CELL THERAPY FOR AUTOIMMUNE DISEASES: 15 YRS EXPERIENCE

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ADWP EBMT Chair
HSCT (BM, PBSC, MSC, CB): RESET of TOLERANCE?

CORTICOSTEROIDS

AZATHIOPRINE  MTX  MMF

HSCT

MSC

CPM po, iv
Anti TNF, Anti-CD20, Anti-Blys

MSC

2005

SSC: 5 yrs Survival (skin + lung / heart / kidney):
30%  ≈ 40%

SLE: 10 yrs Survival / End Stage Renal Failure (neuro + kidney / heart / lung):
70 / 50% 90 / 35%

MS: DMD (IFN, Copaxone) escalation (natalizumab, mitoxantrone, fingolimod, cladribine)
A NEW THERAPEUTIC OPTION:
- Depend HSC (exp, AB) => RESET IR
- Resistant to classical tt => New INDICATIONS
- Feasibility established: TRM risk / benefit
- Benefit from myeloablation+IS
  - HSC? BM, PBSC, MSc…CB
  - Auto / Allo : remission / cure
- => Conditioning regimen : ATG? Irradiation?
- => Cell selection?
- => MAINTENANCE Therapy

SHORT and LONG TERM EFFICACY:
- Ongoing phase 3 trials
- Cost effectiveness
- New trials
- Combined efforts: EBMT, US, Asia
T-cell dysfunction is associated with both the involutionary changes occurring in the thymus of the AI-prone mice and to abnormalities that reside in the stem cells. 

**BMT** could be considered as an approach for treating life threatening AD in humans.
PRECLINICAL EXPERIMENTS

V BEKKUM Best Pract Res 2004; 17; 201

1985 * : 1st successful treatment by allogeneic BMT in LUPUS mice
(better results with fetal bone fragments: stromal cells? or MSC)

<table>
<thead>
<tr>
<th>Autoimmune strain</th>
<th>Disease</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD</td>
<td>Diabetes</td>
<td>Resolution of insulinitis</td>
</tr>
<tr>
<td>B/W, BXSB</td>
<td>Glomerulonephritis</td>
<td>Regression of glomerular damage; reduction in circulating immune complexes or complete cure</td>
</tr>
<tr>
<td>MRL/lpr</td>
<td>Glomerulonephritis</td>
<td>Complete cure</td>
</tr>
<tr>
<td>MRL/lpr</td>
<td>Glomerulonephritis and arthritis</td>
<td>Complete resolution of glomerulonephritis arthritis and correction of immunological abnormalities</td>
</tr>
<tr>
<td>Old MRL/+</td>
<td>Pancreatitis and sialoadenitis</td>
<td>Cure of pancreatitis and sialoadenitis, normalization of T- and B-cell functions</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disease</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjuvant arthritis(^a) (rats)</td>
<td>Complete remission</td>
</tr>
<tr>
<td>Collagen induced arthritis(^b) (mice)</td>
<td>No remission, complete prevention of progression</td>
</tr>
<tr>
<td>Experimental allergic encephalomyelitis(^b) (rats)</td>
<td>Complete remission, few relapses</td>
</tr>
<tr>
<td>Biozzi mice(^b)</td>
<td>Treated in acute phase: Complete remission, few relapses</td>
</tr>
<tr>
<td></td>
<td>Treated in chronic phase: No effect</td>
</tr>
</tbody>
</table>

(syngeneic BMT : Negative ? Controls)

• 1 Inflammatory AID \(\Rightarrow\) initiated + maintained by activated T cells \(\Rightarrow\) Eliminate
• 2 Cy alone < Cy + TBI
• 3 Relapse \(\Rightarrow\) memory T cells \(\Rightarrow\) Radiation > Cy
• 4 Search for specific lymphocytolytic agents: Fludarabine, ATG?
• 5 Immune reconstitution (? stem cell): recapitulation of ontogenesis
Tolerance induction by bone marrow transplantation in a multiple sclerosis model

Martin M. Herrmann, Susanne Gaertner, Christine Stadelmann, Jens van den Brandt, Robert Böscke, Wilfried Budach, Holger M. Reichardt, and Robert Weissert

Blood 2005; 106: 1875

Myelin Oligodendrocytes
Glycoprotein (MOG) EAE
=> RR disease
-DA susceptible rats
-ACI resistant rat

↓ Ig G levels
↓ %CD4+, C8+

CD4+ CD25+ T cells
Fox P3 expression

Induction of CD4+CD25 bright regulatory T cells in rats undergoing BMT
REVERSAL of new-onset TYPE 1 DIABETES in mice by SYNGENEIC BONE MARROW TRANSPLANTATION


Pancreatic histological analysis (day 120 after BMT)– 10d DD and 10d-ND show no lesions

Table 1

<table>
<thead>
<tr>
<th>Days after T1D onset when receiving BMT</th>
<th>Donor</th>
<th>Group</th>
<th>No.</th>
<th>Mean FBG (mmol/L)</th>
<th>Lowest FBG (mmol/L)</th>
<th>Highest FBG (mmol/L)</th>
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<tbody>
<tr>
<td>10</td>
<td>Diabetic</td>
<td>106-DD</td>
<td>7</td>
<td>1004</td>
<td>8.7</td>
<td>11.4</td>
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<tr>
<td>10</td>
<td>Normal</td>
<td>106-ND</td>
<td>6</td>
<td>103</td>
<td>7.5</td>
<td>13.7</td>
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<tr>
<td>No BMT</td>
<td></td>
<td>NB</td>
<td>8</td>
<td>26.08</td>
<td>18.3</td>
<td>28.3</td>
</tr>
<tr>
<td>40</td>
<td>Normal</td>
<td>406-ND</td>
<td>7</td>
<td>&gt;24.88</td>
<td>13.4</td>
<td>&gt;33.3</td>
</tr>
</tbody>
</table>

* Difference was significant compared to group NB (p < 0.0001)

Normal: normal mice
NB: no treatment
10d-DD: BM from fraternal inbred SZ-diabetic mice
10d-ND: BM from normal mice
40d-ND: syn-BM from normal mice on day 40 after T1D onset
Haematopoietic stem cell transplantation (HSCT) in severe auto-immune diseases (ADs): updated guidelines written on behalf of the European Group for Blood and Marrow Transplantation (EBMT) JA Snowden, R Saccardi, M Allez, S. Ardizzone, R Arnold, R Cervera C Denton, JM van Laar, M Labopin, G Mancardi, R Martin, JJ Moore, J Passweg, C Peters, M Rabusin, M Rovira & D Farge on behalf of the EBMT Autoimmune Disease (ADWP) and *Paediatric Diseases (PDWP) Working Parties (BMT submitted) Per Ljungmann BMT 2009

Level II = at least one well designed clinical trial without randomisation: cohort or case controlled analytical studies (preferably from more than one centre), multiple time series studies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Sib donor</th>
<th>Well matched unrelated</th>
<th>Mismatched donor</th>
<th>Autologous</th>
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<tbody>
<tr>
<td>MS</td>
<td>D/III</td>
<td>GNR/III</td>
<td>GNR/III</td>
<td>CO/II</td>
</tr>
<tr>
<td>SSc</td>
<td>D/III</td>
<td>GNR/III</td>
<td>GNR/III</td>
<td>CO/II</td>
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<tr>
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<td>D/III</td>
<td>GNR/III</td>
<td>GNR/III</td>
<td>CO/II</td>
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<tr>
<td>Crohn’s</td>
<td>GNR/III</td>
<td>GNR/III</td>
<td>GNR/III</td>
<td>CO/II</td>
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<tr>
<td>RA</td>
<td>GNR/III</td>
<td>GNR/III</td>
<td>GNR/III</td>
<td>CO/II</td>
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<tr>
<td>Vasculitis</td>
<td>GNR/III</td>
<td>GNR/III</td>
<td>GNR/III</td>
<td>CO/II</td>
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<td>Polymyositis-Dermatomyositis</td>
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<td>GNR/III</td>
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<td>GNR/III</td>
<td>GNR/III</td>
<td>D/III</td>
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<tr>
<td>RCD Type II</td>
<td>GNR/III</td>
<td>GNR/III</td>
<td>GNR/III</td>
<td>D/III</td>
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</table>

HSCT for ADs: EBMT Registry

- Patients: 1262
- Male/Female %: 38/62
- Centres /Countries: 211/29
- Transplant procedures: 1294
- Overall Follow up: 2.93y (<1-24)

<table>
<thead>
<tr>
<th></th>
<th>Autografts n=1211</th>
<th>Allografts n=82</th>
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<tbody>
<tr>
<td>First</td>
<td>1199</td>
<td>62</td>
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<tr>
<td>Second</td>
<td>12</td>
<td>16</td>
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<tr>
<td>Third</td>
<td></td>
<td>4</td>
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<tr>
<td>Age at transplant</td>
<td>36y (2.7-76)</td>
<td>16y (0.4-57)</td>
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</table>
### Number of HSCT: 1271 - EBMT Registry
#### February 2011

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
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<tbody>
<tr>
<td><strong>MULTIPLE SCLEROSIS</strong></td>
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<tr>
<td><strong>CONNECTIVE TISSUE D.</strong></td>
<td>398</td>
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<tr>
<td>SSc</td>
<td>260</td>
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<tr>
<td>SLE</td>
<td>101</td>
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<td>PM-DM</td>
<td>16</td>
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<td>Sjogren</td>
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<td>Antiphosph. syndrome</td>
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<tr>
<td>Other/Unknown</td>
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<td><strong>ARTHRITIS</strong></td>
<td>169</td>
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<tr>
<td>Rheumatoid arthritis</td>
<td>88</td>
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<td>Juvenile chronic arthritis:</td>
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<tr>
<td>- Systemic JIA</td>
<td>47</td>
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<tr>
<td>- Other JIA</td>
<td>28</td>
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<tr>
<td>Psoriatic arthritis</td>
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<tr>
<td>Other</td>
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<tr>
<td><strong>INFLAMMATORY BOWEL</strong></td>
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<td>Crohn's disease</td>
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<td>Ulcerative colitis</td>
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<td>Other</td>
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<td><strong>HAEMATOLOGICAL</strong></td>
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<td>25</td>
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<td>Evan’s</td>
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<td>AIHA</td>
<td>17</td>
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<tr>
<td>Other</td>
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<td><strong>VASCULITIS</strong></td>
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<tr>
<td>Wegener’s</td>
<td>10</td>
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<td>Behcet’s</td>
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<td>Takayasu</td>
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<tr>
<td>Microscopic poly. nodosa</td>
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<tr>
<td>Classical poly. nodosa</td>
<td>1</td>
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<tr>
<td>Chura-Strauss</td>
<td></td>
</tr>
</tbody>
</table>
INDICATIONS HAVE CHANGED:
no more RA...MS, SSc, SLE, Crohn’s.......Immune Cytopenia, NIDD others?

By disease 1999

By disease 2003-2004
ADs: HSCT per Country

- Italy: 274
- Germany: 158
- France: 104
- Netherlands: 98
- Spain: 91
- United Kingdom: 90
- Sweden: 66
- Czech Republic: 55
- Australia: 52
- China: 48
- Russia: 46
- Greece: 46
- Belgium: 29
- Poland: 24
- Hungary: 23
- Israel: 18
- Switzerland: 17
### ALLOGENEIC HSCT for AID (T Draikeler BMT 2008)

#### DIAGNOSIS

<table>
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<tr>
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<td>MS</td>
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<tr>
<td>SLE</td>
<td>1</td>
</tr>
<tr>
<td>PM-DM</td>
<td>1</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>3</td>
</tr>
<tr>
<td>IBD</td>
<td>2</td>
</tr>
<tr>
<td>Behcet</td>
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</tr>
<tr>
<td>AI unspecified</td>
<td>3</td>
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**Hematologic**

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<td>PWCA</td>
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<tr>
<td>PRCA</td>
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<tr>
<td>AIHA</td>
<td>7</td>
</tr>
<tr>
<td>ITP</td>
<td>2</td>
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<tr>
<td>Evans</td>
<td>1</td>
</tr>
<tr>
<td>Unspecified</td>
<td>5</td>
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</tbody>
</table>

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*Overall Survival after HSCT (n=33)*

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*BMT 2008*

80 ± 3% SSc  (n = 137)
93 ± 2  % MS   (n = 345)
87± 4  % SLE   (n = 85)
82 ± 5  % JIA   (n = 65)
80 ± 7 % HIC (n=37)
98 ± 2 % RA    (n = 89)

Overall TRM: 5 ± 1%

CENTER EFFECT : AHSCT AD > 13N

Farge et al Haematologica 2010
Progression Free Survival at 3 yrs (n= 900)

Time to objective disease progression or death (EBMT guidelines)

63 ± 4 % SSc (n = 137)
55 ± 3 % MS (n = 345)
54+ 6 % SLE (n = 85)
52 ± 7% JIA (n = 65)
34 + 9 % HIC (n=37)
23 + 5 % RA (n = 89)
HSCT in MS - Which phase?

- Inflammation
- Degeneration
- Response to treatment
- Disability

R.R.

S.P.

EDSS 6.5

EDSS 3.0

Clinical

Time

SEPTEMBER 9–12, 2009 DÜSSELDORF
**HSCT FOR MS PATIENTS**

**Nash RA, et al.**  
91%

![Graph showing overall survival over time](image)

**Saccardi R, et al.**  

![Graph showing disease activity-free survival](image)

**Burt RK, et al.**  

Progression free Survival at 3 years 100%

### Mobilization of PBSC
- **16 μg/kg G-CSF**
- **CD34+ ex-vivo selection**
  - Age: 41 years (range 27-60)
  - EDSS: 7.0 (5.0 – 8.0)
  - Dis. Dur.: 7 years (1 – 23)

### Conditioning Regimen
- **TBI, 800 Gy Total dose**
- **60 mg/kg Cyclophosphamide**
- **90 mg/kg/day Equine ATG**

### Mobilization of PBSC
- **2 g/m² Cyclophosphamide in 1 day**
- **5 μg/kg G-CSF (Filgrastim) from day +2**
- **No Graft Manipulation**

### Conditioning Regimen
- **BEAM (BCNU, Melphalan, ARA-C, Etoposide)**
- **Rabbit ATG 10 mg/kg**
- **SP PP RR**

### Mobilization of PBSC
- **79%**
- **21%**
- **31%**
- **4%**
- **65%**

### Conditioning Regimen
- **200 mg/kg Cyclophosphamide in 4 days**
- **20 mg Alemtuzumab (n17) or 6 mg/kg Rabbit (n4)**

### Progression Free Survival at 3 years 100%
HSCT for MS: Progression-Free Survival (PFS) according to age and years from diagnosis

Mancardi GL, Saccardi R. Lancet Neurol 2008; 7: 626–36
HSCT in MS: ACHIEVEMENTS and PRESENT

- Most of pts are SP (~50-70%) in EBMT registry
- PFS survival around 50% at 5 years
- Low incidence of clinical relapses
- Efficacy on MRI parameters
- A minority of pts in prospective studies
- Lack of comparative trials
- Lack of clinical details on long-term survivors

Non intervention study for active RR- and SP-MS with failure of first line therapy:
- efficacy and mechanism/s of action of aHSCT
- acceptance of HSCT by neurologists
- further evidence to support AHSCT as treatment option in this group of pts.
Diffuse Cutaneous
Limited Cutaneous
pulmonary hypertension
malabsorption

joint contractures,
GI, lung, heart, kidney

N risk
t factors
Total no. of
pts
Nb / RR
of deaths

0 509 12 7.1

1 349 45 22.8

2 168 55 54.8

3 23 7 100.0

J. Fransen, D. EUSTAR 2010
**ASTIS: 2001-2011**

Pts rapidly progressive or severe SSc (n = 156)

- $\leq$ 4 yrs + skin score $\geq$ 15 (0-51) + involvement heart/lung/kidney
- $\leq$ 2 yrs + skin score $\geq$ 20 + ESR>25mm/1$^{st}$ hr and/or Hb<11 gr/dL

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**Immunoaablation + AST =**

1. **Mobilisation**  
   CYC4 g/m$^2$, G-CSF 10 µg/kg
2. **Leukapheresis /CD34-selection**
3. **Conditioning**  
   CYC 200 mg/kg, ATG 7.5 mg/kg  
   Reinfusion CD34+ cells

**Standard-therapy**

- 12x monthly  
  i.v. pulse CYC 750 mg/m$^2$

**EFS = survival minus persistent major organ failure (heart, lung, kidney)**

**Exclusion criteria:**  
- PHT > 50 mmHg, DLCO < 40%, creat.cl. < 40 ml/min.  
- LVEF < 45%; uncontrolled arhythmia; cardiac tamponade  
- Infection, etc. previous treatment with CYCLO: >5 gr iv, >3 mths po
156 SSc: 79 SCT+77 controls in 27 centers

France: 49; Netherlands: 54
Germany: 20; Italy: 16
Switzerland: 7, UK: 5
Austria: 3, Belgium: 1
Canada: 1
Greece: 1
Randomized (n=156)

Not started treatment (n=6):
- Low DLCO (n = 2)
- Died (n = 1; disease progression)
- MOF (n=1)
- Withdrawal (n=2) → died later (1)

Started Treatment (n=150)

Early Termination (n=32):
- Died (n=15):
  - Disease progression (n=5)
  - Procedure-related (n=8)
  - Others (n=2)
- Non-compliance (n=7, 2 died later)
- AEs (n=6 → 3 died later)
- MOF (n=4 → 3 died later)

Completed 24M Study (n=95)

Died >24M et < 84M (n=9):
- Disease progression (n=3)
- MOF (n=4)
- Sudden death (n=2)

Still in 24M FU (n=23)

Completed 84M Study (n=17)

Still in 84M FU (n=69)

Died >84M FU (n=1):
- Secondary leukaemia
Overall survival ASTIS -cohort, Oct 2010

AE episodes ~ WHO toxicity grading

<table>
<thead>
<tr>
<th>Grade</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>32</td>
</tr>
<tr>
<td>Grade I</td>
<td>40</td>
</tr>
<tr>
<td>Grade II</td>
<td>119</td>
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<tr>
<td>Grade III</td>
<td>53</td>
</tr>
<tr>
<td>Grade IV</td>
<td>44 incl</td>
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</table>

2x EBV lymphoma (1 fatal, 1 treated), Respiratory failure due to ATG

Number of pts with SAE
23/43 in TP group vs 16/48 in control group
DETERMINANTS OF CLINICAL RESPONSE?

Kératinocytes

Mononucléaires/Macrophages

Fibroblastes

Lymphocytes B et T

Cellules endothéliales

Fibrose

Matrice extracellulaire

Myofibroblastes

TGF-β, IL-1β, EGF

TGF-β, PDGF

TGF-β, CTGF, PDGF, IGF-2, VEGF, IL-6, IL-4, IL-17, IL-1β

Figure 2. T cell receptor (TCR) β-chain spectratyping. Shown is the third complementarity-determining region size distribution of selected BV families before (Pre) and 12 months after (Post) hematopoietic

Verrecchia F Rheumatology 2007

Aschwanden Daikeler et al ARD 2008

Launay D J Rheumatol 2009

Farge Arthr Rheum 2005
SECONDARY AIDs
Blood 2007 n = 6 /155
16 % (4/25) alemtuzumab, 1.9% (2/102) ATG
0% (0/28) no lympho-depleting A
NEJM 2007, Human Immunol 2010
⇒ Daikeler (submitted)
7.9 + 1 % at 3 yrs, 9.2 + 2 % at 5 yrs

LATE VIRAL INFECTIONS: VZV
n= 5 / 36 (Nash Blood 2007), n= 2 /18 ISAMAIR

MYELODYSPLASIA:
n= 1 at 76 mths (Nash Blood 2007)

4. SUCCESSFUL PREGNANCY
1 case St Louis H (Clin EXp Rheum 2008)
IMPROVED THERAPY OVER THE LAST 20 YRS in SLE

81% 1st remission, 1/3 relapse,
5- 10 % ERD at 5 - 10 yrs,
10 yr survival : 92 %

TT TOXICITY: infection
+ metabolic, bone, ovary dysfunctions

PRONOSTIC FACTORS:

Compliance,
Response 1st treatment **,
Race, socio-economic factor, HBP
Activity / Chronicity Index, SAPL
Initial Renal failure, Relapse nephritic ∑

* Cook J Rheumatol 2000
** Houssiau Arth Rheum 2004
(±35% disease, activity, 64 %infection )

SLEDAI       RR*
1-5           1.3
6-10          2.2
11-19         4.7
20+           14.1

Survival °  
n= 207
AutoHSCT in SLE: a worldwide overview

AutoHSCT:

- **Northwestern**: 50+ patients, open label
- **NIH**: 8 patients, open label
  - Several open label studies closed or no enrollment
- **LIST**: multicenter, RCT closed: lack of enrollment after sites opened
- **ASSIST**: Berlin open phase 2 (n = 15)
- **Allo HSCT**: few open label pilot studies

Main obstacle: **difficulty recruiting**

- Reluctance of rheumatologists to refer patients
- Concerns about mortality (patients and rheumatologists)
- Competing (sometimes overhyped) studies with other agents

**Randomized study**: pt objection to being randomized to standard of care
Burt R 50 pts 1997 – 2005 Mob 2.0 g /m2 + G-CSF 5 m/m2
HSCT 200 ug/kg + ATG 90 mg/Kg 2% TRM

*JAMA 2006*

**Figure 1. Probability of Survival and Relapse in Lupus Patients Undergoing Hematopoietic Stem Cell Transplantation (HSCT)**

- Survival After HSCT
- Time to Relapse After HSCT

*Blood 2005*

**Figure 1. Anti-cardiolipin IgG antibodies before and serially after stem cell therapy in 7 SLE patients with positive antibody before transplantation.**

<table>
<thead>
<tr>
<th>Number</th>
<th>Time last f/u (month)</th>
<th>SLEDAI</th>
<th>NYHA class</th>
<th>LVEF (%)</th>
<th>RWMA</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Last</td>
<td>Pre</td>
<td>Last</td>
<td>Pre</td>
</tr>
<tr>
<td>1</td>
<td>36</td>
<td>27</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Global hypokinesia</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>2</em></td>
<td>9 (death at 11.5 month)</td>
<td>16</td>
<td><em>II</em></td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>10</td>
<td>0</td>
<td><em>II</em></td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Mild septal hypokinesia</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>30</td>
<td>3</td>
<td><em>II</em></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Inferior and posterior wall akinesia</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12 (death at 19 month)</td>
<td>17</td>
<td>IV</td>
<td>25</td>
<td>30-35</td>
</tr>
<tr>
<td></td>
<td>Extensive global hypokinesia</td>
<td>Global hypokinesia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>6</em></td>
<td>24</td>
<td>16</td>
<td>14</td>
<td><em>II</em></td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Not evaluated</td>
<td>Not evaluated</td>
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</table>
# Auto HSCT in SLE: Major series

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Follow-up (months)</th>
<th>TRM</th>
<th>Disease-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBMT/EULAR¹ RETROSPECTIVE</td>
<td>53</td>
<td>23</td>
<td>12%</td>
<td>*63%</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td></td>
<td>7%</td>
<td>*59%</td>
</tr>
<tr>
<td>Northwestern University² PROSPECTIVE</td>
<td>50</td>
<td>29</td>
<td>2%</td>
<td>**65% (n=48)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>**50% (n=48)</td>
</tr>
</tbody>
</table>

*<10 mg prednisone, SLEDAI<3  
**<10 mg prednisone or HCQ

TRM: transplant-related mortality

² Burt et al, JAMA 295:527-535, 2004
<table>
<thead>
<tr>
<th>Patient number</th>
<th>Time free from insulin (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Period free from insulin</td>
</tr>
<tr>
<td>0,2 IU/Kg/day (basal + prandial) + sitagliptin</td>
<td></td>
</tr>
<tr>
<td>0,30 IU/Kg/day (only basal insulin) + sitagliptin</td>
<td></td>
</tr>
<tr>
<td>0,15 IU/Kg/day (only basal insulin) + sitagliptin</td>
<td></td>
</tr>
<tr>
<td>0,30 IU/Kg/day (only basal insulin) + sitagliptin</td>
<td></td>
</tr>
<tr>
<td>0,15 IU/Kg/day (only basal insulin) + sitagliptin</td>
<td></td>
</tr>
<tr>
<td>0,14 IU/Kg/day (only basal insulin) + sitagliptin</td>
<td></td>
</tr>
</tbody>
</table>

**Patients continuously insulin-free since AHSCT**

- Mean 52.4 mo

- Total: 13 patients
- All patients without previous DKA

**13 Patients transiently free from insulin along the follow-up**

- Period free from insulin
- Period using insulin

**Courtesy of J Voltarelli**
Preliminary Results – Summary

Of 22 patients without previous DKA

- 8 continuously free from insulin
- 13 transiently free from insulin, but 2 resumed insulin independence after sitagliptin use

Of 2 patients with previous DKA

- 1 patient continuously using insulin – corticoids?
- Both continuously using insulin

Patients who never experienced any period free from insulin

- Patient # 1 (male, 24y): DKA + Hydrocortisone
- Patient # 19 (female 16y): DKA
- Patient # 21 (male, 13y): Hydrocortisone

Courtesy of J Voltarelli
Immune depletion does not explain long-term clinical stabilization

From Roth et al.
Naïve T cell expansion following long-term immune reconstitution after myeloablative conditioning

Hematopoietic Stem cell → Lymphoid Progenitor cell → Pre-T cell → Naïve T cell → Thymus → Recent Thymic Emigrant (RTE) T cell

TCR rearrangement:
- \( \alpha \) locus
- \( \delta \) locus
- \( V_\alpha \), \( V_\delta \), \( \delta_{Rec} \), \( D_\delta \), \( J_\delta \), \( C_\delta \), \( y_J \alpha \), \( J_\alpha \), \( C_\alpha \)

Muraro and Uccelli 2009
Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients

Paolo A. Muraro,1 Daniel C. Douek,4 Amy Packer,1 Katherine Chung,1 Francisco J. Guenaga,4 Riccardo Cassiani-Ingoni,1 Catherine Campbell,2 Sarfraz Memon,5 James W. Nagle,3 Frances T. Hakim,5 Ronald E. Gress,5 Henry F. McFarland,1 Richard K. Burt,6 and Roland Martin1

1. TREC assay
   (Douek)

2. Phenotype-based Enumeration
   (Kimmig, Thiele)

Diversification and renewal of T cell repertoire following auto-HSCT in MS patients

Nucleotide sequencing

Patient 1, CD4+, TCRBV1 (TRB09*)

Pre-Tx

CDR3 spectratyping

Pre-transplant

6 months

1 year

2 years

T CELL REPERTOIRE and TREC values AFTER ABMT IN SSC

A (CR, PR) vs B (NR, relapse) (n=14 CY alone)  *Farge Arthr Rheum* 2005; 52: 1555

<table>
<thead>
<tr>
<th>Normal values</th>
<th>At inclusion</th>
<th>6–8 months after HSCT</th>
<th>10–12 months after HSCT†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
<td>Group A</td>
</tr>
<tr>
<td>Polyclonal BV families, %</td>
<td>70.30 ± 19.85</td>
<td>43.17 ± 7.84</td>
<td>22.50 ± 15.58</td>
</tr>
<tr>
<td>Skewed BV families, %</td>
<td>22.80 ± 20.02</td>
<td>39.78 ± 2.25</td>
<td>58.25 ± 13.20</td>
</tr>
<tr>
<td>Negative BV families, %</td>
<td>6.90 ± 7.81</td>
<td>37.53 ± 35.71</td>
<td>19.25 ± 14.70</td>
</tr>
<tr>
<td>TREC /μg CD3+ cell DNA</td>
<td>694 ± 776.85</td>
<td>50.75 ± 51.05</td>
<td>112.75 ± 180.68</td>
</tr>
</tbody>
</table>

*TREC /CRP: r = -0.41, p < 0.001, TREC / CD19+: r = 0.35, p < 0.001 (RA, SEP)*

Figure 2. T cell receptor (TCR) β-chain spectratyping. Shown is the third complementarity-determining region size distribution of selected BV families before (Pre) and 12 months after (Post) hematopoietic

Sustained altered T cell homeostasis and abnormal Repertoire (Crit Rev Immunol 1995)

Persistence of underlying disease mechanism after HSCT?maintenanceimmunosuppression
Recovery of CD4+CD25bright T-cell frequency after ASCT

A

B


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Autologous HSCT in SLE → long-term remission
Restoration of a tolerant immune system

Materials:
- 7 patients with SLE
- Follow-up: 8 years after HSCT

Memory phenotype: CD45RO⁺CD45RA⁻ is the predominant CD4⁺ T-cell subset

↑ naive T cells after HSCT

→ immune reconstitution

CD45RA⁺ CD45RO⁻ naive
CD45RO⁺ CD45RA⁻ memory

Alexander et al., Blood 2009
Recovery of Foxp3$^+$ T-cell and CD19$^+$ B-cell subsets in SLE patients treated by HSCT

↑ T regulatory cells Foxp3$^+$

↑ naive B cells after HSCT

CD19$^+$ B cells

Alexander et al., Blood 2009
The role of CD8^+ Treg in HSCT therapy 15 pre and 15 post-HSCT SLE (8yr FU)


Short-term T cell lines from post-transplant lupus patients

After HSCT, CD8^+ T cells have stronger suppressive function than autologous CD4^+ CD25^{high} Treg subset

Cell contact independent → TGF-β dependent
T-cell clones persisting in the circulation after autologous hematopoietic SCT are undetectable in the peripheral CD34+ selected graft

AN Dubinsky, RK Burt, R Martin, and PA Muraro

CD34+ selected auto-graft

TCR B (auto-graft)

No T cells

CDR3-length (aa)

Mobilisation

Conditioning

Post-transplantation

Pre-mobilisation

Day 0

6 mo

2 yrs

TRBV9*01, TRBJ2-5*01, TRBD1*01 CASSVARETQY
TRBV9*01, TRBJ2-5*01, TRBD1*01 CASSVARETQYF
TRBV9*02, TRBJ2-5*01, TRBD1*01 CASSPARGETNYF
TRBV9*01, TRBJ1-1*01, TRBD1*01 CASSPPGTGVEAFF

TRBV19*01, TRBJ2-1*01, TRBD2*01 CASSSGDQMRFQFF
TRBV19*01, TRBJ1-2*01, TRBD1*01 CASSIDLQTSYGYSF
TRBV19*02, TRBJ1-5*01, TRBD1*05 CASSTSGPSNQPQHF
TRBV19*01, TRBJ1-5*01 CASSIDANNQPQHF
TRBV19*01, TRBJ1-2*01, TRBD1*01 CASTPDRGGGYTFF

Dubinsky et al. BMT 2009
Immune reconstitution after AHSCT: renewal of the immune repertoire

**Type I**: replacement of mature T/B memory repertoire with naïve, non-pathogenic cells

**Type II**: reinstatement of Immune Regulation increased nb and/or function of regulatory cells

Muraro and Douek, 2006
IMMUNE RECONSTITUTION:
YES WE CAN INDUCE REST OF TOLERANCE

Radbruch A Ann Rheum Dis 2004; 63: 96
The effects of MSCs on immune cells

Possible mechanisms of the interactions between MSCs and cells of the innate and adaptive immune systems

MCS and AE murines (EAE): yes

(-) T, B, macrophages / effet trophique sur neurones + oligodendrocytes

effet (+) sur démyelinisation (a,b), infiltration T (c, d), perte axonale (e,f)

CSM et LED murin: rôle ++ ds formes sévères
MLR/lpr Mice by BMT by via portal vein Kushida T. Stem Cells 2001

Irradiation (5.5 Gy x 2) + GM allogénique
C57BL6 mice BMCs (3.10^7)
± cultured stromal cells (0.3. 10^7) PV

° GM conventionnelle iv :
100% + avec 8.5 Gy , 70% + avec 5.5 Gy x 2

° GM 5.5 Gy x 2 (PV) :
30% survie, 100% survie stromal cells

Ishida T J immunol 1994 Requirement of donor derived stroma cells for successful allogeneic BMT
Marked improvement of severe progressive SSc after TP of MSC from an allogeneic haploidentical-related donor mediated by ligation of CD137L

Christopeit M Leukemia, 1Nov 2007

- 41 yr old F patient,
- 4 yr duration SSC
- **Before MSC:**
  mRSS: 25, skin ulceration, severe acral sclerosis,
  7.5 mg pred + 100mg AZA/d
- Allogeneic IV$10^6$ MSCs /kg BW
- **After MSCs :**
  7 mths: 5/6 skin ulcerations recovery
  1 yr: mRSS 11, VAS 2 (vs5), no organ dysfunction

**NO DETAIL ON HLA MATCHING?**
A new Bone Marrow Transplantation Method for Stem cell Disorders…
PERFUSION METHOD + INTRA BONE IKEHARA S Ann N Y Acad Sci 2009; 1173: 774

A new concept for AD disorders
3 non interventional studies: MS, SSc, Crohn’s

5 Retrospective studies: Infection, Pediatrics, Immune Biology, HLA, Economy

5 prospective trials: 3 on-going or closed Phase III
ASTIC (36/48 Recruiting closed) on Crohn’s disease
ASTIS on scleroderma (156 pts recruited, end of follow up: Oct 2011)
ASTIMS on multiple sclerosis (21 pts recruited, end of follow up: June)

2 in preparation
ASTIL Phase II (EBMT approved 80 000€ credited, ethics committee submission)
ASTID Phase III on diabetes (50 000 €) with Chicago and Brazil

EBMT Joint educational meeting
ADWP /AAA/ LEWP
6 November 2011 Barcelona Spain

THANKS EBMT OFFICERS
Paris, London, Barcelona, Leyden