The impact of thymoglobulin prior to paediatric unrelated umbilical cord blood transplantation on immune-reconstitution and clinical outcome

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• **Delayed T-cell recovery** & skewed TCR repertoire \((Komanduri et al, Blood 2007)\);

• **CD4+ T-cell recovery** after 12 months post UCBT \((Eurocord analysis, Nieheus et al. BJH 2001)\) and dependent on return of thymopoiesis;

• Lack of transfer of **antigen-experienced** lymphocytes;

• **Deficient cytokine production** \((Lewis et al, J.Clin.Inv 1991)\);

• **High early infection-related mortality** \((Delaney et al, BJH 2009)\) → up to 50% **TRM** \((Rubinstein et al, NEJM 1998)\).
Role of *in vivo* T-cell depletion

- Prolonged *in vivo* purging of donor T-cells might contribute to delayed immune reconstitution (more infections / loss of GvL);

- ATG or Alemtuzumab are commonly used to reduce the risk of GvHD post HCT;

- ATG or Alemtuzumab can be detected for weeks after HCT (*Waller et al, 2003; Call et al, 2009; Chakraverty et al, 2010*);

- Differences in dose/timing of ATG or Alemtuzumab might impact on outcome after HCT.
Poor immune reconstitution/infections

GVHD

ATG

No ATG
IMPACT OF rATG prior to UCBT

Retrospective analysis of clinical outcome and immune reconstitution in children undergoing 127 UCBT in London and Utrecht.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tbody>
<tr>
<td>n= 33 (Utrecht)</td>
<td>n= 48 (Utrecht)</td>
<td>n= 46 (London)</td>
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### EARLY rATG

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<tr>
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<th>D-7</th>
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### LATE rATG

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### NO ATG

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<tr>
<td>PATIENTS’ CHARACTERISTICS</td>
<td>NO ATG (n= 46)</td>
<td>EARLY ATG (n= 33)</td>
<td>LATE ATG (n= 48)</td>
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<td><strong>Age (years) median (range)</strong></td>
<td>1.8 (0.1-12.2)</td>
<td>5.5 (0.1-22.7)</td>
<td>2.3 (0.2-21.2)</td>
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<tr>
<td><strong>TNC (x 10^7/kg)</strong></td>
<td>8.1</td>
<td>5.8</td>
<td>7.3</td>
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<tr>
<td><strong>CD34+ (x 10^5/kg)</strong></td>
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<td>1.4</td>
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<td><strong>6/6 HLA matched</strong></td>
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<td><strong>5/6 HLA mismatched</strong></td>
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<tr>
<td><strong>4/6 HLA mismatched</strong></td>
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<td>7</td>
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</table>
NEUTROPHIL / PLT ENGRAFTMENT

NEUTROPHIL RECOVERY

PLATELET RECOVERY

Log rank
NONE-EARLY  p = 0.029
EARLY-LATE   p = 0.19 (NS)
NON-ENGRAFTMENT / REJECTION

NON ENGRAFTMENT

REJECTION

One Minus Cum Survival

Time_EFS

11% +/- 4%
5% +/- 3%
3% +/- 2%

11% +/- 4%
3% +/- 2%
CD3+ T-cells @ 1 month post UCBT
CD4+ T-cells @ 2 months post UCBT

- NO ATG
- EARLY ATG
- LATE ATG

p < 0.001
p = 0.006
EARLY CD4+ T-CELL EXPANSION WITH NO ATG
median CD4+T-cell / TRECs count after UCBT (n=33)

Chiesa et al, 2012
CD4+ T-CELL RECOVERY

median CD4+ T-cells after UCBT

MONTHS POST UCBT

CD4+ T-cell counts

- none
- early serotheraphy
- late serotheraphy
B and NK cell RECOVERY post UCBT

B cells

NK cells

months post SCT

none
early ATG
late ATG
VIRAL REACTIVATIONS AFTER UCBT
(CMV, AdV, EBV)

p = 0.022
EARLY FUNCTIONAL T-CELL RESPONSES WITH NO ATG
@ 2-4 months post UCBT (n=16)

**PHA / ELISPOT**

**INFγ ELISPOT**

- CD4+ T-cells
- CD8+ T-cells

**Spot forming cells (x 10^5 cells)**

- PHA
- CMV
- ADENOVIRUS

- Related peptide
- Unrelated peptide
- Hexon
Post UCBT MORTALITY

Non relapse mortality

23% +/- 8%
22% +/- 6%
13% +/- 6%

Relapse mortality

18% +/- 7%
9% +/- 6%
## CAUSES of DEATH post UCBT

<table>
<thead>
<tr>
<th>Cause</th>
<th>NO ATG n= 11/46</th>
<th>EARLY ATG n= 10/33</th>
<th>LATE ATG n= 18/48</th>
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</thead>
<tbody>
<tr>
<td>Leukaemia relapse</td>
<td>6/21 (28%)</td>
<td>2/13 (15%)</td>
<td>7/16 (43%)</td>
</tr>
<tr>
<td>Infection</td>
<td>2 (1 PH)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Idiopathic pneumonia</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Cardiac arrest / toxicity</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>GvHD</td>
<td>0</td>
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<tr>
<td>M.O.F.</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Leukoencephalopathy</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
acute GvHD

GvHD II-IV

Log rank
none-early: p = 0.002
early-late: p = 0.003
none-late: p < 0.001

61% +/- 9%
43% +/- 9%
17% +/- 5%

GvHD III-IV

Log rank
none-early: p < 0.001
early-late: NS p = 0.14

31% +/- 9%
16% +/- 8%
5% +/- 3%

Time_to_aGvHD (days)

One Minus Cum Survival
chronic GvHD

Log rank
none - early: NS (p=0.054)
none - late: NS (p=0.19)
SURVIVAL

Log rank NS

71% +/- 8%
71% +/- 8%
65% +/- 7%
Conclusions (1)

• Omission of *in vivo* T-cell depletion in UCBT leads to significantly quicker CD4+T-cell reconstitution up to 6 months post UCBT;

• This leads to reduced viral reactivations;

• However this is associated with increased aGVHD (not chronic);

• No difference in survival.
Conclusions (2)

• Early rATG has a better immune reconstitution in the first 2 months post UCBT compared to late;

• This is associated with more aGVHD, however with similar rates of severe (gr III-IV) aGVHD.
Discussion

- **NO ATG** may be the best option for patients with ID +/- active viral infections and malignancies;

- **Tailor made ATG dosing** (early pre UCBT) may be the best option for:
  - Immunodeficiency with inflammation (HLH)
  - Metabolic diseases
  - SAA
  - Hemoglobinopathies
Acknowledgements

GOSH
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Kimberley Gilmour
Waseem Qasim
Cathy Cale
Bobby Gaspar
Graham Davies
Austen Worth
Alison Jones
Adrian Thrasher
Amel Hassan
Siobhan Burns

UTRECHT
BMT/Immunology

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Jaap Boelens
Marc Bierings
Birgitta Versluys
Arianne de Wildt
Corinne Gerhardt
NON-INFECTIONOUS LUNG INJURY

Log rank NS

24% +/- 6%

18% +/- 7%

Log rank NS
T-CELL RECOVERY

median CD3+/CD4+/CD8+ T cells after UCBT

LYMPHOCYTE COUNT (X 10⁹/L)

1 month n= 39
2 months n= 39
3 months n= 36
6 months n= 32
12 months n= 27

median CD4+ cells @ 1 and 2 m post SCT: 250 and 550 x 10⁶/L (range 60-1890)
Median time to normal NK counts: 1 month (range, 1-3)
Median time to normal B-cells: 2 months (range, 1-12)
IMMUNE RECONSTITUTION AFTER UCBT

63 children studied: CD4+ cell recovery @ 12 months post SCT
CD8+ cell recovery @ 8 months post SCT
NK cell recovery @ 3 months post SCT
CD19+ cell recovery @ 6 months post SCT

Eurocord analysis. Nieheus et al. BJH 2001

12 children studied: 4/12 with CD4+ count \( \geq 0.2 \times 10^9/L \) @ 2 months


27 patients studied: median CD4+ count @ 2 months: 0.15 \( \times 10^9/L \)

Thomson et al. Blood 2000
SHIFT CD4+ NAÏVE > MEMORY CELLS

% OF TOTAL CD4+ T-CELL COUNT

months post UCBT

naive
central memory
effector memory
Mold et al. Fetal and adult hematopoietic stem cells give rise to distinct T cell lineages in humans. Science 2010
Spectratype @ 1 and 2 months post CBT (pt LS)

<table>
<thead>
<tr>
<th>Vβ1</th>
<th>Vβ2</th>
<th>Vβ3</th>
<th>Vβ4</th>
<th>Vβ5</th>
<th>Vβ6A</th>
<th>Vβ6B</th>
<th>Vβ7</th>
<th>Vβ8</th>
<th>Vβ9</th>
<th>Vβ11</th>
<th>Vβ12</th>
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<tbody>
<tr>
<td><img src="image1" alt="Graph at 1 month" /></td>
<td><img src="image2" alt="Graph at 1 month" /></td>
<td><img src="image3" alt="Graph at 1 month" /></td>
<td><img src="image4" alt="Graph at 1 month" /></td>
<td><img src="image5" alt="Graph at 1 month" /></td>
<td><img src="image6" alt="Graph at 1 month" /></td>
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<td><img src="image11" alt="Graph at 1 month" /></td>
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<th>Vβ14</th>
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<th>Vβ16</th>
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<td><img src="image13" alt="Graph at 1 month" /></td>
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<td><img src="image35" alt="Graph at 2 months" /></td>
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</table>
Spectratype post UCBT (Dkl score)
n=20
Regulatory T-cells

- Not possible to analyse T-regs by conventional intracellular staining of FOXp3;

- **Surrogate marker:** CD4+ CD25+ CD127dim;
  

- @1 and 2 months post UCBT: 7.4% and 8.1% CD4+ cells express CD4+CD25+CD127dim phenotype;

- Measurement of FOXp3 mRNA in PB within 3 months post UCBT showed levels between 5-1422% (median 37%) of expression levels found in healthy children;

Data suggestive of EARLY Treg RECOVERY
CONCLUSIONS
UCBT with no *in vivo* T cell depletion

- rapid peripheral expansion of CB CD4+ T-cells;
- rapid shift from naïve > memory;
- reduced virus-related morbidity;
- early detectable virus specific CTLs;
- Higher steroid responsive aGvHD;
- good engraftment rate/donor chimerism;
- Poor outcome in “*inflammatory diseases*” (HLH)
## REGIMEN RELATED TOXICITY

<table>
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<tr>
<th>Condition</th>
<th>Incidence</th>
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<tbody>
<tr>
<td>Gram POS sepsis (Strept. viridans)</td>
<td>9/46</td>
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<tr>
<td>Adenoviraemia</td>
<td>6/46</td>
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<tr>
<td>CMV viraemia</td>
<td>5/46</td>
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<tr>
<td>HHV6 encephalitis</td>
<td>1/46</td>
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<tr>
<td>RSV/Paraflu3/Rhinovirus URTI</td>
<td>8/46</td>
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<tr>
<td>Candida osteomyelitis</td>
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<td>Aspergillosis</td>
<td>1/46</td>
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<tr>
<td>Cardiac arrest</td>
<td>2/46</td>
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<tr>
<td>Lung injury</td>
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*Updated from Chiesa et al. BJH 2012*