Acquired Aplastic Anemia:
The Ideal Conditioning Regimen

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Special Considerations when Planning HSCT for Bone Marrow Failure Disorders

**AIM : RESTORE ACTIVE HEMATOPOIESIS**

- No Malignancy to Eradicate with GvL
  - NO BENEFIT OF GVHD / No GvL
- Myeloablation not required (Aplastic)
  - USE RIC REGIMEN
- Need to Secure Engraftment
  - MAXIMAL LYMPHO-/IMMUNO ABLATION
- Young Patients
  - FERTILITY PRESERVATION
Actuarial probability of graft failure in AA patients according to number of pretransplant transfusions

MONTHS

Fig 2. Actuarial probability of graft failure in patients receiving cyclophosphamide alone for pretransplant conditioning according to (A) whether corticosteroids were used to treat aplastic anemia prior to transplantation and (B) number of pretransplant transfusions.

Period: 1978-1986

Champlin et al., Blood 73: 606-613 (1989)
• Matched Sibling HSCT
• Matched Unrelated Donor HSCT
• Cord Blood Transplantation
Matched Sibling Donor HSCT for Aplastic Anemia

Cyclophosphamide only conditioning (CY only)

Cyclophosphamide and ATG (CY/ATG)

Fludarabine based (FLU/CY)+/-ATG

Alemtuzumab based conditioning

Elderly patients
Cyclophosphamide Only Conditioning

CY is considered the traditional backbone for conditioning of bone marrow failure

CIBMTR prospective randomized study

CY 50 mg/kg          D-5 to D-2
Add Horse ATG 30 mg/kg     D-5 to D-3

5 years probability of survival is 74% for CY and 80% for CY/ATG (P=0.44)

GF, GVHD, similar on both groups

The study was not adequately powered to detect significant differences between the two groups

Antithymocyte Globulin (ATG) Based Conditioning - I

ATG based conditioning:
First explored in Boston and Seattle group
In context of transplants in patients with graft failure but later on applied to first transplants

Subsequent studies:
CY/ATG combination for upfront HSCT: excellent engraftment rate, (>90%) relatively low incidence of both aGVHD and cGVHD with Excellent long term overall survival (80-90% at 10 years)

# ATG Preparations

<table>
<thead>
<tr>
<th>Commercial Name</th>
<th>Manufacturer</th>
<th>Source</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoglobulin</td>
<td>Genzyme</td>
<td>Horse</td>
<td>Discontinued</td>
</tr>
<tr>
<td>ATGAM</td>
<td>Pharmacia/UpJohn Now Pfizer</td>
<td>Horse</td>
<td>Commercially available</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not available in Europe</td>
</tr>
<tr>
<td>Thymoglobulin</td>
<td>Sanofi</td>
<td>Rabbit</td>
<td>Available</td>
</tr>
<tr>
<td>ATG-Fersenius</td>
<td>Fersenius</td>
<td>Rabbit</td>
<td>Available</td>
</tr>
</tbody>
</table>
ATG Preparations

- Rabbit and equine ATG have different pharmacokinetics profile:
  - Rabbit ATG (rATG): 29.8 days
  - Horse ATG (hATG): 5.7 days
- Consequently:
  - rATG can deplete transplanted donor T cells in vivo much more efficiently as opposed to hATG
  - Can prevent acute GVHD but at the expense of possibly higher incidence of graft rejection and delayed immune reconstitution.

Vo PT, Pantin J, Hematol Oncol. 2015; 26;8:78
Alemtuzumab Conditioning Regimens

- Alemtuzumab (CAMPATH) monoclonal Ab against CD52
- CD52, a GPI-linked membrane protein expressed on almost all WBC but not on CD34 HSC
- Potent lympholytic agent
- Detected in the plasma for several weeks after administration resulting in depletion of recipient auto reactive lymphocytes
  - Prevents GVHD by depletion of donor allo-reactive T-cells

Alemtuzumab (CAMPATH-IG) Conditioning Regimens

- Use of Alemtuzumab was consistently associated with high incidence of mixed chimerism which tips the balance away from GVHD

- High survival of early patients with graft failure was in part due to a high incidence of autologous recovery

Alemtuzumab Conditioning Regimens

- Significant proportion (20-40%) of patients with AA have an associated PNH clone with defective expression of CD52 on T-cells.

- In these patients ATG is considered as an alternative
King’s FCC conditioning for idiopathic SAA

- FLU 30mg/m2 x 4
- CY 300mg/m2 x 4
- Alemtuzumab (Campath-1H) 0.2mg/kg x 5
- Post graft ciclosporin (no methotrexate needed)
- For MUD HSCT, no irradiation needed

N = 45, transplanted 2007-2015

Grimaldi et al, unpublished data 2016
FCC HSCT - Patient outcomes

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N=</td>
<td>45</td>
</tr>
<tr>
<td>Median days to ANC &gt; 0.5x10^9/l</td>
<td>12 (10-22)</td>
</tr>
<tr>
<td>Median days to platelets &gt; 20x10^9/l</td>
<td>12 (9-61)</td>
</tr>
<tr>
<td>Graft failure (primary)</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>1 year TRM</td>
<td>3 (6.6%)</td>
</tr>
<tr>
<td>Acute GVHD</td>
<td>6 (13.6%)</td>
</tr>
<tr>
<td>- 5/6 grade I/II, skin only</td>
<td></td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td>6 (13.3%)</td>
</tr>
<tr>
<td>Mild/Moderate/Severe</td>
<td>4/1/1</td>
</tr>
</tbody>
</table>

Cumulative incidence of GVHD (acute + chronic)

16% at 1yr

Grimaldi et al, unpublished data 2016
Persistent mixed T-cell chimerism despite CsA discontinuation

Grimaldi et al, unpublished data 2016
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>No.</th>
<th>Age</th>
<th>Donor type, n (%)</th>
<th>Conditioning</th>
<th>OS</th>
<th>EFS</th>
<th>aGVHD</th>
<th>cGVHD</th>
<th>infections</th>
<th>Graft failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>MARSH et al, 2011</td>
<td>retrospective multicenter study</td>
<td>50</td>
<td>35 (8-62)</td>
<td>MSD N= 21 (42%) MUD n= 29 (58%)</td>
<td>fludarabine 30 mg/m2 IV/d x 4 CY 300 mg/m2 IV /d X 4 Alemtuzumab 40-100 mg/d iv or s.c x4</td>
<td>88% @2 yrs</td>
<td>80 % @2 yrs</td>
<td>N= 7 (13.7%)</td>
<td>N= 2 (4%)</td>
<td>EBV 4(8%) CMV 9(19%) ADENOVIRUS 7 (15%)</td>
<td>N= 6 (12%)</td>
</tr>
<tr>
<td>Samarasinghe et al, 2012</td>
<td>multicentre retrospective study</td>
<td>43</td>
<td>11 (0-5-17)</td>
<td>MUD</td>
<td>Flu 30 mg/m2 x 5 CY 120-200 mg/kg X 2-4 d Campath (0-9-1 mg/kg)</td>
<td>95% @5 yrs</td>
<td>NA</td>
<td>2-3%</td>
<td>2-3%</td>
<td>CMV (22-7%)</td>
<td>0</td>
</tr>
<tr>
<td>Hamad et al 2014</td>
<td>retrospective study</td>
<td>41</td>
<td>37 (17-59)</td>
<td>MSD MUD MMRD</td>
<td>Cy 22% Cy/flu 71% Flu/BU 2% Flu/ Bu /TB 4% GVHD prophylaxis CAMPATH/ CSA 37 (90) CAMPATH/ tacrolimus 3 (7) CAMPATH/ MMF 1 (2) CAMPATH dose 60 mg 35 (85) 50 mg 6 (15)</td>
<td>85 % @3 yrs</td>
<td>N/A</td>
<td>NONE &gt;G1</td>
<td>1/33 (3%)</td>
<td>Bacterial sepsis 21 (51%) CMV 19/25 (79%)</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>Gupta et al.2004</td>
<td>retrospective study</td>
<td>33</td>
<td>16 (4-45)</td>
<td>N/A</td>
<td>CY 50 mg/kg x 4 0.75 -1 mg/kg x 4-8 d</td>
<td>81% @5 yrs</td>
<td>4 (13%)</td>
<td>0 (0%)</td>
<td>15 (45%)</td>
<td>8/33 (24%)</td>
<td></td>
</tr>
<tr>
<td>Novitsky et al, 2013</td>
<td>Prospective study</td>
<td>30</td>
<td>19 (7-60)</td>
<td>MSD</td>
<td>fludarabine 30 mg/m2 x 5 days cy 60 mg/kg x 2 days Campath “in the bag”</td>
<td>100% @1 yr</td>
<td>96% @1 yr</td>
<td>0</td>
<td>0</td>
<td>7 (23)</td>
<td>2/30</td>
</tr>
<tr>
<td>Kanda et.al. 2013</td>
<td>retrospective study</td>
<td>15</td>
<td>34 (20–46)</td>
<td>MUD</td>
<td>Fludarabine 30 mg/kg x 4 CY 25 mg/kg x 4 Campath 0.16 mg/kg/day x 6 TBI 2 Gy</td>
<td>83.3 % @1 yr</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>8.3 %</td>
</tr>
<tr>
<td>Siegal et al 2008</td>
<td>retrospective study</td>
<td>10</td>
<td>40 (25–56)</td>
<td>MRD 8 (80) Alternative donor (MMFD, MUD) 2 (20)</td>
<td>High-dose CY based 3 (30) Fludarabine based 7 (70)</td>
<td>7/10 (70) @1 yr</td>
<td>7/10 (70) @1 yr</td>
<td>1/9 (11%)</td>
<td>0</td>
<td>Bacterial 6/10 (60) Fungal 2/10 (20) Viral CMVreactivation 5/6 (83) H.Z 2/10 (20)</td>
<td>1/10 (10%)</td>
</tr>
<tr>
<td>Gupta et al, 2005</td>
<td>retrospective study</td>
<td>7</td>
<td>13 (8-35)</td>
<td>MUD</td>
<td>Alemtuzumab 0.2 mg/kg/day x 5 Flu 30 mg/m2 x 5 CY 20 mg/kg x 4</td>
<td>6/7 @10 mo</td>
<td>6/6 @6mos</td>
<td>3/7</td>
<td>1/6</td>
<td>CMV 1/6</td>
<td>0</td>
</tr>
</tbody>
</table>
Radiation-based Conditioning Regimens for MSD HSCT

- Lower rates of graft failure, at the expense of:
  - Significant early toxicities like GVHD and pneumonitis
  - Late toxicity including:
    - Secondary malignancies and
    - Reduced growth and development in children

Justified in alternate donor or allo-immunized

Fludarabine based conditioning

**FLU/CY**

- increasingly used in situations where ATG is not available or cannot be used
- Extremely low toxicity and low risk of graft failure
- Possible increase in GVHD risk remains an important concern

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Number</th>
<th>Age</th>
<th>Donor type</th>
<th>Conditioning</th>
<th>OS</th>
<th>EFS</th>
<th>aGVHD</th>
<th>cGVHD</th>
<th>Graft failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacigalupo et al, 2005</td>
<td>Retrospective</td>
<td>38</td>
<td>14 (3-37)</td>
<td>MMRD</td>
<td>Flu/Cy/ ATG</td>
<td>29/38</td>
<td>92% at 1 year</td>
<td>2/35</td>
<td>2/35</td>
<td>7/38</td>
</tr>
<tr>
<td>Bacigalupo et al, 2010</td>
<td>Retrospective</td>
<td>100</td>
<td>13 (3=51)</td>
<td>Alternate donor</td>
<td>Flu/Cy/ ATG n=52 Flu/Cy/ATG/TBI n=48</td>
<td>73%</td>
<td>79%</td>
<td>-</td>
<td>18%</td>
<td>1/52 4/48 17%</td>
</tr>
<tr>
<td>Maury et al, 2009</td>
<td>Prospective</td>
<td>30</td>
<td>46 (31-66)</td>
<td>MSD</td>
<td>Flu/Cy±ATG n=30 Cy±ATG n=239</td>
<td>77%</td>
<td>60% at 5 years</td>
<td>3 (10%)</td>
<td>3 (13%)</td>
<td>3 (13%) 0% 11% 1ry GF</td>
</tr>
<tr>
<td>Alzahrani et al, 2011</td>
<td>Retrospective</td>
<td>38</td>
<td>20 (14-36)</td>
<td>MSD</td>
<td>Flu/Cy</td>
<td>79% at 43 months</td>
<td>-</td>
<td>4 (11%)</td>
<td>8 (25%)</td>
<td>1 (3%) at 43 months</td>
</tr>
<tr>
<td>George et al, 2007</td>
<td>Retrospective</td>
<td>35</td>
<td>20.8(5-43)</td>
<td>MSD</td>
<td>Flu/Cy±ATG</td>
<td>29/35 (82.8%)</td>
<td>29/35 (82.8%)</td>
<td>9/31 (29%)</td>
<td>8/25 (32%)</td>
<td>3/35 (8.5%)</td>
</tr>
<tr>
<td>Go´mez-Almaguer et al.2006</td>
<td>Retrospective</td>
<td>23</td>
<td>25(4-65)</td>
<td>MSD</td>
<td>Flu/Cy±Bu</td>
<td>88% at 1500 days</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>8.7% at 1500 days</td>
</tr>
<tr>
<td>Xue et al. 2015</td>
<td>Prospective</td>
<td>20</td>
<td>7.5 (3-14)</td>
<td>MSD n= 13 MUD n=7</td>
<td>Flu/Cy/ ATG</td>
<td>19/20</td>
<td>18/20</td>
<td>0</td>
<td>0</td>
<td>1/20</td>
</tr>
<tr>
<td>Kang et al, 2014</td>
<td>Prospective</td>
<td>28</td>
<td>13.5(1-30)</td>
<td>MUD</td>
<td>Flu/Cy/ ATG</td>
<td>67.9%</td>
<td>-</td>
<td>13/28</td>
<td>2/25</td>
<td>0</td>
</tr>
<tr>
<td>Kudo et al, 2015</td>
<td>Retrospective</td>
<td>55</td>
<td>9 (1-15)</td>
<td>MSD MMRD MUD CB</td>
<td>Flu/Cy± ATG± TBI</td>
<td>45/55 (82.9%) at 4 years</td>
<td>81.2%</td>
<td>12/55</td>
<td>7/55</td>
<td>9/55</td>
</tr>
<tr>
<td>Mahmoud et al, 2015</td>
<td>Retrospective</td>
<td>273</td>
<td>19.7(1.5-51)</td>
<td>MSD</td>
<td>Flu/Cy/ATG n=181 Cy/ATG n= 92</td>
<td>74% at 8 years</td>
<td>-</td>
<td>42/273 (15%)</td>
<td>70/248 (28%)</td>
<td>3/273</td>
</tr>
</tbody>
</table>
KFSHRC FLU/CY Experience: Overall Survival

OS at 5-years = 80%

N = 81
Age = 14-50 yrs
Neutrophil engraftment = 19 days
Platelet engraftment = 20 days

KFSHRC FLU/CY Experience

**Acute GvHD (≥grade II)**

Cumulative Incidence:
- \( aGvHD (≥\text{grade II}) = 20.9\% \)
- Death w/o \( aGvHD \) = 5%

**Chronic GvHD**

Cumulative Incidence:
- \( cGvHD = 31\% \)
- Death w/o \( cGvHD \) = 8%

**Elder patients with SAA**

*Seminars in Hematology 2000; 37: 69*

<table>
<thead>
<tr>
<th>Age PMN</th>
<th>10 yy</th>
<th>20 yy</th>
<th>30 yy</th>
<th>40 yy</th>
<th>50 yy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24</td>
<td>20</td>
<td>14</td>
<td>16</td>
<td>-2</td>
</tr>
<tr>
<td>100</td>
<td>19</td>
<td>14</td>
<td>8</td>
<td>1</td>
<td>-7</td>
</tr>
<tr>
<td>200</td>
<td>14</td>
<td>9</td>
<td>3</td>
<td>-4</td>
<td>-11</td>
</tr>
<tr>
<td>300</td>
<td>16</td>
<td>5</td>
<td>-1</td>
<td>-7</td>
<td>-14</td>
</tr>
<tr>
<td>400</td>
<td>6</td>
<td>1</td>
<td>-4</td>
<td>-10</td>
<td>-16</td>
</tr>
<tr>
<td>500</td>
<td>3</td>
<td>-2</td>
<td>-7</td>
<td>-12</td>
<td>-17</td>
</tr>
</tbody>
</table>

**Positive values:** BMT has superior (+) survival compared to IS

**Negative values:** BMT has inferior (-) survival compared to IS
conditioning MSD in older patients

• Standard CY/ATG

• Limited data on the advantage of including FLU in the conditioning in these patients

• May receive a reduced intensity conditioning regimen using FLU/CY alone or more optimally Flu/CY with adjusted doses of ATG or Alemtuzumab (FCC)

Conditioning for MUD HSCT

• MUD second line treatment and possibly upfront treatment for young patients with very severe aplasia.

• Induction of engraftment against immune barriers

• Standard CY/ATG did not provide sufficient immuno and lympho ablation for sustained engraftment in MUD HSCT

• Addition of TBI to CY/ATG in MUD: Best survival was achieved with a TBI dose of 200 rads

Conditioning for MUD HSCT

- Having established the optimal dose of TBI of 200 rads, a North American study looked at the optimal dose of Cyclophosphamide with fixed doses of ATG, Fludarabine and TBI 200.
- CY 0, 50, 100, 150 mg/kg were tested
- CY 0 closed after 3 graft failures
- CY 150 closed due to excess toxicity
- Leaving CY 50-150 as the possible dose choice
- More recent study, CY 50 vs CY 100 in combination with fixed doses of ATG, Fludarabine and TBI 200

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cyclophosphamide 50 mg/kg (n=38)</th>
<th>Cyclophosphamide 100 mg/kg (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft failure, primary and secondary</td>
<td>3 (8%)</td>
<td>6 (15%)</td>
</tr>
<tr>
<td>Survival</td>
<td>37 (97%)</td>
<td>39 (95%)</td>
</tr>
<tr>
<td>Major regimen-related toxicity* (grade 3 or higher)</td>
<td>4 (11%)</td>
<td>9 (22%)</td>
</tr>
<tr>
<td>Alive and engrafted</td>
<td>35 (92%)</td>
<td>35 (85%)</td>
</tr>
</tbody>
</table>

Anderlini P, et al. Lancet Haematol. 2015; 2(9); 367-375
Conditioning for MUD HSCT

1. CY is important component of conditioning, 50-100 mg (data support 50)

2. TBI helps to promote engraftment and prevent rejection, but should be limited to 200 rads

3. Cy> 100 should not be used in combination with TBI

4. Fludarabine and ATG help in promoting engraftment and preventing rejection

5. The combination of CY 50/TBI 200/Flu/ATG is the most optimal conditioning at present

6. FCC is a strong emerging radiation free alternative with encouraging results.
CBT for Acquired Aplastic Anemia

1. Only few reports, poor outcome. Good outcome in related CBT

2. Possible option for patients who had failed ISP, no MUD with available CB units with adequate TNC

3. Large cohort of 31 Japanese patients, 2 years OS 41%.

4. Eurocord 71 patients (1996-2009)

   Median age 13 years, 28 adults (9 with PNH), RIC regimen was used on 68%, 3 years OS 38%

Yoshimi A, et al, BBMT 2008;
Conditioning for Unrelated CBT
Ongoing French Society of SCT and Eurocord (APCORD-Protocol)
Prospective Phase II Study on Unrelated CBT

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fludarabine</td>
<td>30 mg/m²</td>
<td>D-6 to D-3</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>30 mg/kg</td>
<td>D-6 to D-3</td>
</tr>
<tr>
<td>Thymoglobulin</td>
<td>2.5 mg/kg</td>
<td>D-3 to D-2</td>
</tr>
<tr>
<td>TBI</td>
<td>200 rads</td>
<td>D-2</td>
</tr>
</tbody>
</table>

3-55 years, failed ISP, no MUD, no clonal evolution, KPS > 60, one or two units CB with total > TNC $4 \times 10^7$.

Result is still pending
Conditioning for Bone Marrow Failure of PNH

- PNH/AA patients transplanted for concomitant AA should follow the same conditioning regimens used for AA for related and MUD HSCT.

- Some investigators suggested that a “graft versus PNH” effect may be needed to eradicate the PNH clone, especially in non-hypoplastic PNH.

- No specific guidelines are available for patients transplanted for thrombosis or hemolytic indications.

- Myeloablative conditioning have been used in non-hypoplastic PNH (even Busulfan-based).

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