London Health Sciences Centre

St JOSEPH's HEALTH CARE LONDON

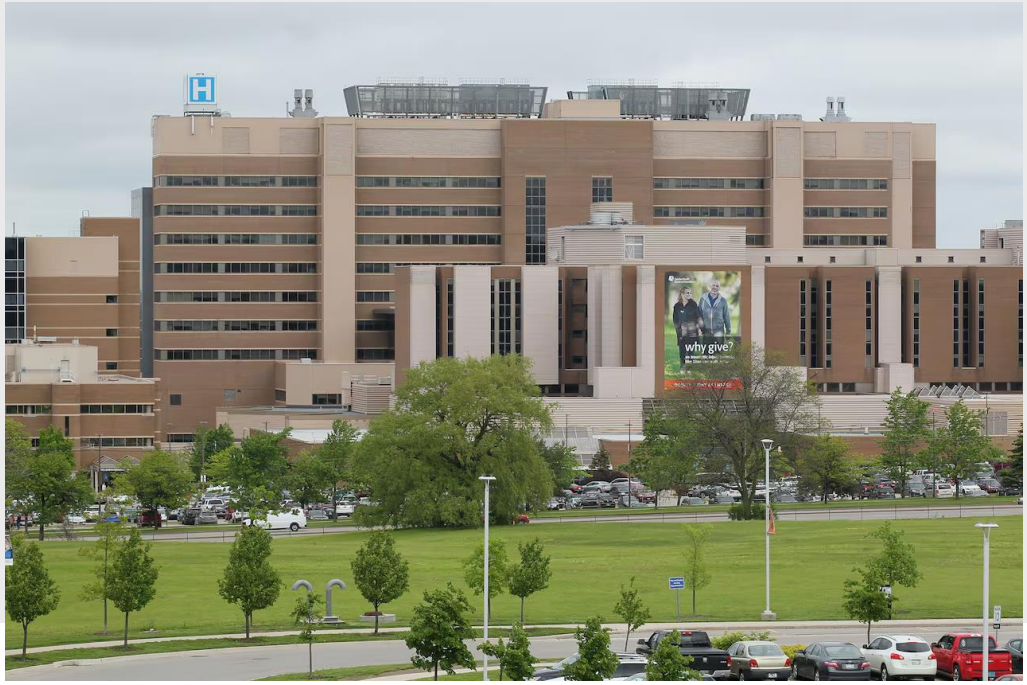
Pathology and Laboratory Medicine

Stem Cell Transplantation in the age of CarT Cell Therapy

Ben Hedley

London Health Sciences Centre

- 1,000-bed tertiary care hospital located on three sites within the city of London, Ontario Canada
- > 700,000 inpatient, outpatient and Emergency visits annually
- Specialized services include critical care/trauma, cardiology, neurology and neurosurgery, cancer care and transplantation (solid organ and stem cell)



Victoria Hospital

- Regional adult and paediatric Hemato-Oncology and stem cell transplantation centre serving Southwestern Ontario
  - ~3 million people
- Specialized Hematology, Hemostasis & Thrombosis, Flow Cytometry in close proximity to Core Laboratory
- Hematology – Symex analyzers
- Flow Cytometry
  - 3 Navios + 1 Navios EX
  - 4 BD Lyric
  - 1 DxFlex
  - 1 FC500
  - 1 Aquios

Flow Cytometry Test Menu

- Leukemia/lymphoma immunophenotyping
- MRD (Lymphoid only)
- HIV
- CD34
- Solid organ Crossmatch
- Transplant monitoring
- PNH
- Hb F
- Basic immunology
- Research applications

Stem Cell Transplantation

- **Content:** History and development of HSCT as a treatment.
- **Key Diseases Treated:** Aplastic anemia, leukemia, lymphoma, multiple myeloma, sickle cell disease.
- **Visual:** Timeline of HSCT milestones or graphical overview of diseases treated by HSCT

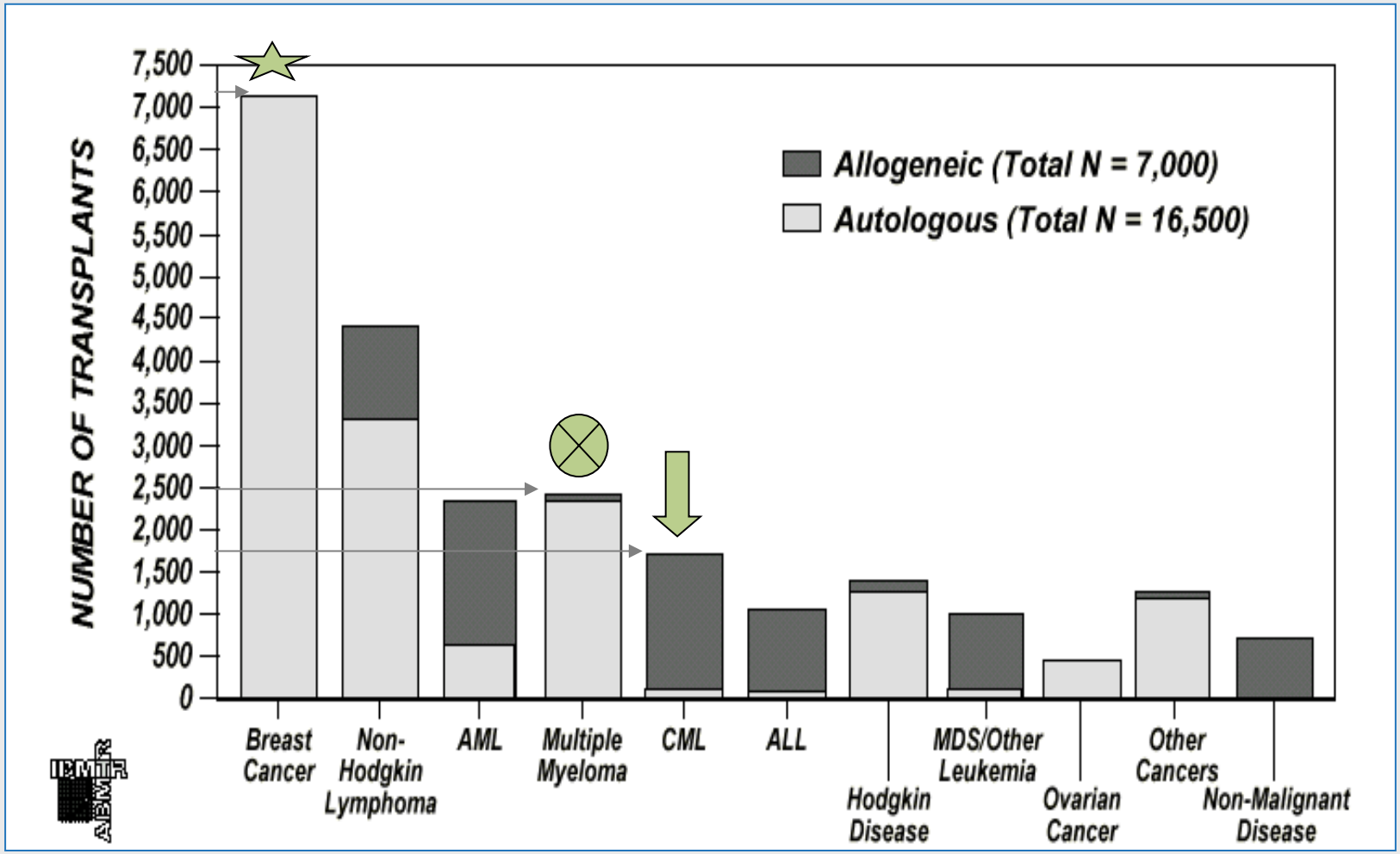
Assessment of CD34+ Cells in Hematopoietic Stem Cell (HSC) Transplants - Overview

- Background and Indications for stem/progenitor cell transplantation
- Why do we count CD34+ cells?
- How do we count CD34+ cells?
- How does freezing/storage impact CD34+ cell viability?
- Poor viability
- Conclusions

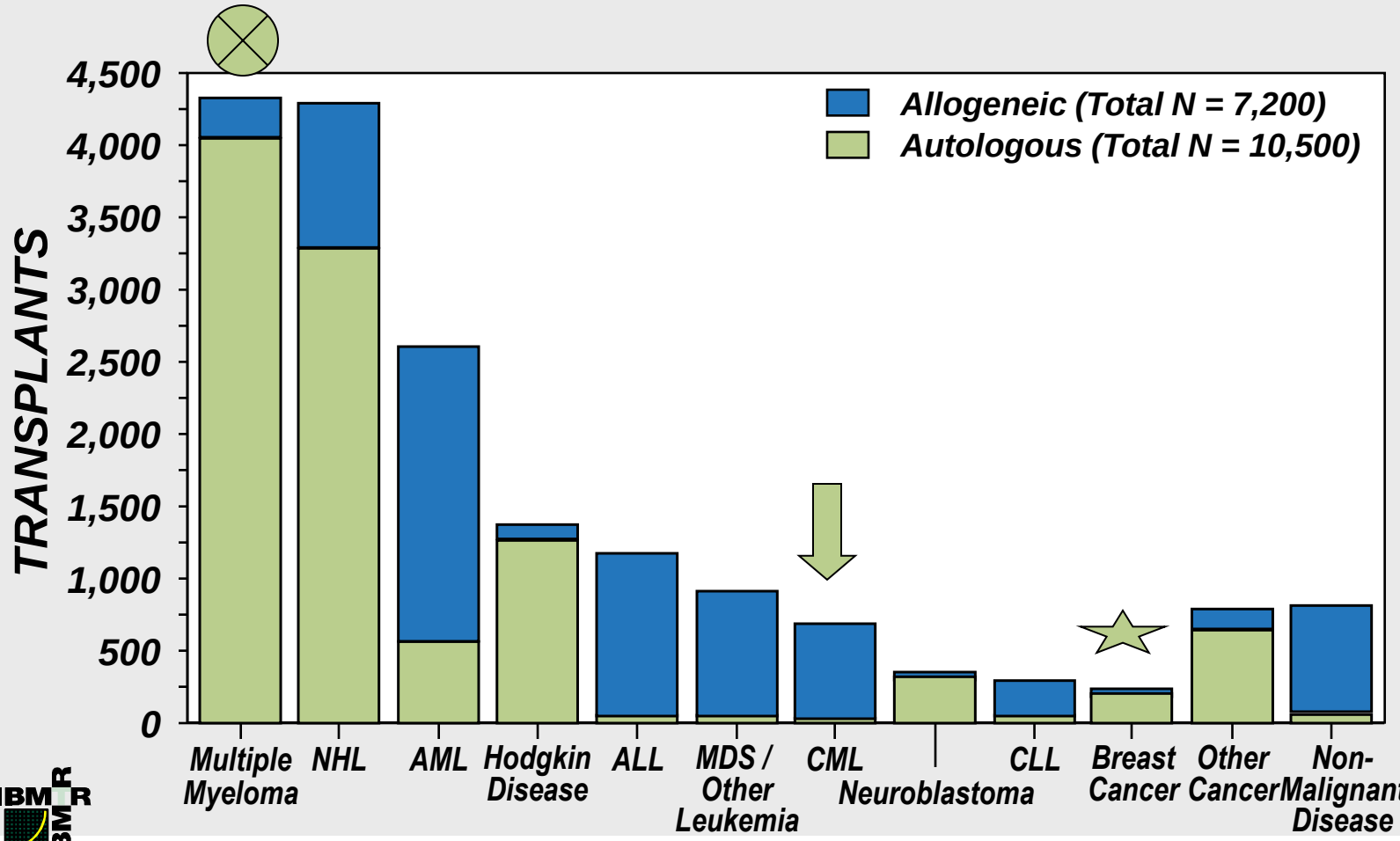
Autologous versus Allogeneic Transplant

- Autologous transplant CD34+ cells collected
  - Bone marrow,
  - Cytokine mobilized CD34+ from peripheral blood
  - Give high doses of radiation and/or chemotherapy
  - “Salvaged” by returning the collected cells.
  - In autoimmune conditions may “reset” the immune system.
- Allogeneic transplant
  - the “graft versus tumor” effect may play an additional curative role over and above reconstitution of the hematopoietic system

1998


NUMBER OF TRANSPLANTS
7,500
7,000
6,500
6,000
5,500
5,000
4,500
4,000
3,500
3,000
2,500
2,000
1,500
1,000
500
0
Allogeneic (Total N = 7,000)
Autologous (Total N = 16,500)
Breast Cancer
Non-Hodgkin Lymphoma
AML
Multiple Myeloma
CML
ALL
Hodgkin Disease
MDS/Other Leukemia
Ovarian Cancer
Other Cancers
Non-Malignant Disease
IBMTR
ABMTR

Transplantation in North America 2002



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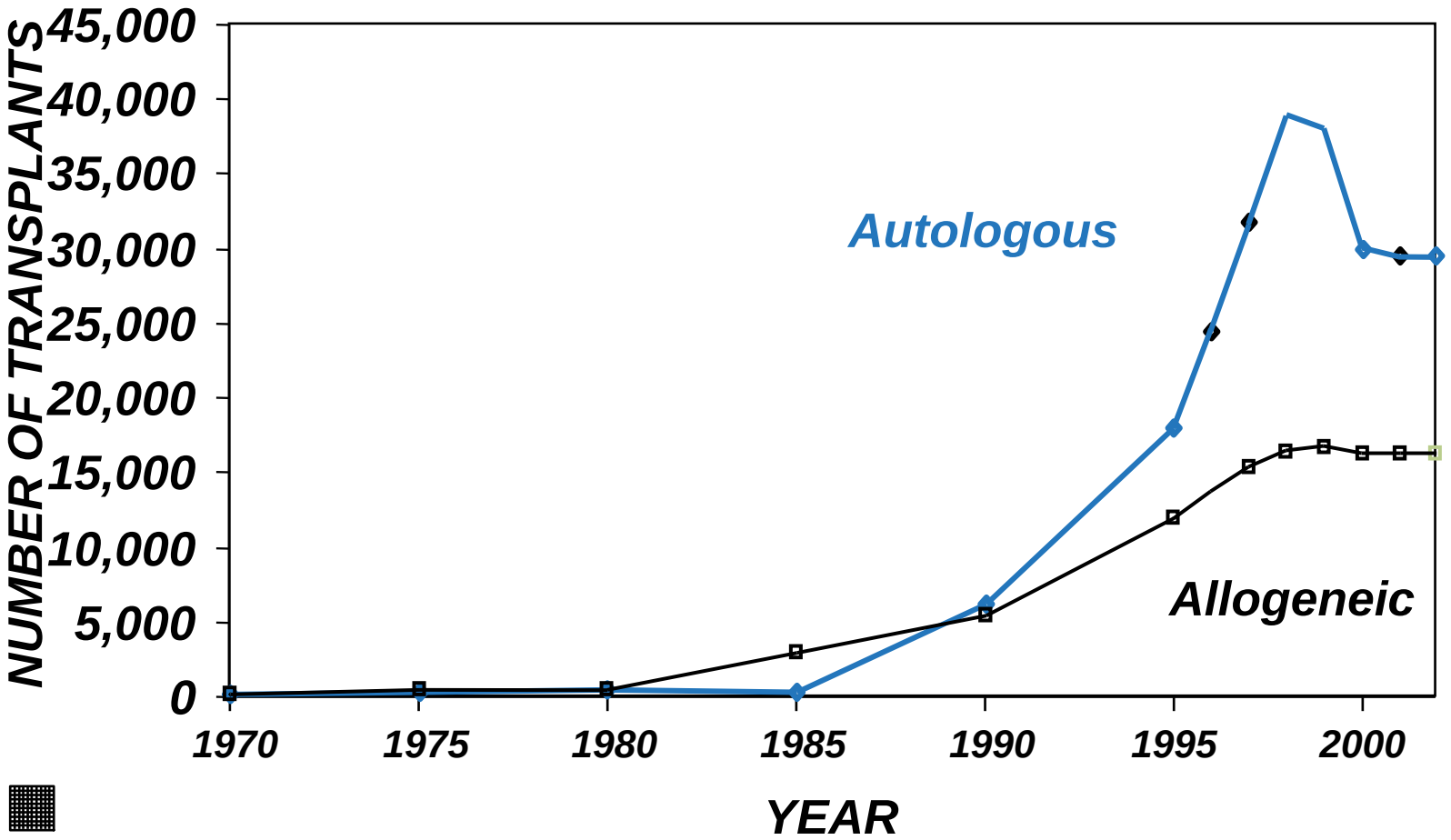
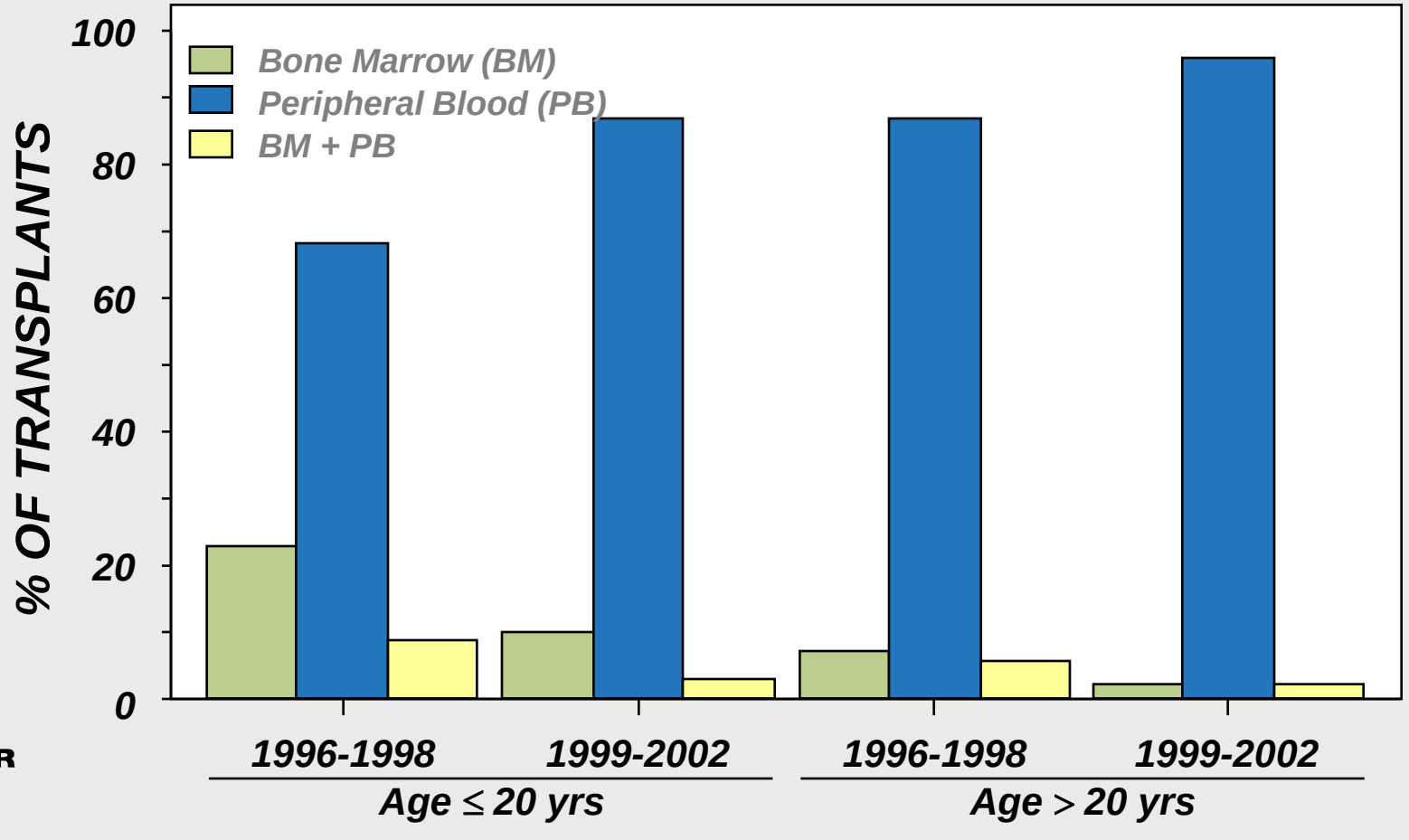
Transplants worldwide 1970-2002



Autologous stem cell by age 1996-2002



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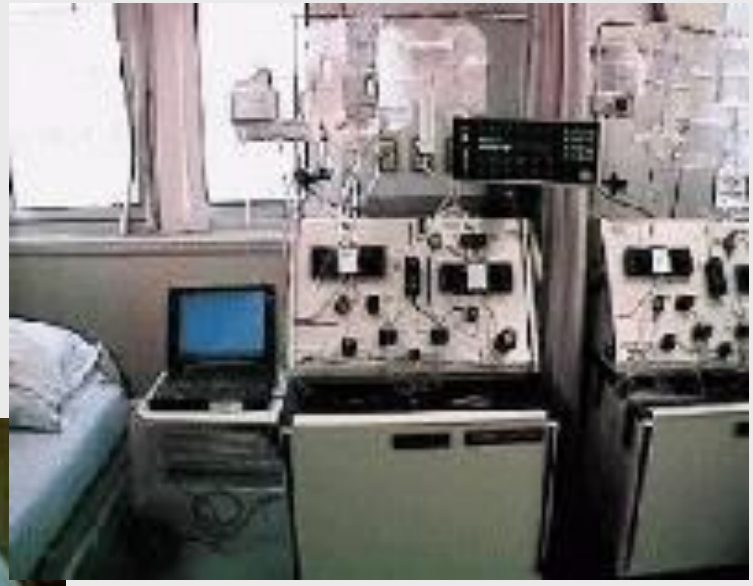
Bone Marrow Harvest vs PB Stem Cell Harvest



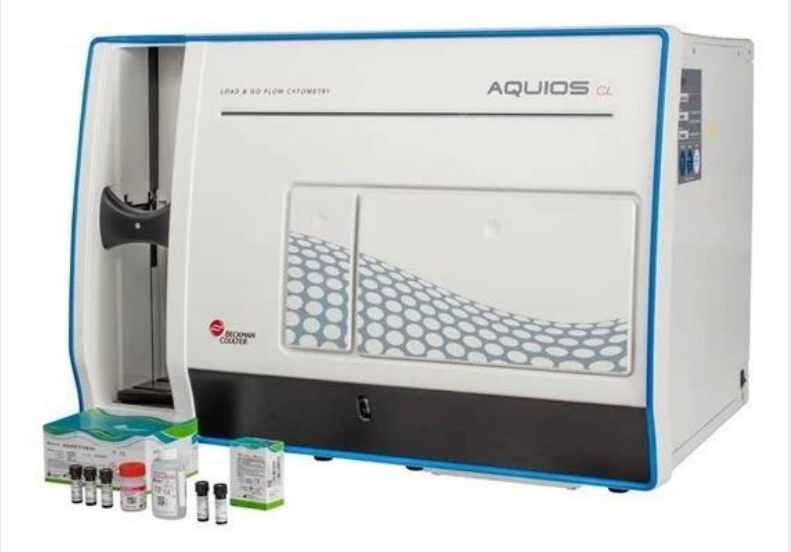
Comparison of Autologous Peripheral Blood Progenitor Cell versus Bone Marrow Transplant

Parameter	PBPC*	BMT	P
Hospitalization (days)	17	23	.002
Neutrophil recovery (days)	11	14	.005
Platelet recovery (days)	16	23	.02
Platelet transfusions (days)	6	10	.001
RBC transfusions (number)	2	3	.002

* G-CSF 10 mcg/kg/day mobilization

Assessing Stem Cell Graft Adequacy

- Mononuclear cell count (marrow)
- Colony-forming assays (CFU)
- CD34+ enumeration
- Patient engraftment

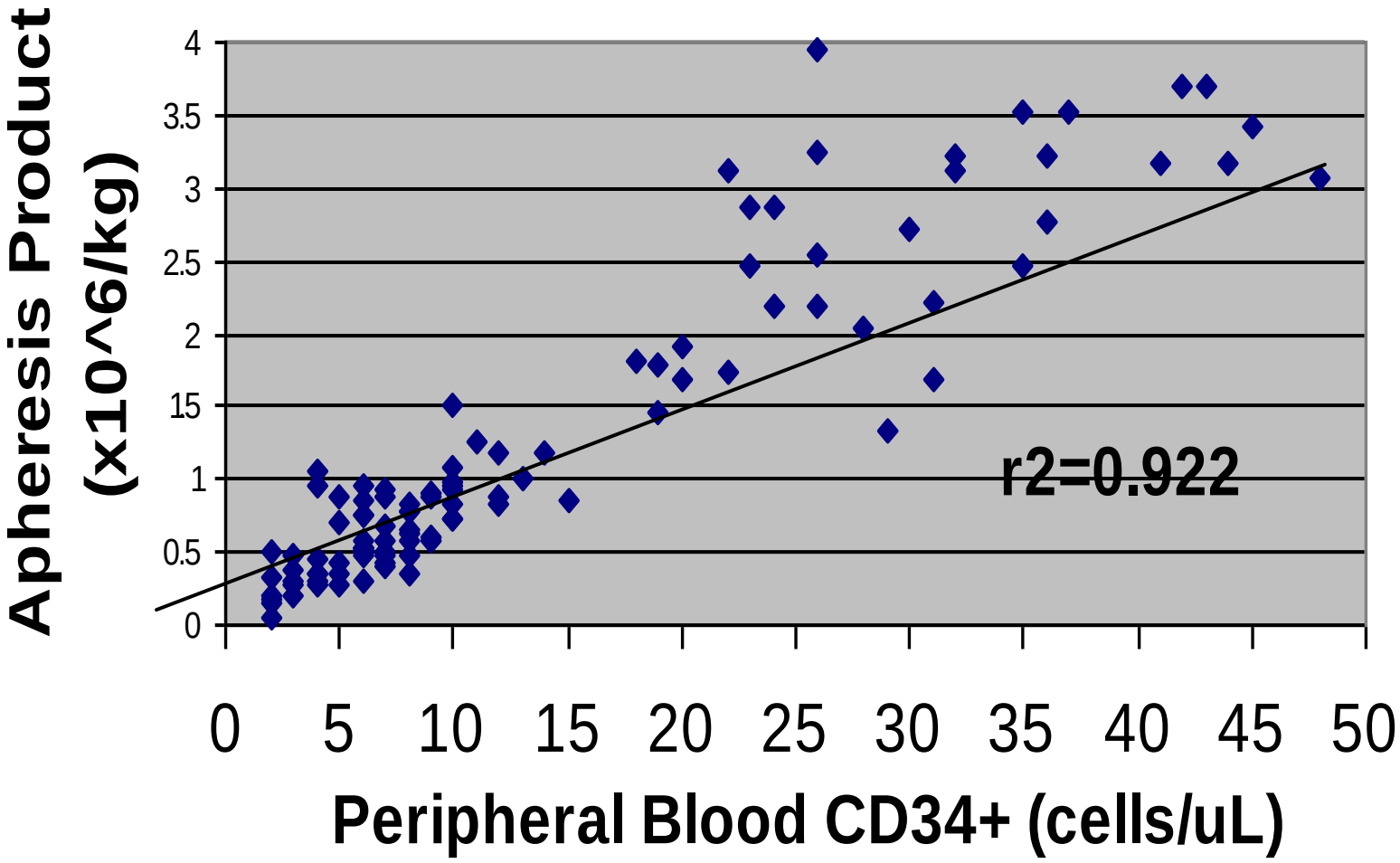


CD34 Antigen

- CD34 is a glycoprotein found on the surface of stem and progenitor cells in blood, bone marrow, apheresis and cord blood.
- Levels in bone marrow are approximately 1-3%
- Levels in un-mobilized peripheral blood usually <0.1%

  - Can be increased to >10% with chemotherapy and or cytokine mobilization.
- CD34 enumeration is useful in monitoring mobilized peripheral blood to predict the likelihood of success of an apheresis procedure
- Many labs use peripheral blood CD34+ cell count to determine if apheresis should proceed
- Peripheral blood CD34+ cell values less than 5/uL predict poor harvest

Hospital CD34 Count



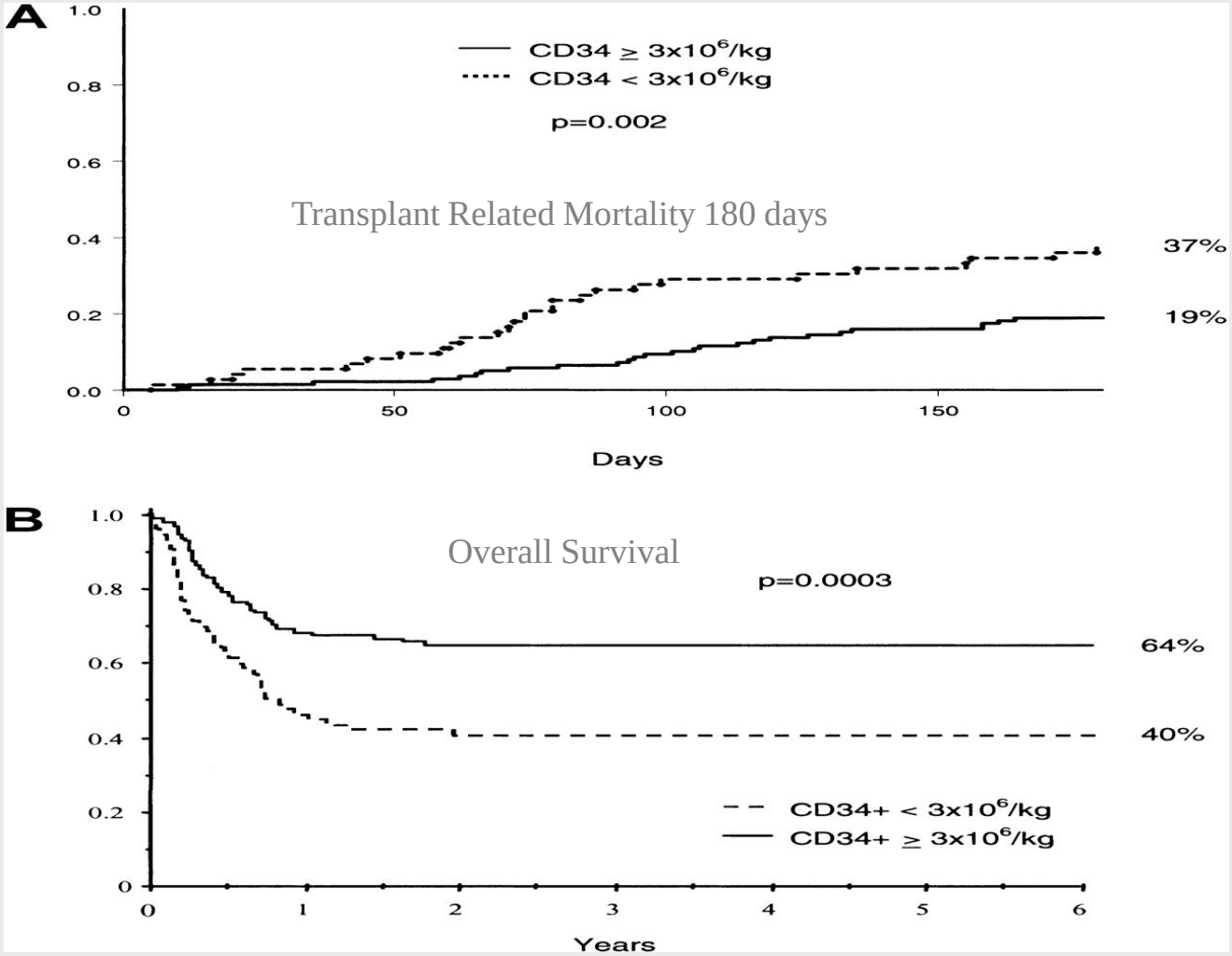
Economic Impact of > 5 X 10^6 CD34+/kg as Optimal Cell Dose In Autologous Transplant

- compared resource utilization among 1,317 PBSC transplant patients based on CD34+ cell dose infused (< 5 versus > 5 x 10^6 CD34+/kg)
  - multiple centres, variety of diagnoses
  - chemotherapy + cytokine G-CSF mobilization

- >5 million CD34+ cells associated with significantly:
  - shorter mean duration of low ANC and PLT
  - fewer PLT (2.5 vs 5) and RBC (1.3 vs 2.3)
  - shorter mean lengths of stay (11 vs 14 Days)
  - lower estimated costs by CA$8,062

Schulman KA et al. JCO, 1999Apr;17(4):1227

CD34 Cell Dose in HLA-Identical Sibling BM Transplants- TRM and Overall Survival



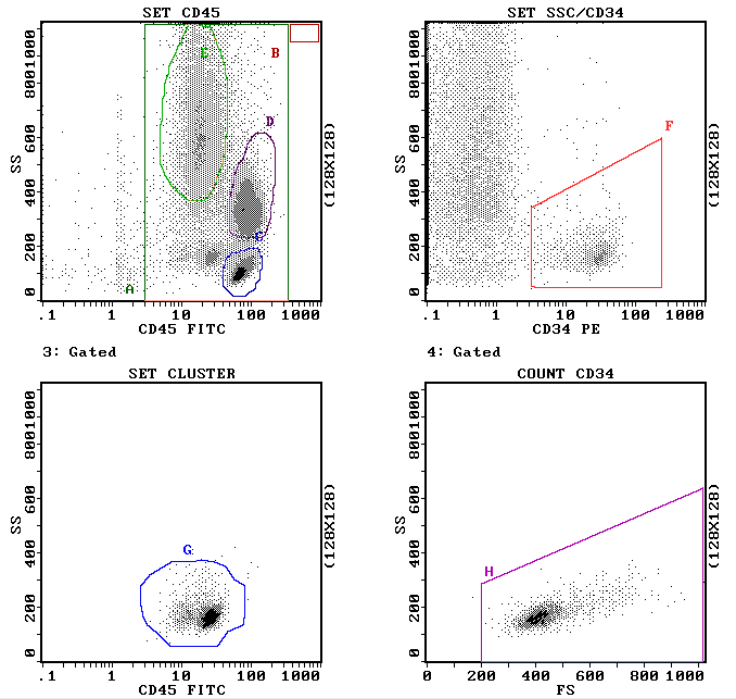
Bittencourt H et al, Blood, 15 April 2002 99;8

Flow Cytometric Methods for CD34 Enumeration

- Milan ------> ISHAGE
- Single parameter ----> Multiparameter
- Dual platform -----> Single Platform
- Automated methods

Criteria For True CD34+ Cells (ISHAGE Guidelines*)

- Positive CD34 Expression
- Characteristic (dim) CD45 Expression
- Low - Intermediate Forward Angle Light Scatter
- Low Side Scatter



* Sutherland et al, Journal of Hematotherapy 1996

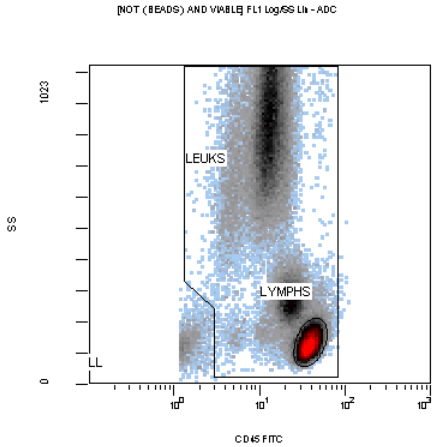
Viability

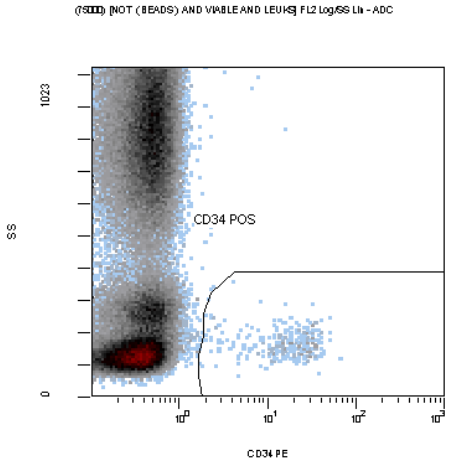
- Storage of product may lead to cell death
- Purging, T cell depletion or other manipulations may negatively impact viability
- Cord blood and bone marrow contain a variable percentage of dead cells
- 7-AAD - viability dye added to single platform ISHAGE method allows direct assessment of cell death

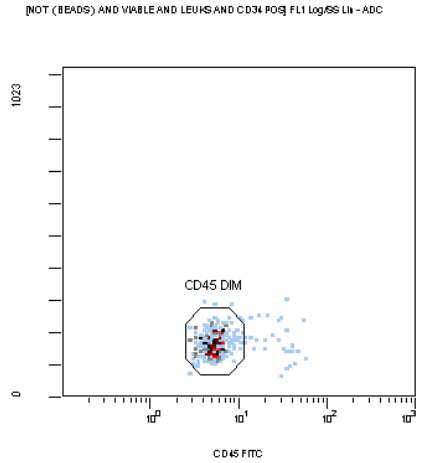
StemONETM Automated Software

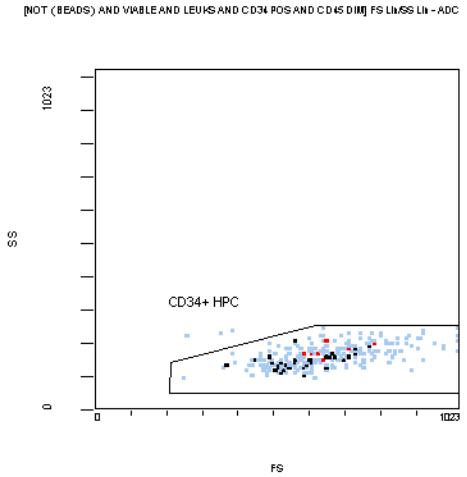
- Post acquisition analysis software designed to work with StemKIT reagents and Coulter System 2 software on an EPICS XL flow cytometry
- Creating protocols with multiple boolean gates is difficult for many labs
- StemONE follows the decision making process of an expert operator in defining limits of the CD34+ population
- Designed to work with all sample types (fresh or frozen) except selected CD34+ products

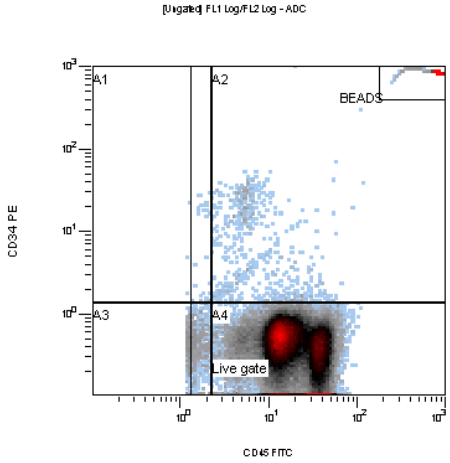
Coulter FC 500 with StemCXP on Cord Blood Sample

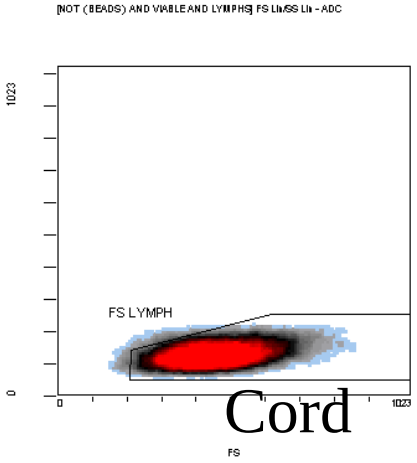


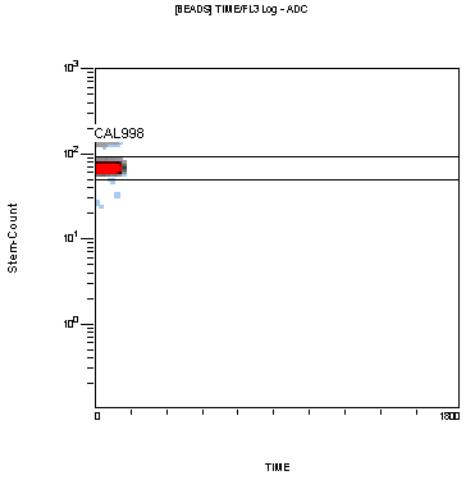


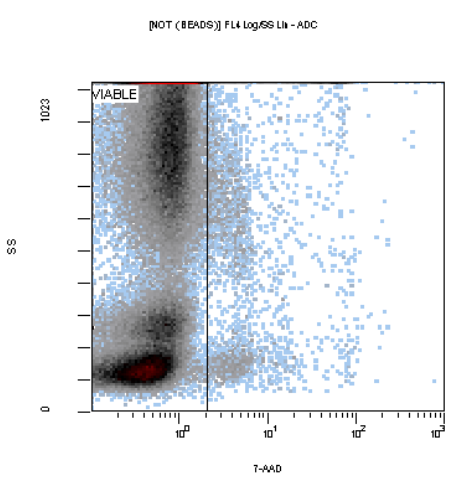












Cord

Impact of Cryopreservation

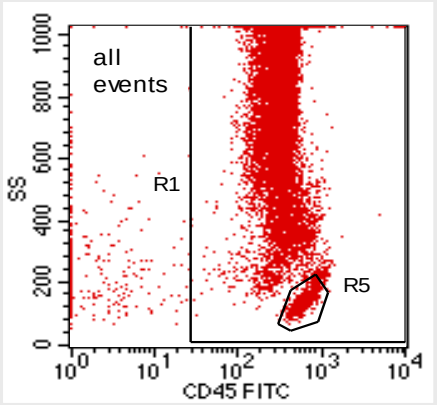
- At time of harvest 92% exceeded standard minimum threshold of 2.0 x 10^6 CD34+ cells/kg
- At time of reinfusion, only 53% met this threshold

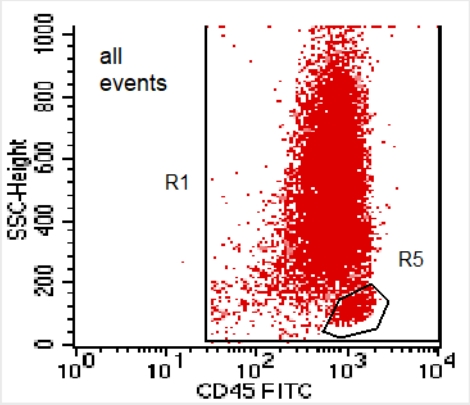
Case Study - Engraftment Failure

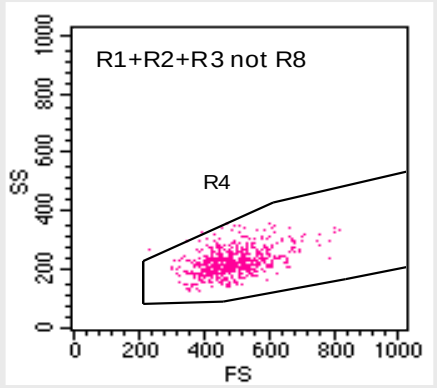
- DY 48 year old male
  - Multiple myeloma
  - Stage III A
  - Mobilization with Cyclophophamide/GCSF/SCF
  - Harvested day 9 and 10
  - Total CD34+ 32 X 10(6)/Kg

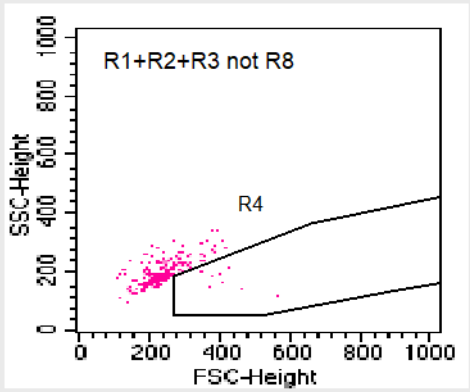
Case Study - Engraftment Failure

- Conditioning regimen
  - Melphalan 180mg/m2 IV
  - 48 hours post melphalan PBSC reinfused
- Prolonged pancytopenia
  - Day 42
    - WBC 0.8 , neutrophils (0.5)
    - Platelets 18



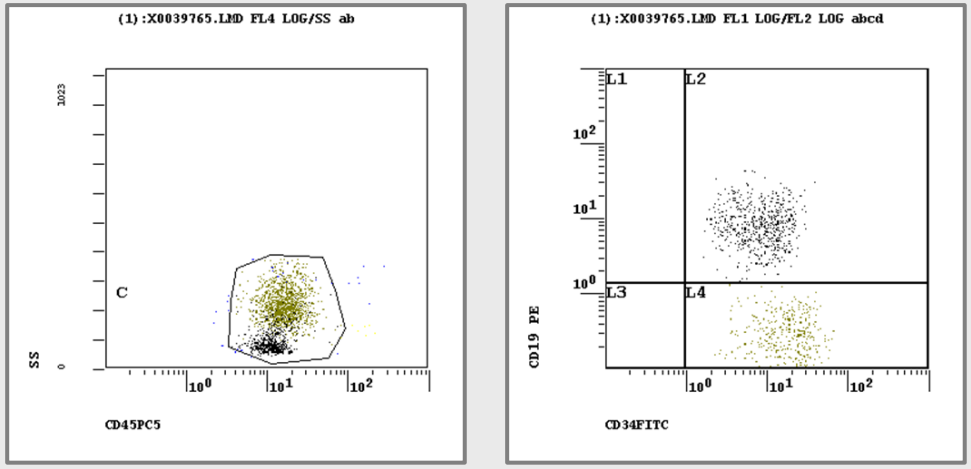






Is measuring CD34 Subsets Clinically Relevant?

- In most cases, probably not!
- The number of viable CD34 cells actually infused is probably the most relevant parameter
- Measuring CD34+CD19+ may be useful in poor mobilisers with marginal collections (< 2 x10 ^{6}/Kg)
- Response to different cytokine regimens



Conclusions

- CD34+ cell transplant are an important treatment option in hematological, genetic and in other malignant/ non malignant conditions
- The flow cytometry laboratory has an important role in the monitoring and assessment of stem cell product collection and manipulation
- Standardized methods are available to enumerate CD34+ cells
- If viability is an issue, method must contain a viability dye to exclude dead cells
- The introduction of automated methods should lead to significant reduction of inter-laboratory variation

People that made all of that possible

<u>London Health Sciences Centre, Canada</u>

Michael Keeney
Ian Chin Yee
Kang Howson Jan
Dave Allan
Anargyros Xenocostas
Karin Weir
Jan Popma
Wendy Brown
Leslie Gray-Statchuk

<u>The Toronto Hospital, Canada</u>

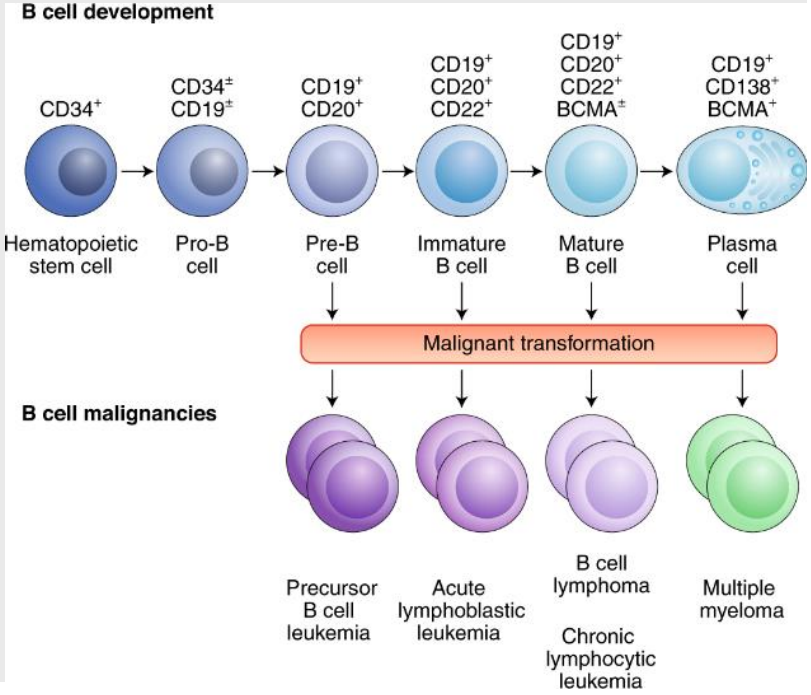
Rob Sutherland
Casey Nayar

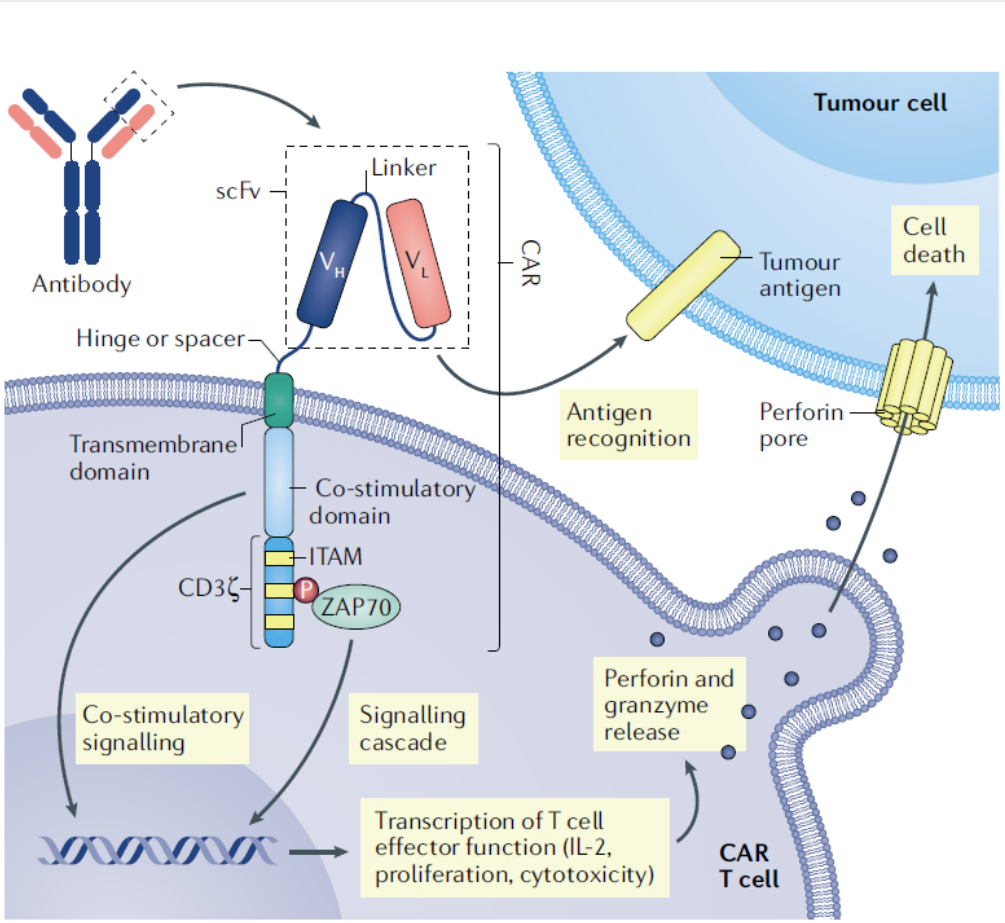
<u>Erasmus MC Daniel den Hoed Cancer Centre, Netherlands</u>

J Gratama
J Kraan

Introduction to CAR T-cell Therapy

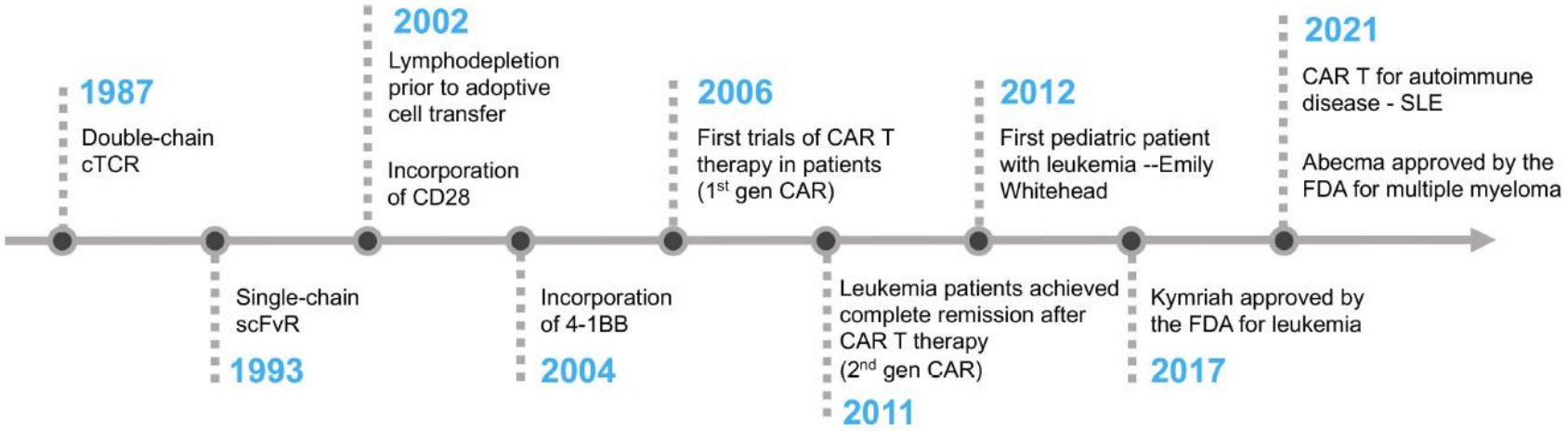
- Background on CAR T-cell therapy and its revolutionary impact.
- Targeted approach to treating hematologic malignancies.





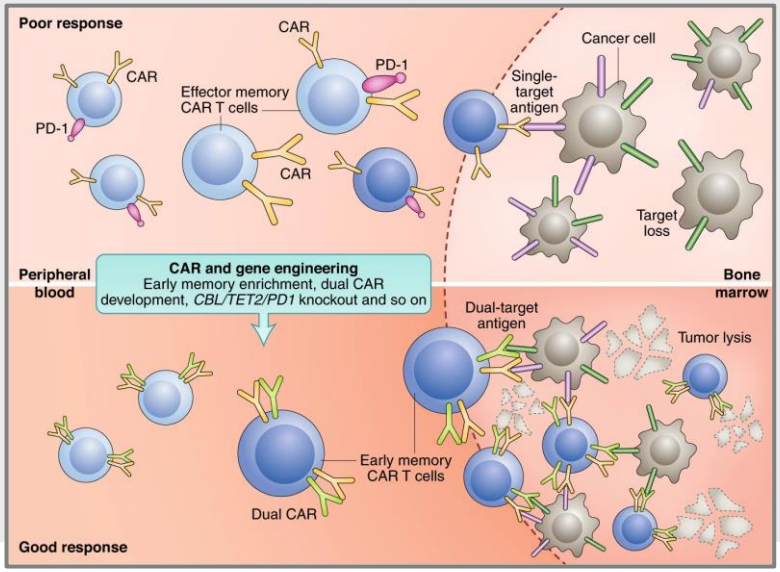
History of Immunotherapy

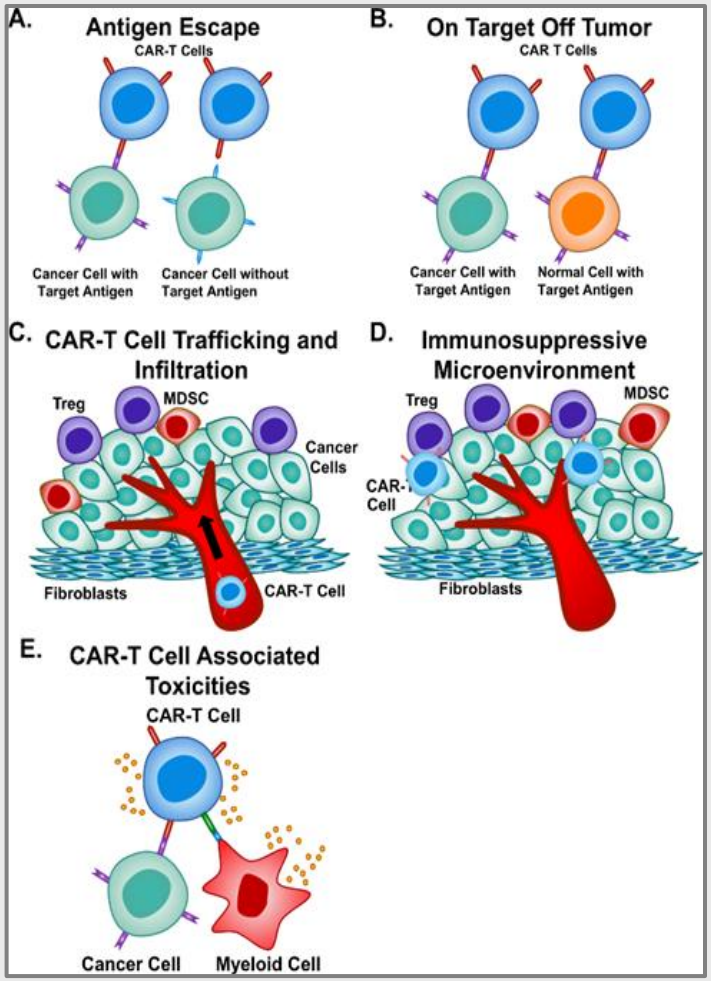
- Monoclonal Ab therapy
- Rituximab first used in 1998 in Australia
  - 2000 Traztuzimab, Blinatumomab 2015, Inotuzumab 2018



Mechanism of Action for CAR T-cell Therapy

- Chimeric antigen receptor (CAR)-T cell therapy has produced remarkably effective and durable clinical responses.
- CARs are engineered synthetic receptors that function to redirect lymphocytes, most commonly T cells, to recognize and eliminate cells expressing a specific target antigen.





Financial Impact to Healthcare

HSCT Costs

- **Autologous** (using the patient's own stem cells): Typically less expensive than allogeneic transplants.

  Cost: $100,000 to $200,000

- **Allogeneic** (using a donor's stem cells): Costs can increase due to the need for donor matching, graft-versus-host disease (GVHD) treatment, and other complications.

  Cost: $200,000 to $500,000

CAR-T Cell Therapy Costs

- CAR-T cell therapy is a relatively newer treatment, and it involves genetically modifying the patient's T cells to fight cancer.

  Cost: $350,000 to $500,000 (for the therapy itself), with additional costs for hospitalization, pre-treatment, and follow-up care, potentially raising the total to $1,000,000 or more.

Conclusion

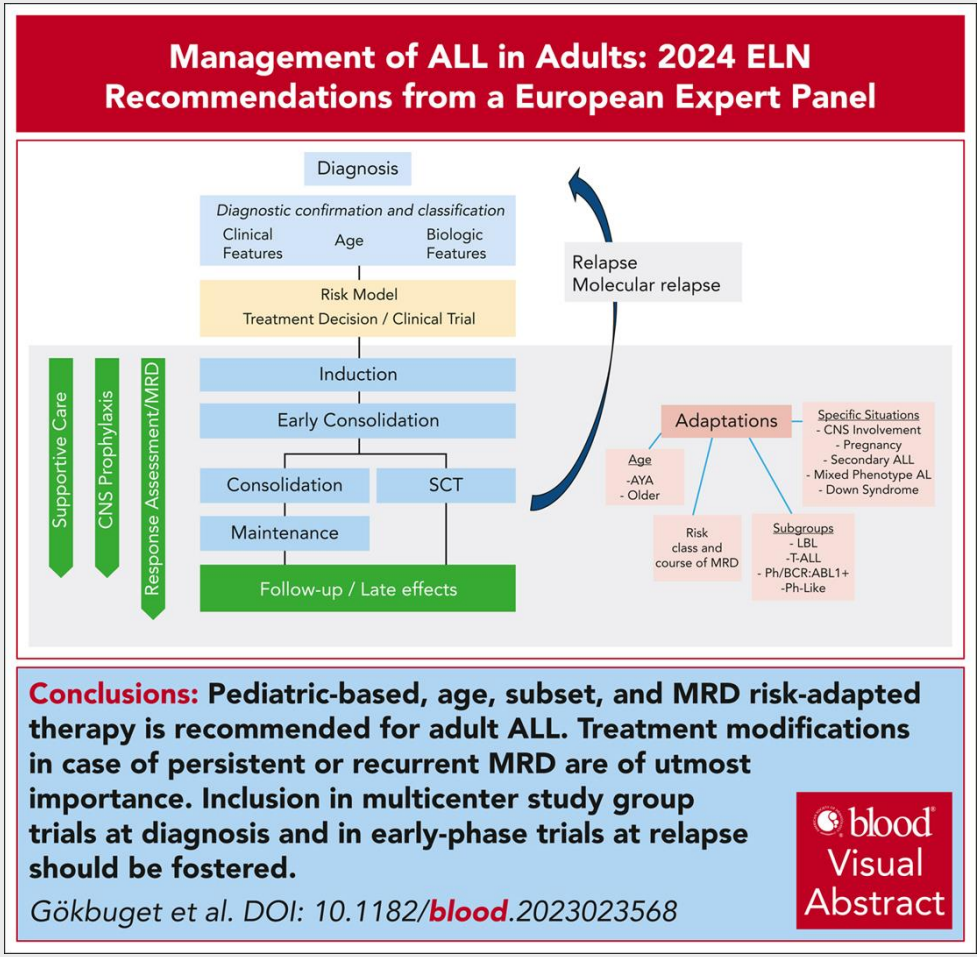
- Stem Cell Transplant: $100,000 to $500,000, depending on type and complications.
- CAR-T Cell Therapy: $350,000 to $1,000,000 or more, with significant upfront costs due to the cutting-edge nature of the treatment.
- CAR-T therapy is generally more expensive than stem cell transplants, but the choice of treatment depends heavily on the patient's condition and eligibility.

	HSCT	Car-T
Hospitalization	Long	Short
Post Treatment	Chronic	Acute

When to use HSCT and CarT

ASH

- BCR-ABL + vs BCR-ABL –
- Addition of Blinatumomab
- Inotuzumab (evidence lacking)
- Daratumomab (evidence lacking)
- BCR-ABL +
  - HSCT after CR1

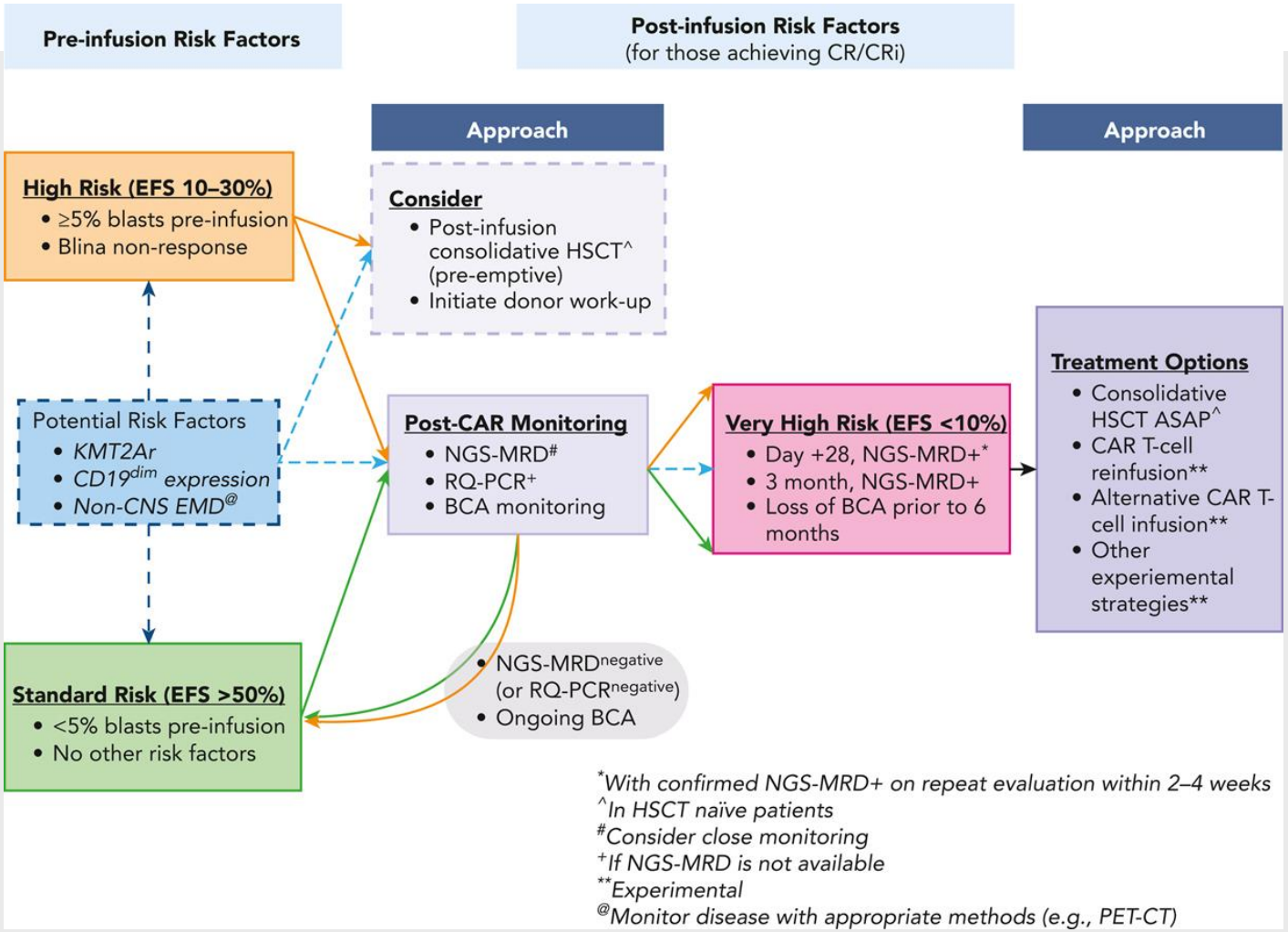


Nicola Gökbuget,Nicolas Boissel,Sabina Chiaretti,Hervé Dombret,Michael Doubek,Adele Fielding,Robin Foà,Sebastian Giebel,Dieter Hoelzer,Mathilde Hunault,David I. Marks,Giovanni Martinelli,Oliver Ottmann,Anita Rijneveld,Philippe Rousselot,Josep Ribera,Renato Bassan, Management of ALL in adults: 2024 ELN recommendations from a European expert panel, Blood, 2024,

Current Evidence and Research

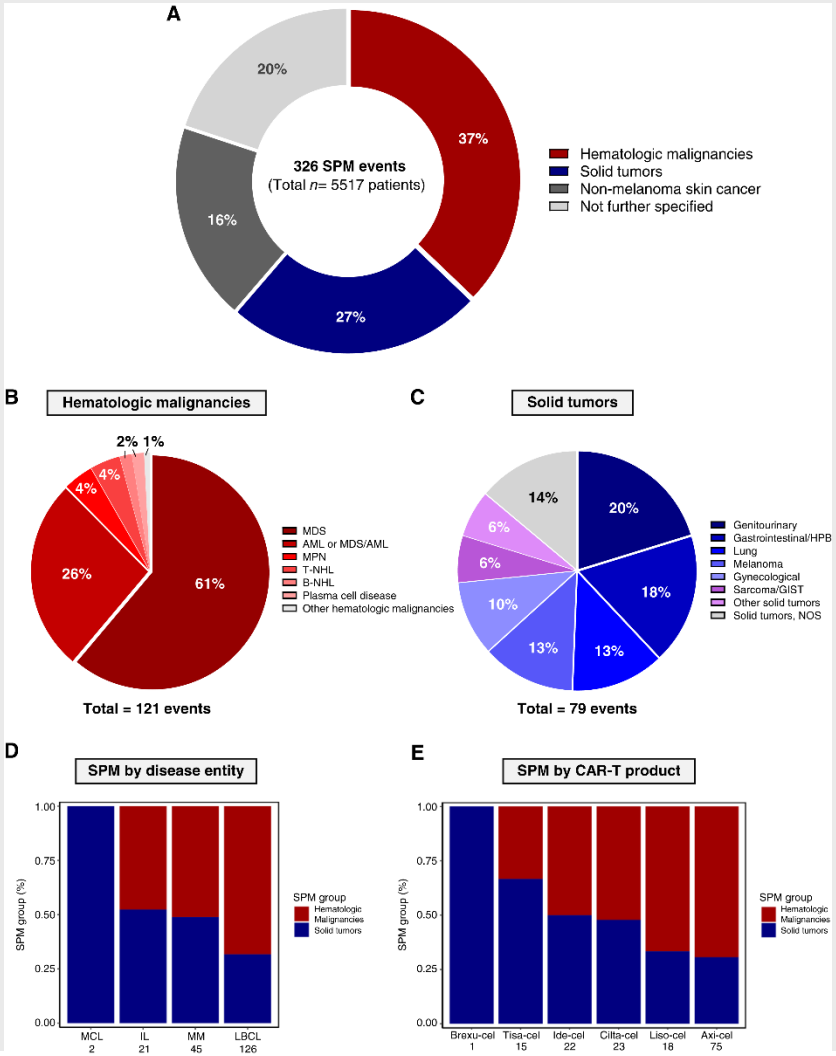
- **Post-CAR-T SCT**: In some cases, patients who achieve remission after CAR-T therapy, especially with allogeneic CAR-T trials, may still undergo SCT to sustain long-term remission.
- **CAR-T After SCT Failure**: Patients who relapse after a stem cell transplant often become candidates for CAR-T therapy, especially if they still have responsive disease.
- **Sequential Use:** In patients with multiple myeloma, researchers are exploring whether combining autologous SCT with CAR-T therapy could improve outcomes.

Risk factors for success or failure of CD19 CAR T cells to guide management of children and AYA with B-cell ALL



risk factors for success or failure of CD19 CAR T cells to guide management of children and AYA with B-cell ALL, Blood, 2023,

Secondary Malignancies post CAR-T



From: **Second Primary Malignancies after CAR T-Cell Therapy: A Systematic Review and Meta-analysis of 5,517 Lymphoma and Myeloma Patients**

Clin Cancer Res. 2024;30(20):4690-4700. doi:10.1158/1078-0432.CCR-24-1798

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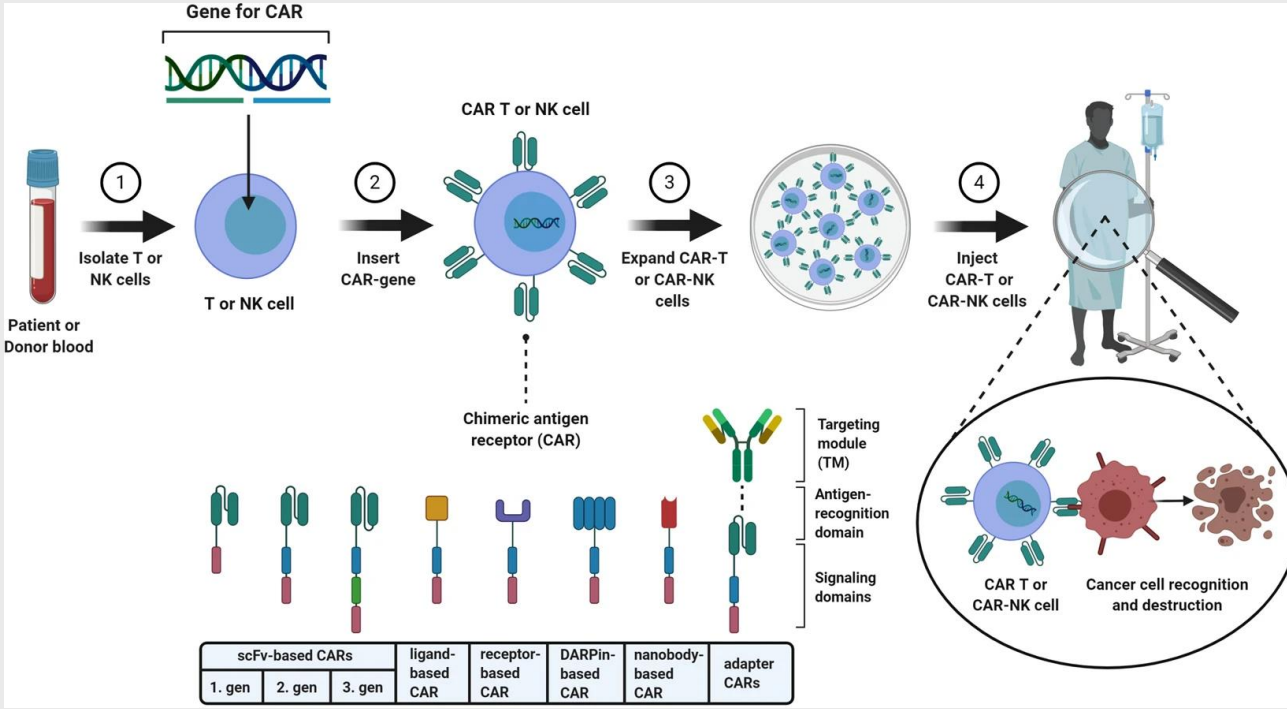
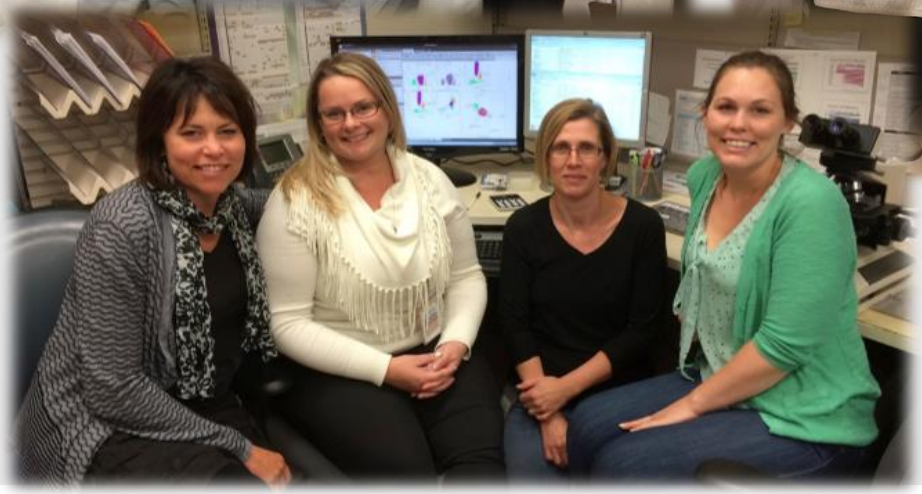
Conclusion

- Roles for HSCT
- Role for CAR T-cell therapy
- There will be patients for which both may be the best option



- Personalized treatment approaches in hematology can leverage both HSCT and CAR T-cell therapy for optimized patient outcomes.

Acknowledgments



- Over the past decade, an astounding series of proof-of-concept trials have taken place, with validation of early results in phase II trials39,44,183,184,185,186 leading to the approval of CD19-specific CAR T cell therapies for select B cell malignancies. Separately, insight into the biology of CRS has led to biomarker-driven trials (NCT02906371) and the discovery and validation of a novel biomarker profile of this potentially lethal toxicity59. Additional observations from routine and translational studies have revealed mechanisms of resistance and response, as well as identification of the natural basis of successful and failed CAR T cell therapy40,66,68. Novel therapies started to incorporate small molecules, which proved to augment T cell function and simultaneously inhibit the malignant population35,93,95,187. Combination trials also targeted more than one surface protein, either on the same target cell (as with CD19 and CD22) or on precursors and progeny of the tumor (as with CD19 and CD20, CD22 or BCMA)185,188,189. In the next few years, we are likely to witness increased efficacy of CAR T cells for solid tumors—a major current focus in this field. However, a better understanding and monitoring of the tumor will be essential for CAR T cell therapy to be offered to patients in the early stages of their disease, before genomic instability and evolution of the tumor complicate treatment.