

# T-CELL DEPLETION: ALEMTUZUMAB IN THE BAG

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# Engraftment variables in Allo SCT

## □ Host

- HLA identity
- Integrity of marrow stroma
- Disease type and status
- Previous chemotherapy
- Effective immunosuppression
  - TBI
  - Calcineurin inhibitors
  - Purine analogues
  - Serotherapy
  - ATG, alemtuzumab

## □ GvHD

- Acute
- Chronic

## □ Graft

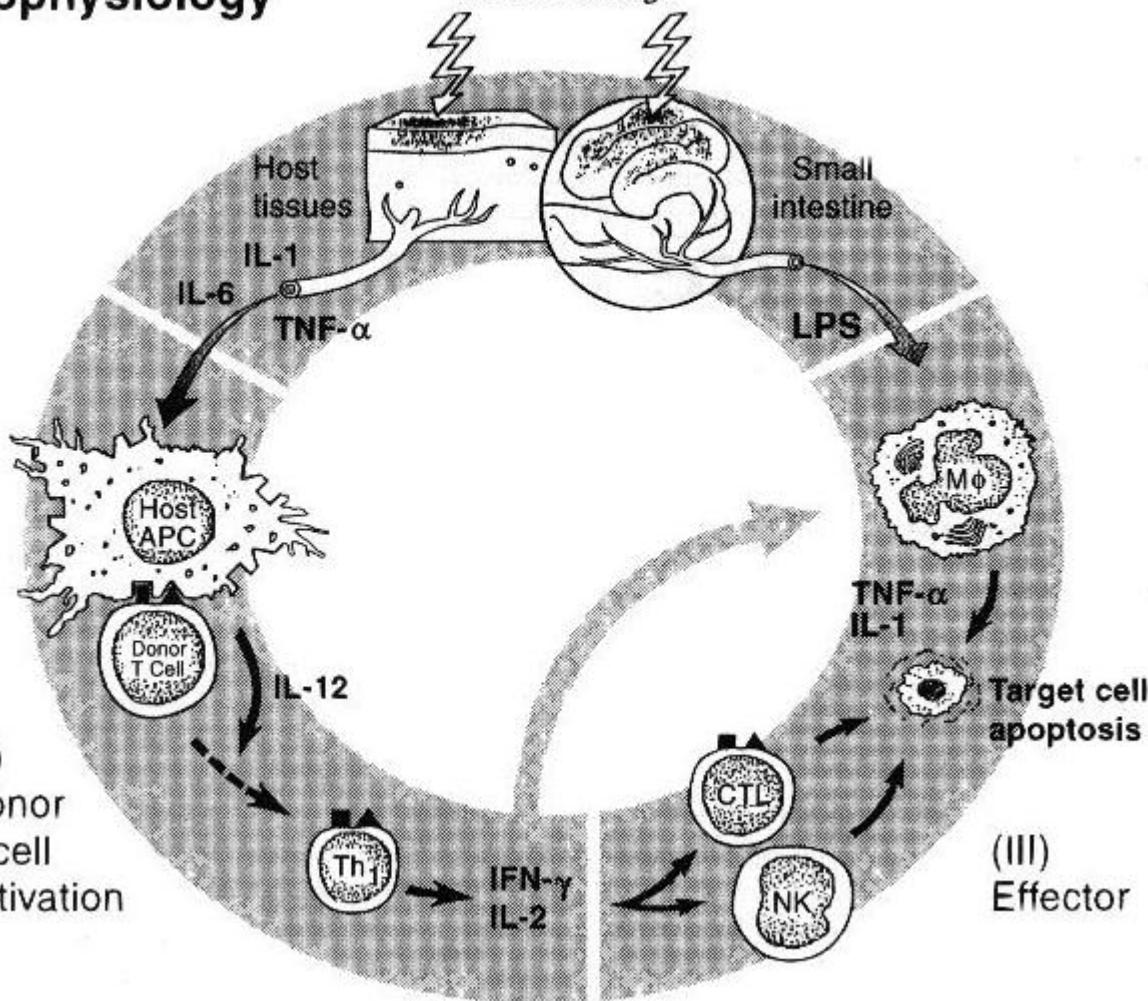
- Origin, gender, age
  - CB, BM, PBPC
- HLA identity
- Progenitor cell number
  - CD34+ cell dose /kg
  - Lymphocytes
    - T-cells
  - Accessory cells
- Graft manipulation
  - T-cell depletion
  - Addition of mesenchymal cells
- Late DLI



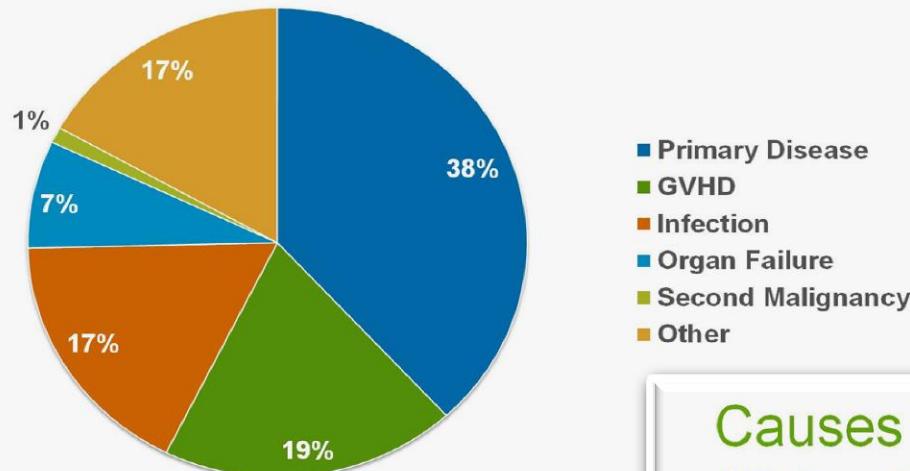
# GVHD Pathophysiology

## (I) Recipient conditioning

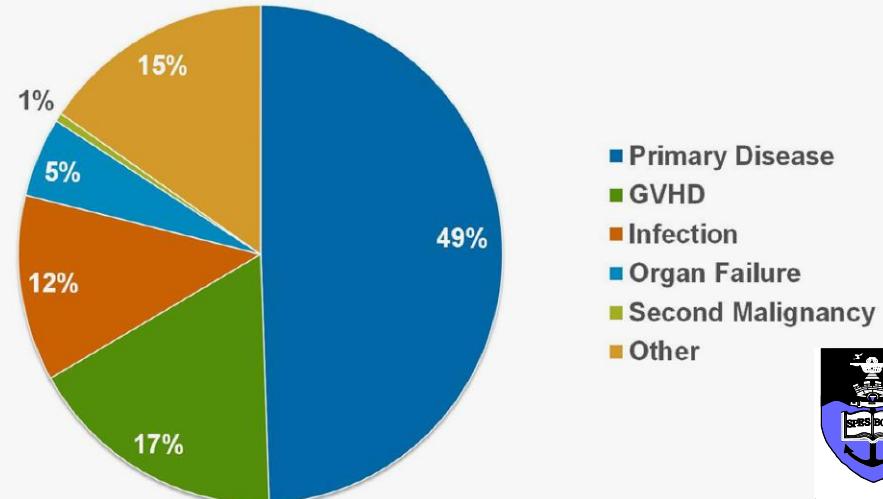
Tissue Damage



## Causes of Death after Unrelated Donor Transplants done in 2010-2011

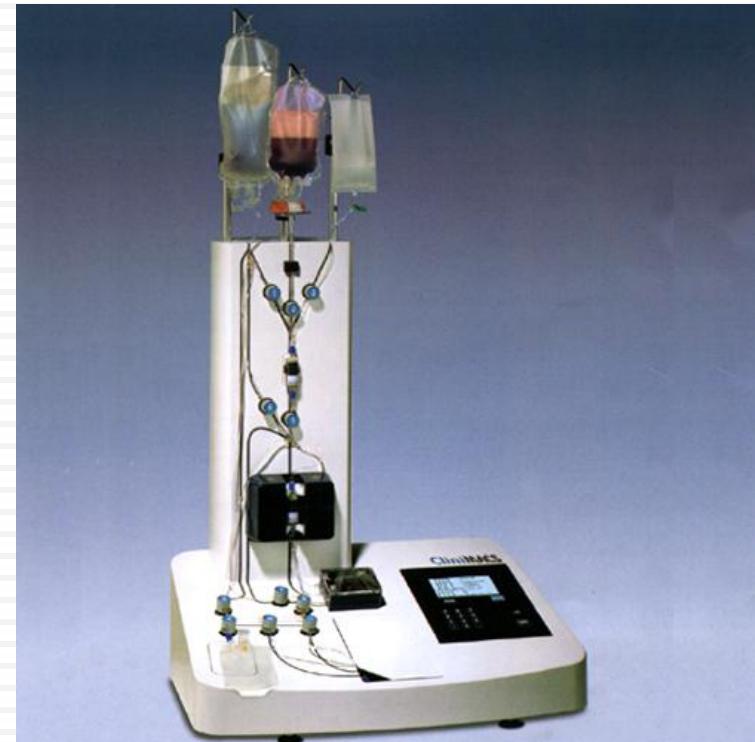


## Causes of Death after HLA-identical Sibling Transplants done in 2010-2011

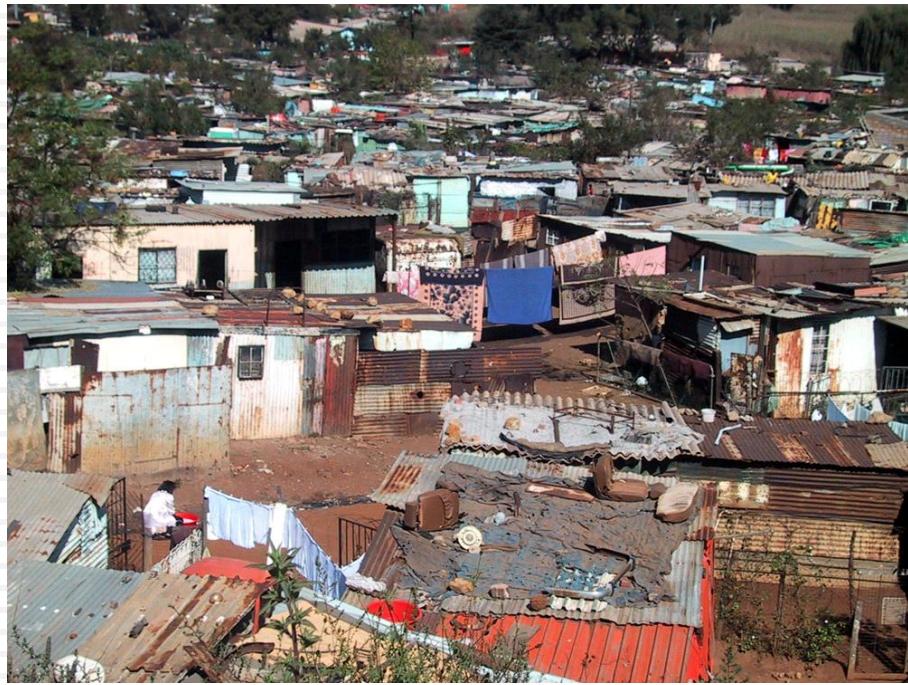
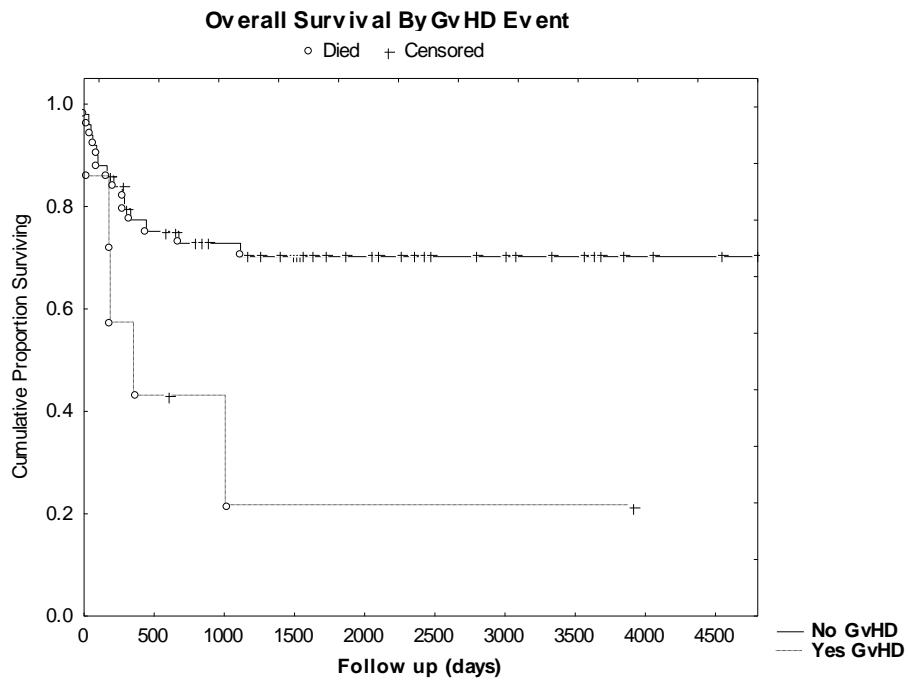


# Preventing GvHD

- Unfractionated grafts
  - Cyclosporin / tacrolimus
  - Methotrexate
    - Maxi / mini
  - Mycophenolate mofetil
  - Rapamycin
- T-cell depletion
  - Anti CD3
  - ATG / ALG
  - Campath / Alemtuzumab
  - Immunomagnetic depletion

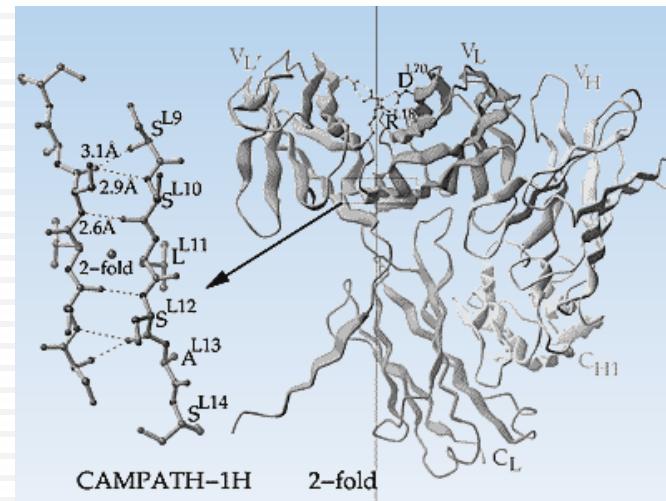
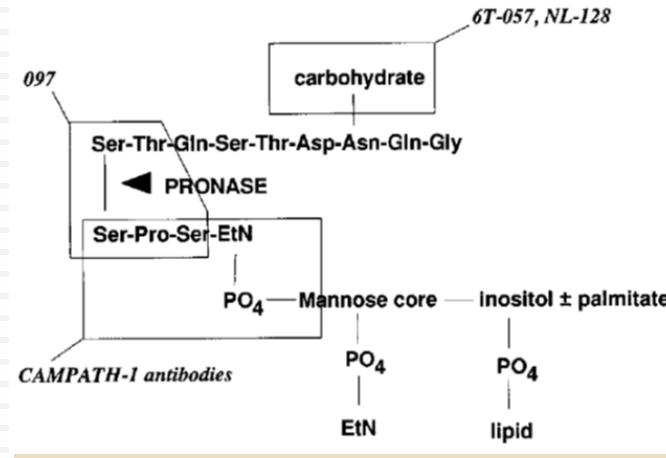


# Outcome of GvHD



# CD52

- Short peptide 35-39 KD
  - 12 aa + GPI anchor
    - Sialylated polylactosamine
    - Fucosylated mannose core
- Gene on chromosome 1
  - 2 alleles known
    - Similar phenotype
- CD52 very abundant on cells
  - Expressed on leukocytes, epithelial cells of epididymis
  - CD 4 cells: 450 K
  - Not on CD34+ cells
- No ligand binding activity
  - “Anti-adhesion function”?



# CAMPATH-1

## □ Campath-1 M

### ■ IgM, murine

- Ex vivo binding to T-cells
- Poor clearance of host T-cells
  - Increase in non-engraftment

## • Campath-1 G (rat IgG-2b)

Good binding to Fc receptor

Cell mediated cell cytotoxicity

Ab dependent cell cytotoxicity

Effective in killing malignant cells expressing CD 52

Rapid clearance of Ab

Immune antigen-globulin response

## □ Campath-1H (Alemtuzumab)

### ■ IgG1- humanised form

### ■ CHO transfectants

### ■ Purified: affinity chromatography

### ■ CD-CC

■ Activates complement

### ■ In vivo: AbD CC (FcIII)

■ Macrophage, NK cells

■ Spleen, liver, marrow, etc.

### ■ Cell kill dependent on density CD 52

### ■ Long half life: months



# CD 52 & CAMPATH-1

- CD 52 expression:

- CD 4; CD4+/CD 25+;  
CD8
- NK cells
- Dendritic cells
- Monocytes; macrophages
- Granulocyte precursors

- Epididymis

- Alemtuzumab

- B-CLL (registration)
- B-PLL
- Waldenstrom Mg
- T-LGL
- Peripheral T-cell NHL

- Off label

- Immuno depletion in  
organ transplantation
- HSCT
- Renal transplantation



# Immunodepletion: Which?

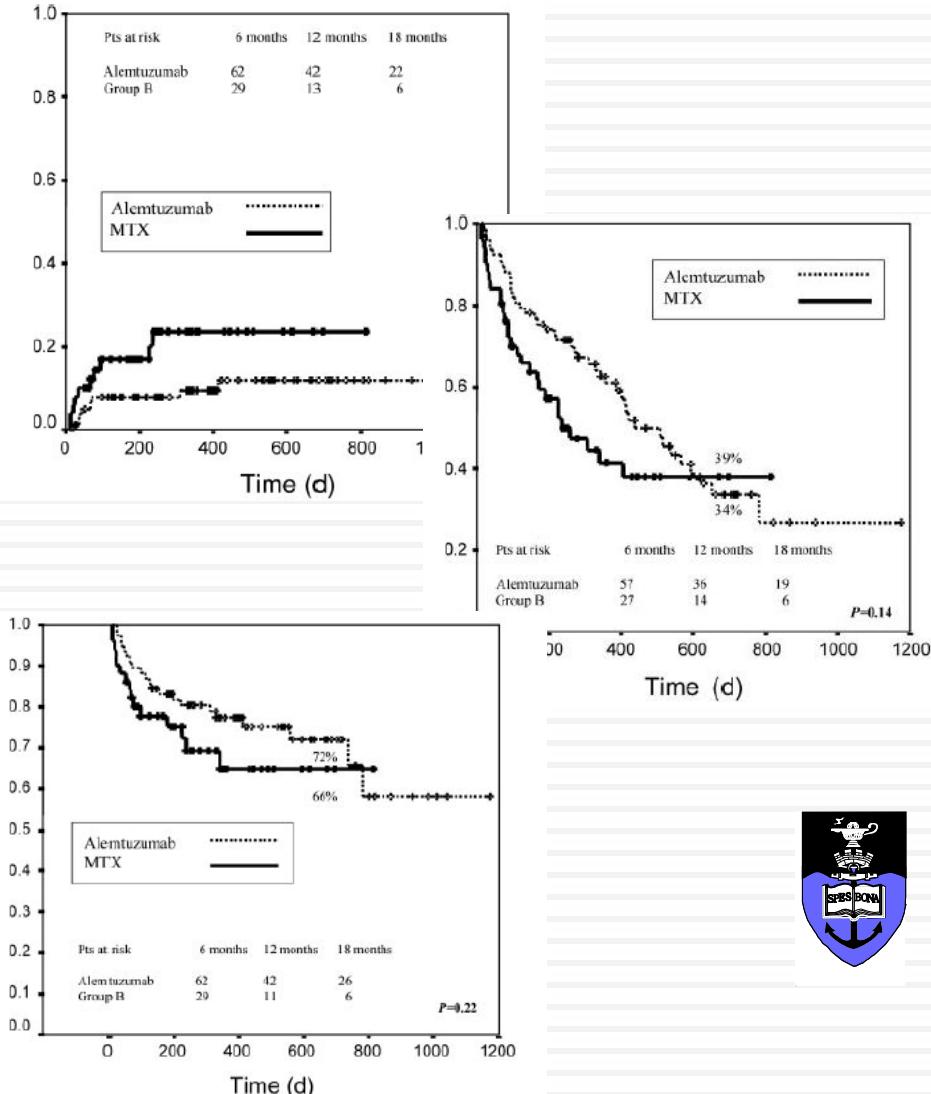
- RIC – Allo (n= 1676)
  - Haematological malignancies
  - Flu – Cy
  - HLA id. Related or MUD
- Alemtuzumab vs. ATG vs. No
  - aGvHD II-IV 19 vs. 38 vs. 40%
  - cGvHD II-IV: 24 vs. 40 vs. 52%
  - Relapse 49 vs. 51, vs. 38%
  - DFS: 30, vs. 25, vs. 39%
  - OS: 50 vs. 38 vs. 46%
- Cautionary note for T-cell depletion



# Immunodepletion: Yes or No?

- Non randomised, retrospective analysis:
  - Lymphoproliferative disorders (n= 129)
    - PBPC grafts
    - RIC with melphalan + fludarabine
    - CAMPATH 100 mg+ CSA
    - Methotrexate (10mg/m<sup>2</sup> x 3)+ CSA
    - Escalated doses of DLI allowed
  - Higher GvHD on MTX group
  - Higher infections (CMV) & relapses in Campath group
  - Similar OS

(Perez-Simon et al, Blood 2002)



# Pharmacokinetics

## □ Stem cell transplantation (n= 10)

### □ Compared *in vivo* + ex vivo

#### ■ (Flu-Mel) + cyclosporin

- 20 mg x 5 days

- in vitro alemtuzumab in RIT

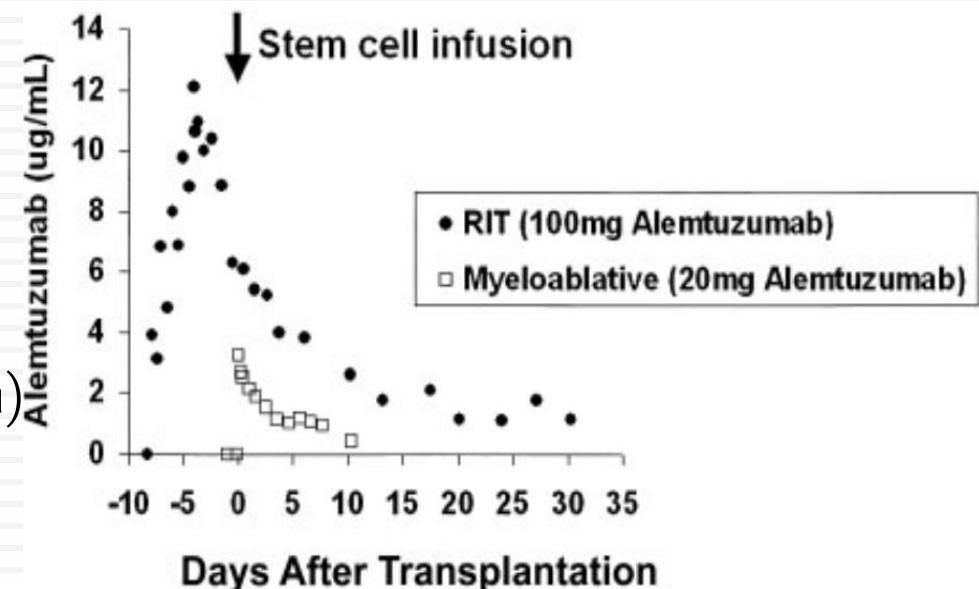
#### ■ 20 mg *ex vivo* (TBI, Cy / Flu)

### □ Median peak:

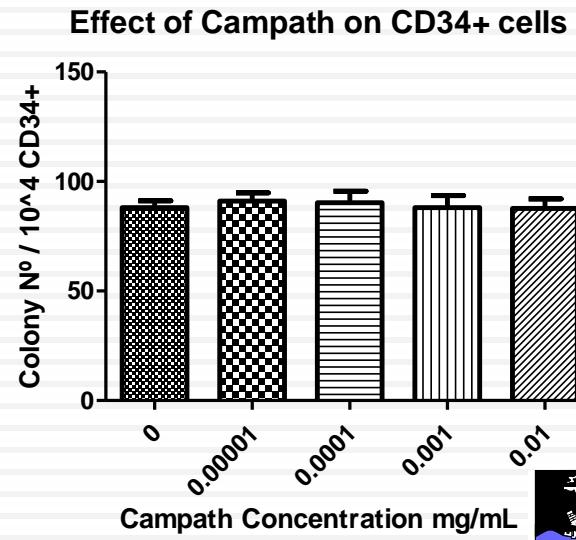
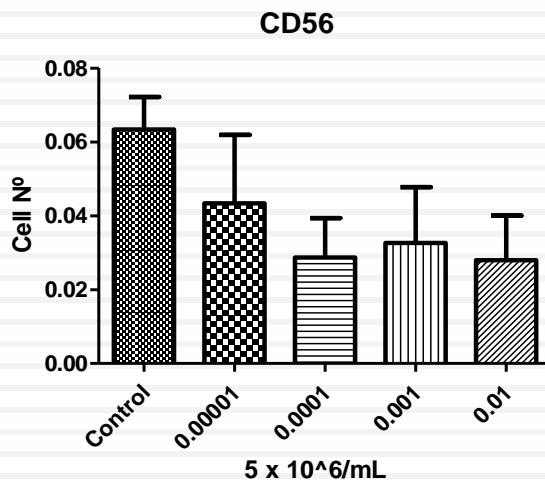
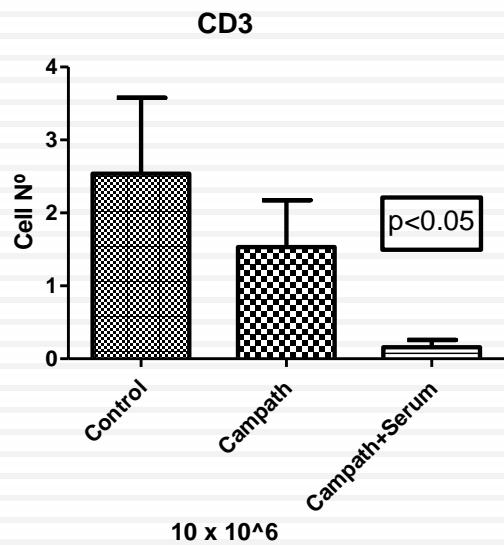
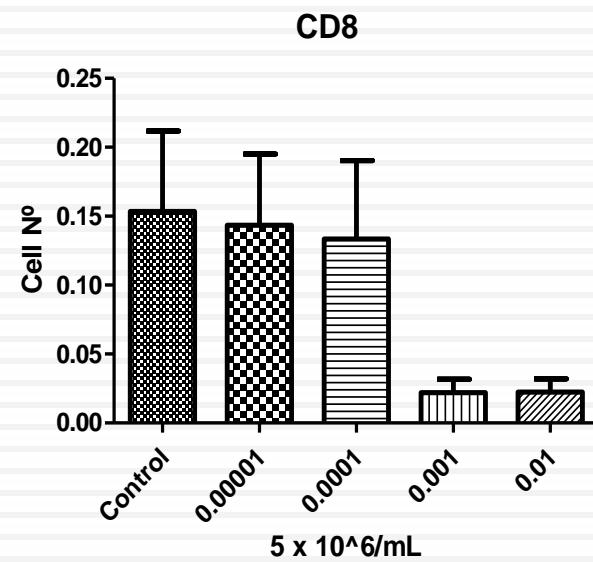
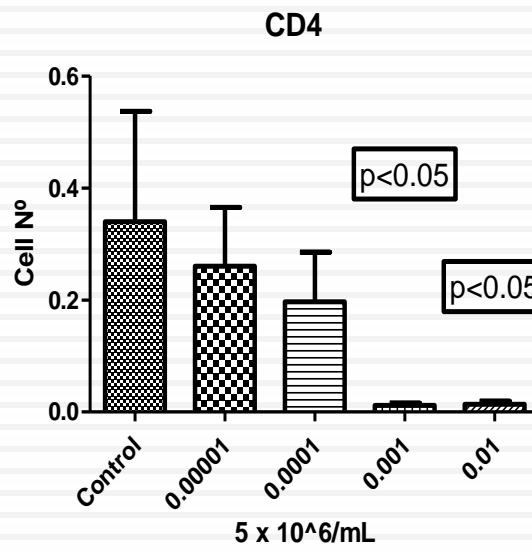
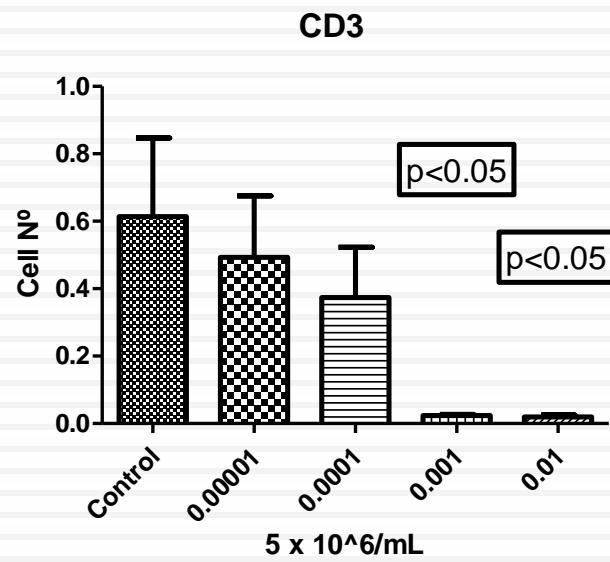
#### ■ RIT: 13.7 ug/mL

- At 30 days: 1.0ug/mL

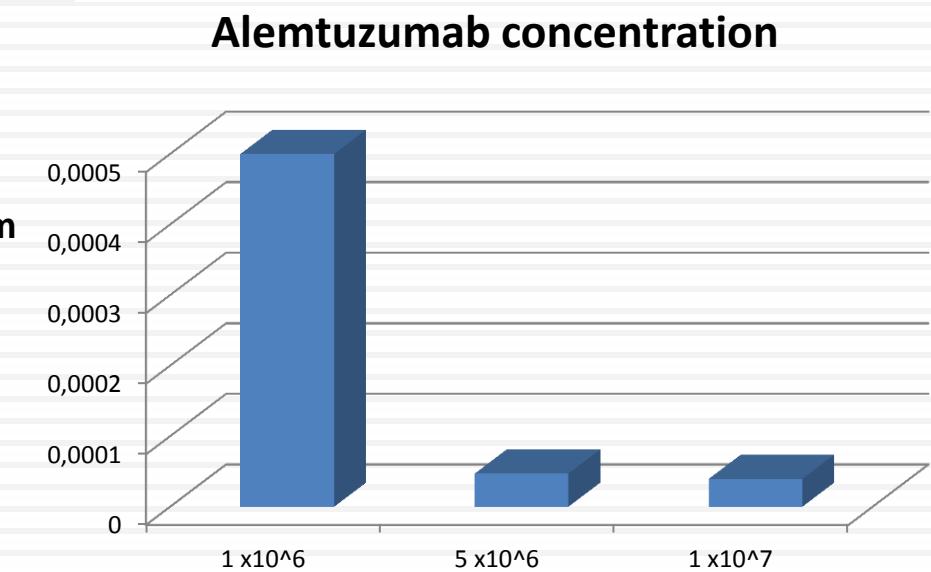
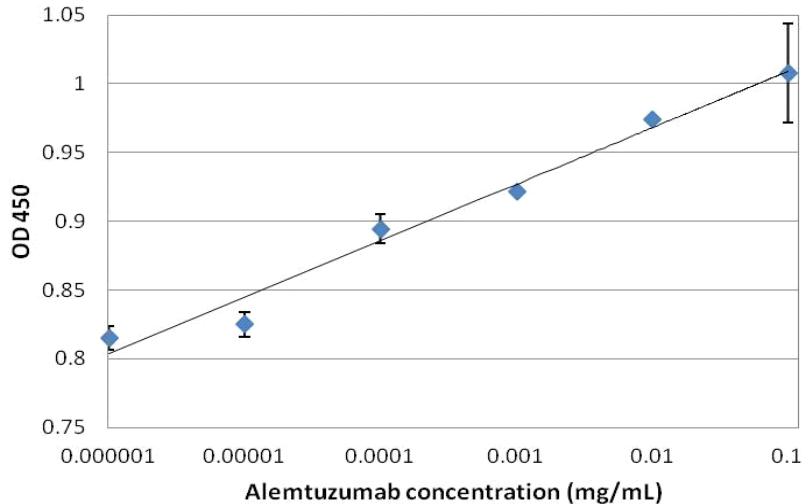
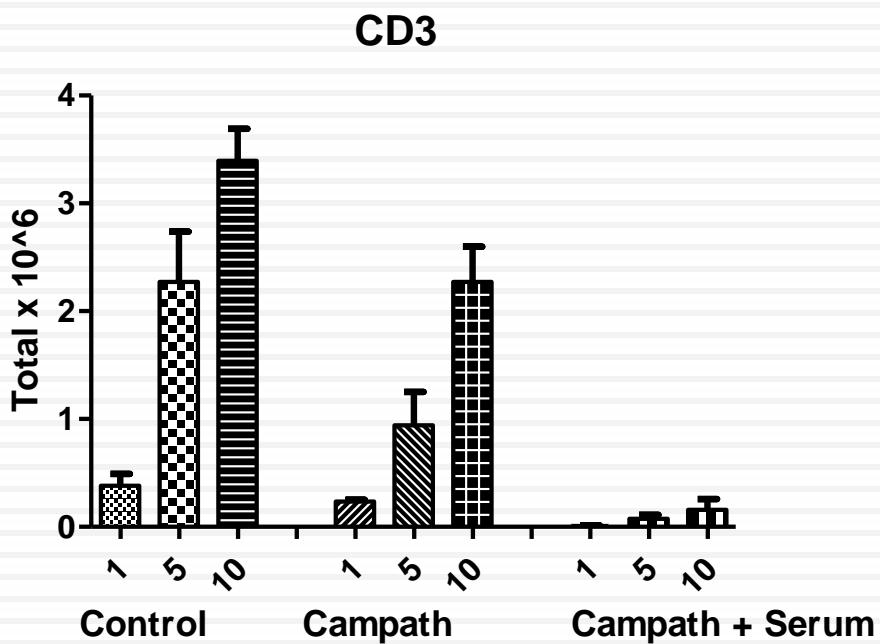
- At 60 days 0.1ug/mL  
(opsonising dose)



(Morris et al, Blood 2003)



# Alemtuzumab in the bag



# Patient population

## Haematological malignancies (n= 103)

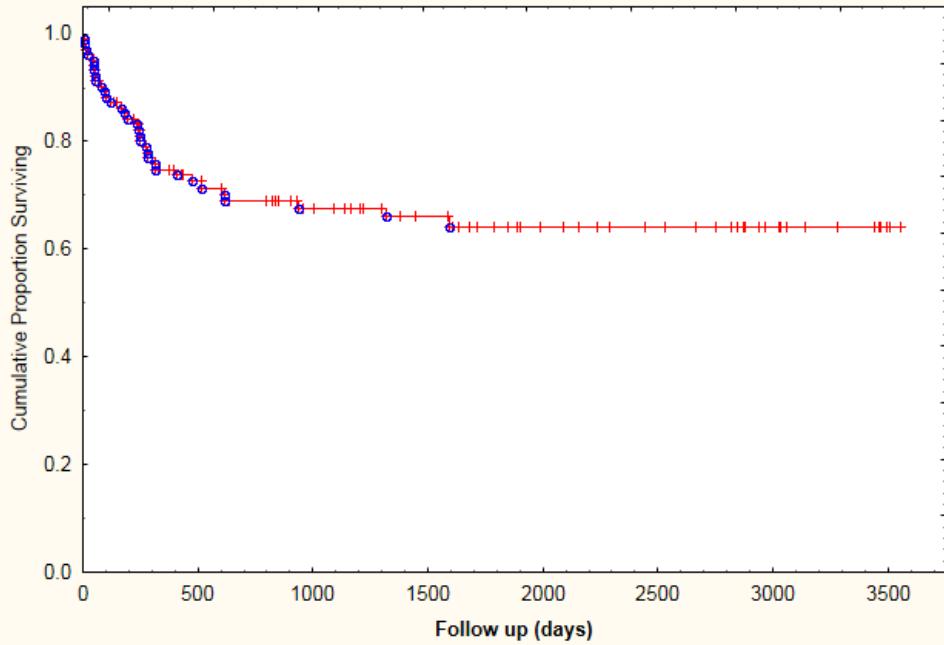
Age median (range)	40.5 (2-60)
Diagnosis	
• AML	16
• ALL	35
• NHL	26
• MDS	9
• MM	10
CD34+ x 10 <sup>6</sup> /kg	6.25
Alemtuzumab dose, median	5 mg
Conditioning	
Bu/Mel/Cy / TBI	81/23
aGvHD (II-IV) / cGvHD	16 / 4
Follow up, median (range)	905 days (11-3551)
LTFU	13
Alive / dead	70/ 33

## Aplastic / Fanconi Anaemia (n= 36)

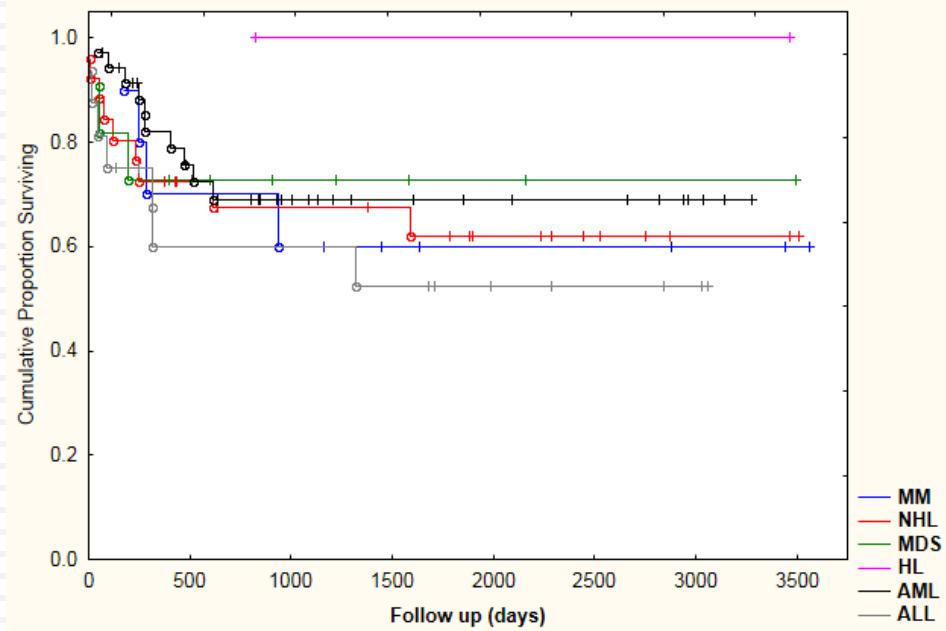
Age, median (range)	32 (5-60)
Female / Male	17 / 19
Conditioning	
SAA (n= 28)	Flu-Cy
Fanconi (n= 8)	Bu-Flu-Cy
CD34+ x 10 <sup>6</sup> /kg	7.12
Alemtuzumab dose	5 mg
Follow up, median (range)	1291 (121-3155)
Retransplant (graft failure)	6
DLI (PRCA)	2
LTFU	2
aGvHD / cGvHD	0 / 0



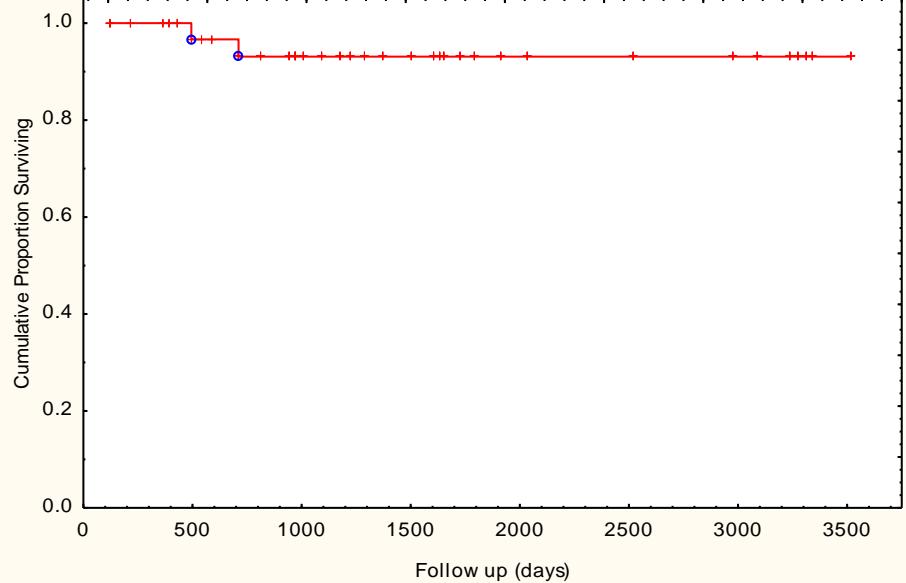
Overall Survival: Malignancies  
○ Died   + Censored



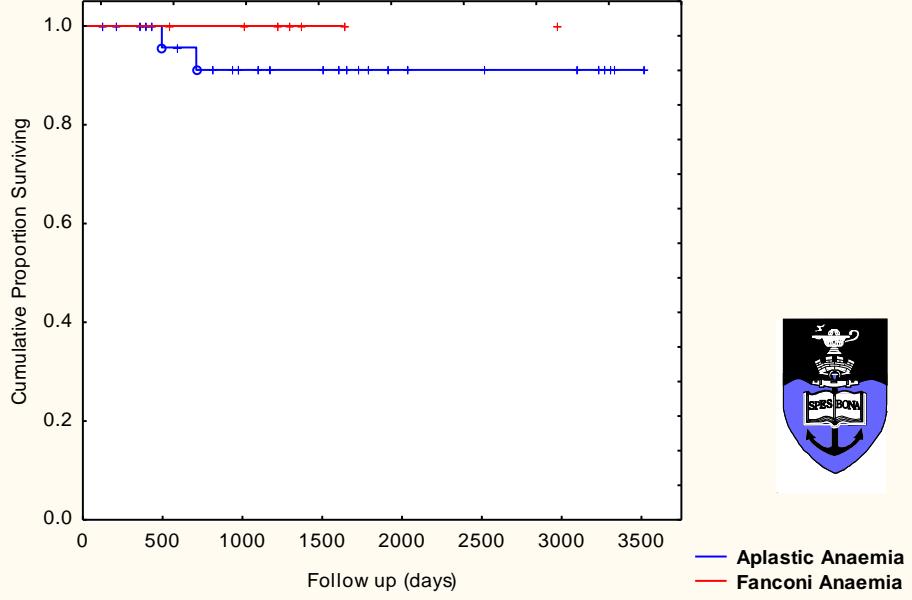
**Overall Survival by Diagnosis**



**Overall Survival**  
○ Died   + Censored



**Overall Survival: Diagnosis**  
○ Died   + Censored



— Aplastic Anaemia  
— Fanconi Anaemia

# Conclusions (1)

- In vivo immunodepletion with alemtuzumab
  - Valuable in preventing GvHD
  - Favours engraftment
    - Effective dose not established
  - Slowed immune reconstitution
    - (high dose)
    - Associated with high infection rates
      - CMV, fungal
  - Higher relapse rates
  - Higher cost
- Ex vivo immunodepletion with alemtuzumab
  - Affect multiple populations,
    - lymphocytes, myeloid cells & probably Ag presenting cells
  - Multiple mechanisms of cell death
    - Requires at least 1-2 ug/mL  $\times 10^{10}$
    - Repletion of complement
    - Cell density may be important
  - Depletion of CD4 cells of 3 log
  - Associated with low GvHD
  - Does not preclude engraftment
  - Low infection rates
    - CMV reactivation 21%



# Acknowledgments

- CAMPATH Study Group
  - Geofrey Hale
  - Herman Waldmann
- Cape Town Group
  - Regana Abdullah
  - Glenda Davidson
  - Shaheen Mowla
  - Valda Thomas
- Clinical Program
- The Doctors
  - Cecile du Toit
  - Andrew McDonald
  - Estelle Verburgh
- Apharesis team
  - Rachel Charles
- Protected environment Team
  - W. Vries

All the patients!



# Conclusions (2)

- CD4 load in PBPC graft
    - 16% of  $2-4 \times 10^{10}$  ( $5 \times 10^9$ )
  - What is the potential T-cell depletion (Eg. CD4)?
    - Ex vivo:  $3 \log^{-10}$ 
      - In vivo of infused with cells?
    - In vivo:  $2 \log^{-10}$
  - Would combination lead to higher CD4 depletion?
    - Potential:  $5 \log^{-10}$
  - Remaining CD4 cells:  $5 \times 10^4$
- 
- Practical use
    - Ex vivo + in vivo (mini):
      - Haemoglobinopathies (adults) SS anaemia
    - Ex vivo + in vivo (maxi)
      - RIC mismatched
      - Haploidentical transplantation

