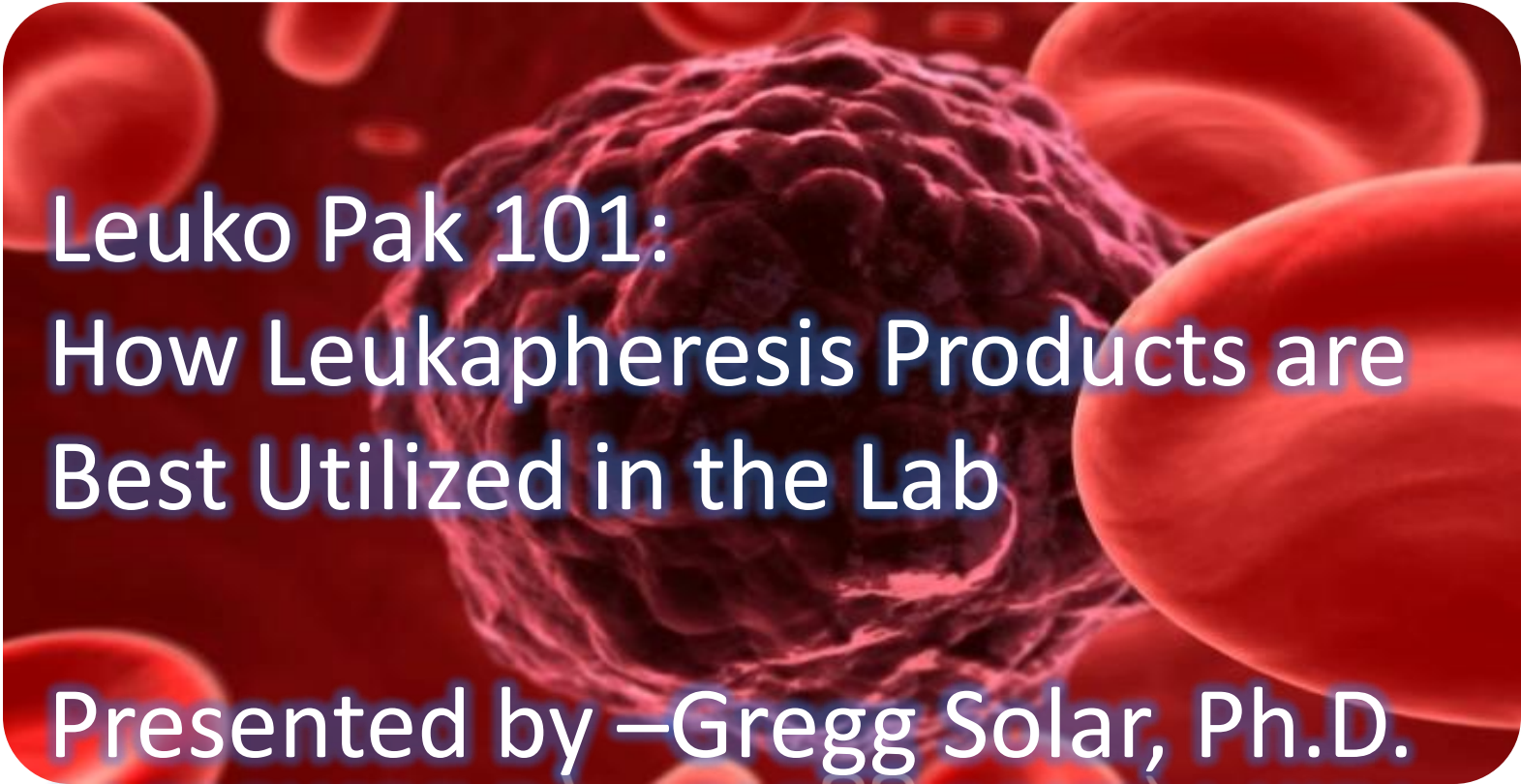


AllCells, LLC – Webinar Presentation

A microscopic view of a cell cluster, likely a leukapheresis product, surrounded by red blood cells. The cell cluster is a large, textured, spherical mass of cells, and the red blood cells are smaller, biconcave discs. The background is a deep red color.

Leuko Pak 101: How Leukapheresis Products are Best Utilized in the Lab

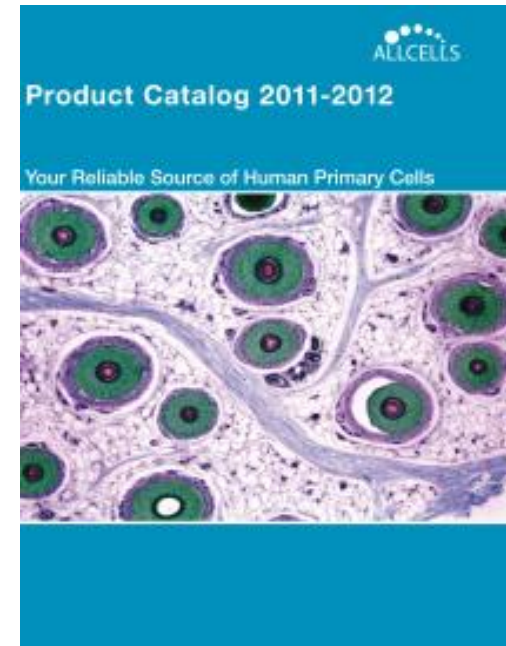
Presented by –Gregg Solar, Ph.D.

Corporate Background

- Founded in 1998
- Centrally located within San Francisco Bay Area (Emeryville, CA)
- Key products (>700)
 - Hematopoietic and Immunological Cells from diseased and healthy donors
 - Mesenchymal Stem Cells
 - Primate, rat and mouse tissue
- AllCells has a dedicated onsite collection facility operating under IRB approved protocols
- Contract Assay Services:
 - Hematopoietic & Immunological Assays
 - Flow Cytometry Services
 - Luminex Multianalyte Assays
 - Cell Based Assays for Drug Discovery
 - Diseased primary cells and cell lines
 - *In vitro* expansion and differentiation of primary hematopoietic cells



Leading Provider of Human Hematopoietic Cells and Services

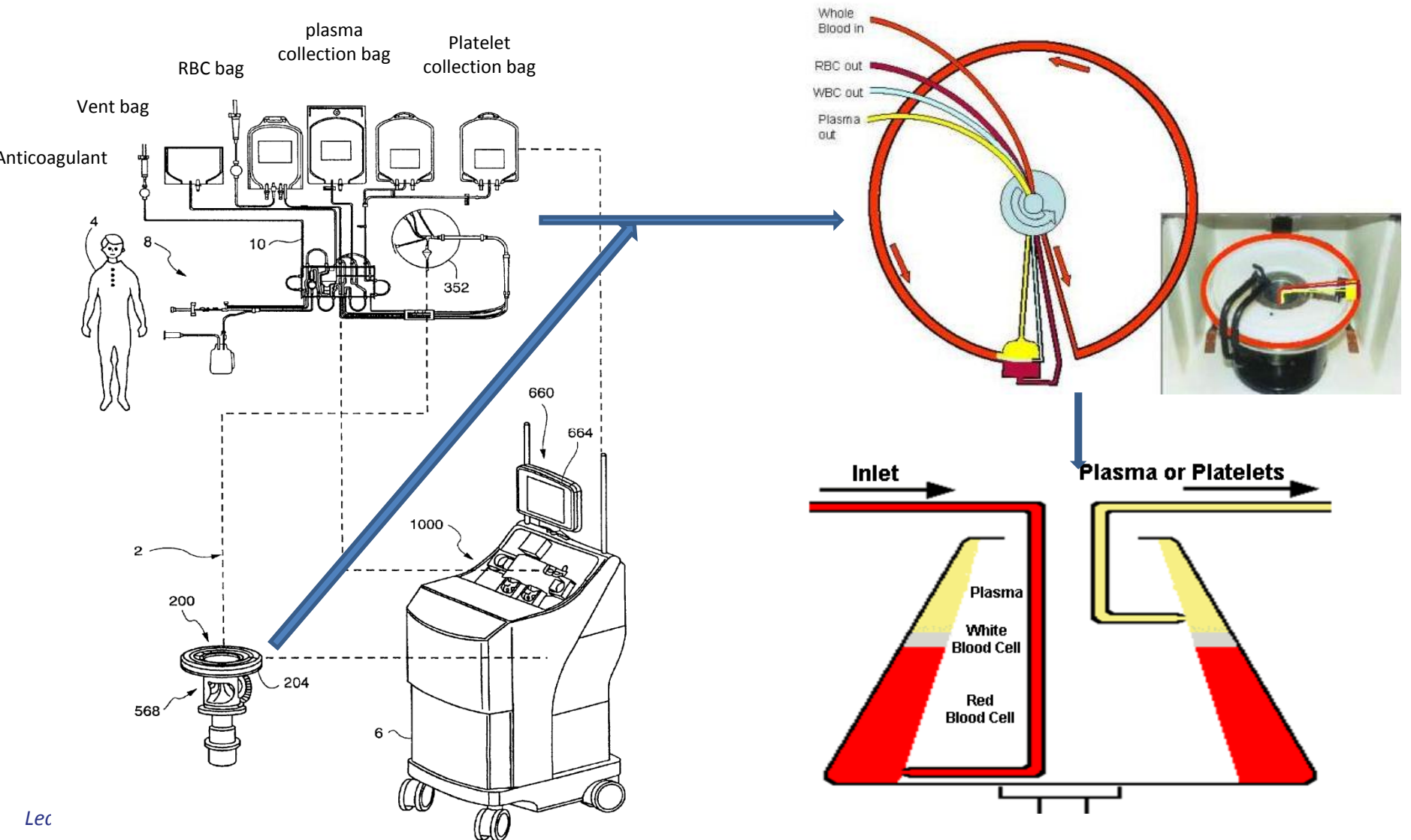


Apheresis

- Derived from Greek, “to carry away”
- A technique in which whole blood is taken and separated extracorporeally, separating the portion desired from the remaining blood
- Performed using Cobe Spectra (Terumo BCT)



Apheresis Principle



Plasmapheresis

- Therapeutic
 - Plasma:
 - Removal (replaced with albumin, plasma and saline solution) helps reduce circulating antibodies and immune complexes
 - Diseases include: Waldenstrom's macroglobulinemia, Myasthenia gravis and others
- Donation
 - Plasma removed to supply blood components such as clotting factors

Plateletpheresis

- Therapeutic
 - Myeloproliferative disorders
 - Thrombocytosis can occur
 - Removal of platelets can help to avoid complications of thrombosis and bleeding
- Donation
 - Most common means for supplying HLA matched platelets to patients who have become HLA sensitized

Therapeutic Leukapheresis

- Performed to decrease a very high white blood cell count
- In hematological malignancies such as AML, WBC counts may be high enough to cause hemostasis and "sludging" in the capillaries
 - Affect retinal vasculature leading to vision changes
 - Pulmonary vasculature leading to shortness of breath from decreased efficiency in oxygen exchange
 - Neurological deterioration of a patient from cerebrovascular compromise
- Stem Cell Harvesting for transplantation

Non-mobilized Leuko Pak

- Donor criteria

- Age: 18-55 years old
- Weight: \geq 140 lbs
- Complete Blood Count (CBC) lab test must meet protocol specifications
- Donors cannot be pregnant
- Donors are screened for HIV, Hepatitis B and C
- Donors must be in good general health
- Donors are required to sign a procedure-specific consent form prior to the collection procedure
- All consent and protocols have been approved by an IRB
- Donors must have adequate peripheral veins

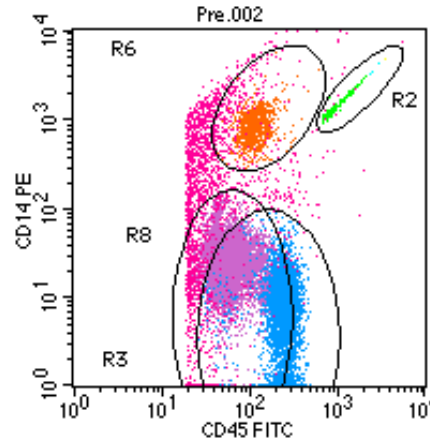
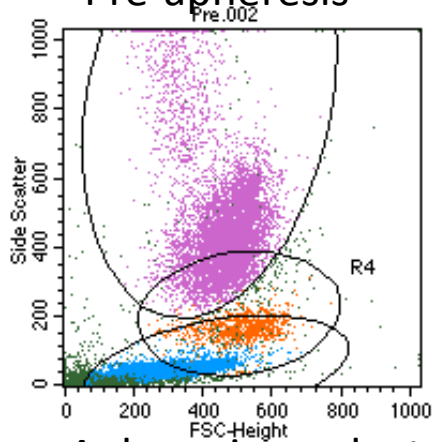
Non-mobilized Leuko Pak

Procedure

- 3 total blood volumes processed
- 4 hours
- Minimum of 6 months between donations
- Life time maximum of 6 donations
- Average yield: 1.5×10^{10} MNCs/bag (1- 3.5×10^{10})
 - ¼ Bag, ½ Bag and full Bag
- Total volume 300 mL

Non Mobilized Leukapheresis

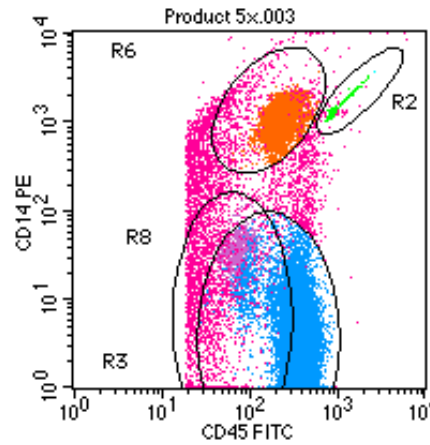
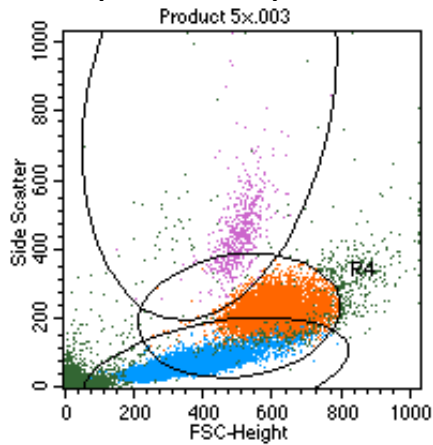
Pre-apheresis



Lymphocytes: 38.2%
 Monocytes: 6.9%
 Granulocytes: 54.9%

WBC 5.17×10^6 /mL

Apheresis product



Lymphocytes: 83.6%
 Monocytes: 15.3%
 Granulocytes: 1.1%

WBC 9.8×10^7 /mL
 Total cell #: 2.94×10^{10}

G-CSF (Neupogen™) Mobilized Leuko Pak

- Granulocyte-Colony Stimulating Factor (G-CSF) also known as Neupogen® a recombinant form of human granulocyte colony stimulating factor (G-CSF)
- Used to treat
 - Chemotherapy
 - Bone marrow transplantation
 - AML
 - Severe/chronic neutropenia
- Used to increase the number of hematopoietic stem cells prior to leukapheresis

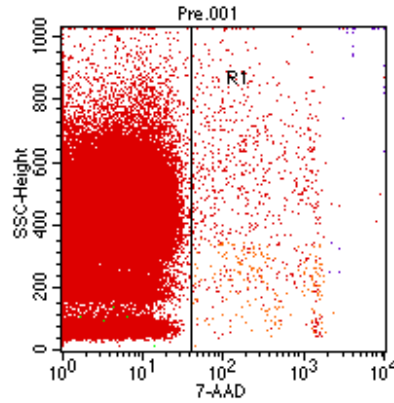
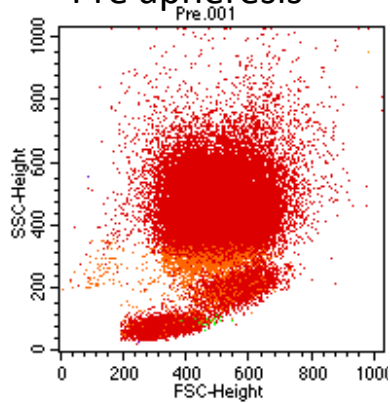
G-CSF (Neupogen™) Mobilized Leuko Pak

Procedure

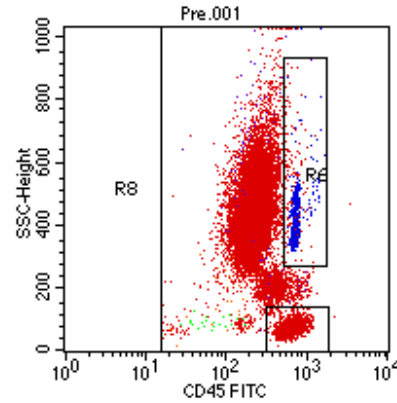
- Donor criteria: 18-40 year old
- 3 total blood volumes processed
- 4 hours
- Minimum of 12 months between donations
- Twice in a lifetime maximum
- Average yield: 2.5×10^{10} MNCs/bag ($0.9-5.6 \times 10^{10}$)
- Total volume: 300 mL
- Three mobilization regimes

G-CSF (Neupogen™) Mobilized Leuko Pak

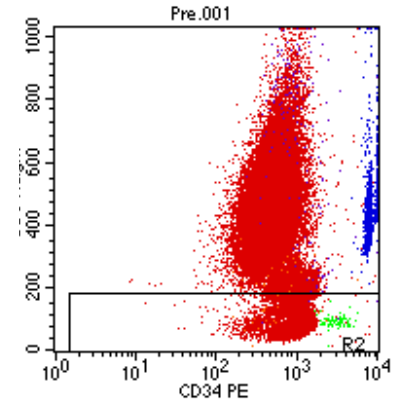
Pre apheresis



Viability: 99.05%

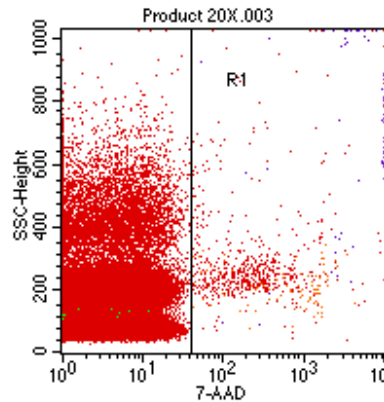
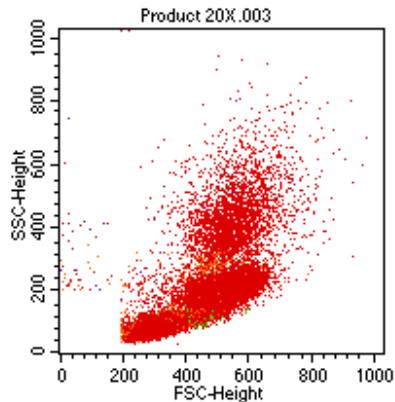


WBC: 3.19×10^7 /mL

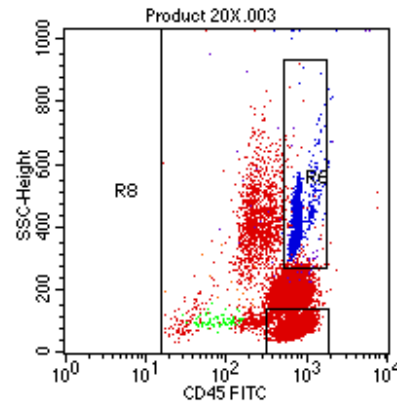


CD34⁺: 3.02×10^4 /mL

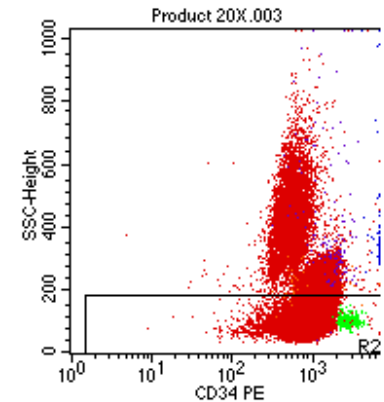
Apheresis Product



Viability: 99.16%



WBC: 1.90×10^8 /mL



CD34⁺: 8.14×10^5 /mL

6 fold increase in WBC
27 fold increase in CD34+ cells

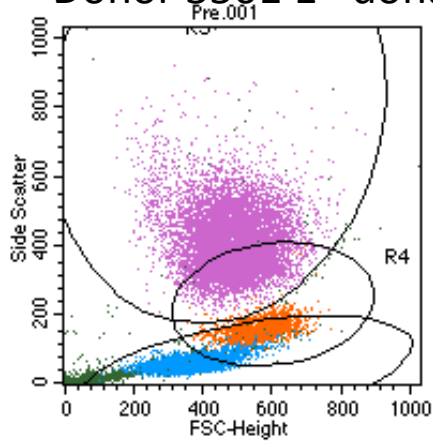


Dual Half Pak-Leukapheresis

- For each donation a half Leuko Pak is collected
- Shorter collection time (< 2 hrs)
- Healthy donor undergoes leukapheresis twice within 6-8 weeks
 - Reduced variability between experiments
 - Potential studies
 - T cell activation/expansion
 - NK cell cytotoxicity

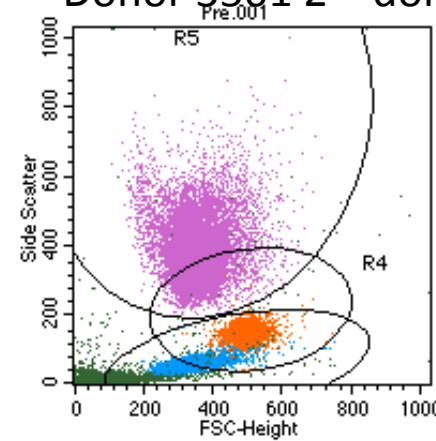
Dual Half Pak-Leukapheresis

Donor 3501 1st donation

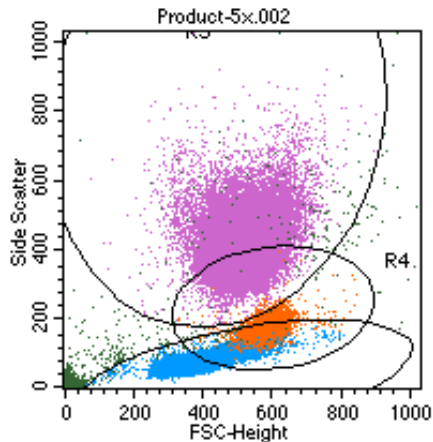


WBC: 7.25×10^6 /mL
% Lymphs: 36.7%
% Monos: 9.8%
% Grans: 53.5%

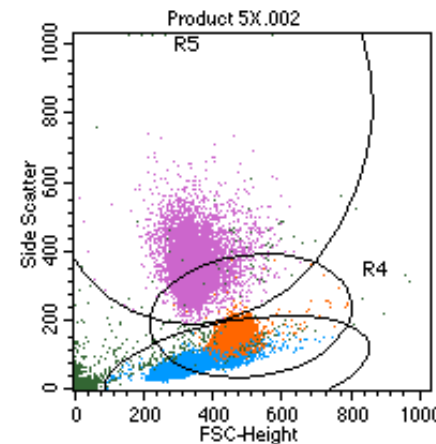
Donor 3501 2nd donation



WBC: 7.36×10^6 /mL
% Lymphs: 34.1%
% Monos: 11.3%
% Grans: 54.6%



WBC: 1.37×10^8 /mL
% Lymphs: 53.2%
% Monos: 15.4%
% Grans: 31.4%



WBC: 1.13×10^8 /mL
% Lymphs: 66.1%
% Monos: 20.9%
% Grans: 13.0%



Total Cell number: 2.2×10^{10}

Total Cell number: 1.8×10^{10}

Application using Dual Half Pak

- NK effector function has traditionally been examined by Cr⁵¹ release assays
- We investigated a non radioactive FACS based assay for cytotoxicity using CD56⁺CD16⁺ NK cells and K562 as the target cell type

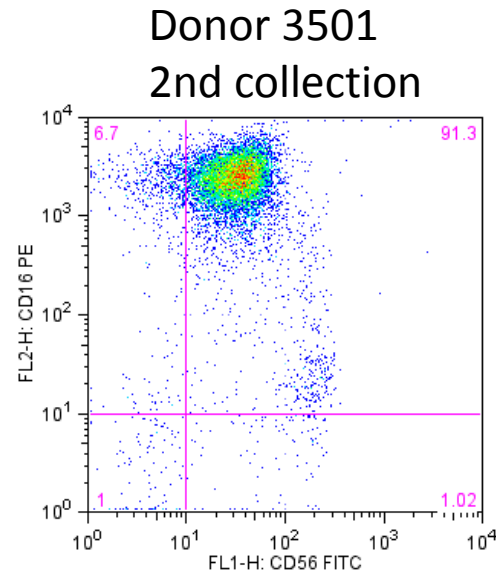
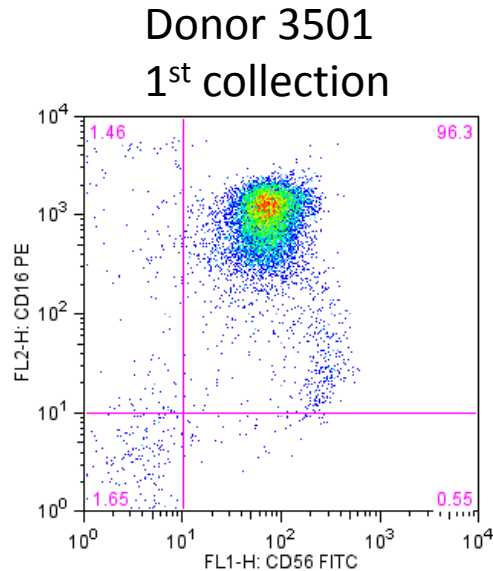
NK Cytotoxicity Assay

Protocol

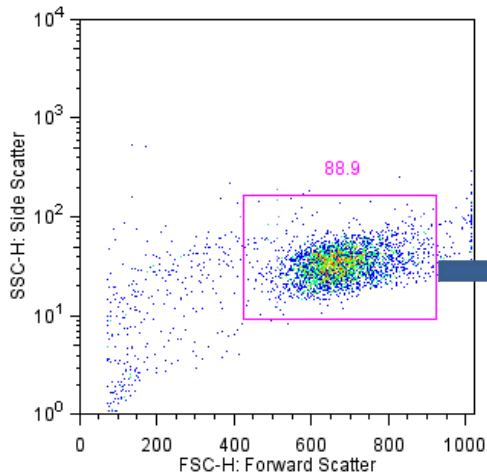
1. NK cells were isolated by immunomagnetic “untouched” selection using Leuko Paks from a healthy donor
2. K562 cells were labeled with 5 μ M CMFDA (CellTracker Green) for 1h at 37°C
3. NK cells were then co-incubated with K562 cells at a E:T ratio of 50:1 and 25:1 for 4 hours at 37°C
4. 7-AAD was then added and the cells were analyzed by flow cytometry
5. Experiment was repeated for 2nd leukapheresis on the same donor 6 weeks after the initial donation

NK Cytotoxicity Assay

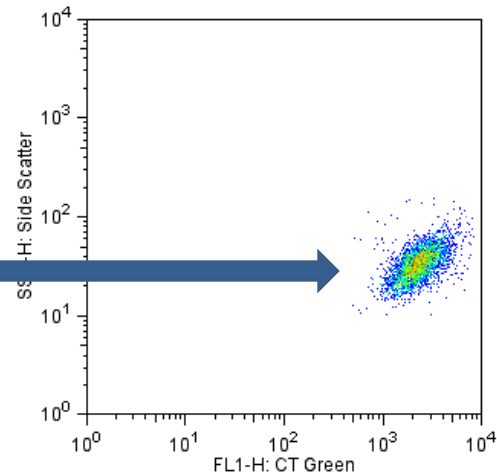
- Collections were performed 6 weeks apart
- Consistent yield and purity between donations



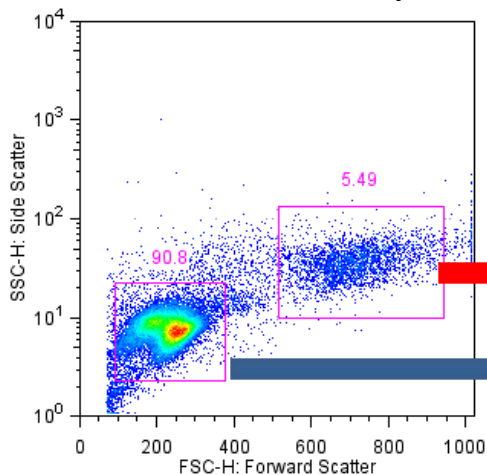
NK Cytotoxicity Assay



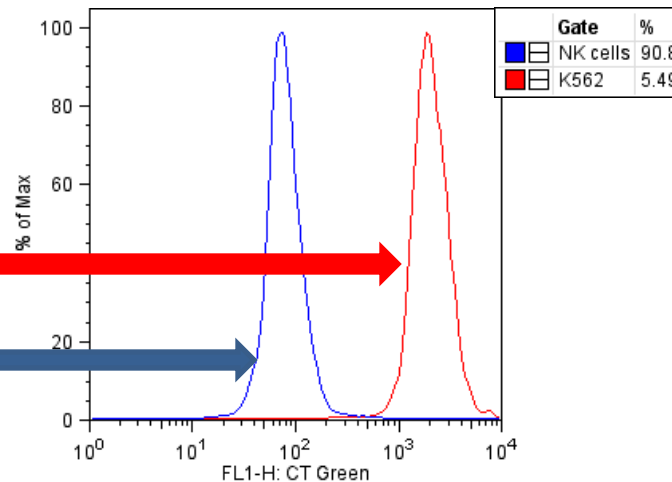
K562 only



CellTracker™ Green CMFDA



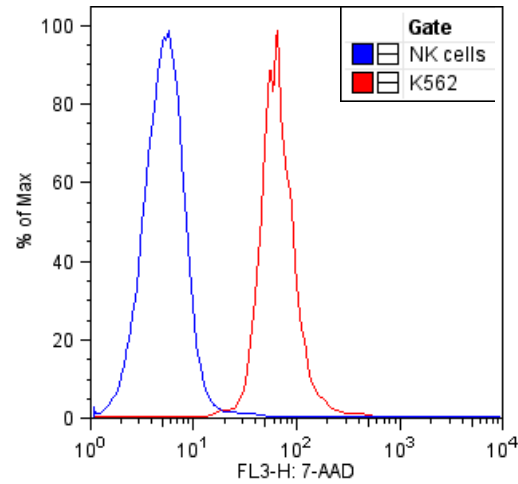
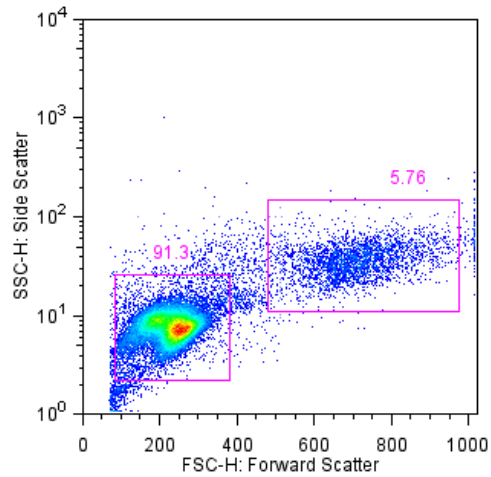
NK cells + K562



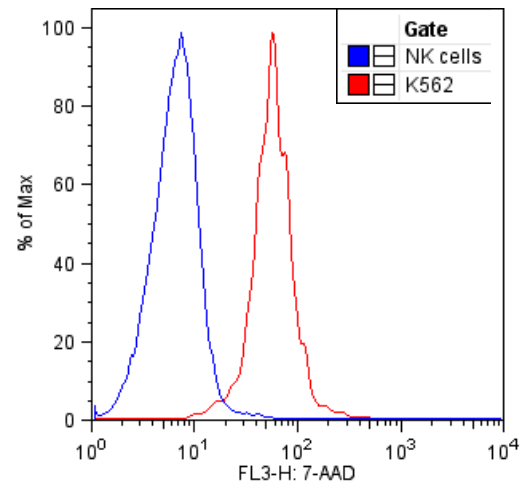
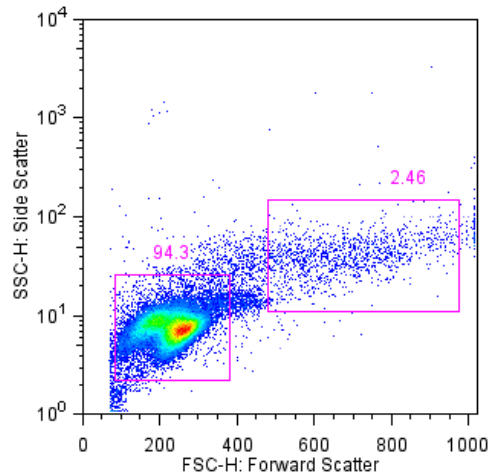
CellTracker™ Green CMFDA

Results: First Donation

E:T=50

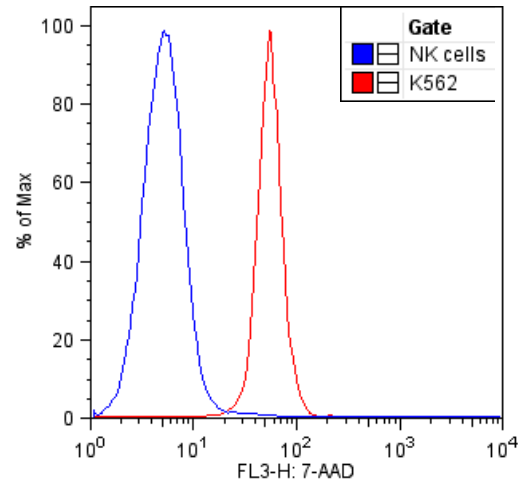
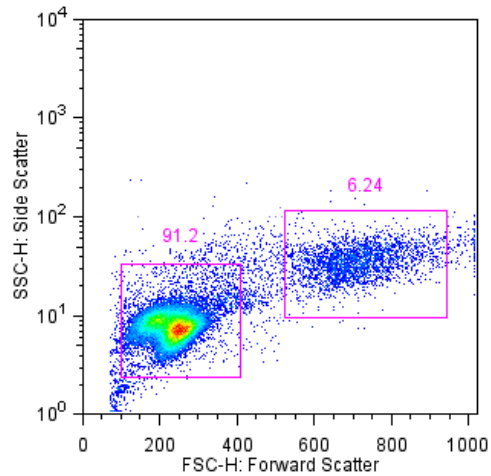


E:T=25

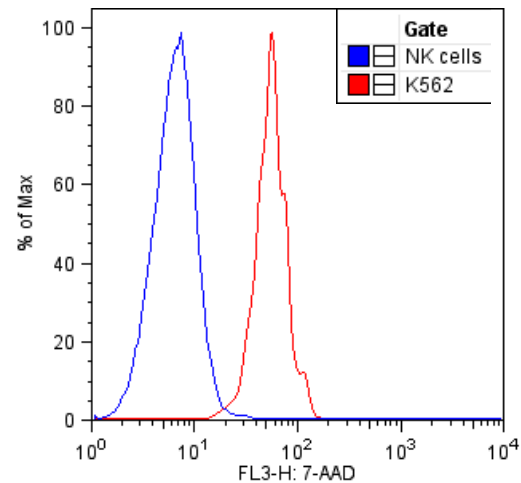
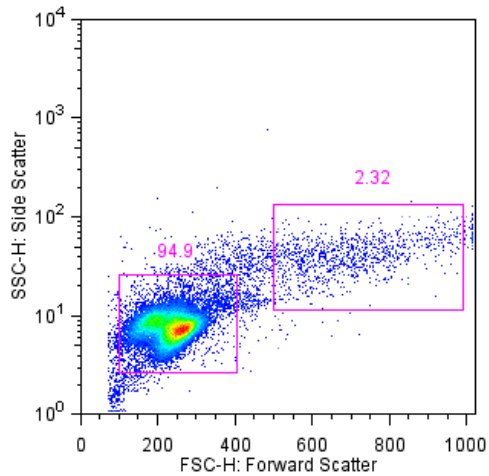


Results: Second Donation

E:T=50



E:T=25

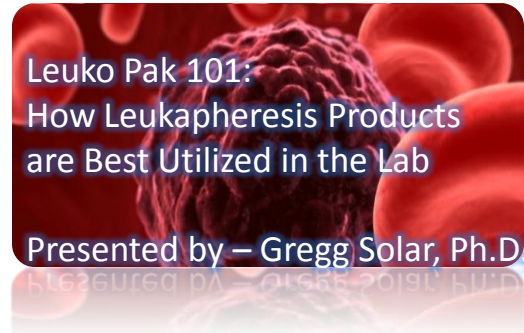


Summary

- AllCells is a leading provider of non-mobilized and G-CSF mobilized leukapheresis products
- We recruit donors directly based on your specific needs
- Leuko Paks can be used as a source for large quantities of primary cells
- Our new Dual Half Pak leukapheresis product
 - Allows for the same individual to donate twice within 6-8 weeks
 - Reduce variability
 - Illicit similar responses



Thank You – Q & A



- Survey to follow
- Discuss your project needs with us:
www.allcells.com/technical_services.php
- Sign-up for our free monthly eNewsletter:
www.allcells.com
- Presentation will be uploaded in 'Support' section