Advanced Graft processing

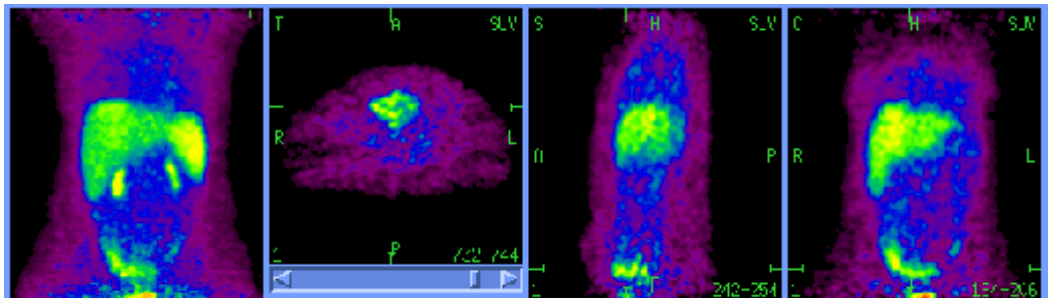
Dominic Wall PhD FFSc (RCPA)

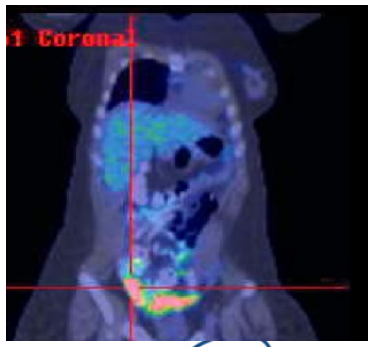
Operations Director
Centre for Blood Cell Therapies

Peter MacCallum Cancer Centre

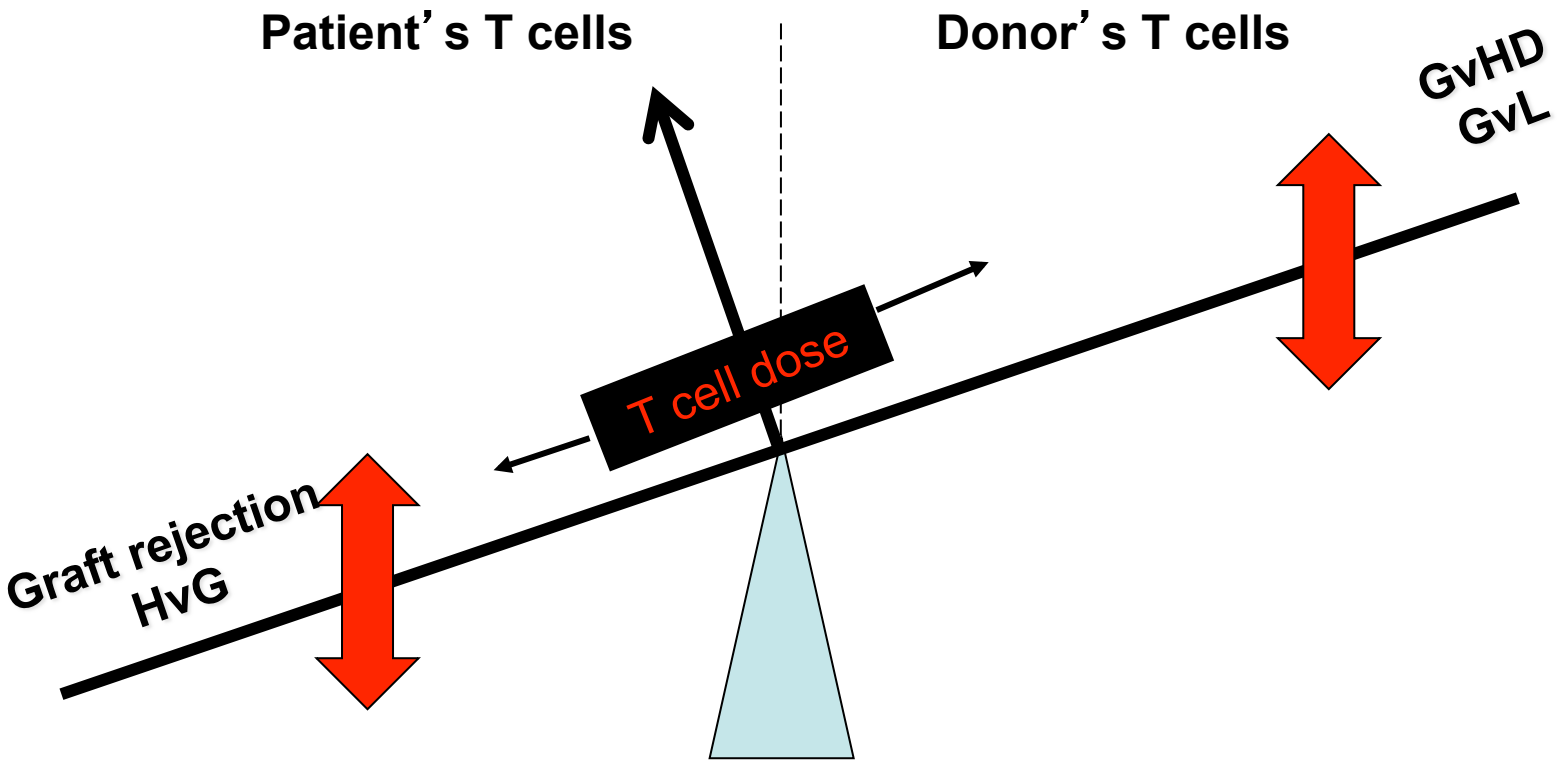
Scope

- Standard of care or experimental/investigational manufacturing?
- Complex graft processing for various indications
- Investigational and experimental approaches
- Future flexibility for new approaches, new technology
- Other specializations required? imaging/ immunology/ others?…





Basic Rationale



Which approaches?

- **CD34**
- **CD3/CD19**
- TCR$\alpha\beta$
- Broad specificity Ab
- Mechanical properties
- Other agents

Methods

T cell depletion Method	Depletion target	Projected T cell depl. (Log)
MAb CD2, CD3, CD5, CD6	T	1 – 2.5
Mab CD4, CD8	T	1 – 2
T10B9 (TCS) + complement	T	1.0 – 1.5
Imm. Magn. Sep. CD3, CD8	T	2
Campath + complement	T, B (DC, SC)	1.7 – 2.5
Elutriation		1.5 – 3.0
SBA + E-Rosetting	T, B, NK	2.5 – 3.0
ISOLEX CD34	All but SC	3.5 – 4.0
CliniMACS CD34	All but SC	4.0 – 4.5

TRANSPLANTATION

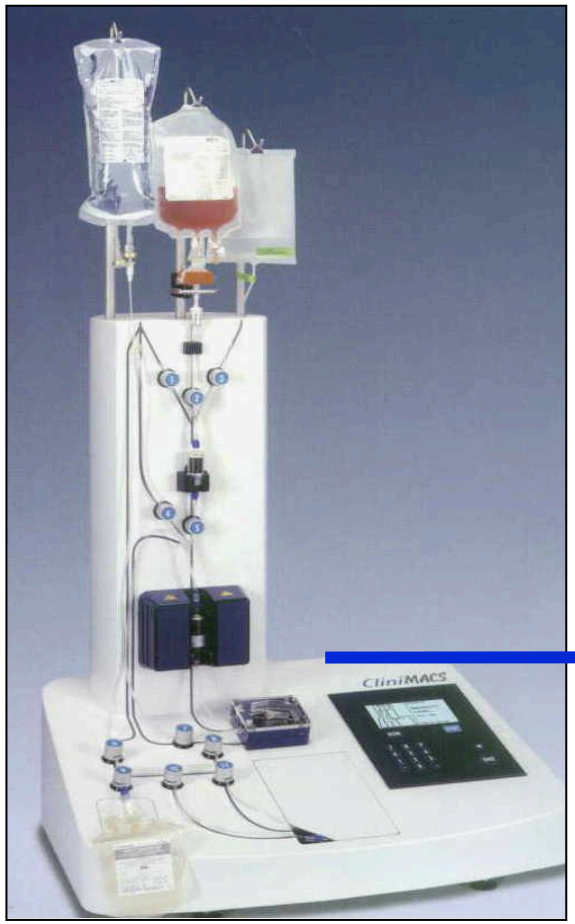
T-cell depletion of bone marrow transplants for leukemia from donors other than HLA-identical siblings: advantage of T-cell antibodies with narrow specificities

Richard E. Champlin, Jakob R. Passweg, Mei-Jie Zhang, Philip A. Rowlings, Corey J. Pelz, Kerry A. Atkinson, A. John Barrett, Jean-Yves Cahn, William R. Drobyski, Robert Peter Gale, John M. Goldman, Alois Gratwohl, Edward C. Gordon-Smith, P. Jean Henslee-Downey, Roger H. Herzig, John P. Klein, Alberto M. Marmont, Richard J. O'Reilly, Olle Ringdén, Shimon Slavin, Kathleen A. Sobocinski, Bruno Speck, Roy S. Weiner, and Mary M. Horowitz

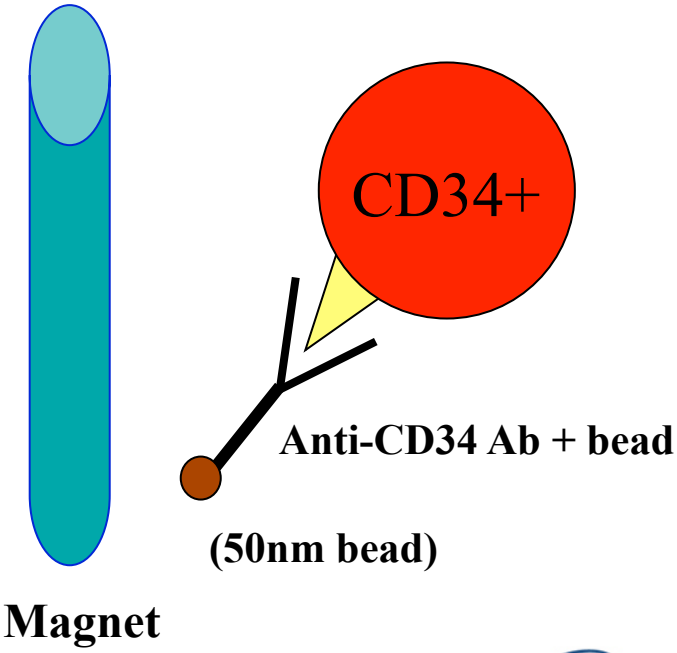
IBMTR (Champlin et al, Blood 2000)

Basis of selection

immunomagnetic bead technology



CliniMACS

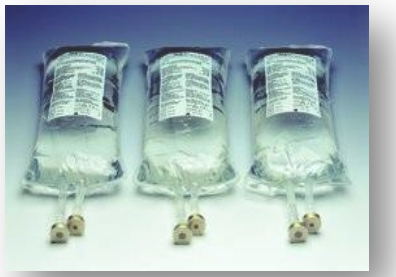


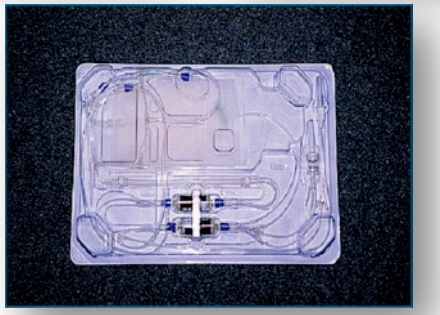
Processing

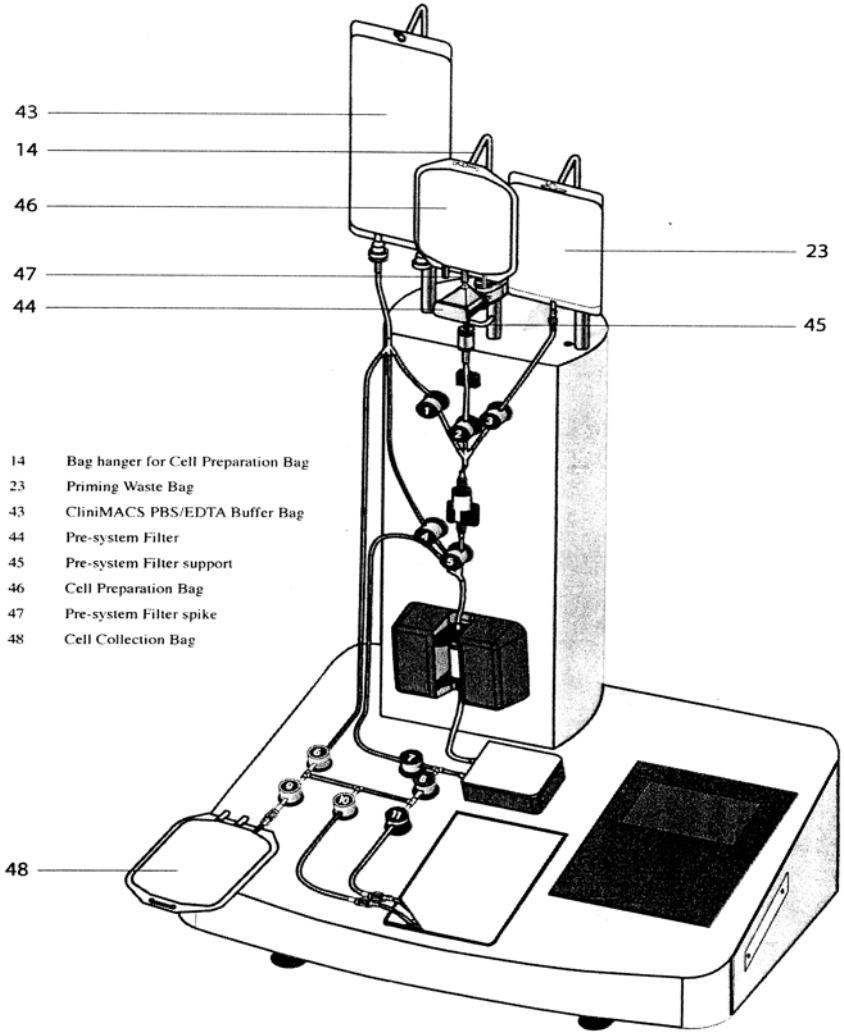
- “Machine time” is only a component of the process
- Pre-processing
  - Washing/platelet removal
  - Volume adjustments
  - Labelling
  - Washing/reagent removal
- Instrument setup
- Tubing set setup
- Automatic processing
- Analysis of cells (counts, flow cytometry)

Impact on processing lab

Specialised Cell Processing
All day procedure
2 staff
± Cryopreservation process

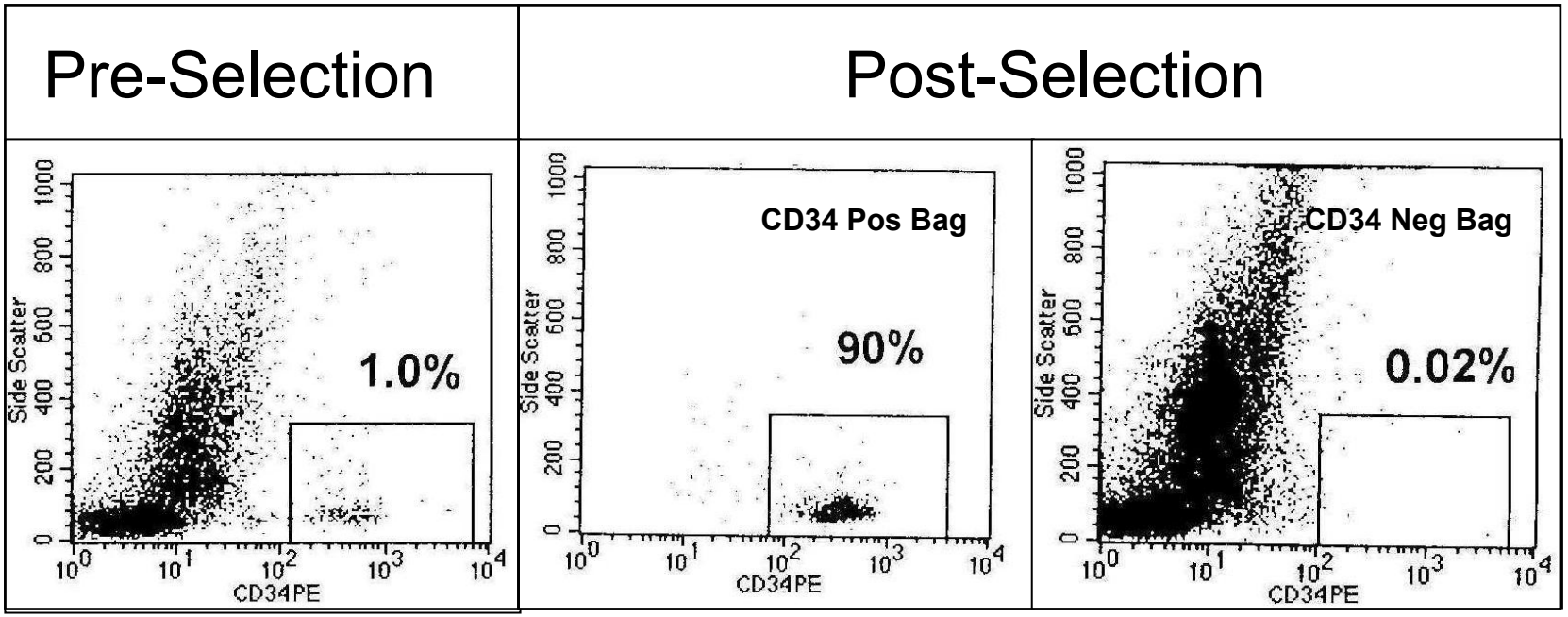






Cost >$8,000 $

CD34 Flow Cytometry Results



CD34: 2.8x10^6/kg

CD3 : 6300x10^5/kg

CD34: 2.1x10^6/kg

CD3 : 0.2x10^5/kg

Mean CD34 Recovery>75% Mean Purity >90%

Miltenyi results

	CD34 Enriched Fraction										Engraftment	
	TNC 10e6	CD34 %	CD34 10e6	CD34 Yield%	CD3 %	CD3 10e5	CD3 Log	CD19 %	CD19 10e5	CD19 Log	D > 1000 WBC	D > 20K PLT
Median	176.73	93.79	162.75	63.24	0.19	2.66	4.55	nd	nd	nd	10	11
Average	236.34	90.10	218.35	63.95	0.33	6.38	4.60	nd	nd	nd	10	11
Min	6.50	19.70	6.24	9.49	0.01	0.06	3.80	nd	nd	nd	10	10
Max	980.40	99.30	973.54	109.80	2.80	86.09	6.07	nd	nd	nd	10	12
Stdev	177.70	11.85	173.60	21.65	0.48	11.00	0.50	nd	nd	nd	0	1

- **Note:**
  - **Results have *not* been cleaned, *nor* manipulated or truncated**
  - **Cell source used: mPB**
  - **Pathologies encompass: NHL, AML, CLL, MDS, ALL with conventional conditioning**

Combined CD3 and CD19 Depletion

- **Rationale**:
  - **To keep graft-enabling and -supporting cells in the cell population**
  - **To reduce the risk for GvHD and EBV-related lymphomas**

- **Described Applications**:
  - **Mismatched/haploidentical stem cell transplantation (combination of CD34 selected grafts and CD3/CD19 depleted grafts)**
  - **Reduction of T and B cells in MUD and HLA-identical sibling Tx**
  - **Enrichment of NK cells for NK-DLI concepts**

CD34 vs CD3/CD19

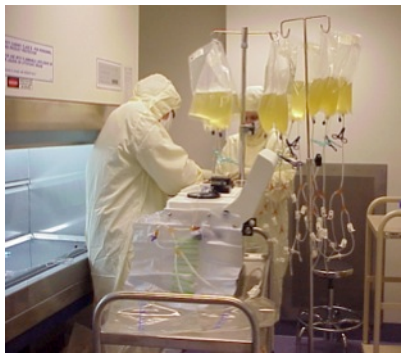
Stem Cell Isolation	T and B Cell Depletion
Highly pure stem cell population	Limited change to stem cell purity
Highly reduced total cell number	Partially reduced cell number
Few/no graft-enabling and facilitating cells	Abundant graft-enabling and facilitating cells
Exclusively $CD34^{pos}$ expressing stem cells	$CD34^{neg}$ stem cells present
No immune competent cells	Immune competent cells present NK cells Monocytes Dendritic cells and DC-precursors
No cytokine producing cells	Cytokine producing cells present
No/few committed progenitors	Committed progenitors present

Other manipulations

- Expansion- precursors, differentiated cells, cord expansion etc
- Adaptive immunotherapeutics

Not standard of care procedures

Require specialized infrastructure/technology, reagents and procedures beyond what is required for closed processing of grafts



Options

- Central facilities for a region?
- Centers of excellence distributed throughout a region?
- Centers of specialization- allo vs auto, unmanipulated vs complex processing, fresh/short term vs long term banked?
- Harvesting and processing- linked or on-site
- Links to treatment centers, blood centers
- Logistics and jurisdictional issues
- Commercial structures, suppliers and sponsors

Acknowledgements

Vicki Antonenas
Westmead Hospital

Paula Stoddart
Miltenyi Biotec Australia

Centre for Blood Cell Therapies

Prof Miles Prince
Dr David Ritchie
Dr Simon Harrison
Gianna O'Donnell
Valerie Costa
Maureen Loudovaris
Alannah Evans
Kate Dunster
Lucy Kravets
Javier Haurat
Gabby Workman
SiChong Zhou
Angela Morgan
Diana Raj
Carmen Chong
Wendy Chung
Lezly Penalver
Nicole McCarthy
Peter Gambell
Kerrie Stokes
Gill Treloar
Dimitrios Tsavios
Martin Bleasdale
Thu Lam

Cell Therapies
ACN 100 285 918

Peter Mac