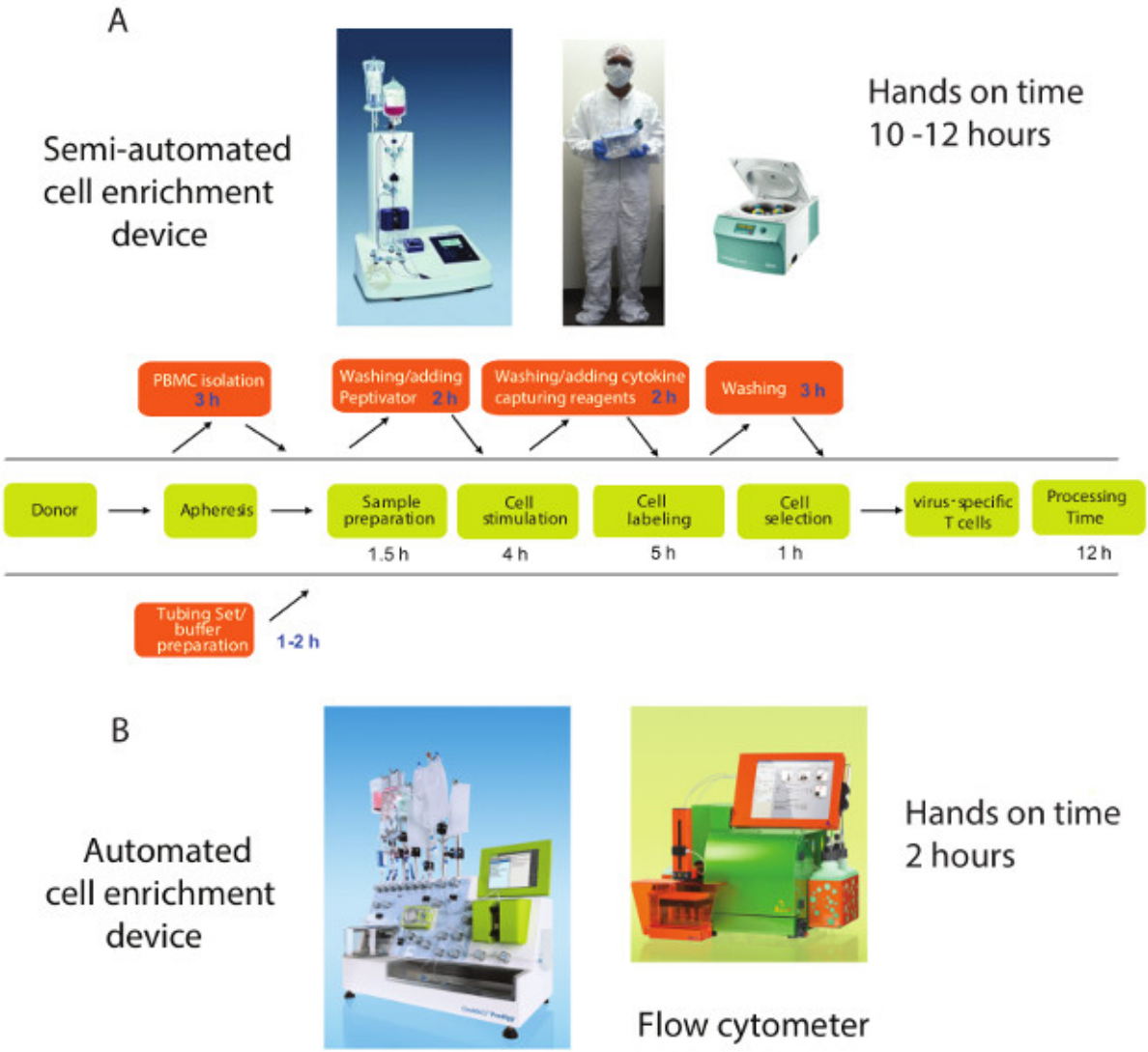

Figure 1. Enrichment of CMV-specific T cells using CCS system. (A) Multiple processing steps involved in the first generation cell enrichment device are handled by skilled professionals. **(B)** Most of the processing steps, except initial tubing setup, are automated in the second generation cell enrichment device which saves 10 - 12 hr of operator handling time in comparison with the first generation device. The enriched CMV-virus-specific T cells are characterized by the flow cytometry cell analyzer. Please click here to view a larger version of this figure.

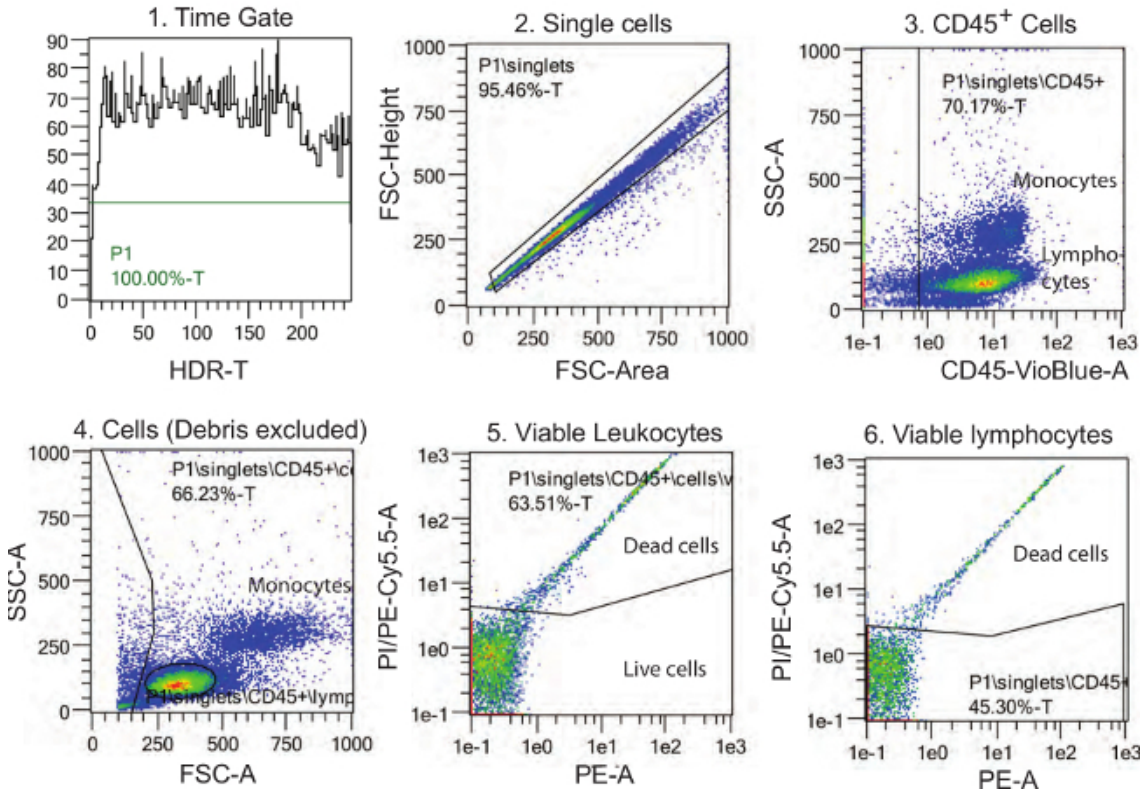

Figure 2. Gating strategy used to determine viable T cells. The numbers 1 through 6 indicate the corresponding gating hierarchy domain in the figure. **(1)** Setting up time gate, **(2)** Removing doublet cells by plotting FSC-height against FSC-area **(3)** Identifying $CD45^+$ cells, **(4)** Removing cell debris **(5)** Selecting viable leukocytes and **(6)** Viable lymphocytes from original population by propidium iodide staining. Please click here to view a larger version of this figure.

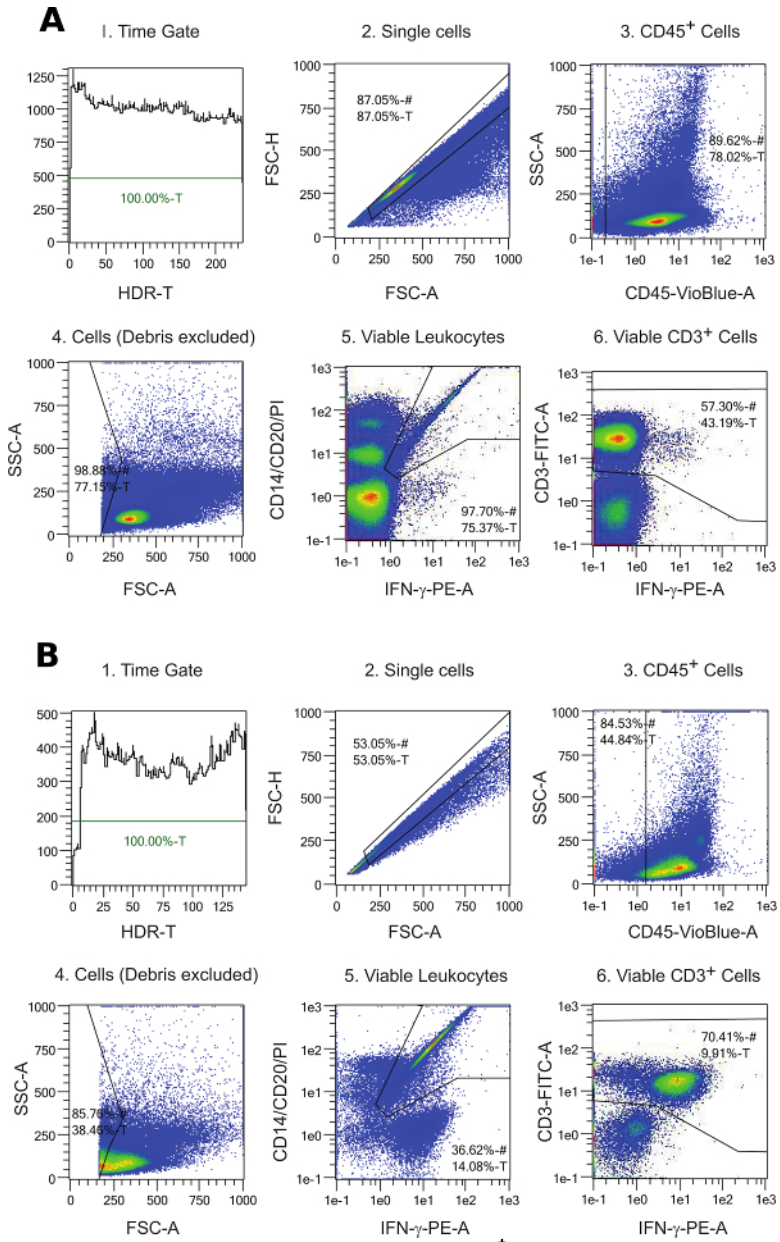

Figure 3. Gating strategy used to determine CD3$^+$ T cells. Flow cytometry blot analysis of CD3$^+$T cells before **(3A)** and after **(3B)** cell enrichment is shown here. It is crucial to determine how many CMV-specific peptide activated T cells are present in the samples before and after the enrichment process. In this figure, numbers 1 through 6 indicate the corresponding cell population either by size or stained by a specific antibody. **(1)** Setting up time-gate, **(2)** Removing doublet cells, **(3)** Selecting CD45$^+$ cells, **(4)** Removing cell debris **(5)** Selecting viable leukocytes and **(6)** Viable CD3$^+$ lymphocytes. Propidium Iodide staining was performed to remove dead cells. Please click here to view a larger version of this figure.

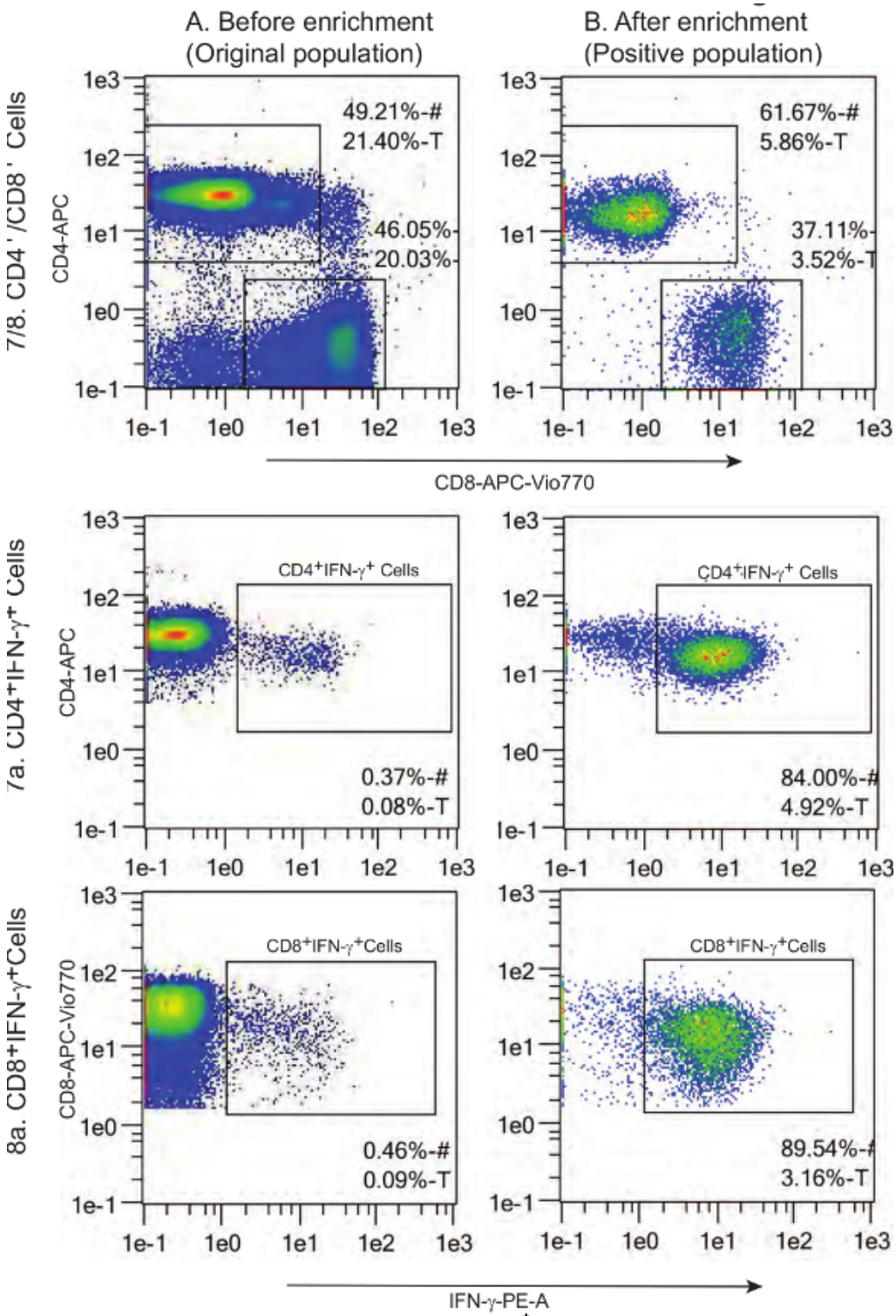

Figure 4. Gating strategy used to determine purity of IFN-γ$^+$ T cells. Activated CMV-specific T cells are critical for controlling CMV infection, so IFN-γ$^+$ expression on T cells were used for gating. IFN-γ$^+$ T cells among CD4$^+$ and CD8$^+$ subsets **(A)** before enrichment and **(B)** after enrichment. **(7/8)** Percentage of CD4$^+$ T cells and CD8$^+$ T cells is shown in **A** and **B**. **(7a)** Percentage of CD4$^+$IFN-γ$^+$ T cells is shown in the square box (a) within the gating area and similarly for CD8$^+$IFN-γ$^+$ T cells in **(8a)**. "T" represents % of cells in total population and "#" represents % of cells in gated population. Propidium iodide staining was performed to gate-out dead cells. Please click here to view a larger version of this figure.

Fraction	Cell count performance	Separation Performance
Original Fraction (QCB)	200 µL	2 mL
Negative Fraction (NTCB)	150 µL	400 µL
Positive Fraction (TCB)	150 µL	500 µL

Table 1. Volumes of the fractions used for cell staining strategy.

total number of viable leukocytes per fraction = number of viable leukocytes/ mL (figure 2, gate 5) × dilution factor × bag volume
total number $CD3^+$ T cells = $\frac{\%\ CD3^+\ T\ cells\ (figure\ 3,\ gate\ 6)}{100\%}$ × total number of viable leukocytes
total number $CD4^+$ T cells = $\frac{\%\ CD4^+\ T\ cells\ (figure\ 4,\ gate\ 7)}{100\%}$ × total number of $CD3^+$ T cells
total number $CD4^+$ IFN-γ^+ T cells= $\frac{\%\ CD4^+\ IFNg^+\ T\ cells\ (figure\ 4,\ gate\ 7a)}{100\%}$ × total number of $CD4^+$ T cells

Table 2. Formulas used for calculation of CD4+ IFN-γ+ T cells after enrichment process. Calculation of CD8+ IFN-γ+ T cells is performed similarly.

Fraction collection	$CD3^+$ T cells	$CD4^+$ T cells	$CD8^+$ T cells	IFN-γ^+ $CD3^+$ T cells	IFN-γ^+ $CD4^+$ T cells	IFN-γ^+ $CD8^+$ T cells
Original fraction (Mean) SD	3.39 x 10^8 ± 3.96 x 10^7	1.66 x 10^8 ± 1.91 x 10^7	1.56 x 10^8 ± 1.63 x 10^7	1.34 x 10^6 ± 1.54 x 10^6	6.17 x 10^5 ± 7.12 x 10^4	7.18 x 10^5 ± 8.28 x 10^4
Positive Fraction (Mean) Std. Deviation	6.01 x 10^6 ± 5.18 x 10^6	3.70 x 10^6 ± 3.2 x 10^6	2.22 x 10^6 ± 1.9 x 10^6	5.11 x 10^5 ± 4.40 x 10^5	3.11 x 10^5 ± 2.68 x 10^5	2.07 x 10^5 ± 1.72 x 10^5
Negative Fraction (Mean) Std. Deviation	3.21 x 10^8 ± 2.76 x 10^7	1.51 x 10^8 ± 1.27 x 10^7	1.56 x 10^8 ± 1.63 x 10^7	3.37 x 10^5 ± 2.85 x 10^5	9.08 x 10^4 ± 7.9 x 10^4	2.48 x 10^5 ± 2.16 x 10^4

Please click here to view a larger version of this table.

Table 3. Total cell counts in T-cell subsets before and after enrichment. Sample #1 results are shown here.

Percentage of purity and recovery	IFN-γ^+ $CD4^+$ T cells among viable $CD4^+$ T cells	IFN-γ^+ $CD8^+$ T cells among viable $CD8^+$ T cells
Before enrichment	0.37	0.46
After enrichment	84	89.5
Recovery from original fraction after enrichment	50.4	27.8

Table 4. Purity and recovery of IFN-γ+ T cell before and after enrichment process of sample #1.

Technology	FACS Technology	MACS Technology	
Instrument	FACS Aria III	CliniMACS	CliniMACS Prodigy
Purity	High grade	Excellent	Excellent
Cell sorting per hour	~10^7	~10^9 - 10^{10}	~10^9 - 10^{10}
Processing time for 10^9 cells	~ 100 hours	~24 hours	~13 - 15 hours
Multi cell sorting	Yes	No	No
Personnel	2 or More	2 or More	1
GMP facility	Yes	Yes	No

Table 5. Cell sorting strategies used in the isolation of clinical grade CMV-antigen specific T cells.[5]

Discussion

Adoptive T-cell therapy has emerged as a viable option to treat B-cell malignancies[4]. Its therapeutic potential is dependent on infusing the desired number of target antigen specific T cells that lack replicative senescence[2]. This can be achieved by sorting out a pure population of antigen specific T cells from expanded T cells in compliance with current good manufacturing practices. Two sorting procedures are widely used, namely, fluorescence-activated cell sorting (FACS) and magnetic activated cell sorting (MACS) to generate CMV antigen specific T cells as we have reviewed recently[5]. The advantage of using one strategy over the other is outlined in **Table 5**. MACS technology offers the highest cell enrichment purity in a cheaper and faster way compared to FACS technology. In addition usage of disposable columns and reagents for cell enrichment totally prevents sample to sample contamination making it easier to apply in clinical settings. The semi-automated cell enrichment device is labor intensive and time consuming so development of the automated cell enrichment device was necessary to feed clinical demand.

The automated cell enrichment CCS device is a versatile instrument for isolating clinical grade cells such as hematopoietic stem cells, somatic stem cells, as well as T cells for adoptive transfer. This automated device integrates cell processing, including fractionation of starting material, cell washing, cell separation, cell culture, and final product formation in a single-use GMP-compliant disposable unit. The closed system reduces

clean-room requirements and minimizes operator involvement for maintaining GMP facilities. Automation reduces the time required for an operator to be present thereby decreasing the cost associated with these laboratory procedures.

To validate the automated cell enrichment device, as compared with the semi-automated cell enrichment device, we isolated CMV-specific T cells after incubating CMV pp65-derived peptides with an apheresis product. GMP grade CMV pp65-derived peptide cocktail (*e.g.* PepTivator) is a peptide pool that consists mainly of 15 mer-peptides with 11 amino acids overlap, covering the complete sequence of the pp65 protein of human cytomegalovirus. Sample recovery yield was ~ 0.3 x 10^6 $CD3^+IFN\text{-}\gamma^+$ T cells from 10^9 TNC. Clinical studies have demonstrated that infusing a few thousand CMV-specific T cells as prophylactic treatment for patients (~ 360 to 4,000 cells/kg body weight) undergoing HSCT resulted in protection against CMV. For example, in order to treat CMV in clinical trials using this technology, a 70 kg adult and a 30 kg child apparently require only 0.26 – 3.0 x 10^5 and 1 x 10^4 – 1.2 x 10^5 cells, respectively, of viral-specific T cells[12-15]. In general, the number of live/dead cells is comparable to the semi-automated cell enrichment device, as shown by Feuchtinger *et al.*[13]. A higher number of viable cells would also be acceptable as this would be associated with enough material for different infusions. However, more viable cells in general also means more cells other than T cells (B, NK, etc). Our data demonstrate that CMV-specific T cells generated on the automated cell enrichment system resulted in clinically-appealing numbers of CMV-specific T cells that may then be infused after allogeneic HSCT.

CMV infection can be a major problem after HSCT resulting in both increased morbidity and mortality. Furthermore, CMV infection is associated with increased costs, despite recent progress in early diagnosis and early treatment with anti-viral drugs[16]. The current treatment using ganciclovir and foscarnet can lead to toxicity in medically-fragile recipients of HSCT. The add-back of donor-derived CMV-specific T cells has been demonstrated to prevent and treat opportunistic infections in recipients of allogeneic HSCT[9]. This approach to adoptive immunotherapy has also been applied to help restore immunity to other pathogens, such as Epstein-Barr virus (EBV), adenovirus[8], and *Aspergillus*[3] by incubating mononuclear cells (MNC) with respective antigen derived clinical grade peptide cocktail reagents (**Materials and equipment's table**). Recently, investigators have safely infused third-party pathogen-specific T cells that are matched with at least one HLA allele in the recipient presenting immunodominant peptide[17]. The automated cell enrichment CCS system may also be used to generate third-party T cells for off-the-shelf applications that will be useful when the donor is CMV-seronegative or unavailable, such as the case with donors for allogeneic umbilical cord blood transplantation.

In summary, we demonstrate the utility of an automated cell enrichment device to generate CMV-specific T cells based on automation of CCS. We believe this device has the potential to lower the threshold for clinical teams to infuse pathogen-specific, as well as tumor-specific, T cells in immunocompromised patients.

Disclosures

Both MD Anderson Cancer Center and Dr. Cooper have a financial interest in ZIOPHARM Oncology, Inc., and Intrexon Corporation. On May 7, 2015, Dr. Cooper was appointed as the Chief Executive Officer at ZIOPHARM Oncology. Dr. Cooper is now a Visiting Scientist at MD Anderson. Dr. Cooper founded and owns InCellerate, Inc. He has patents with Sangamo BioSciences with artificial nucleases. He consults with Targazyme, Inc. (formerly American Stem cells, Inc.), GE Healthcare, Ferring Pharmaceuticals, Fate Therapeutics, Janssen Pharmaceuticals, and Bristol-Myers Squibb. He is on the Scientific Advisory Board of Cellectis. He receives honoraria from Miltenyi Biotec.

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References

1. Syed, B. A., Evans, J. B. From the Analyst's Couch Stem Cell Therapy Market. *Nat Rev Drug Discov.* **12**, (3), 185-186 (2013).
2. Maus, M. V., *et al.* Adoptive Immunotherapy for Cancer or Viruses. *Annu Rev Immunol.* **32**, 189-225 (2014).
3. Kumaresan, P. R., *et al.* Bioengineering T cells to target carbohydrate to treat opportunistic fungal infection. *Proc Natl Acad Sci U S A.* **111**, (29), 10660-10665 (2014).
4. Singh, H., *et al.* Redirecting specificity of T-cell populations for CD19 using the Sleeping Beauty system. *Cancer Res.* **68**, (8), 2961-2971 (2008).
5. Kumaresan, P. R., *et al.* Automating the manufacture of clinically appealing designer T cells. *Treatment Strategies-BMT.* (1), 55-59. Available from: http://www.cambridgeresearchcentre.co.uk/all-publications/blood-and-marrow-transplantation/ (2014).
6. Einsele, H., *et al.* Adoptive transfer of CMVpp65-peptide loaded DCs to improve CMV-specific T cell reconstitution following allogeneic stem cell transplantation. *Blood.* **100**, (11), 214a-214a (2002).
7. Blyth, E., *et al.* Donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic stem cell transplantation. *Blood.* **121**, (18), 3745-3758 (2013).
8. Gerdemann, U., *et al.* Safety and clinical efficacy of rapidly-generated trivirus-directed T cells as treatment for adenovirus, EBV, and CMV infections after allogeneic hematopoietic stem cell transplant. *Mol Ther.* **21**, (11), 2113-2121 (2013).
9. Meij, P., *et al.* Effective treatment of refractory CMV reactivation after allogeneic stem cell transplantation with in vitro-generated CMV pp65-specific CD8+ T-cell lines. *J Immunother.* **35**, (8), 621-628 (2012).

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10. Lee Buckler, J. Enal Razvi,. Rise of Cell-Based Immunotherapy : Personalized Medicine Takes Next Step Forward. *Genetic Engineering & Biotechnology News.* **33**, (5), 12-13 (2013).
11. Apel, M., *et al.* Integrated Clinical Scale Manufacturing System for Cellular Products Derived by Magnetic Cell Separation, Centrifugation and Cell Culture. *Chem-Ing-Tech.* **85**, (1-2), 103-110 (2013).
12. Brestrich, G., *et al.* Adoptive T-Cell Therapy of a Lung Transplanted Patient with Severe CMV Disease and Resistance to Antiviral Therapy. *Am J Transplant.* **9**, (7), 1679-1684 (2009).
13. Feuchtinger, T., *et al.* Clinical grade generation of hexon-specific T cells for adoptive T-cell transfer as a treatment of adenovirus infection after allogeneic stem cell transplantation. *J Immunother.* **31**, (2), 199-206 (2008).
14. Peggs, K. S., *et al.* Directly selected cytomegalovirus-reactive donor T cells confer rapid and safe systemic reconstitution of virus-specific immunity following stem cell transplantation. *Clin Infect Dis.* **52**, (1), 49-57 (2011).
15. Tischer, S., *et al.* Rapid generation of clinical-grade antiviral T cells: selection of suitable T-cell donors and GMP-compliant manufacturing of antiviral T cells. *Journal of Translational Medicine.* **12**, (1), 336 (2014).
16. Svahn, B. M., Remberger, M., Alvin, O., Karlsson, H., Ringden, O. Increased Costs after Allogeneic Haematopoietic Sct Are Associated with Major Complications and Re-Transplantation. *Biol Blood Marrow Transplant.* **18**, (2), S339-S339 (2012).
17. Leen, A. M., *et al.* Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. *Blood.* **121**, (26), 5113-5123 (2013).

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