Consistently and accurately measure CAR T cell concentration and viability using Cellometer Image Cytometry for adoptive cell transfer therapy

Dmitry Kuksin[1], Scott McMenemy[1], Scott Cribbes[1], and Leo L. Chan[1]

[1]Nexcelom Bioscience LLC, 360 Merrimack St. Building 9, Lawrence, MA 01843

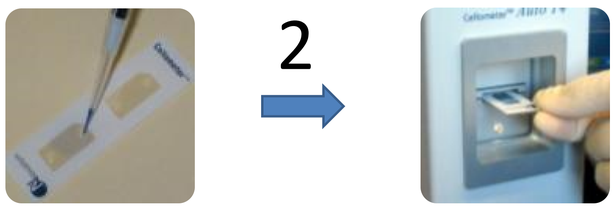
1. ABSTRACT

Cancer immunotherapy research consists of multiple strategies that are aimed at enhancing immune responses, such as vaccine, antibody, immune checkpoint inhibitor, and adoptive cell transfer (ACT) therapies. Specifically for ACT therapy, it focuses on utilizing patient's own cells for therapeutic treatment. Chimeric-antigen receptor (CAR) has become a major player in ACT, where T cells can be genetically engineered to express tumor-associated antigen using the *Sleeping Beauty* transposon system. In the general ACT process for CAR, the T cells from a patient are first collected and purified. Next, these T cells are genetically engineered to express the tumor-associated antigen and then expanded through cell culture. After expansion, the T cell function is tested and conditioned prior to infusing back into the patient for treatment. In each step described above, T cell concentration and viability must be measured to ensure the quality of the cells to go back to the patients. Traditionally, concentration and viability of the T cells are measured using manual hemacytometer with Trypan Blue, but this method is time-consuming and has user-dependent variation. More recently, automated Trypan Blue image-based cell counters have been widely adopted for cell-based assays. However, using bright-field imaging with Trypan Blue can have inaccurate measurement due to cellular debris, platelets, and residual RBCs. In addition, distinction between live and dead cells may vary due to the viability of cells, which makes it more difficult for an accurate cell count. In this work, we demonstrate a robust dual-fluorescence-based concentration and viability method using acridine orange (AO) and propidium iodide (PI). This image cytometry method can be utilized at every step of the ACT process and generate accurate and consistent T cell concentration and viability measurement, in order to propagate to the next step, and eventually infusing back to the patients.

References

Singh et al. Cancer Gene Therapy (2015) 22, 95-100, Park et al. Trends Biotechnol (2011), 29 (11), 550-557

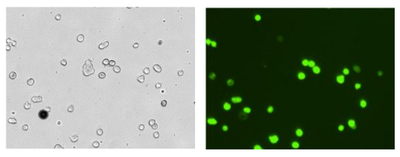
2. CELLOMETER IMAGE CYTOMETRY FOR CELL CONCENTRATION AND VIABILITY



Results:

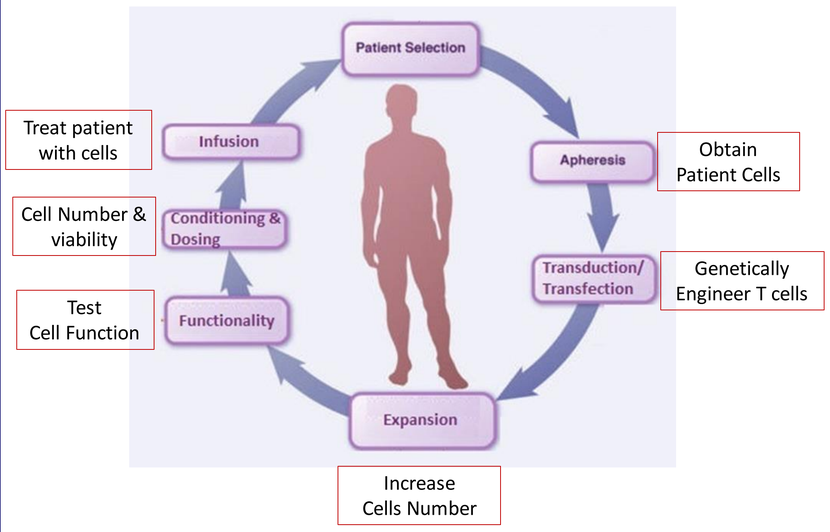
Count	Concentration
Total: 1035	1.16x10^7 cells/mL
Live: 954	1.07x10^7 cells/mL
Dead: 81	9.06x10^5 cells/mL

Viability: 92.2%



1. Cellometer Image Cytometer is a slide-based cytometer that can capture bright-field and fluorescent images in a disposable counting chamber
2. The captured images are analyzed with the Cellometer software to measure size, morphology, cell count, and fluorescent intensity
3. The measured parameters are used to generate GFP/RFP expression, DNA cell cycle analysis, apoptosis, and cell viability results

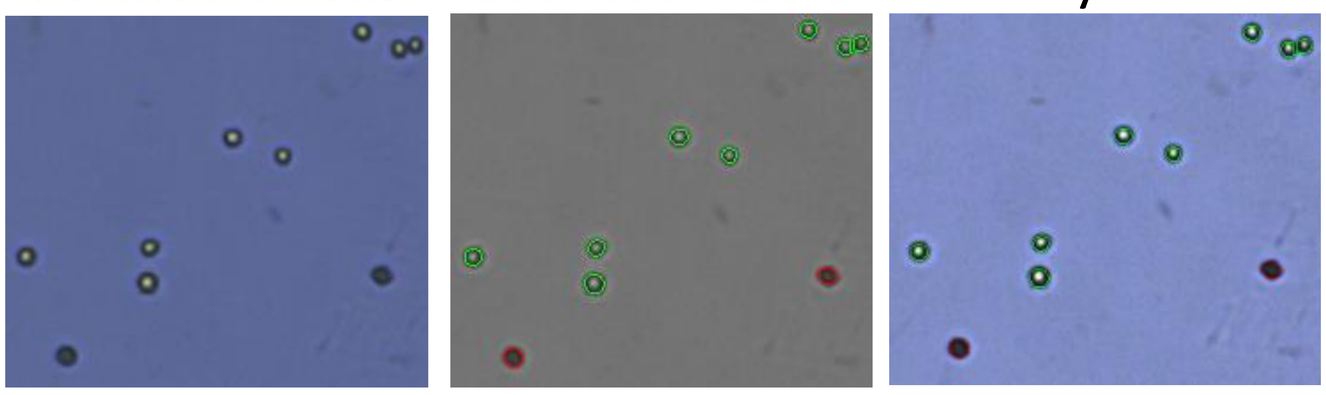
3. ADOPTIVE CELL TRANSFER THERAPY PROCESS USING CELLOMETER



- Cellometer image cytometer can be used to measure cell concentration and viability during the entire ACT process
  1. Obtaining patient immune cells
  2. Genetically engineering T cells
  3. Culture to increase T cell count
  4. Test T cell cytotoxicity function
  5. Ensure high viability and cell count
  6. Treat patients with modified T cells

4. MEASURING T CELL COUNT AND VIABILITY USING TRYPAN BLUE

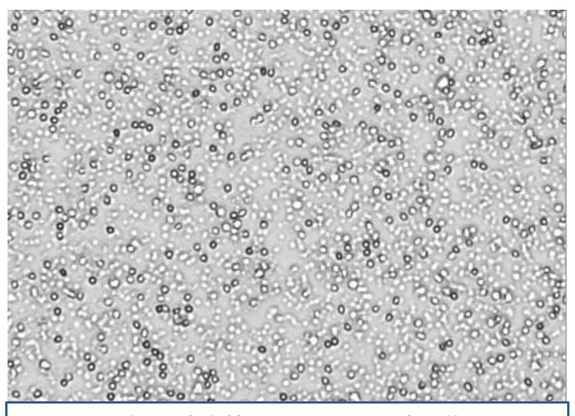
Purified T cells | Counted T cells | Overlay Marker



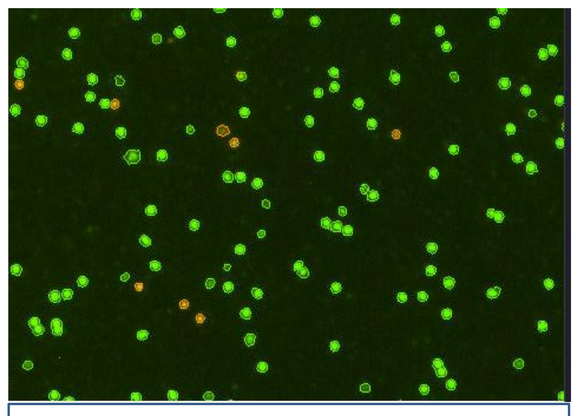
- Cellometer can be used to perform cell count and viability using Trypan Blue for isolated T cells
- Secretion of IFNγ by TIL indicates tumor specific response by the isolated T cells. An ELISA assay is performed to test for high levels of interferon-γ (IFNγ)
- Patient's purified T cells are often cryopreserved before they are genetically engineered with the cancer-specific T cell receptor
- It is extremely important to measure T cell viability and concentration of isolated human T cells before and after the cryopreservation, electroporation, cell expansion, and for plating step during ACT process

5. MEASURING T CELL COUNT AND VIABILITY USING AOPI

Fresh LeukoPak | AOPI Combined



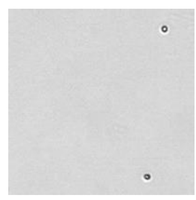
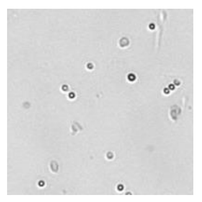
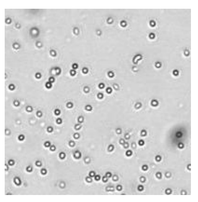
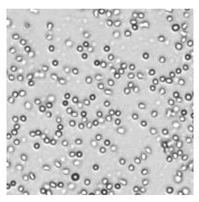
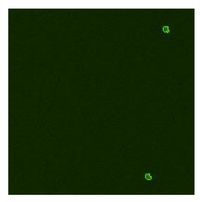
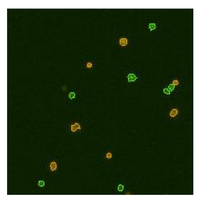
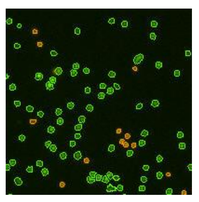
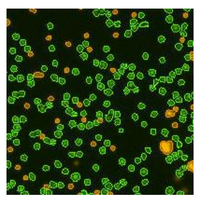
A lot of different types of cells Very difficult to count



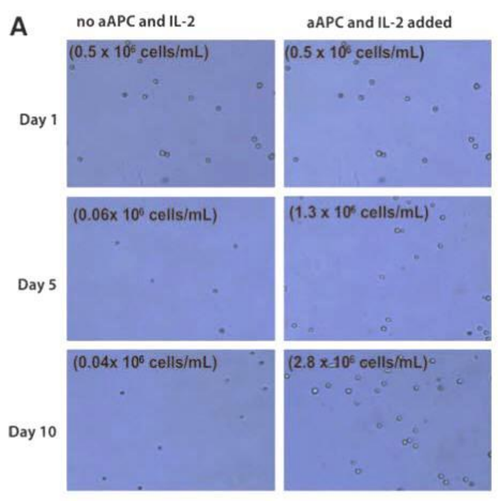
Stain only nucleated cells: live and dead

- Cellometer can be used to perform cell count and viability using AOPI for freshly collected patient immune cells
- These cells requires initial purification from the red blood cells and platelets, however, high amount of red blood cells, platelets, as well as cellular debris still remain
- By using acridine orange (AO) and propidium iodide, live (AO) and dead(PI) immune cells are nuclear stained to emit bright green and red fluorescence, respectively
- Using AOPI allows accurate and consistent measurement of concentration and viability in the presence of RBCs, platelets, and cellular debris
- This eliminates the need to further purify the sample using RBC lysing buffer or cell sorting

6. LARGE COUNTING DYNAMIC RANGE USING AOPI METHOD

	Sample # 7	Sample # 6	Sample # 2	Sample # 5
Total cell count	49	1419	10635	24245
Live cell concentration	1.07E+05	2.84E+06	3.11E+07	6.75E+07
Viability	63.20%	58.00%	84.40%	80.30%
Bright field cell image				
Counted live /dead cell image				

7. CAR T CELL COUNTING EXAMPLE: CAR T CELL PROLIFERATION



***piggyBac* Transposon/Transposase System to Generate CD19-Specific T Cells for the Treatment of B-Lineage Malignancies**
Hum Gene Ther. 2010 April; 21(4): 427–437

- In this work, Cellometer was used to measure CAR T cell proliferation over time
- Simultaneously, Trypan Blue is used to measure the cell viability of the CAR T cells
- This work was performed in MD Anderson under Dr. Laurence Cooper, who has pioneered CAR T cell therapy for cancer treatment