The Challenge of Variability in CART Cell Manufacturing

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Disclosures

I have no relevant financial conflicts to disclose.

Objectives

1. **Mechanism of apheresis collection of mononuclear cell (MNC) products**

2. **Many sources and downstream effects of variability in the CART manufacturing process**

3. **Understand our approach to limit variability**

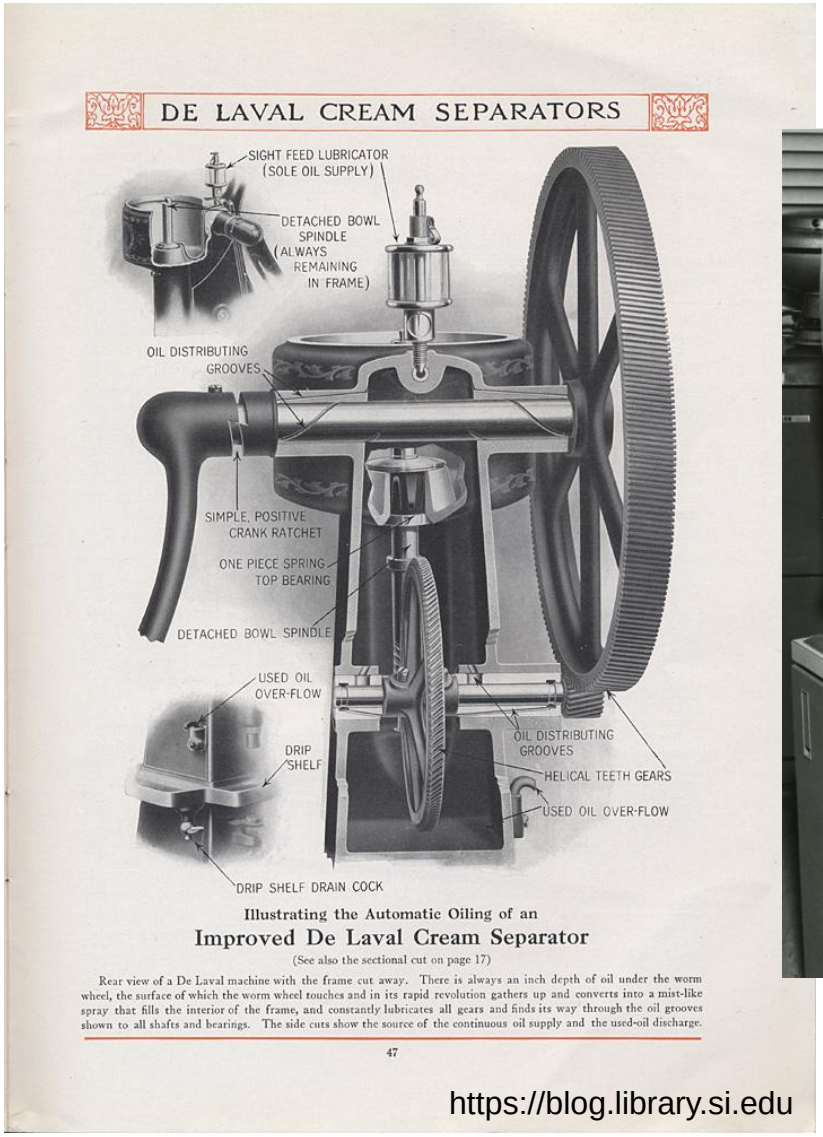
Apheresis - Mononuclear Cell Collection

- **Apheresis is a continuous or semi-continuous method of isolating peripheral blood cells based on density.**

- **Mononuclear cell collection can be a reliable method for collecting large numbers of mononuclear cells (eg. lymphocytes and monocytes) while excluding non-MNCs (eg. granulocytes, PLTs, RBCs).**

- **Steady state MNC collection (non-mobilized) is the most common source of starting material for CART manufacturing.**

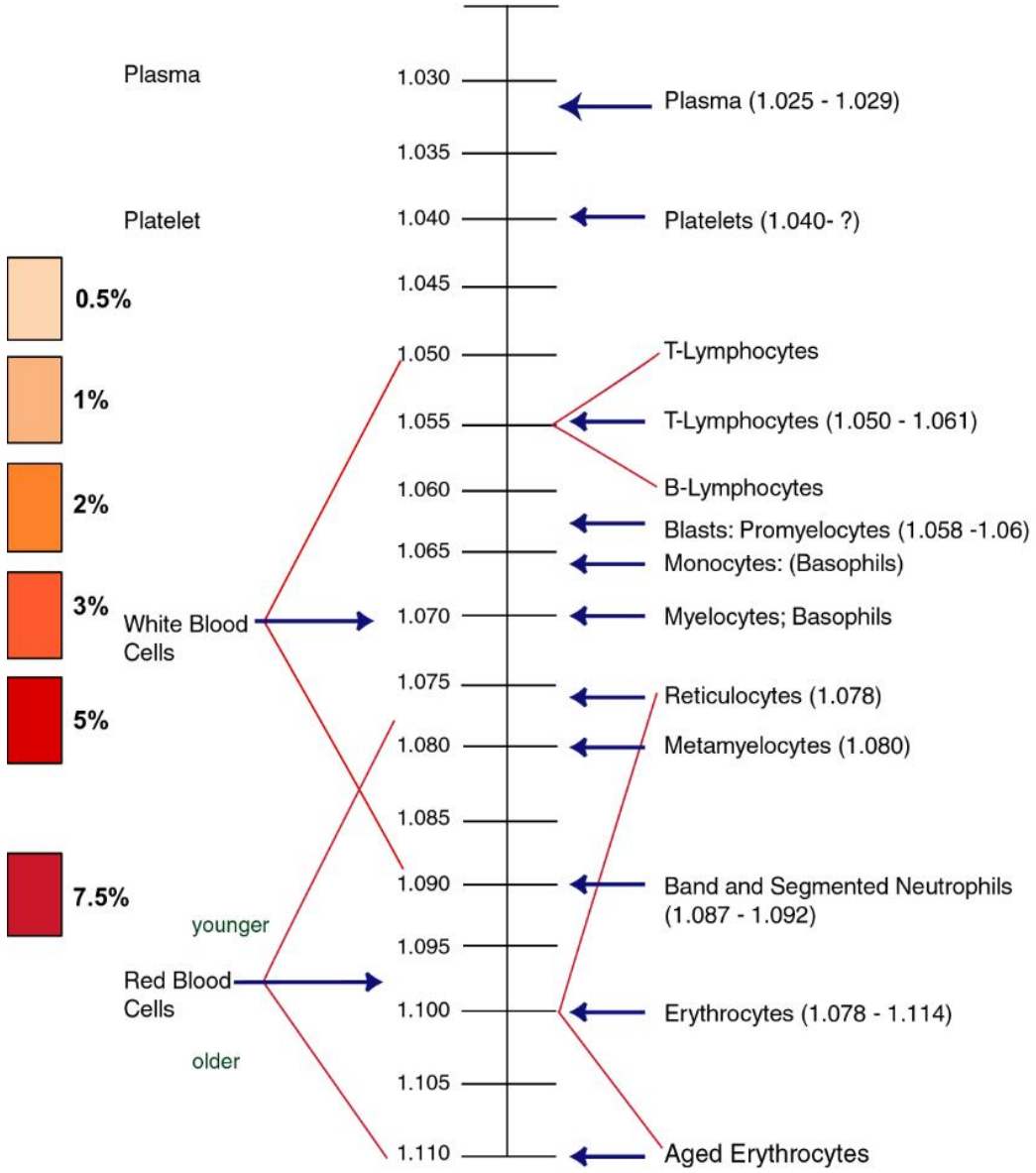
Continuous Flow Separation Machines



https://blog.library.si.edu

https://www-03.ibm.com

Specific Gravity of Blood Components

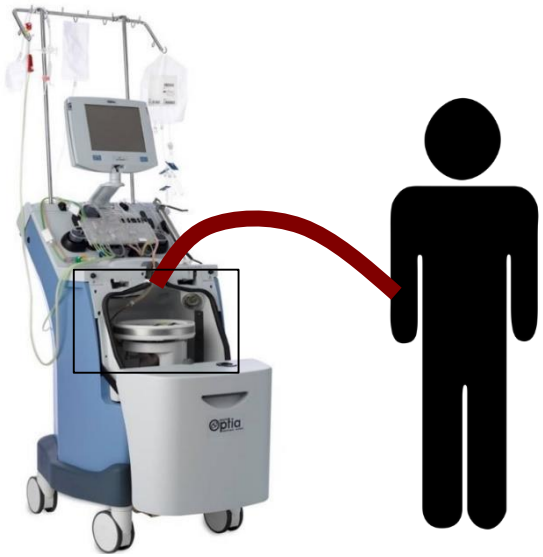


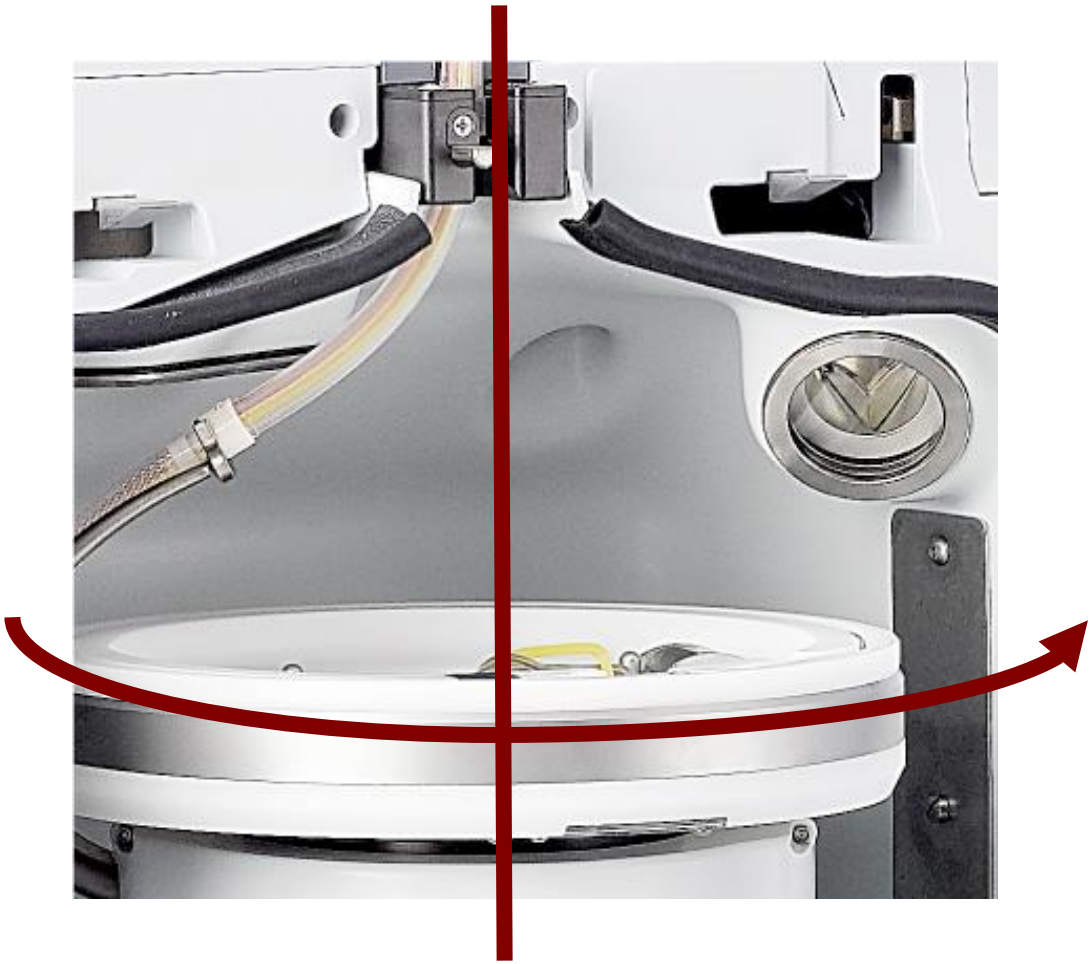
Plasma

T, B Lymphocytes

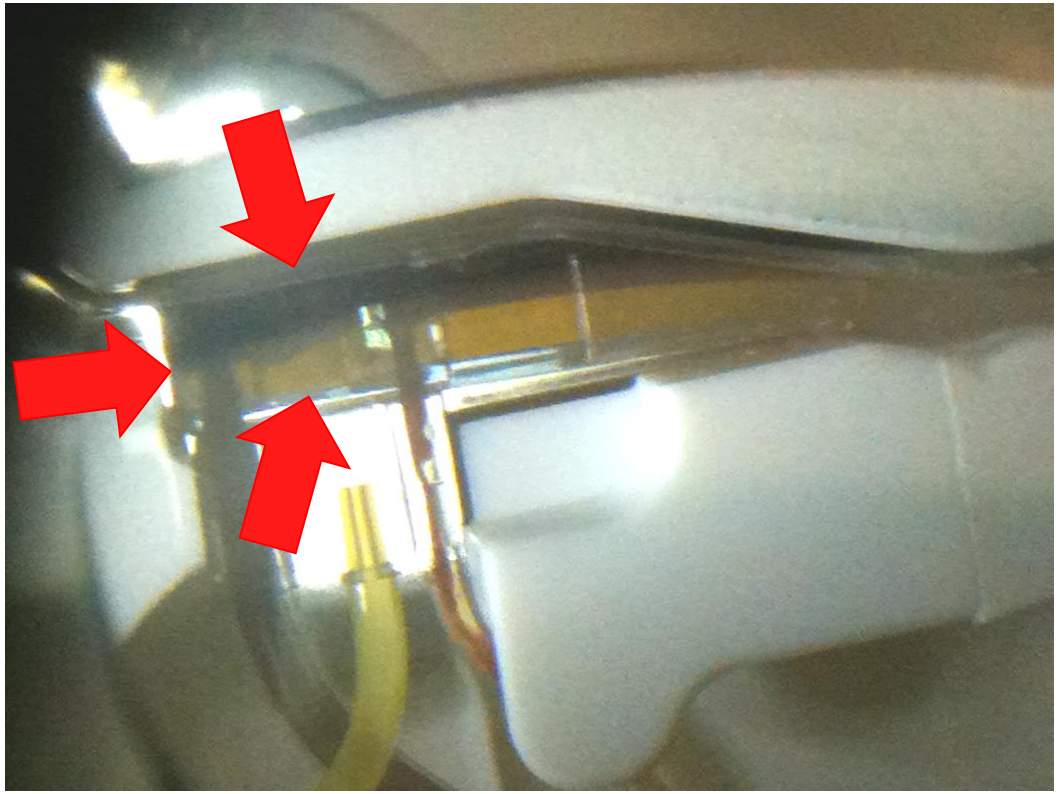
Red blood cells

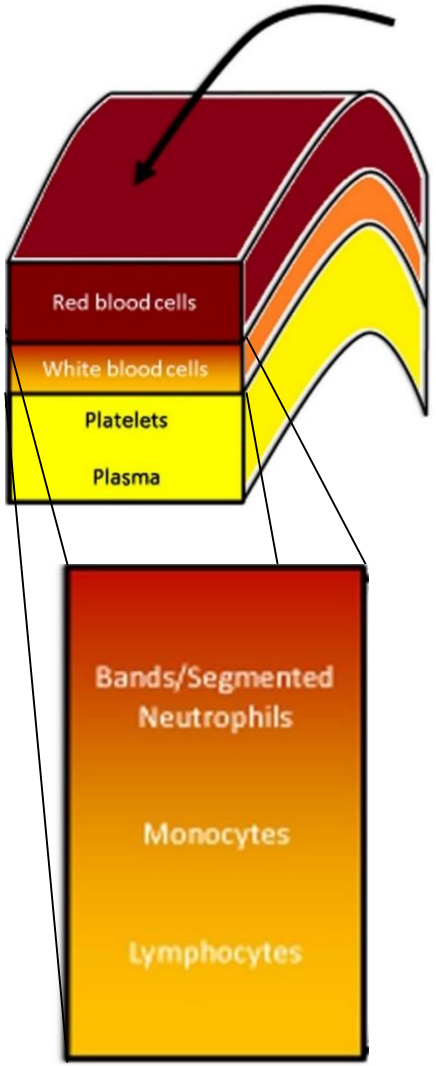
Mononuclear Cell Collection by Apheresis



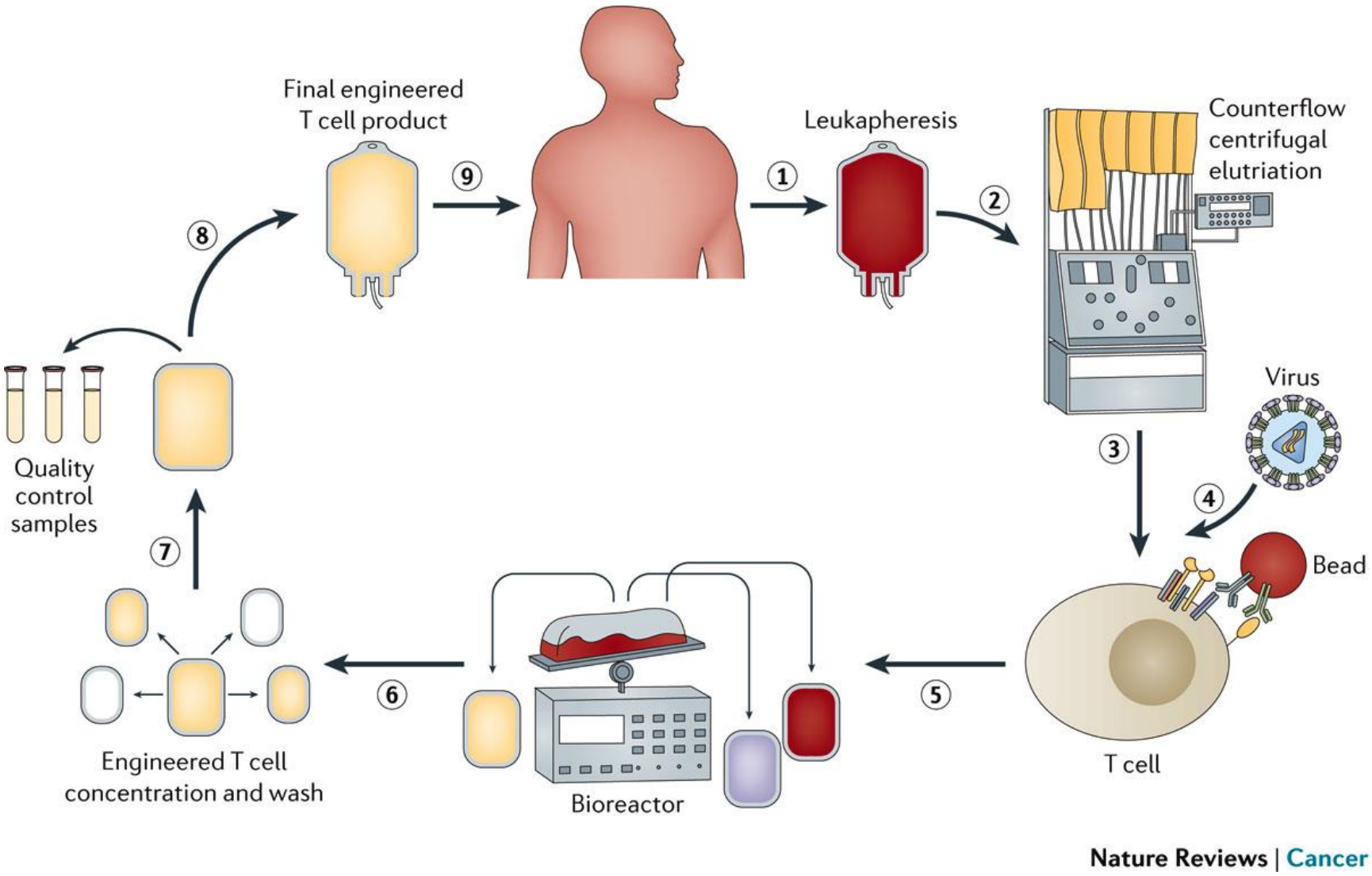


Mononuclear Cell Collection by Apheresis

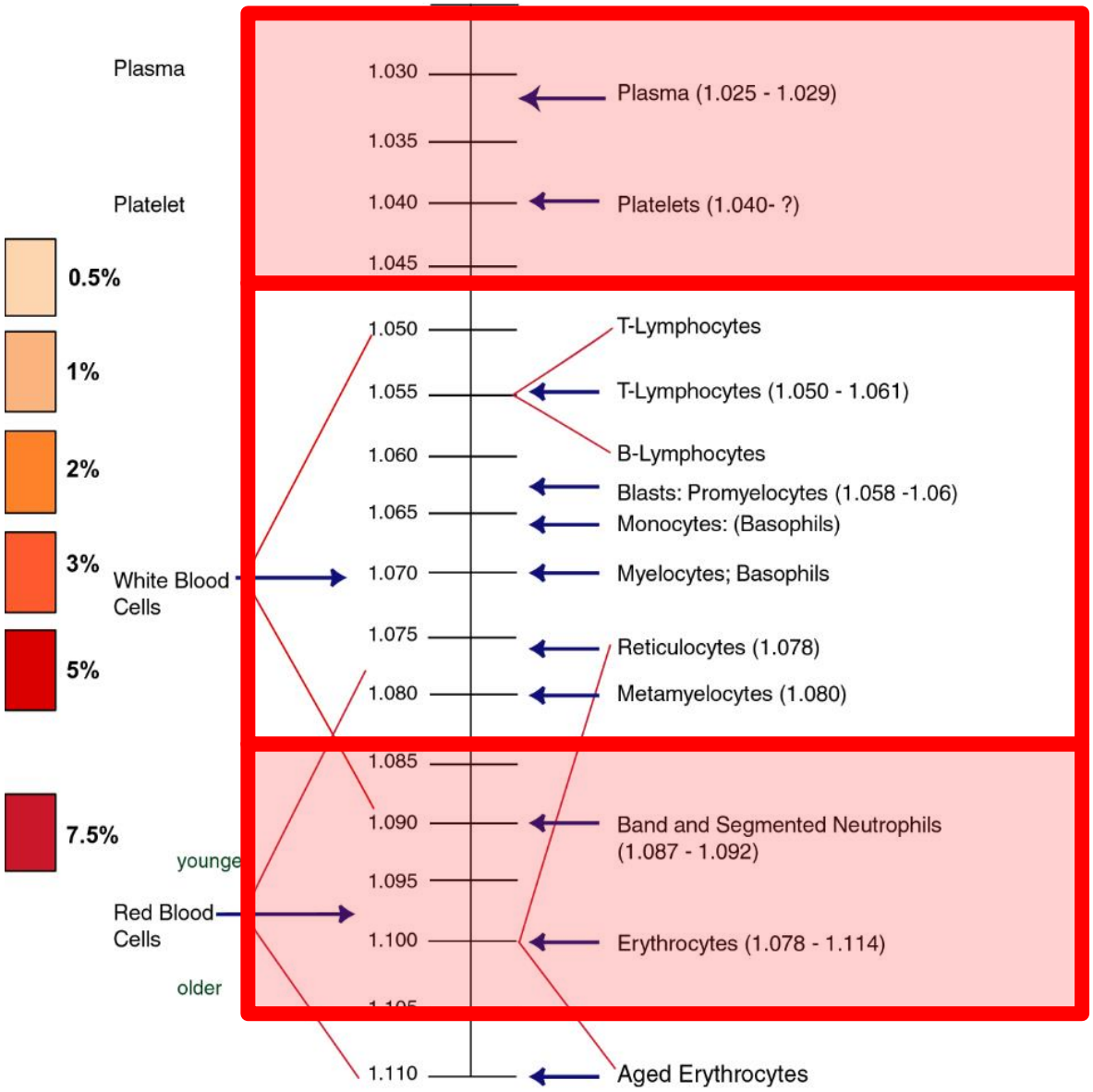




CART Manufacturing Begins with MNC Collection



Limited Ability of Apheresis to Resolve Cell Types

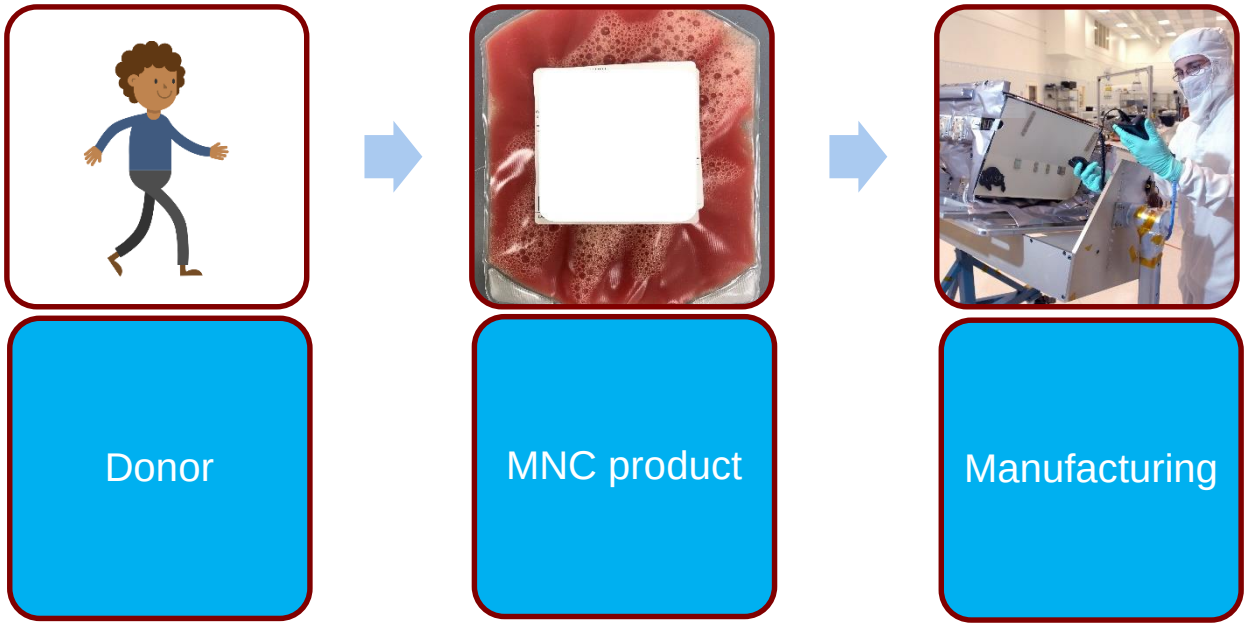


Non-T cell contaminants negatively impact manufacturing

- **Granulocytes suppress T cell proliferation, cytokine synthesis (Munder et al. 2006)**
- **RBCs T cell proliferation in vitro (Bernard et al. 2010)**
- **PLTs degranulate and can lead to clumping**
- **Blasts secrete soluble inhibitor of T cell proliferation (Orelans-Lindsay et al 2001) and suppress lymphocyte activation (Chiao et al 1986)**
- **Monocytes selectively induce apoptosis of activated T cells (Munn et al. 1996)**
- **Myeloid-derived suppressor cells are associated with poor T cell expansion (Leskowitz et al. 2017)**
- **High NK cell% in PB is associated with low CD3% and CD3 abs count in MNC product (Allen et al. 2017)**

Donor is Primary Driver of Manufacturing Variability

- **MNC products are a reflection of what is circulating in that donor during the collection.**
- **Donor variability drive MNC product variability which drives CART manufacturing variability.**



Characterizing CART manufacturing variability

Sources

- **Pre-collection**
  - Patient demographics
  - Clinical indication
  - Prior treatment
- **Collection**
  - Access type
  - Procedure duration
  - Procedure tolerance

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- **There many possible sources of variability prior to, at collection.**
- **Such variability can impact multiple downstream parameters.**

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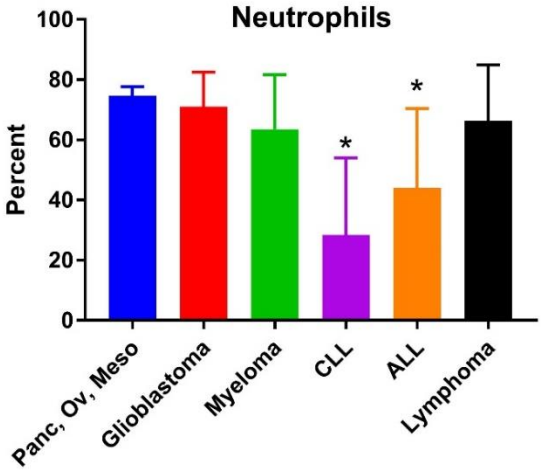
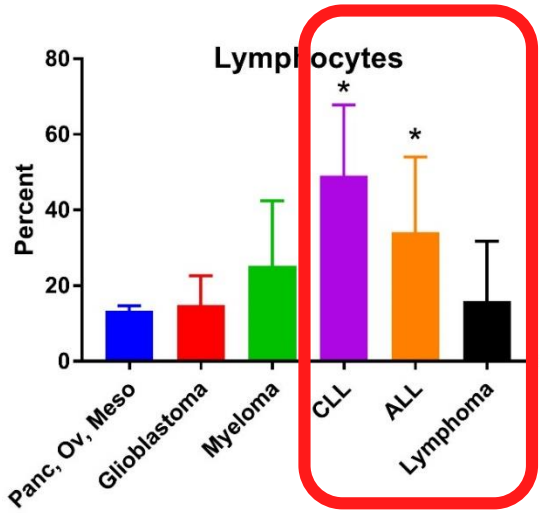
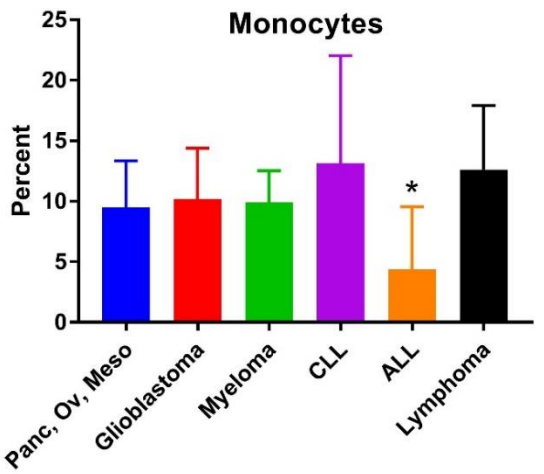
Peripheral Blood Counts Differ by Clinical Indication



Ref: 36-75%



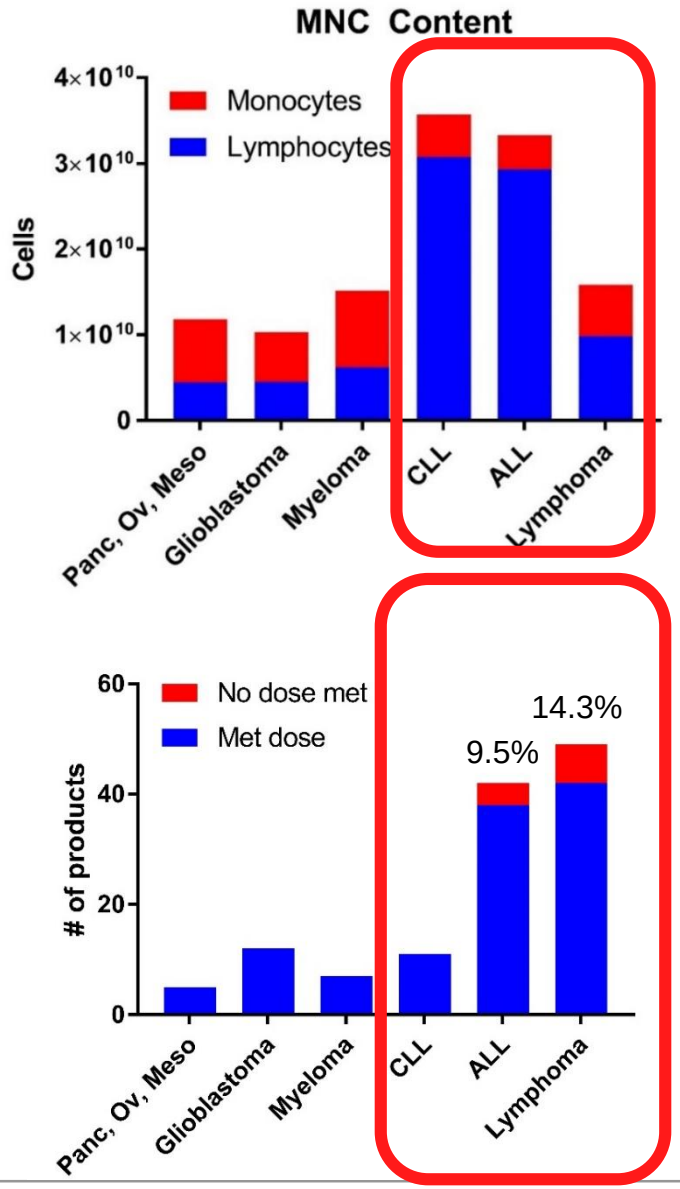
Ref: 20-50%

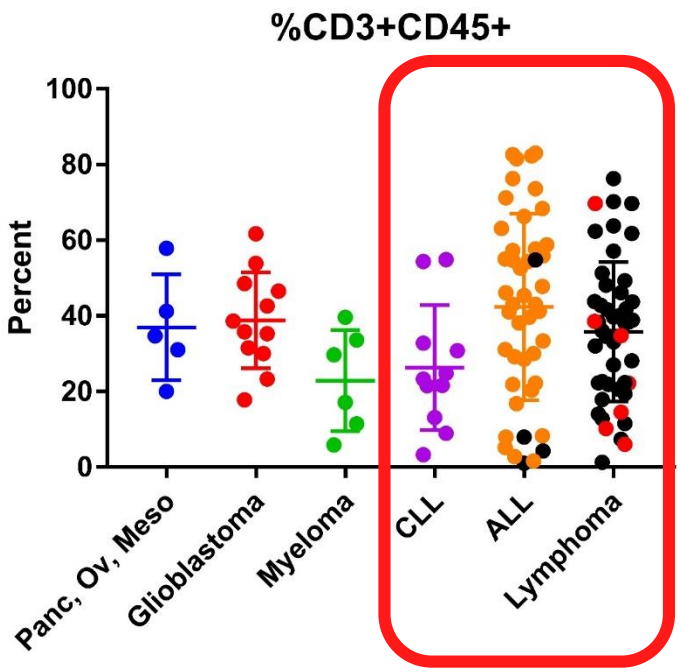


Ref: 3-10%

Reference ranges: http://www.med.umich.edu/digitallab/m2pathlabs

MNC Product Content, Success Rate Differ by Indication

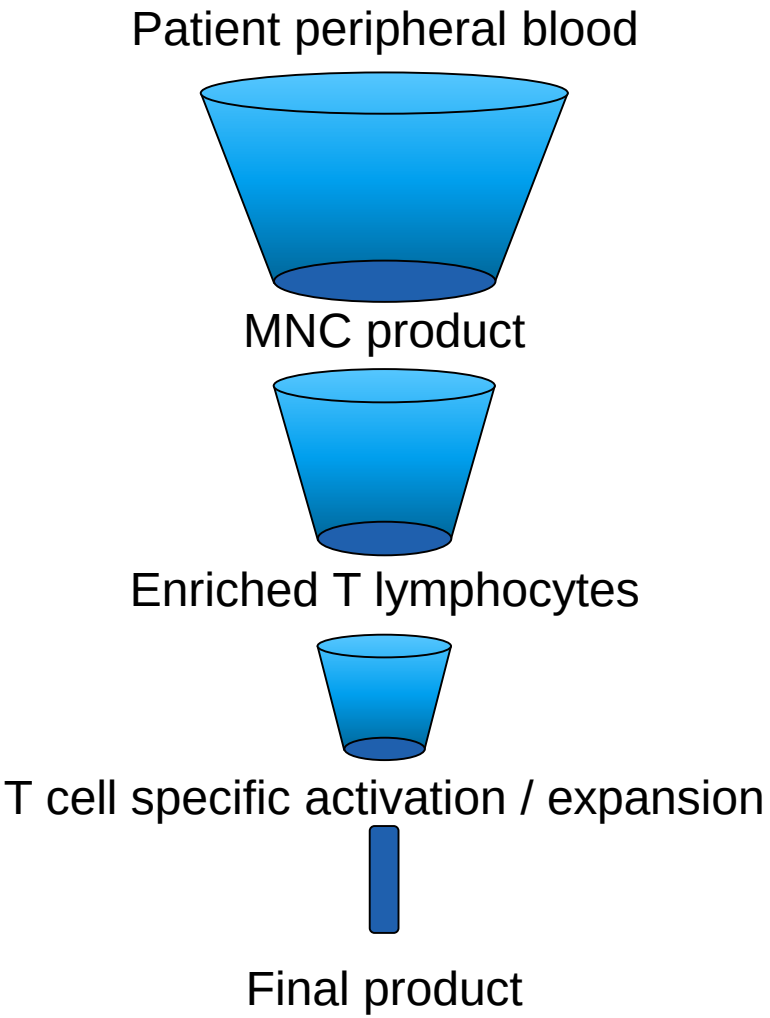




- **Because MNC product content varies by indication, impact on manufacturing success may be indication specific.**

Sequential reduction of variability throughout the process

- **<u>Variation makes standardization</u> challenging**
  - Stepwise reduction of variability throughout process
  - Effective, but inefficient and at times unpredictable



Limitations of current/future mitigation strategies

- **Too few T cells? ---> Collect longer**
  - Procedural intolerance
  - Diminishing returns of single collection

- **Too many non-T cells? ---> Better enrichment**
  - Limited GMP grade reagents/techniques available
  - Higher purity often associated with lower yield

- **Suboptimal T cells? ---> Collected earlier, allogeneic donor**
  - Infrastructure does not exist for prophylactic collections
  - Allogeneic CART risks GVHD, rejection

Summary

- **MNC products are a snapshot of the donor**
- **Donor -> MNC product -> CART manufacturing**
- **Many sources impact many downstream parameters**
  - eg. MNC product content differs by indication and may lead to different manufacturing success rates.
- **Sequential processing is effective, inefficient at reducing variation**
- **Variation mitigation strategies are limited but evolving**

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