Current issues in Severe Aplastic Anaemia (SAA) and Bone Marrow Failure (BMF)

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Overlapping bone marrow failure syndromes

FA, Fanconi anaemia
DC, dyskeratosis congenita
SDS, Schwachman Diamond syndrome
NSAA, non-severe; SAA, severe; VSAA, very severe AA
Medical history and examination for a new patient with aplastic anaemia

**Extended family history**

- Low blood counts, AA, leukaemia
- Cancers: age onset, type eg head and neck
- Lung problems: fibrosis, emphysema *(telomeropathy)*
- Liver problems: cirrhosis: don’t dismiss “due to alcohol”! *(telomeropathy)*
- Consanguinity
- Draw the family pedigree

**Direct questions and examination**

- Preceding jaundice
- Drug history
- Nails: fingers *and* toes *(DC/telomeropathy)*
- Skin rashes *(Fanconi anaemia, telomeropathy)*
- Lymphoedema, warts, deafness *(GATA2 deficiency)*
- Hair greying: age onset, family history *(telomeropathy)*
- GI malabsorption *(SDS)*

SDS, Schwachman Diamnd syndrome  
DC, dyskeratosis congenita
How I investigate a new AA patient

History, clinical exam.

Confirm diagnosis

Determine disease severity

Tissue bank: blood, BM, skin

Look for abnormal clones

Exclude constitutional AA

HLA tissue typing

Check platelet count increment

MDT review

Blood
FBC & film
Reticulocyte count
LFTs, Hep B, C, HIV
ANA
Immunoglobulins

PNH, T-LGL

Bone marrow
Asp + biopsy
Cytogenetics/FISH
Emerging tests:
SNPA karyotyping
Myeloid gene panel

Radiology
CT chest
CT/US abdomen

Cardiac
ECG and ECHO

DEB test, SBDS
Emerging tests:
Telomere length
Constitutional gene panels/GATA2, RUNX1, telomere genes

Emerging tests:
SNPA karyotyping
Myeloid gene panel

Emerging tests:
Telomere length
Constitutional gene panels/GATA2, RUNX1, telomere genes
Is it Aplastic Anaemia or Hypocellular MDS?

**Morphology***
- Presence of blasts
- >10% dysplastic granulocytic cells or megakaryocytes, megakaryocyte clusters
- Abnormal sideroblasts
- Two or more ALIP

**Immunohistochemistry***
- ↑CD34, CD117, MPO
- CD61
- ↑ Reticulin

**Metaphase cytogenetics, interphase FISH, SNPA karyotyping**
- 12% AA have an abnormal clone
- Metaphase cytogenetics often fail
- Interphase FISH may be informative
- Good prognosis +8, del13q; poor prognosis -7

**DNA diagnostics**
- Somatic mutations recurrent in MDS/AML?
  - DNMT3A, ASXL1, PIGA, BCOR/BCORL1
  - CHIP: Significance of low level clones?
- Telomere length

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* Bennett and Orazi, Haematologica 2009;94:264
When should I treat?

SAA
Hypocellularity (<30%) and at least 2/3 criteria:
- PNN < 0.5x10^9/L
- Platelets < 20x10^9/L
- Reticulocytes < 20x10^9/L

VSAA
PNN < 0.2x10^9/L

Moderate/Non severe AA
Not all criteria for SAA
PNN > 0.5x10^9/L

Transfusions?

- Yes
- No

Treatment

Follow-up

SAA, severe AA; VSAA, very severe AA
Treatment Options

Transplant
- Matched sibling donor
- Unrelated donor
- Haploidentical donor
- Cord blood

Non-transplant
- Immunosuppressive therapy
- Eltrombopag
- Androgens
- Developmental
Treatment of acquired severe aplastic anaemia

- **Age of the patient?**
  - ≤ 35yr
  - 35-50yr
  - > 50yr

- **HLA ID sibling donor**
  - Yes: HLA matched sibling HSCT
  - No: Children

- **Horse ATG (ATGAM) with CSA**
  - No response at 3-6 months

- **Unrelated donor HSCT**

*EBMT SAAWP and BCSH 2015 guidelines*
Case study

• 49y male
• No FH, normal clinical examination
• Hb 71g/l, WBC 2.1, platelets 22, neutrophils 0.57, reticulocyte count 85 x 10^9/l
• PNH clone: 21% granulocytes, 1.7% red cells
• BM hypocellular (5% cellularity), no dysplastic features, normal reticulin, cytogenetics normal
• No co-morbidities
• Brother not a match, sister in Australia
• FBC worsened : neuts 0.3, regular blood and platelet transfusions
What treatment I would give

ATG with ciclosporin or HSCT ?
Prospective randomized trials of ATG-based first line treatment of severe aplastic anemia

Androgens  | German trial ATG ± CSA  | ± GCSF  | ± Sirolimus or MMF  | Horse vs rabbit ATG  | ± Eltrombopag

40% 56% | 65% 31% | 60-70% | 60-70% | 68% | 37% | 87% 39%

Response at 6 months

HDMP 5-10mg/kg

Prednisolone 1mg/kg short course

Horse Antithymocyte globulin (ATG)

Late 1970s  | TIME | 2017

EBMT trial is in progress

Horse versus Rabbit Antithymocyte Globulin in Acquired Aplastic Anemia

Phillip Scheinberg, M.D., Olga Nunez, R.N., B.S.N., Barbara Weinstein, R.N., Priscila Scheinberg, M.S., Angélique Biancotto, Ph.D., Colin O. Wu, Ph.D., and Neal S. Young, M.D.

Table 2. Hematologic Response at 3 and 6 Months to Horse ATG and Rabbit ATG.

<table>
<thead>
<tr>
<th>Response</th>
<th>Horse ATG (N=60)</th>
<th>95% CI</th>
<th>Rabbit ATG (N=60)</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. (%)</td>
<td></td>
<td>no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 3 mo</td>
<td>37 (62)</td>
<td>49–74</td>
<td>20 (33)</td>
<td>21–46</td>
<td>0.002</td>
</tr>
<tr>
<td>At 6 mo</td>
<td>41 (68)</td>
<td>56–80</td>
<td>22 (37)</td>
<td>24–49</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

A Data Censored for Stem-Cell Transplantation

B Data Not Censored for Stem-Cell Transplantation
Factors impacting on outcome after ATG and ciclosporin in SAA

Severe AA

Very severe AA

All patients (n=192)

![Graph showing outcomes and survival rates for different severity levels and age groups.](image-url)
Events Following IST for Severe Aplastic Anaemia: 1st Line

**No Response**
- 30%

**Response**
- 70%
  - CR rates only 10%
  - CSA dependency: 26% at 6 months
    - 14% at > 5 years

**Other events**
1. Infections is the most common cause of death
2. Cardiac events*: Myocardial infarct, dysrrhythmias

Other events following IST for SAA: 1st Line

- Relapse • 35%
- Haemolytic PNH Clone • 10%
- PNH Clone • 45-50%
- Cytogenetic Abnormality • 20%
- Somatic Mutation
  - Myeloid Specific 20-25%
  - PIG-A 8%
- Clonal evolution to MDS/AML 15-20% at 10-yrs (36% if somatic mutation present)

EBMT study, n=860

Socie et al NEJM 1993;329:1152
Mutational profile of AA and its evolution following IST

**B** Frequency of 4 Commonly Mutated Genes in Aplastic Anemia From the Two Studies

<table>
<thead>
<tr>
<th>Top 4 Genes</th>
<th>Kulasekararaj et al</th>
<th>Yoshizato et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT3A</td>
<td>8.3%</td>
<td>8.4%</td>
</tr>
<tr>
<td>ASXL1</td>
<td>8%</td>
<td>6.2%</td>
</tr>
<tr>
<td>BCOR/BCORL1</td>
<td>4%*</td>
<td>9.3%</td>
</tr>
<tr>
<td>PIGA</td>
<td>NA</td>
<td>7.5%</td>
</tr>
<tr>
<td>Median VAF</td>
<td>20%</td>
<td>9.3%</td>
</tr>
</tbody>
</table>

*Kulasekararaj et al. Blood 2014; 124: 2698*  *Yoshizato et al. NEJM 2015; 373:35*
Overall survival after immunosuppressive therapy for SAA - Impact of somatic mutations

**B Overall Survival**

- **‘Favourable’ mutations**
- **‘Unfavourable’ mutations**

All patients

**Table:**

<table>
<thead>
<tr>
<th>No. at Risk</th>
<th>PIGA, BCOR or BCORL1</th>
<th>Unmutated</th>
<th>DNMT3A, ASXL1, TP53, RUNX1, CSMD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIGA, BCOR or BCORL1</td>
<td>34</td>
<td>34</td>
<td>33</td>
</tr>
<tr>
<td>Unmutated</td>
<td>176</td>
<td>142</td>
<td>116</td>
</tr>
<tr>
<td>DNMT3A, ASXL1, TP53, RUNX1, CSMD1</td>
<td>30</td>
<td>24</td>
<td>17</td>
</tr>
</tbody>
</table>

**D Overall Survival in Patients <60 Yr of Age**

- Potential candidates for early HSCT in future studies?

All patients aged < 60 years

**Table:**

<table>
<thead>
<tr>
<th>No. at Risk</th>
<th>PIGA, BCOR or BCORL1</th>
<th>Unmutated</th>
<th>DNMT3A, ASXL1, TP53, RUNX1, CSMD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIGA, BCOR or BCORL1</td>
<td>33</td>
<td>31</td>
<td>30</td>
</tr>
<tr>
<td>Unmutated</td>
<td>152</td>
<td>119</td>
<td>101</td>
</tr>
<tr>
<td>DNMT3A, ASXL1, TP53, RUNX1, CSMD1</td>
<td>25</td>
<td>19</td>
<td>14</td>
</tr>
</tbody>
</table>

*Yoshimato et al. NEJM 2015;373:35*
Current lack of robust predictive factors for response to ATG
Predictors of response to ATG

- **Age**: Response independent of age in adults
- **Non-severe AA**: Higher response
- **FBC**: Retic count ≥ 20, lymphs ≥ 1.0 x10^9/l
- **PNH clone**: Good risk: +8, del13q
  - Poor risk: -7
- **Immune signature**: Using high sensitivity flow cytometry
- **Somatic mutation**: Good risk: PIGA, BCOR
  - Poor risk: DNMT3A, ASXL1
- **Cytogenetic clone**: Good risk: +8, del13q
  - Poor risk: -7
- **Future testing of Treg subsets?**
Immune pathogenesis of aplastic anaemia

NK cells

Cytokines

Memory-like Tregs ‘B’

Naïve-like Tregs ‘A’

Reduced number and function of Tregs and aberrant composition

Th1 and Th17 expansion CD4+ T cells

IFN-γ

TNF-α

CD8+ T-cell

CD8

CD8

CD8

FasL

FasR

ICE

IRF-1

IRF-2

eIF-2 RNAse

Stat1

Stat3

NO

BM failure

Haemopoietic stem cell

Marsh, Kulasekararaj, Mufti, Oxford Textbook of Medicine 2013
Deep phenotyping of Tregs identifies an immune signature for aplastic anaemia and predicts response to treatment

Identification of Treg subsets by automated clustering (viSNE)

Low dose Interleukin-2 in aplastic anaemia patients failing one course of IST

For those who fail IL-2 could move across to expanded autologous Treg Trial

- CYTOF Profiling at baseline, 1, 3 and 6 months+
- Myeloid Gene Panel Baseline, 3 and 6 and 12 months +
- Cytokine Luminex Panel Baseline, 1, 3, 6 months
- Safety and QOL routine
Novel Therapeutic Trials in Aplastic Anaemia
Expanding functional autologous AA Tregs

1. Remain responsive to IL-2
2. They are >90% FOXP3+ after expansion
3. Expand at a similar rate to normal Tregs
4. Can suppress Tcon proliferation in both autologous and allogeneic setting

Novel Therapeutic Trials in Aplastic Anaemia

Expandability and type of expanded Tregs

Treg specific demethylated region, TSDR

Expanded AA Tregs are stable

Expanded Tregs are polyclonal

Expanded Tregs are more similar to Treg B subpopulation

Should this patient be treated with upfront matched sibling donor SCT?
Transplant conditioning protocols for SAA

**MSD**

*Age < 30yr*

- **High dose cyclophosphamide**
  - CY 200mg/kg
  - ATG / Campath

*Age > 30yr*

- **‘FCATG’**
  - Fludara 30mg/kg x 4
  - CY 300mg/m² x 4
  - ATG

- **‘FCC’**
  - Fludara
  - CY
  - Campath

*MUD*

- **FCATG + 2Gy TBI**
  - or
  - FCC alone

*Post graft immune suppression:*

- For 9 months, then 3 month taper
- To prevent late graft failure

**Stem cell source and dose:**

- BM for ATG-based regimens
- PB (or BM) for Campath regimens
- Low dose is associated with graft failure

MSD = HLA matched sibling; CY = cyclophosphamide; CSA = ciclosporin; MTX = methotrexate; Campath = Alemtuzumab
Alemtuzumab vs ATG based conditioning for SAA: retrospective BSBMT study

All patients

Matched sibling donors

Unrelated donors

Case study: treatment decision

- 49y male
- No FH, normal clinical examination
- Hb 71, WBC 2.1, platelets 22, neuts 0.57, retic 55
- PNH clone: 21% granulocytes, 1.7% red cells
- BM markedly hypocellular (5% cellularity), no dysplastic features, normal reticulin, cytogenetics normal
- No co-morbidities
- Brother not a match, sister in Australia
- FBC worsened: neuts 0.3, regular blood and platelet transfusions

- Sister HLA typed, a match

Patient and MDT decision: FCC sibling transplant

Sustained engraftment, no PNH, no GVHD, 2 years post SCT
Danazol and telomere length

- Phase I/II design

- Age adjusted TL ≤1st percentile +/- identified mutations

- ≥1 abnormal blood count: Hb < 95 g/L, N<1 x 10⁹ /l, plts < 30 x 10⁹ /l

- Pulmonary fibrosis

- Danazol 800 mg daily x 2 yrs

- Landmark visits at 6, 18, 24 mos

- Hematological responses 19/24 (79%) at 3mo

- RR at 2 yrs = 84% (10/12)

- Almost all responses in <3 months

- Elevated liver enzymes (41%) and cramps (33%)


Mean increase 386 bp/year
Heterozygous RTEL1 variants in bone marrow failure and myeloid neoplasms - King’s-NIH study

- Pathogenic variants in 1.7% patients; 5.5% of suspected constitutional BMF patients
- Additional missense variants with strong evidence of pathogenicity
- Most common presentation: non-severe AA, hypoMDS, ICUS

Importance of not missing a diagnosis of constitutional AA in adults

• Avoid inappropriate treatment eg ATG
• For HSCT
  o Avoid use of stem cells from asymptomatic affected family donor
  o Impact on conditioning regimen
  o Specific post transplant complications
• Family screening and genetic counselling
• Need for long term cancer surveillance
• Monitor long term non-haematological complications (lung and liver fibrosis)
Supposing the 49yr old patient with SAA did not have a matched sibling donor, so was treated with ATG and ciclosporin but did not respond. What treatment would you give next?

1. Second course of ATG?
2. Eltrombopag?
3. Matched unrelated donor transplant?
How best to manage refractory Aplastic Anaemia?

Persistence of severe cytopenia(s) after one course of ATG/CSA
No matched sibling or unrelated donor

What next?

• Re-evaluate the patient
• Continue best supportive care
• Second course ATG
• Alemtuzumab
• Androgens
• Alternative donor HSCT
• New therapies eg eltrombopag

Marsh and Kulasekararaj, Blood 2013
Impact of improved supportive care

Improved survival of severe aplastic anaemia patients not responding to ATG

Survival probability

HSCT censored

1989-1996

1996-2002

2002-2008

Group 1
5-yr survival = 23%

Group 2
5-yr survival = 35%

Group 3
5-yr survival = 57%

Non-responders to IST N=174
P < 0.001

Time (years)
Repeat ATG+CSA for refractory SAA

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>ATG 1(^{st}) course</th>
<th>ATG 2(^{nd}) course</th>
<th>Response at 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheinberg, 2006</td>
<td>22</td>
<td>Horse (+/- MMF or sirolimus)</td>
<td>Rabbit</td>
<td>30%</td>
</tr>
<tr>
<td>Scheinberg, 2012</td>
<td>27</td>
<td>Horse</td>
<td>Rabbit</td>
<td>33%</td>
</tr>
<tr>
<td>Scheinberg, 2014</td>
<td>19</td>
<td>Rabbit</td>
<td>Horse</td>
<td>21%</td>
</tr>
<tr>
<td>Cle, 2015</td>
<td>22</td>
<td>Rabbit</td>
<td>Rabbit</td>
<td>30%</td>
</tr>
</tbody>
</table>

\textit{2\(^{nd}\) course of ATG is a risk factor for later MDS/AML}

Eltrombopag in Aplastic Anaemia

- Direct stem cell stimulation. Binds to MPL on HSC and BM progenitors
- Small molecule, direct local effect or indirect paracrine effect on BM microenvironment?
- Off target immunomodulatory effect?

Desmond R et al, Blood 2014; 123: 1818-25
## Clonal evolution on eltrombopag (8/43, 19%)

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age</th>
<th>Response</th>
<th>Baseline</th>
<th>At evolution</th>
<th>Time on drug (months)</th>
<th>Dysplasia</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>NR</td>
<td>46XY[20]</td>
<td>−7[20]</td>
<td>3</td>
<td>N</td>
<td>Died of progressive cytopenias</td>
</tr>
<tr>
<td>8</td>
<td>17</td>
<td>NR</td>
<td>No metaphases</td>
<td>+1,der(1;7) [4]/46XY[16]</td>
<td>3</td>
<td>N</td>
<td>Transplanted successfully</td>
</tr>
</tbody>
</table>

5/8: abnormalities of chromosome 7, mostly -7; two del(13q) and one +8

*Desmond. Blood 2014; 123: 1818*
Unrelated donor SCT for severe aplastic anaemia

What is the ideal timing?
Outcomes of matched sibling vs unrelated donor HSCT using ATG-based conditioning

Overall survival

Acute II-IV GVHD

Chronic GVHD

Risk score based on: Age > 20yr, time Dx-Tx > 6mo, CMV -/-, ATG in conditioning, BM stem cells

Bacigalupo et al. Haematologica 2015;100:696
Alemtuzumab with fludarabine and cyclophosphamide reduces chronic graft-versus-host disease after allogeneic stem cell transplantation for acquired aplastic anemia


King’s FCC conditioning
Fludarabine 30mg/m² x 4
CY 30mg/kg x 4
Alemtuzumab 0.2mg/kg x 5
Ciclosporin alone; no methotrexate
Irradiation-free regimen

Acute GVHD
13.5% (16.5% CI at 1yr)

Chronic GVHD
4% (7% CI at 1yr)

Graft failure
6 (12%), 3 primary, 3 secondary
9.5% MSD, 14.5% UD
King’s FCC experience: Patient characteristics
Transplanted 2007-2015

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>59</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (yr)</td>
<td>42 (15-72)</td>
</tr>
<tr>
<td></td>
<td>Number aged &gt; 50yr</td>
</tr>
<tr>
<td></td>
<td>Number aged &gt; 60 yr</td>
</tr>
<tr>
<td>PNH clone</td>
<td>28 (47%)</td>
</tr>
<tr>
<td></td>
<td>- median clone size (granulocytes)</td>
</tr>
<tr>
<td>HLA alloimmunised</td>
<td>13 (22%)</td>
</tr>
<tr>
<td>Donor</td>
<td>16 (27%)</td>
</tr>
<tr>
<td></td>
<td>- MSD</td>
</tr>
<tr>
<td></td>
<td>- MUD</td>
</tr>
<tr>
<td></td>
<td>- 9/10 UD</td>
</tr>
<tr>
<td>Stem cell source</td>
<td>52 (88%)</td>
</tr>
<tr>
<td></td>
<td>- PB</td>
</tr>
<tr>
<td></td>
<td>- BM</td>
</tr>
</tbody>
</table>

Update of Grimaldi et al. BBMT 2017; 23:293-299
Outcomes of FCC transplants for SAA

Age <50yr (n = 39) 97%
Age >50yr (n = 20) 85%

Overall survival

P=0.06

Donor type

MSD (n = 16) 100%
MUD (n = 34) 91%
9/10 UD (n=9) 89%

Overall survival

P=0.46

Graft failure 1 (1.7%)
1yr TRM 6.7% (5% for FCC)
Acute GVHD To follow!
Chronic GVHD To follow!

Update of Grimaldi et al. BBMT 2017; 23:293-299
Persistent mixed T-cell chimerism despite ciclosporin discontinuation following FCC HSCT for SAA

Grimaldi et al. BBMT 2017; 23:293-299
Mixed T-cell chimerism at 1 year principally due to persistence of patient CD8+ T-cells with notable contribution of effector subset

Basis for low incidence of GvHD and prolonged mixed T-cell chimerism following FCC HSCT appears to be multifactorial:

- Sustained low T cells numbers
- CD4+ T-cell recovery donor-derived with thymic education
- Recipient-derived effector CD8+ T-cells shape mixed CD3+ chimerism
- CD4+ T cells with regulatory phenotype present
- High number IL10 producing B cells

Grimaldi et al. BBMT 2017; 23:293-299
Is there now a case for upfront MUD HSCT as an option for adults with SAA?
FCC HSCT vs Immunosuppressive Therapy for SAA

**FCC HSCT**
- Potential cure of SAA
- Low 1yr TRM (5%)
- Low risk of GVHD
- Graft failure 2%-15%
- Infections
- $2^\circ$ autoimmune disease

**ATG+CSA**
- Lower 1yr mortality? No!
- No risk of GVHD
- MDS/AML 15%; acquired somatic mutations 20-25%
- Relapse 35%
- Infections
- Cardiotoxicity of ATG
What are the current outcomes of alternative donor HSCT?

1. Cord blood
2. Haploidentical donor
**Unrelated cord blood transplantation in acquired refractory AA**

<table>
<thead>
<tr>
<th>Conditioning regimen</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fludarabine, CY, TBI</td>
<td></td>
</tr>
<tr>
<td>ATG 5mg/Kg</td>
<td></td>
</tr>
</tbody>
</table>

**Anti CD20: 150mg/m² (D5)**

**G-CSF (D5)**

**UCB**

**Number of patients**

<table>
<thead>
<tr>
<th></th>
<th>29</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNC</td>
<td>$3.7 \times 10^7$/kg</td>
</tr>
<tr>
<td>CD34</td>
<td>$1.4 \times 10^5$/kg</td>
</tr>
<tr>
<td>Primary graft failure</td>
<td>10%</td>
</tr>
<tr>
<td>Cl acute GVHD II-IV</td>
<td>40% (8/0/2: II/III/IV)</td>
</tr>
<tr>
<td>Cl chronic GVHD</td>
<td>27% (severe in 2/5)</td>
</tr>
<tr>
<td>2yr overall survival</td>
<td>81%</td>
</tr>
</tbody>
</table>

RP Delatour, Blood 2016; 2671. ASH Annual Meeting abstract
Non-myeloablative peripheral blood HSCT for refractory SAA using post transplant CY


**Johns Hopkins protocol**

Stem cell infusion

Luznik. BBMT 2008; 14: 641
<table>
<thead>
<tr>
<th>Age/donor</th>
<th>Disease</th>
<th>Previous therapy</th>
<th>Previous HSCT</th>
<th>CD34 dose (x $10^6$/kg)</th>
<th>Engraftment (neut, platelet)</th>
<th>GVHD</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>19y, mother</td>
<td>SAA-MDS</td>
<td>ATG + CSA x 3</td>
<td>Cord-GF</td>
<td>6.7</td>
<td>D+18, D+ 21</td>
<td>None</td>
<td>Alive</td>
</tr>
<tr>
<td>51y, sister</td>
<td>$^2$ SAA</td>
<td>ATG+CSA</td>
<td>MUD-GF</td>
<td>4.5</td>
<td>D+18 plt engraftment</td>
<td>None</td>
<td>Dead 22mo GBS, sepsis</td>
</tr>
<tr>
<td>23y, sister</td>
<td>SAA/PNH</td>
<td>ATG+CSA</td>
<td>None</td>
<td>5.8</td>
<td>D+16, D+26</td>
<td>Gd II acute</td>
<td>Alive Healthy baby</td>
</tr>
<tr>
<td>57y, brother</td>
<td>SAA/PNH</td>
<td>ATG+CSA, danazol, eltrombopag</td>
<td>MUD-GF MUD-GF</td>
<td>4.9</td>
<td>D+20, D+27</td>
<td>None</td>
<td>Alive</td>
</tr>
<tr>
<td>22y, brother</td>
<td>VSAA</td>
<td>ATG+CSA</td>
<td>None</td>
<td>6.9</td>
<td>D+23, D+27</td>
<td>None</td>
<td>Alive</td>
</tr>
<tr>
<td>20y, father</td>
<td>VSAA</td>
<td>CSA, ATG, MMF, androgens</td>
<td>None</td>
<td>6.7</td>
<td>Non-engraftment HLA Ab +</td>
<td>N/A</td>
<td>Dead: GF</td>
</tr>
<tr>
<td>50y, son</td>
<td>$^2$ SAA</td>
<td>None</td>
<td>MUD-GF MUD-GF</td>
<td>8.3</td>
<td>D+19, D+25</td>
<td>None</td>
<td>Alive</td>
</tr>
<tr>
<td>41y, mother</td>
<td>VSAA/PNH</td>
<td>ATG+CSA x2</td>
<td>None</td>
<td>1.8</td>
<td>Non-engraftment HLA Ab +</td>
<td>N/A</td>
<td>Dead: GF</td>
</tr>
</tbody>
</table>

Recipient HLA antibody(ies) directed against donor precludes use of that donor

*Clay et al. Biol. Blood Marrow Transplant 2014; 20: 1711*
Novel approach to first line immunosuppressive therapy

Combination of ATG and ciclosporin with eltrombopag
**RACE study**

Prospective Randomized multicentre study comparing horse ATG, Ciclosporin ± Eltrombopag as front-line therapy for SAA

- **TREATMENT Scheme**

  - Steroids
  - Cyclosporin A
  - hATG
  - Eltrombopag

  Randomisation

  - +1
  - // +14
  - // +3m
  - +24m

  Primary endpoint 3m CR

  No CR
  CR

  continue
  stop

Central Lab for Research Studies at King’s College
- Molecular biomarker study to detect early evolution to MDS/AML
- Immunological signature for response
Eltrombopag added to standard IST for aplastic anaemia

**Median time to ANC >0.5 x10^9/L 47 days for all cohorts**

- **Time to TI:** PI 32 days, RBC 39 days
- Combination well tolerated; 2 patients discontinued eltrombopag
- Six withdrawals before 6-month time point (4 refractory patients; 2 patients experienced clonal evolution)

**Overall Survival**

OS at 2 years = 97%

---

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1 n=30</th>
<th>Cohort 2 n=31</th>
<th>Cohort 3 n=31</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3 months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>23 (77)</td>
<td>24 (77)</td>
<td>23/25 (92)</td>
</tr>
<tr>
<td>CR</td>
<td>5 (17)</td>
<td>8 (26)</td>
<td>11/25 (44)</td>
</tr>
<tr>
<td><strong>6 months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>24 (80)</td>
<td>27 (87)</td>
<td>19/20 (95)</td>
</tr>
<tr>
<td>CR</td>
<td>10 (33)</td>
<td>8 (26)</td>
<td>12/20 (60)</td>
</tr>
</tbody>
</table>

---

**Primary endpoints:** CR rate and safety at 6 months

**Secondary endpoints:** OR, PR, survival, clonal evolution and relapse

CR = ANC ≥1 x10^9/L, Hb ≥10 g/dL, PL ≥100 x10^9/L

PR = Blood counts no longer meeting criteria for SAA or CR

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**Figure A:** Bone Marrow Cellularity

**Figure B:** CD34+ Cell Count
Conceptual approach to treatment

- Does the patient have AA or hypoMDS?
  - If it is AA, is it acquired or constitutional?
    - Constitutional AA
      - Danazol
    - Acquired AA
      - HSCT
      - Is the treatment ATG or HSCT?
        - If HSCT, what type?

Future clinical trials:
- Low dose IL-2
- Expanded autologous Tregs
- Danazol
Assessing the aetiology of bone marrow failure

- Telomeropathy
- ATG
- Cyclosporin
- Immunomodulation
- TPO mimetics
- HMAs
- HSCT
- Investigational agents

<table>
<thead>
<tr>
<th>Immune-mediated</th>
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</thead>
<tbody>
<tr>
<td>Cytogenetics</td>
</tr>
<tr>
<td>del(13q) only</td>
</tr>
<tr>
<td>Mutations</td>
</tr>
<tr>
<td>6pUPD, PIGA, BCOR, BCORL1</td>
</tr>
<tr>
<td>(+)</td>
</tr>
<tr>
<td>(+)</td>
</tr>
<tr>
<td>(-)</td>
</tr>
<tr>
<td>Increased percentage of PNH-type cells (+)</td>
</tr>
<tr>
<td>HLA-class I allele-lacking leukocytes (+)</td>
</tr>
<tr>
<td>Increased percentages of bone marrow CD34+ or CD42b+ cells (-)</td>
</tr>
<tr>
<td>Increased plasma TPO levels (&gt; 320 pg/ml)28 (+)</td>
</tr>
<tr>
<td>HLA-DR15 (ref. 29) (+)</td>
</tr>
</tbody>
</table>