



T Cell Collection for CAR T-Cell Therapy

*Hans Vrielink, MD, PhD
Sanquin Blood Supply Foundation, The Netherlands*



h.vrielink@sanquin.nl

For Life.

Disclosure of COI

The scientific planning committee (SPC) :

has reviewed all disclosed financial relationships of speakers, moderators, facilitators and/or authors in advance of this CPD activity and has implemented procedures to manage any potential or real conflicts of interest.

The SPC members

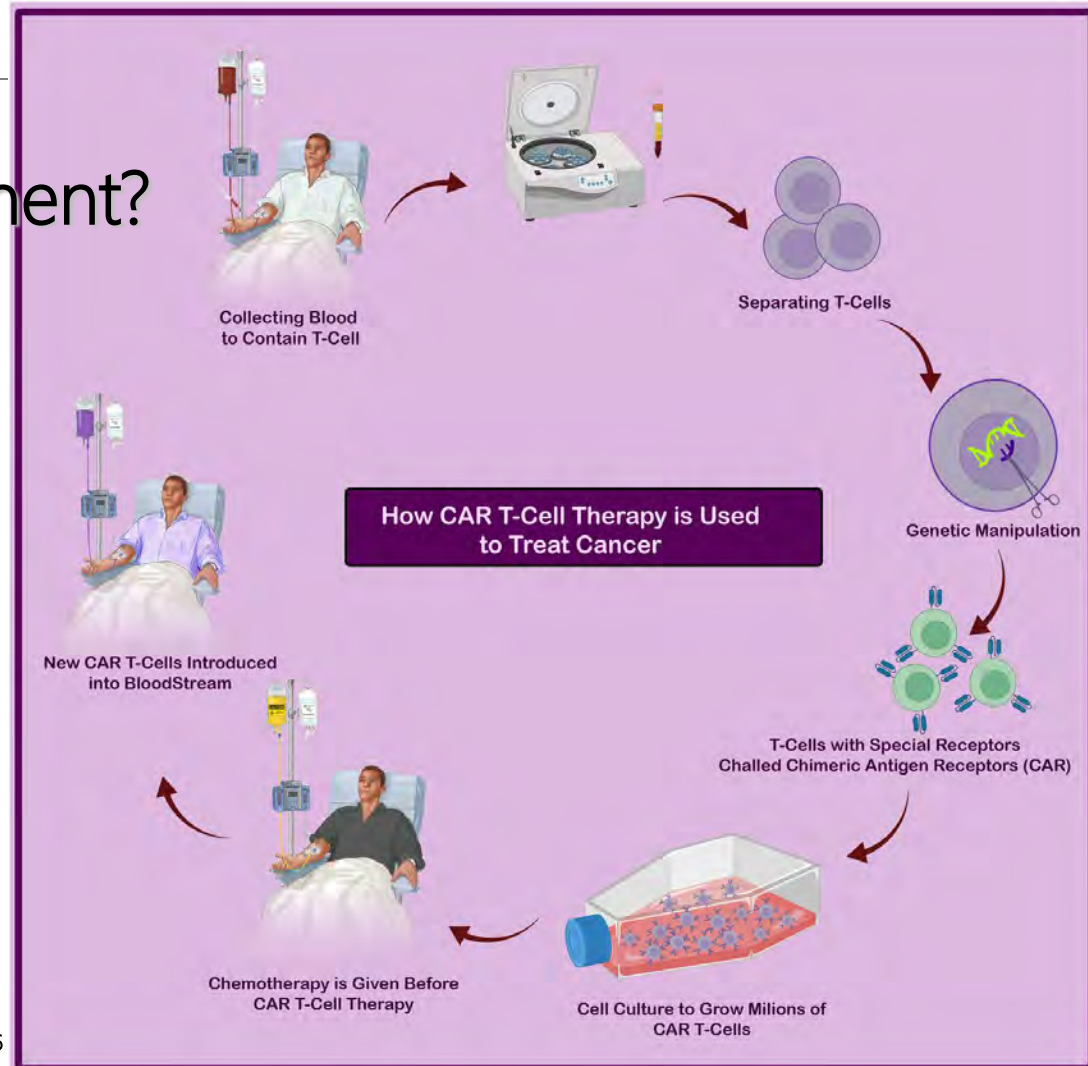
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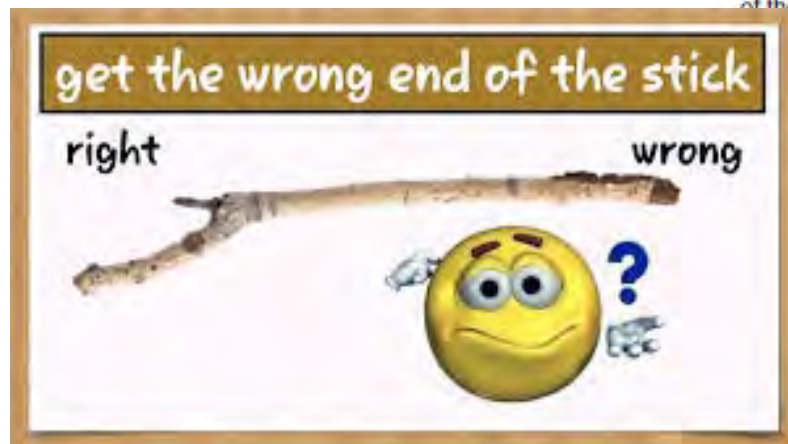
Disclosure

No conflict of interest / disclosure to make for this presentation

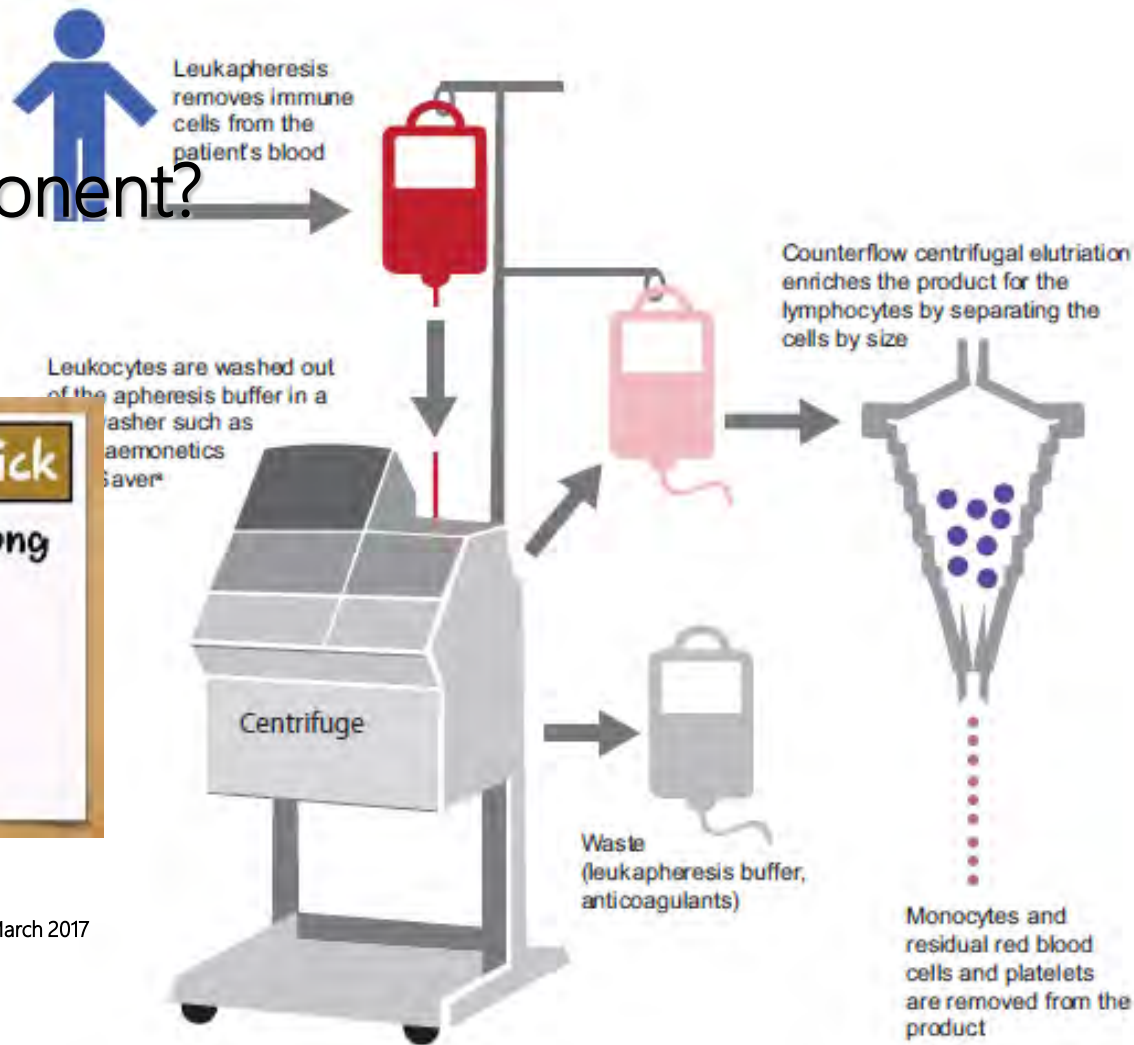
Collection right component?

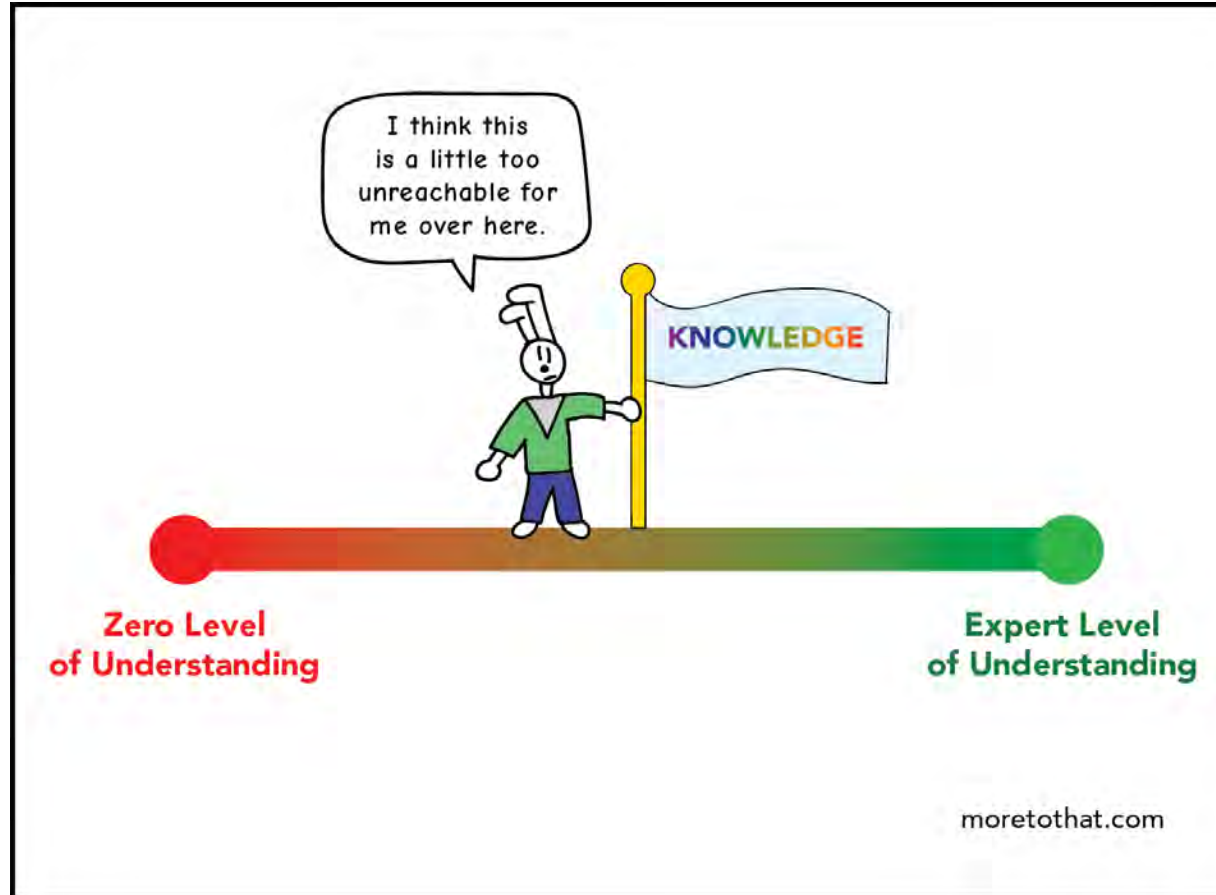


Collection right component?

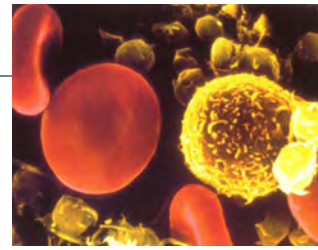


Levine, et al. Molecular Therapy: Methods & Clinical Development Vol. 4 March 2017





- Clinic
- Collection centers
- Processing facilities
- Manufacturers of specific products

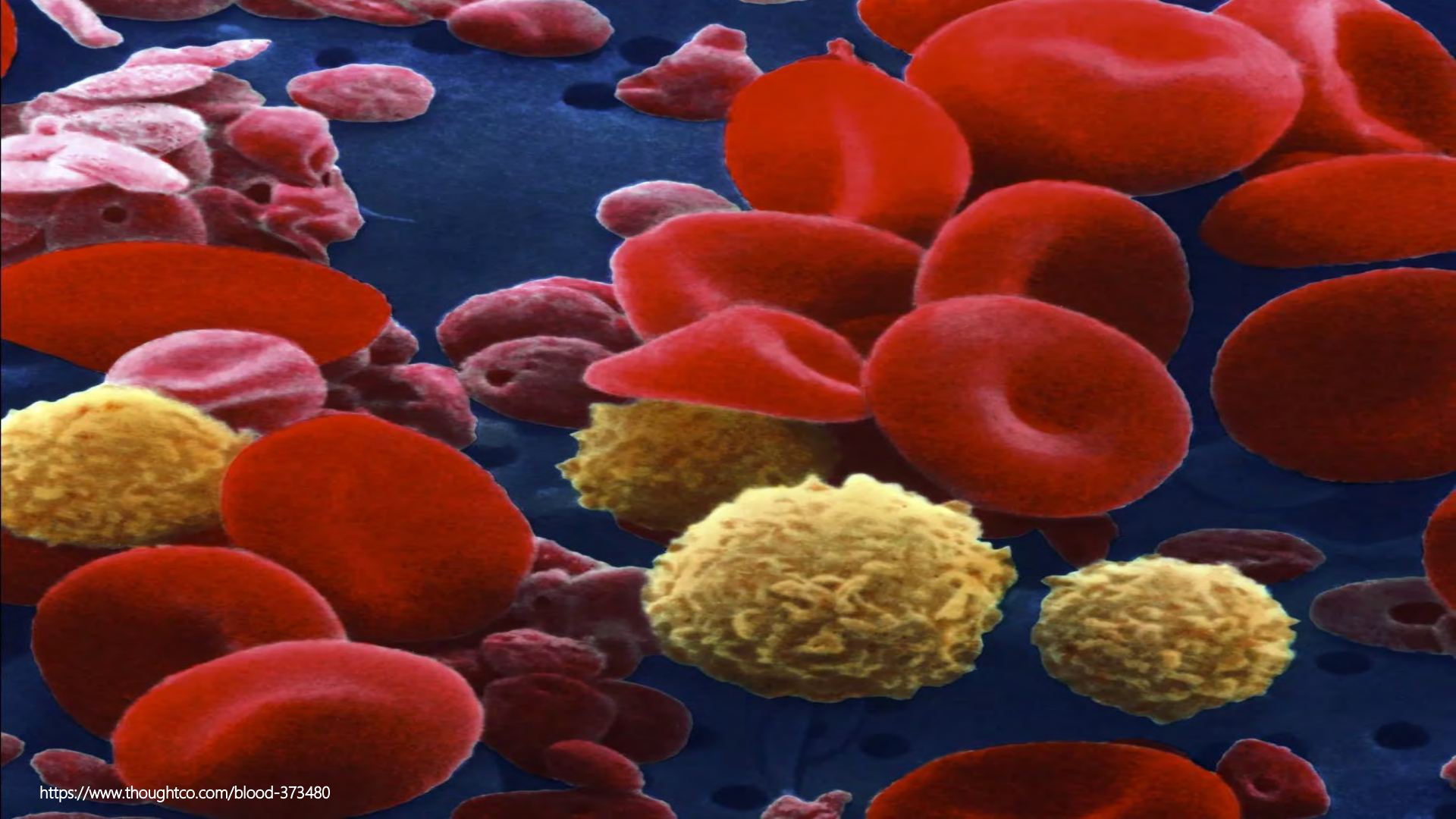


Apheresis

Method of obtaining one or more blood components by machine processing of whole blood in which the residual components of the blood are returned to the donor / *patient* during or at the end of the process.

Reference
Guide to the preparation, use and quality assurance of blood components
European committee on Blood Transfusion
2023-21th Ed.







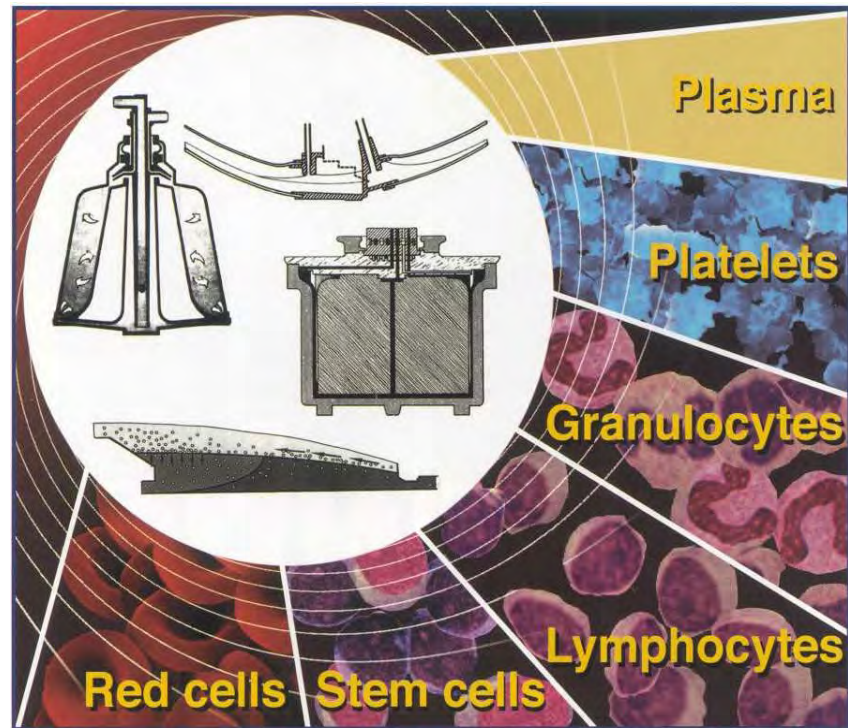
Apheresis of blood components

Plasmapheresis

Cytapheresis

- Platelet apheresis
- Lymphocyte apheresis
- Stem cell (HPC) apheresis
- Monocyte apheresis
- Granulocyte apheresis
- Erythrocyte apheresis

Multicomponent apheresis





All blood cells are different

	Specific weight	Size
Plasma	1.026	
Platelets	1.040	1-4 μm
Lymphocytes	1.050-1.061	6-10 μm
Monocytes	1.077	10-30 μm
Basophils	1.080	10-15 μm
Eosinophils	1.082	9-15 μm
Neutrophils	1.088	12-15 μm
Erythrocytes	1.100	6-8 μm

Apheresis equipment

Fresenius Kabi
Haemonetics
Terumo BCT

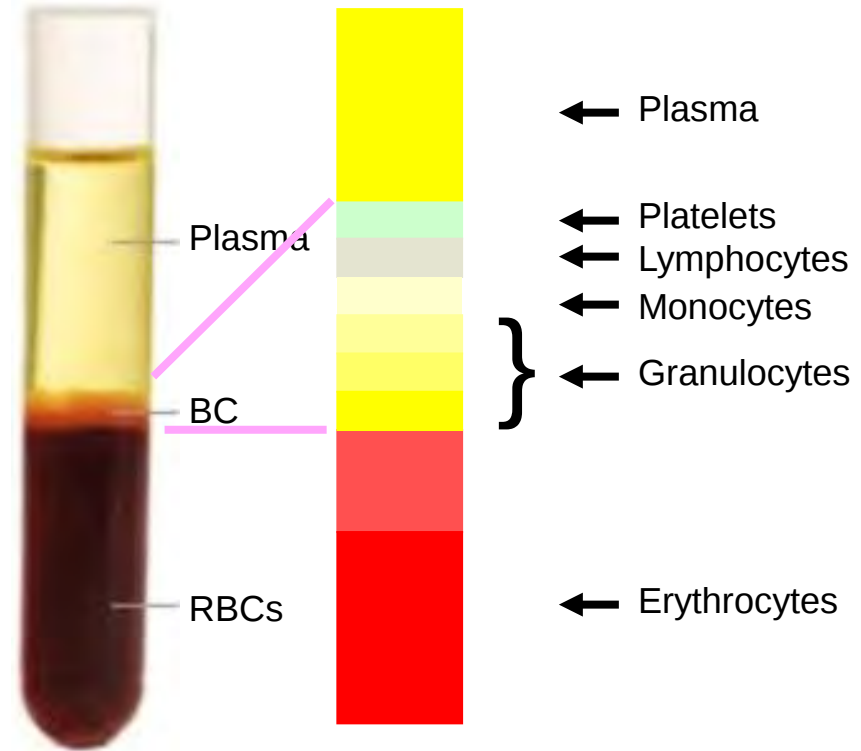


Centrifugal techniques to
collect the cells



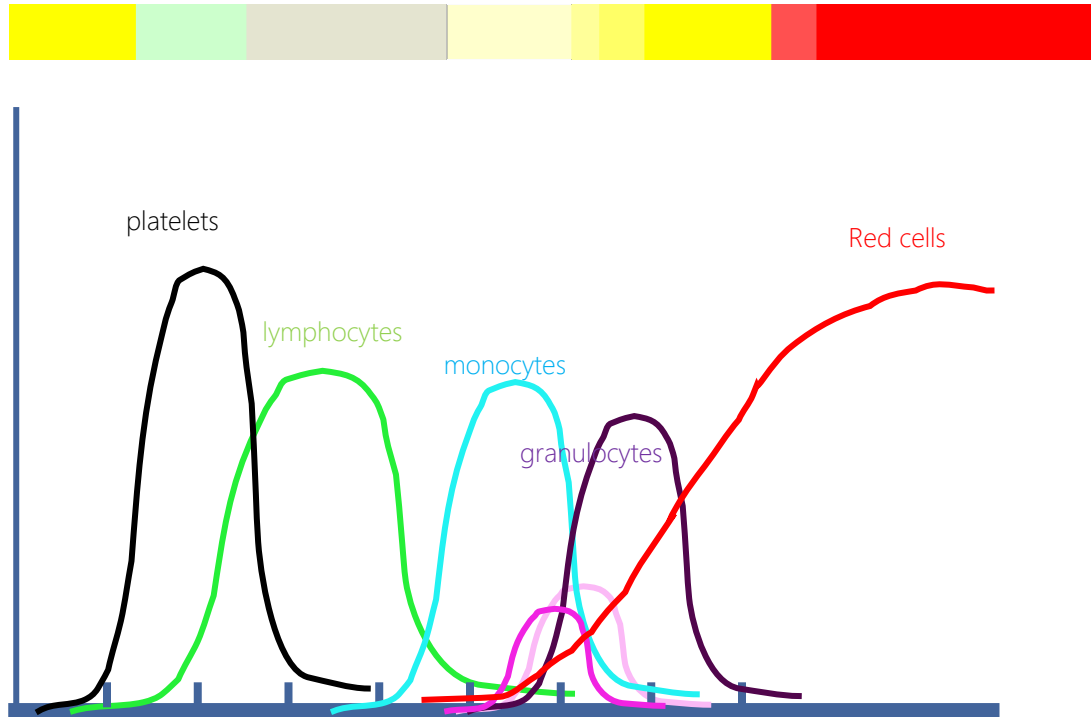
Separation in the centrifuge

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Plasma	1.026
Platelets	1.040
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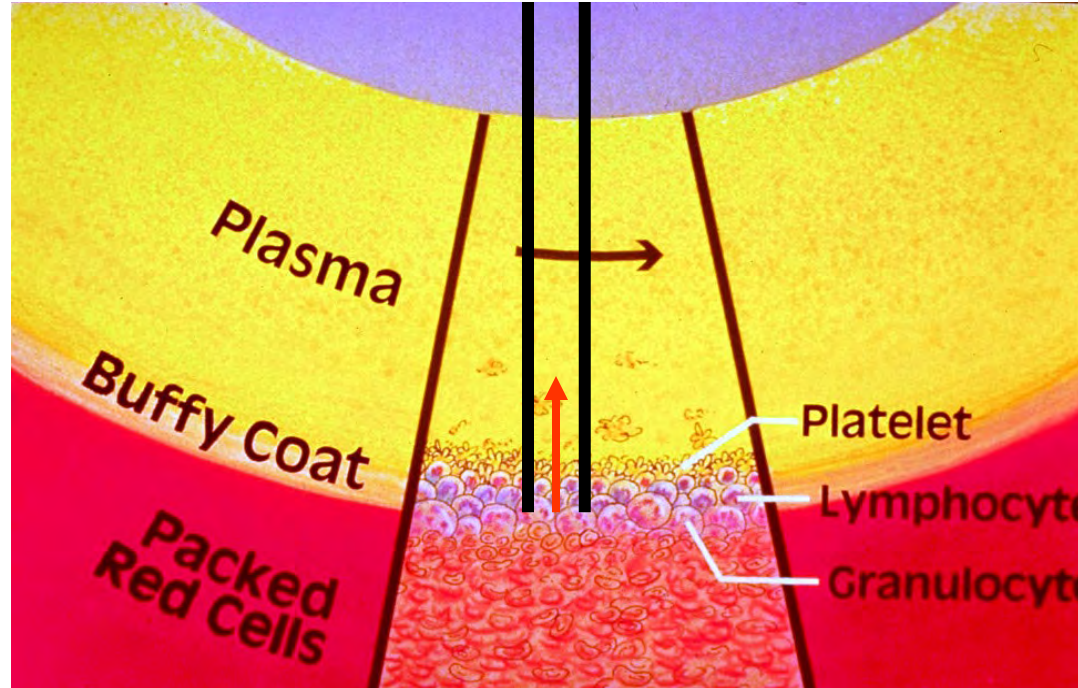
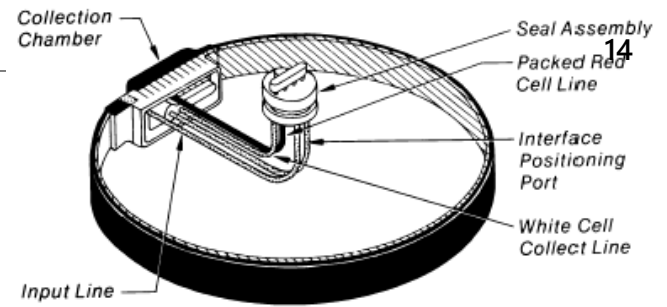


Centrifugal techniques



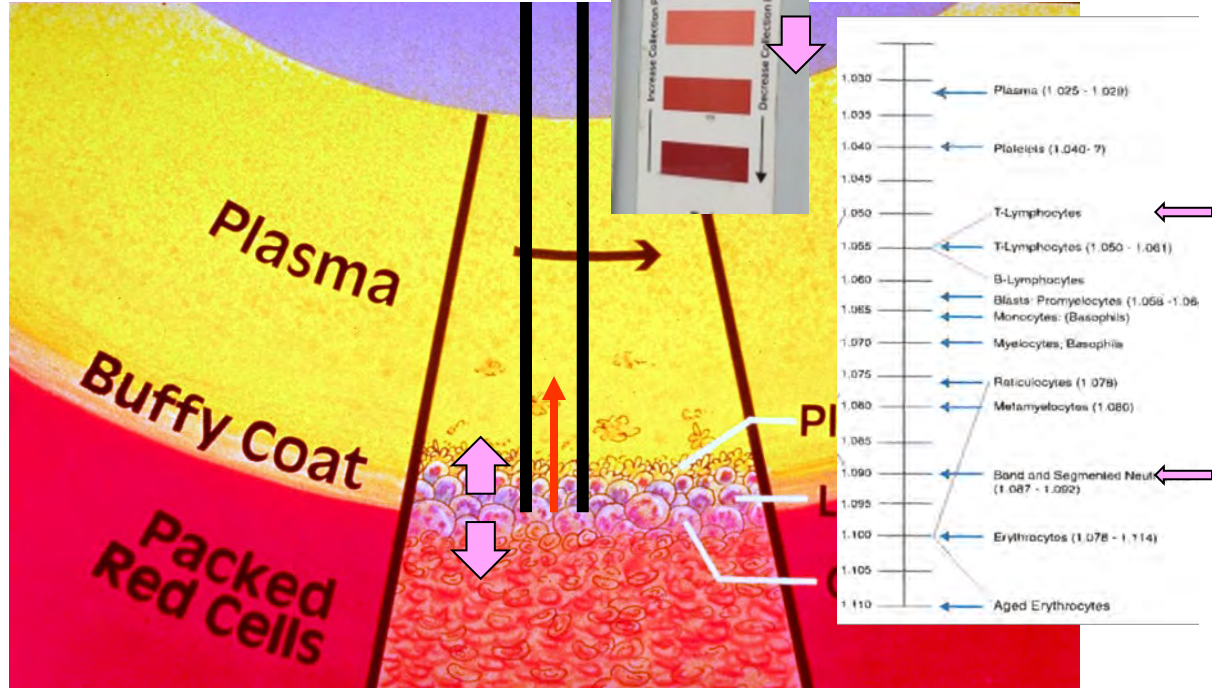
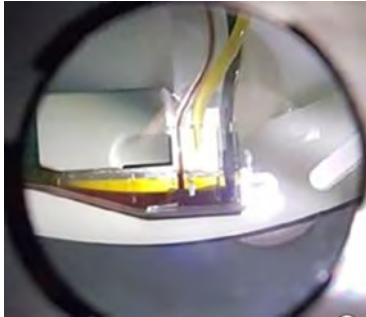


Separation in centrifuge belt



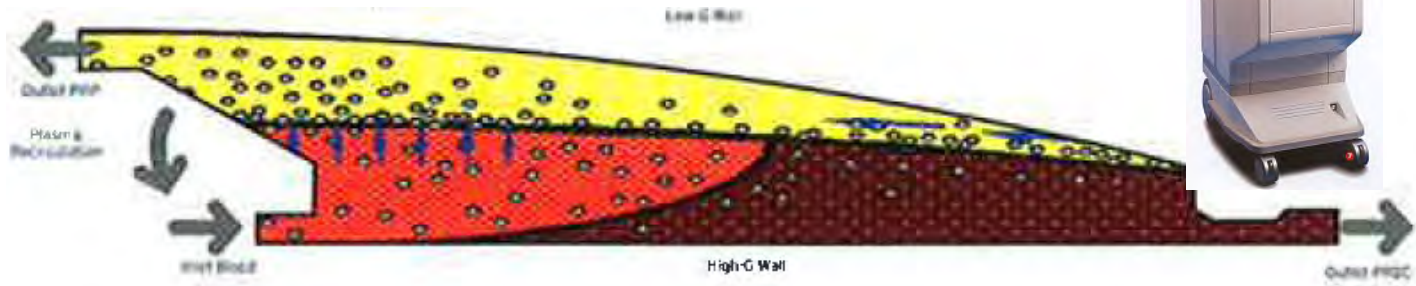


Separation in centrifuge belt





Separation in Amicus / Amicor system





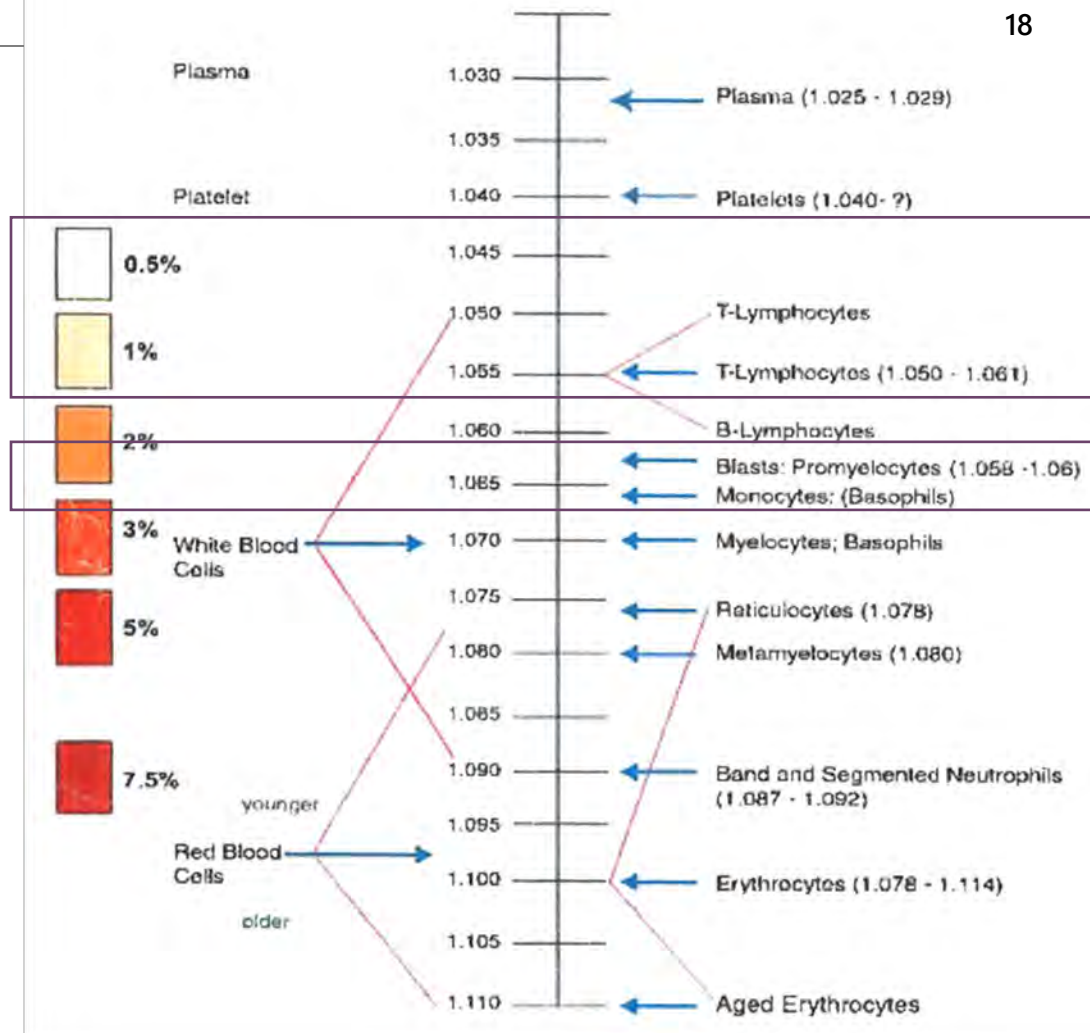
Collection of cells for cellular therapies – MNCs

CD34+ Hematopoietic progenitor cell transplant

CD3+ Source for CAR-T cell therapy

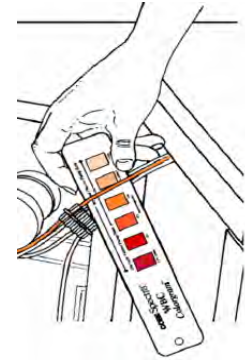
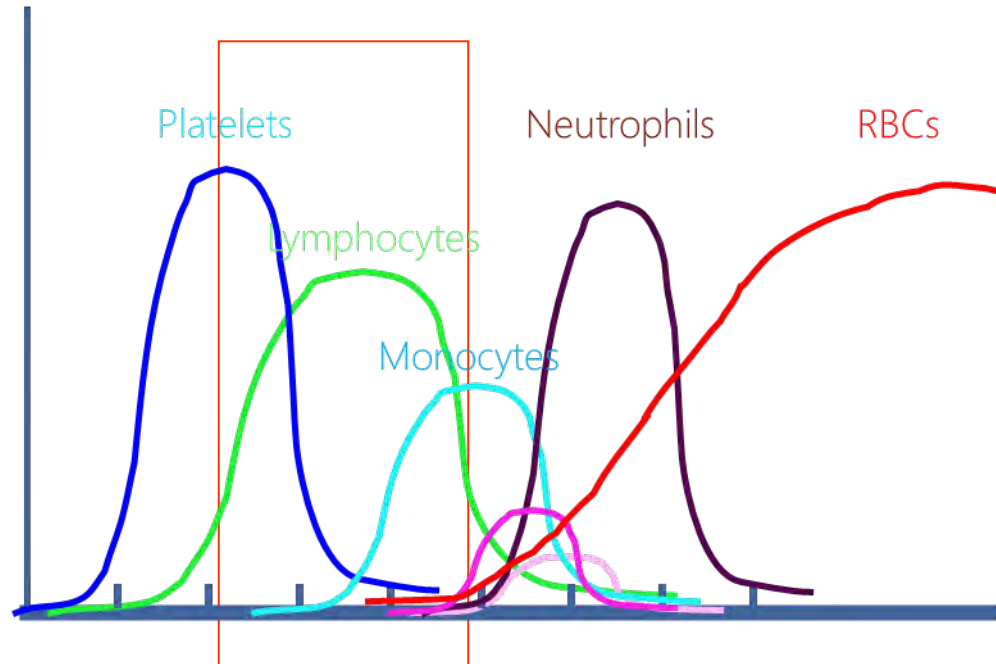
CD3+ cells

CD34+ cells





MNC collections → T – cells / CD3+ cells

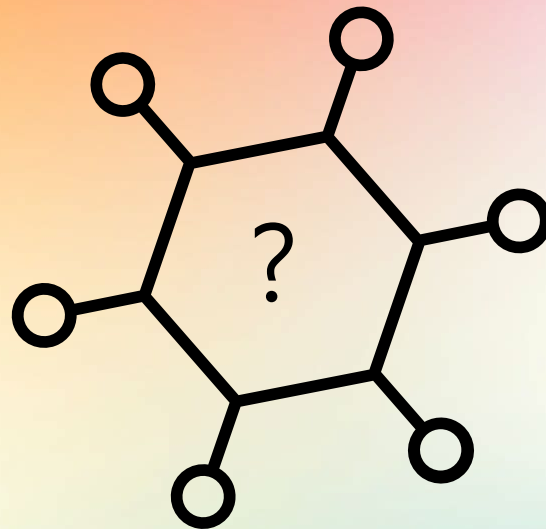


Color 0.5-1.5



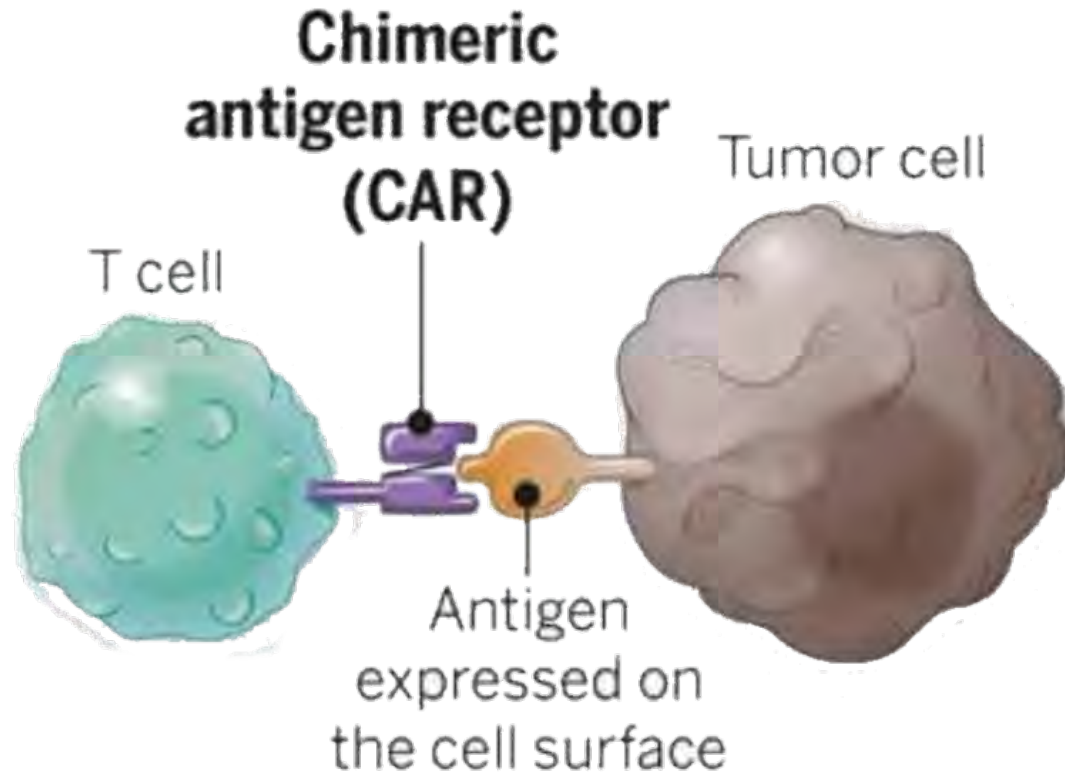
Apheresis prescription to get the right cell

- What cell?
 - ✓ CD3+
 - ✓ ~~CD4+~~
 - ✓ ~~MD~~
 - ✓ ~~TNC~~
- What amount?
 - ✓ $10^6/\text{kg}$ or $10^9/\text{unit}$
 - ✓ ~~liters or x times TBV~~
- Which concentration?
 - ✓ Concurrent plasma collection



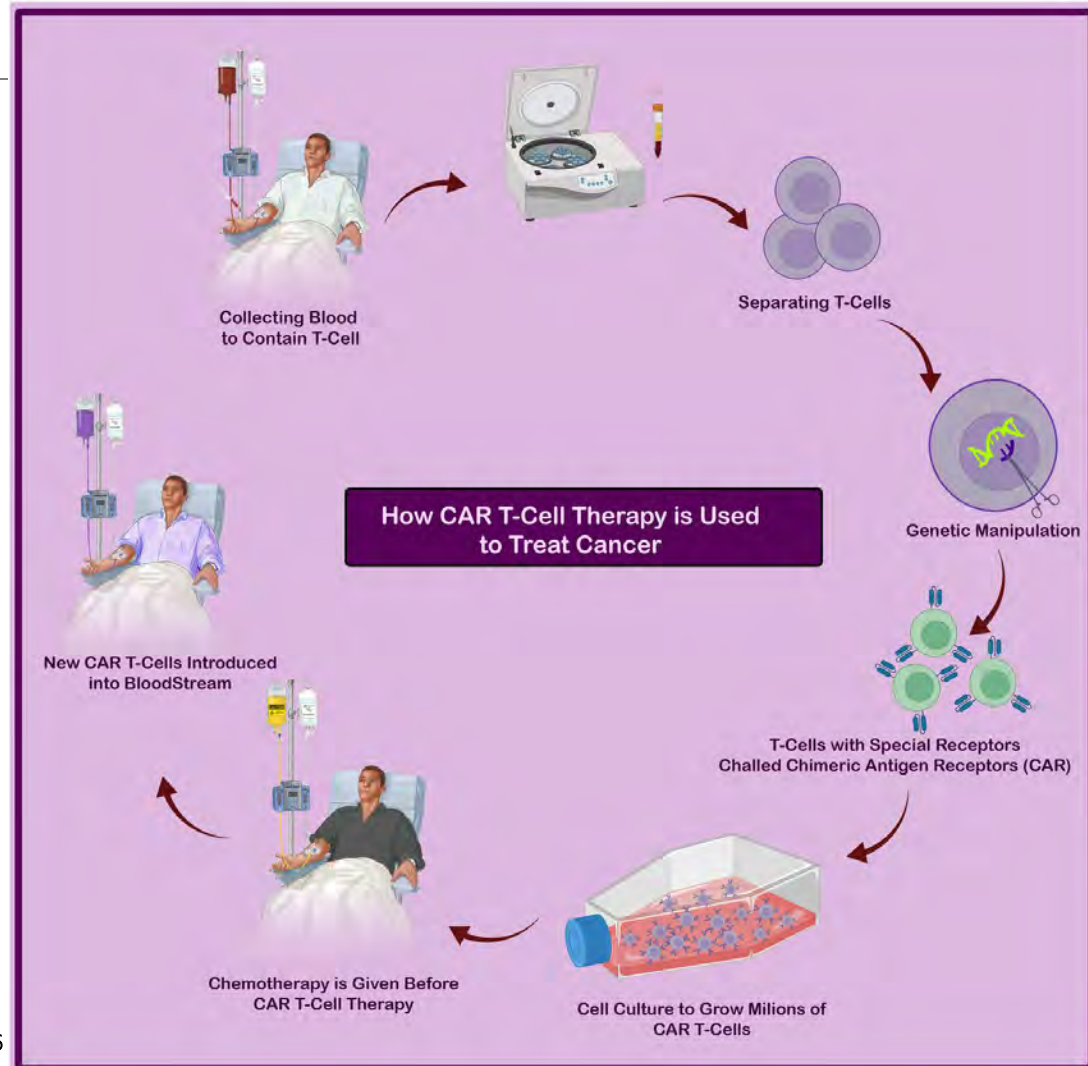


CAR-T cells



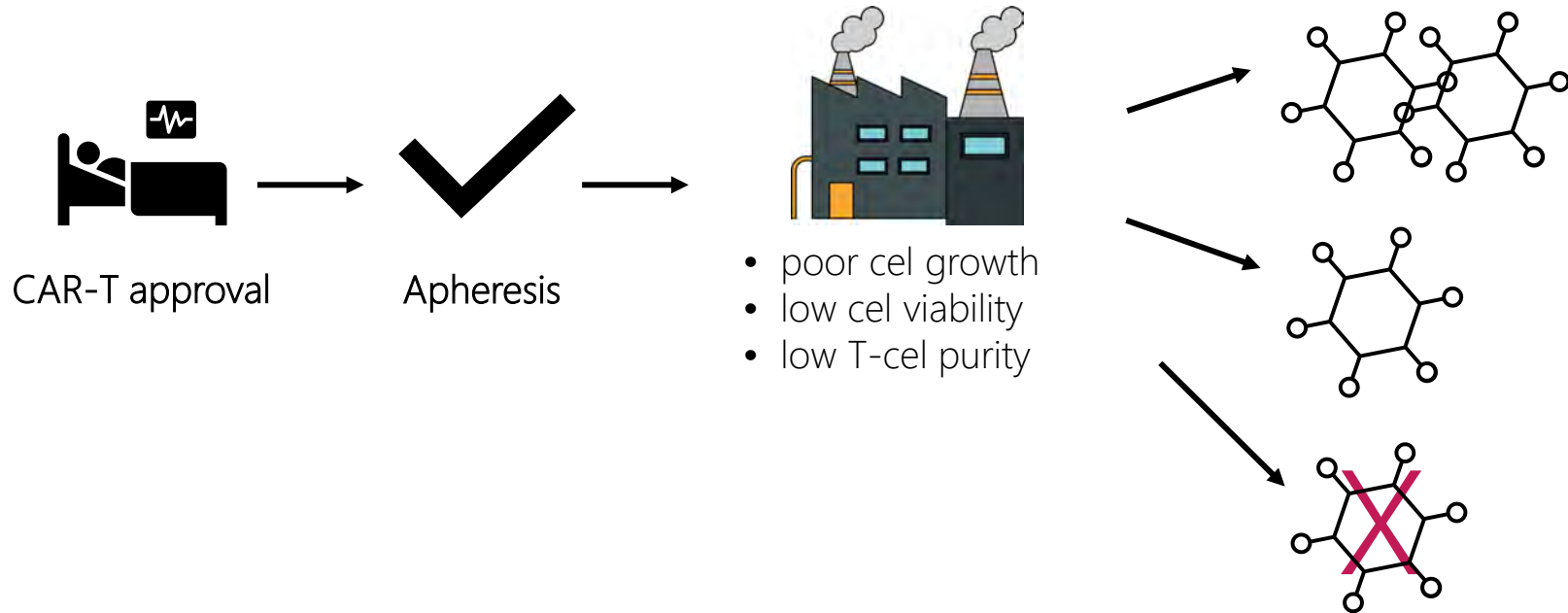
CAR-T cell process

- Process of days - weeks





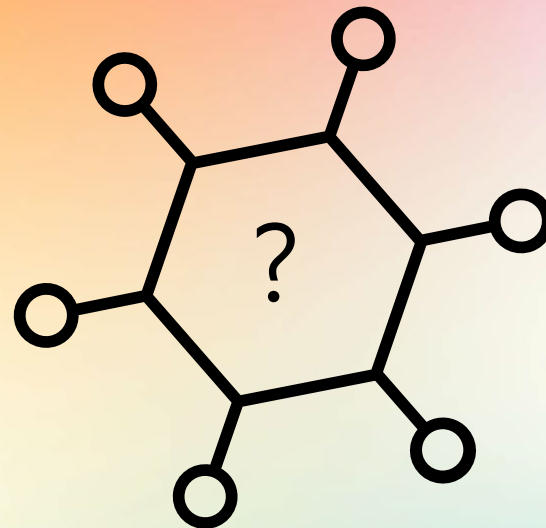
Risk Factors for CAR-T manufacturing failure



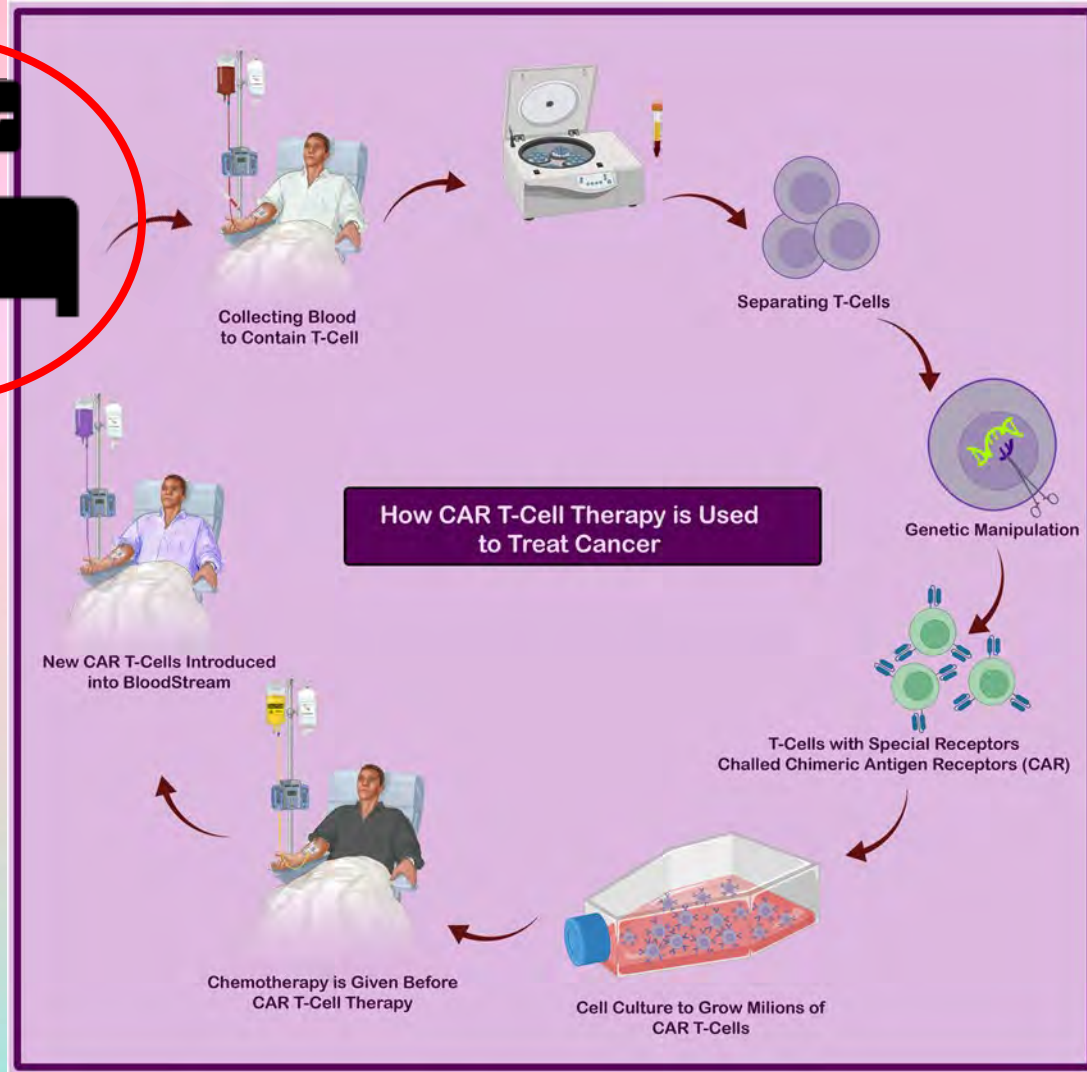


Apheresis prescription to get the right cell

- What cell?
 - ✓ CD3+ (no need of mobilization)
- What amount?
 - ✓ 3×10^9 /unit
- Which concentration?
 - ✓ 100 mL Concurrent plasma



Right cell? quantitative

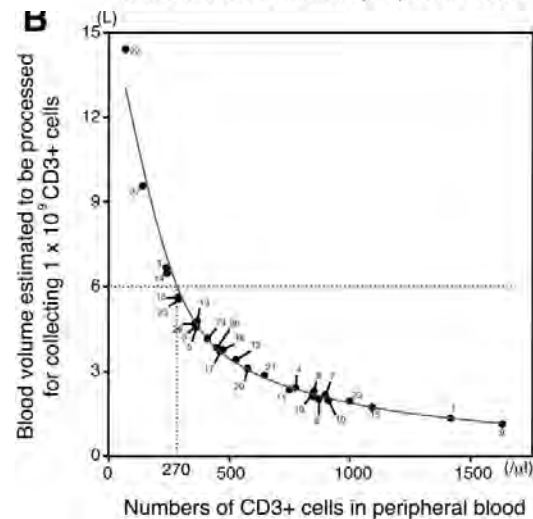
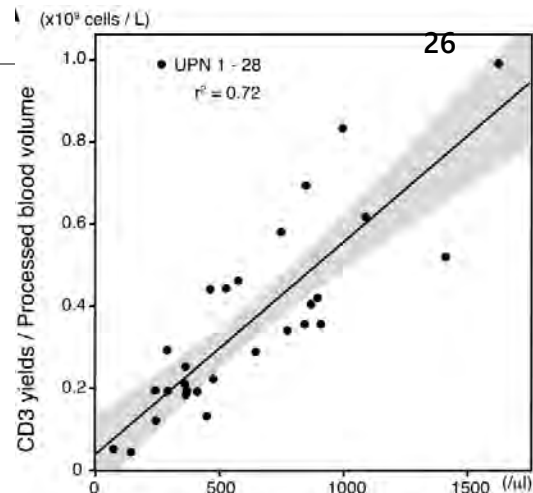




Factors that affect CD3+ yield



Parameters	Log Worth		p value
CD3 numbers in PB	2.288		**0.00515
Flow rate	0.541		0.28775
Processed BV	0.516		0.30506
Weight	0.476		0.33398
% Lymphocytes in PB	0.441		0.36201
White blood cells in PB	0.414		0.38584
Age	0.393		0.40480





Lymphocytes

T-lymphocytes

B-lymphocytes

Normal range

Children 3,000 - 9,500 / μL

Adults 1,000 - 4,800 / μL



Sangui

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T-lymphocytes

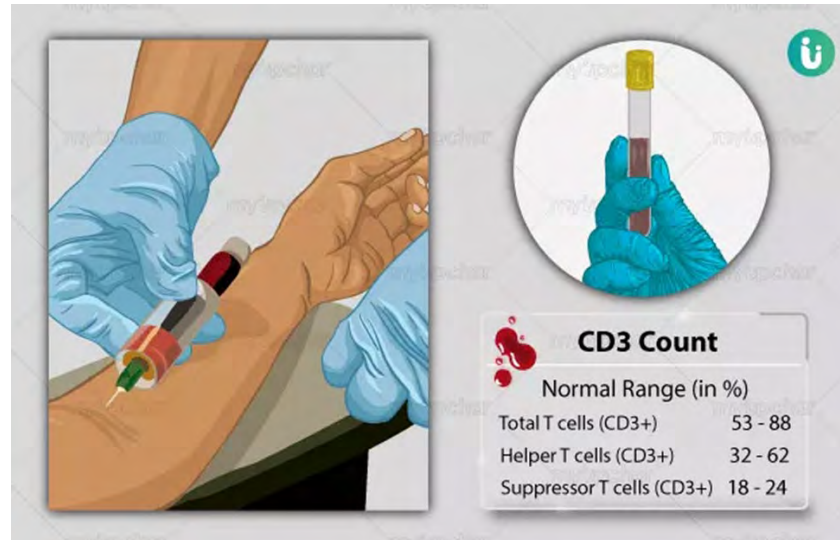
- T-helper (CD4+ve) → activate and regulate B-cells
- CD8+ve → viruses and tumor cells
- $\gamma\delta$ T-cell → first line of defense
- Regulatory T-cells → prevents autoimmunity and back regulation to normal
- Natural killer cells → viruses and tumor cells

*CD3+ cells are all T-lymphocytes, which includes both
CD4+ and CD8+ lymphocyte cells*



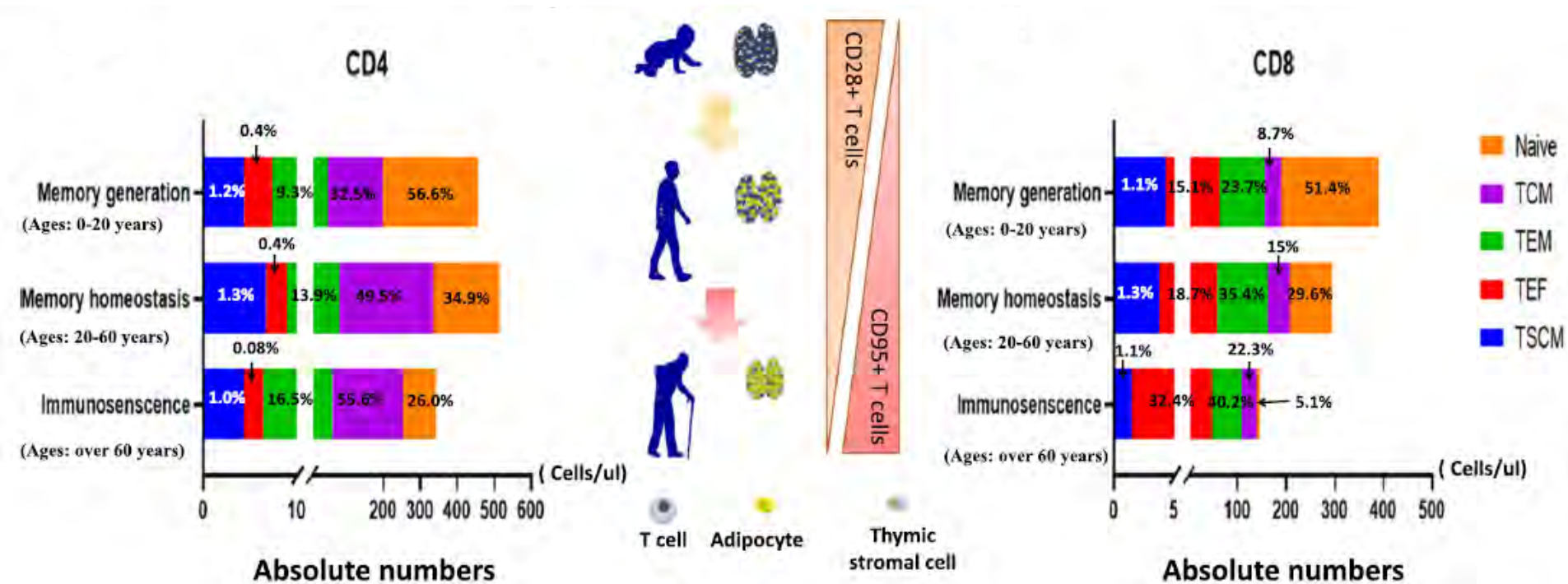
Percentage of lymphocytes of children and adults = CD3+

Population	0-3 m	3-6 m	6-12 m	1-2 yr	2-6 yr	6-12 yr	12-18 yr	>18 yr
	53-84	51-77	49-76	53-75	56-75	60-76	56-84	50-84





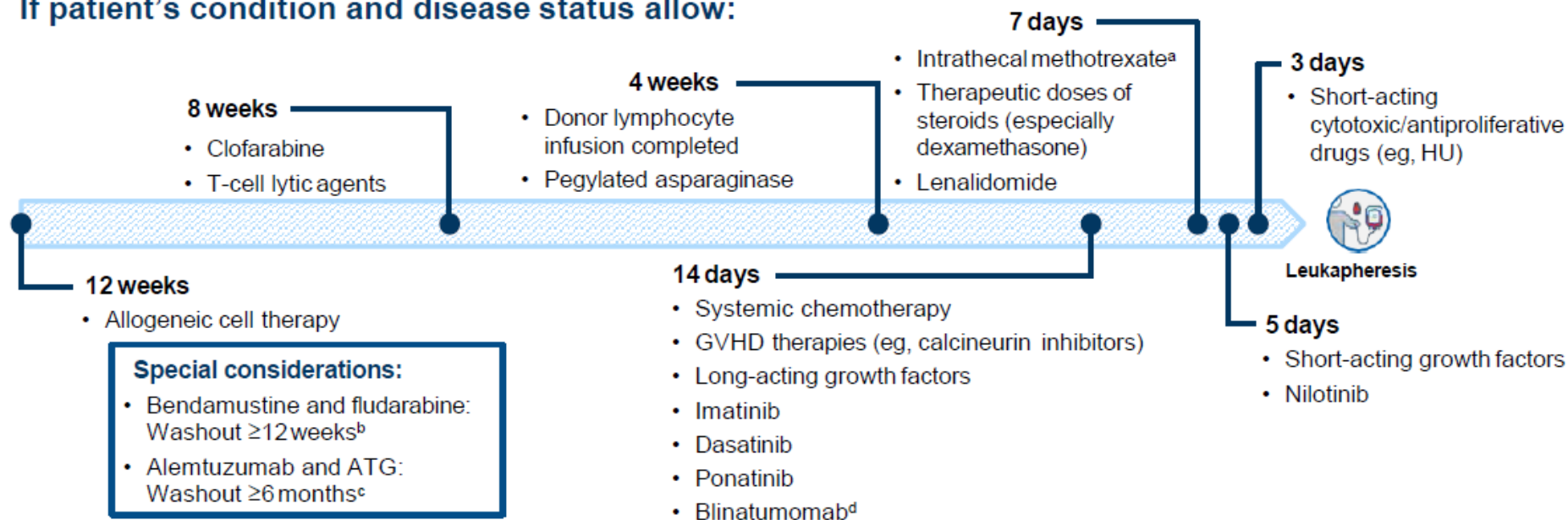
CD3+ cell count - Age



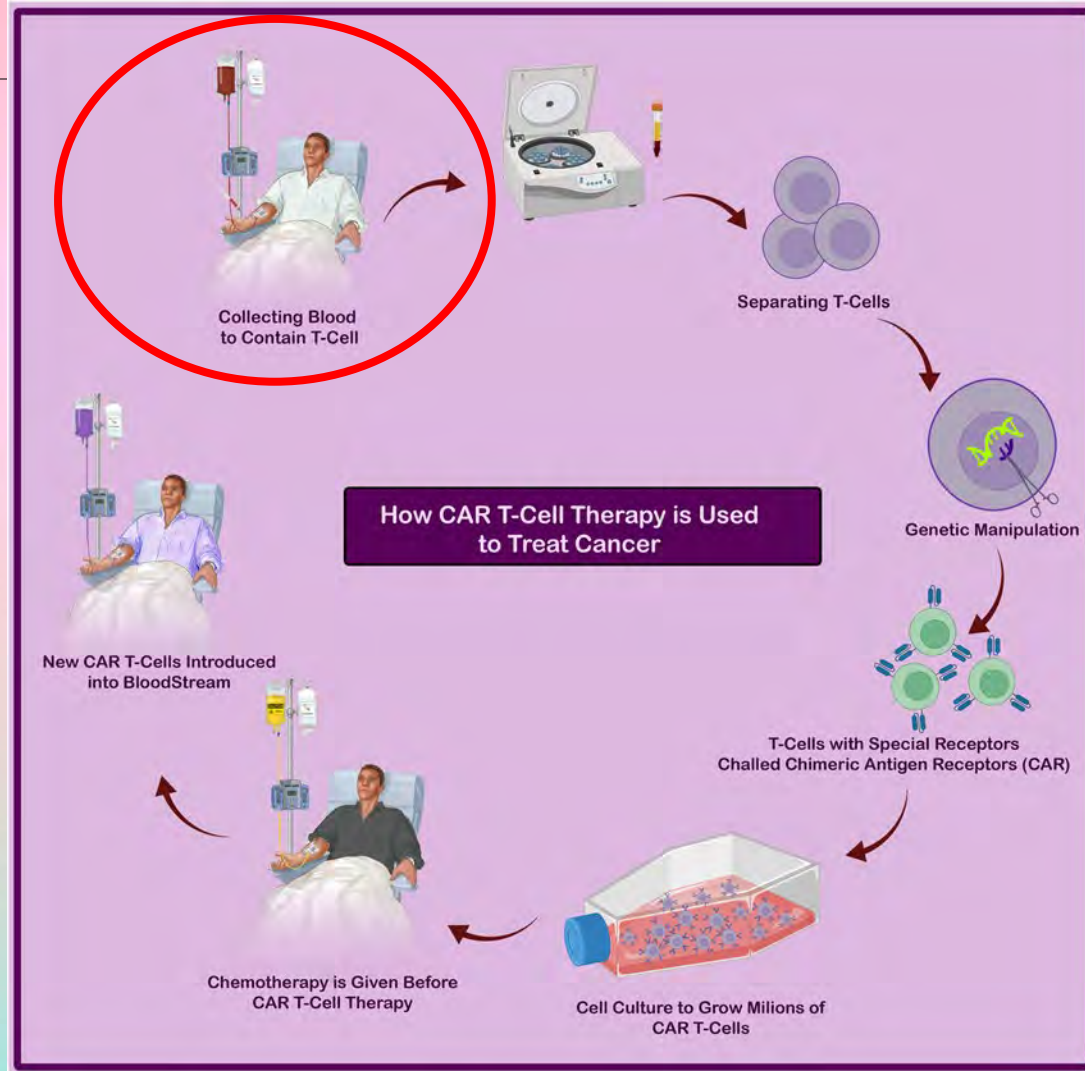


CD3+ cell count - medication prior to apheresis

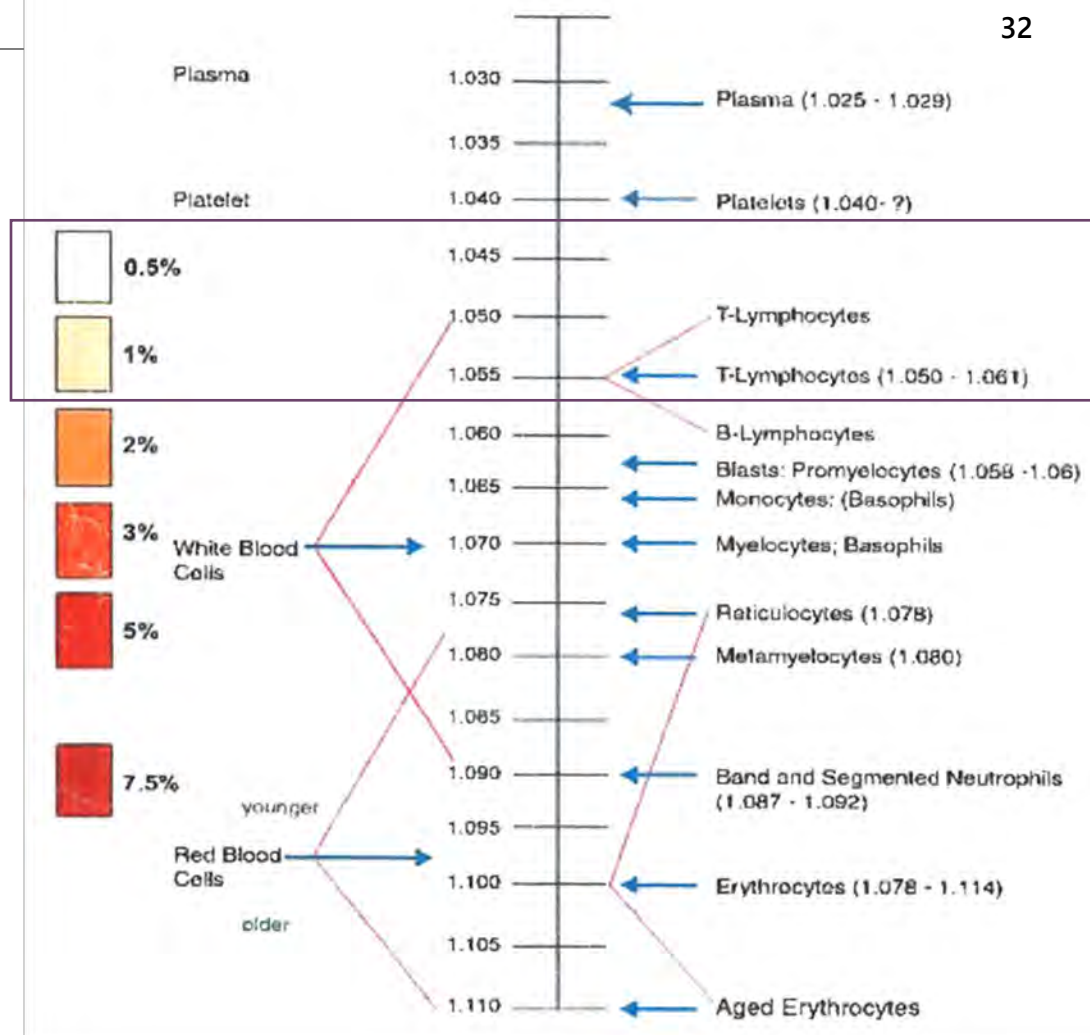
If patient's condition and disease status allow:



Right cell? qualitative



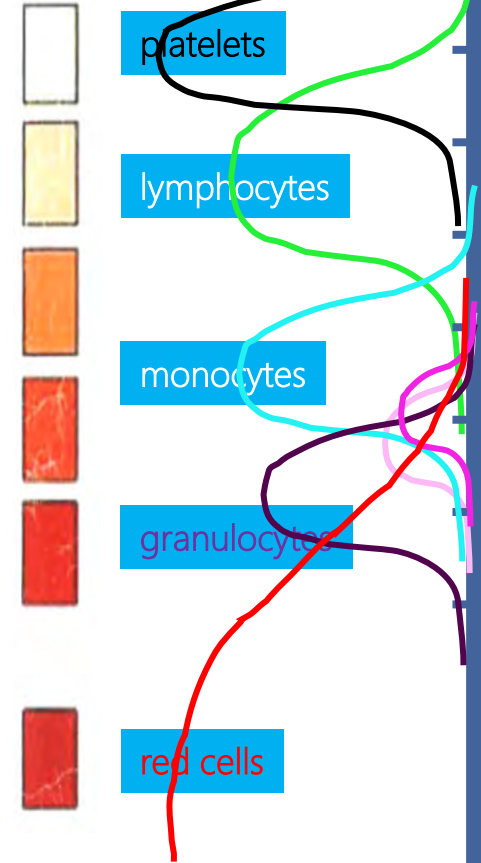
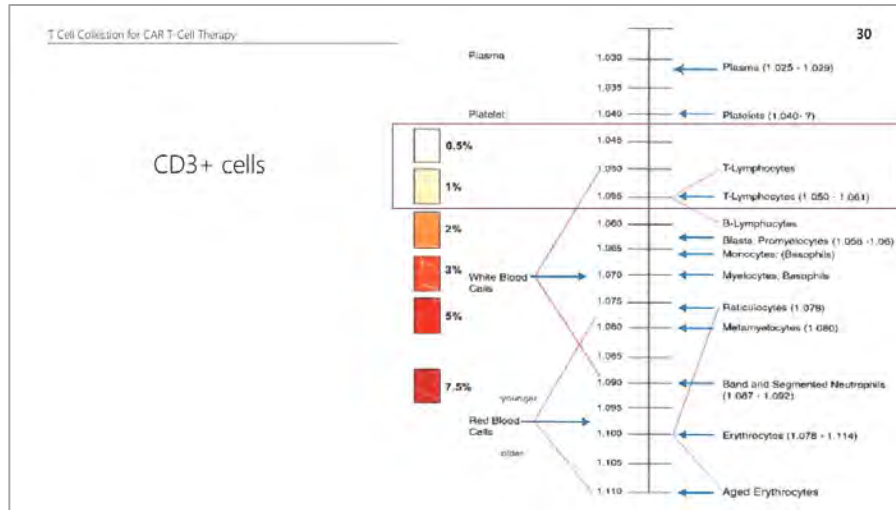
CD3+ cells





The right cell: Qualitative measures

- ENOUGH T-cells (industry must disclose target dose)
- PURE (low monocytes; low granulocytes if cryopreservation intended)





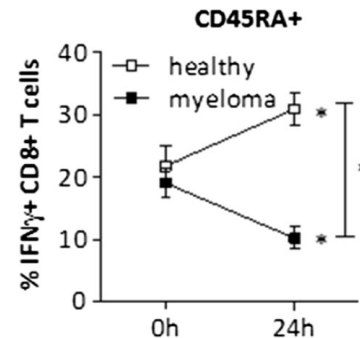
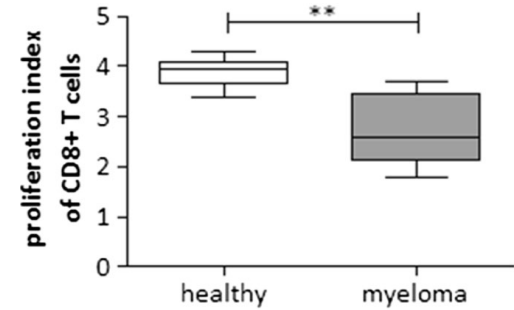
The right cell: Qualitative measures

- BALANCED (approximately physiological) ratio between CD4 and CD8
- HIGH fraction of T-cells with immature phenotypes (Tn or Tscm)
- LOW fraction of Treg, of T-cells expressing immune checkpoints
- HIGH „fitness“, proliferation capacity, healthy metabolic profile, healthy mitochondria, ... (targets unknown)



The right cell: Qualitative measures

- Reduced T-cells, especially CD4+ T-cells¹
- Excessive TCR $\gamma\delta$ T-regs which suppress anti-tumor responses²
- Attenuated proliferation capacity and effector function of T-cells of MM patients³



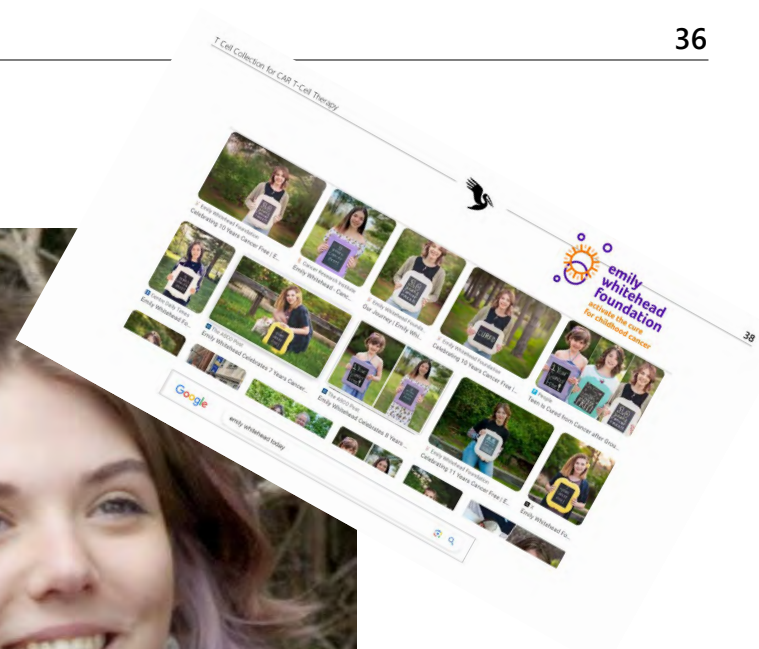
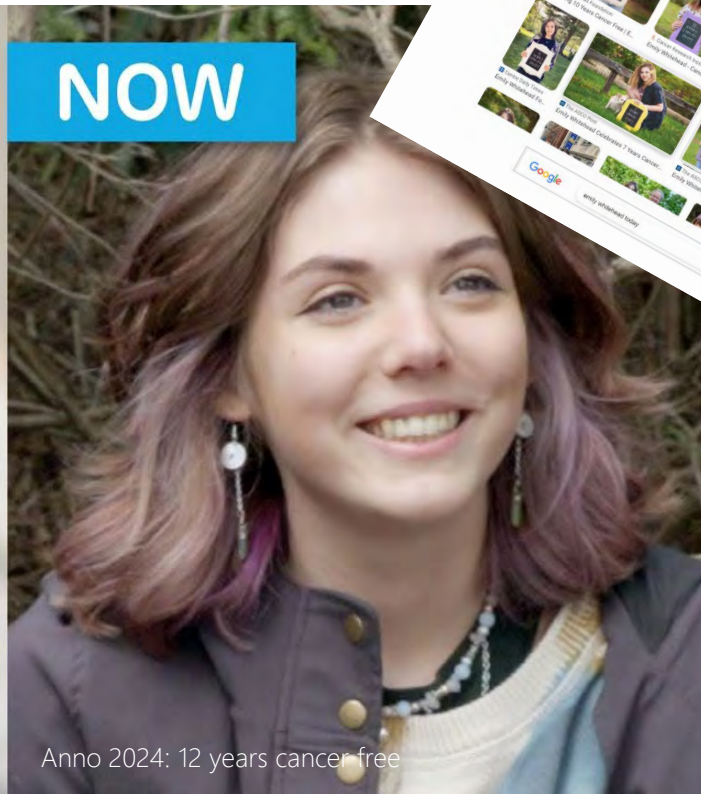
1. Bergmann L, et al. Cancer Immunol Immunother. 1984;17(2):112-6.

2. Ma Y, et al. Biochem Biophys Res Commun. 2016;480(4):594-601.

3. Zelle-Rieser C, et al. J Hematol Oncol. 2016;9(1):116.



Emily Whitehead





Quality in a chain





In summary for CD3 collections

For Optimal CD3+ cellular collection by apheresis

- Precise prescription
- Collection with the correct color
- Reconsider the CD3+ collection timing in the patient's journey
- Collaboration between all departments
- More data and research is needed for the effects of apheresis on the cellular product



Sanquin

*Thank
you!*