Aplastic Anemia: Current Thinking on Disease, Diagnosis and Non-Transplant Treatment

Bogdan Dumitriu, MD
Hematology Branch
National, Heart, Lung and Blood Institute
National Institutes of Health

Today's agenda

- Aplastic Anemia – general overview
- Non-transplant treatment options
- Novel agents and active research
CLINICAL MANIFESTATIONS OF BONE MARROW FAILURE

anemia, bleeding, infection

AGE AT DIAGNOSIS
Aplastic Anemia Admissions to NIH Clinical Center

Severity Criteria (two of three):
- platelets <20k/uL
- reticulocytes <1% (<60k/uL)
- ANC <500/uL

Super-severe: ANC <200/uL

% Surviving

Utah, total (n = 99)

AA Study Group, non-transplanted (n = 63)

*NATURAL HISTORY* OF APLASTIC ANEMIA

Camitta et al, Blood 53:504, 1979

Utah, extrapolated severe
Most of the cases of Aplastic Anemia have no identifiable cause.

Pregnancy, eosinophilic fasciitis, and seronegative hepatitis are associated with AA.

Drugs and chemicals have been reported as well (Benzene, Chloramphenicol).

All identifiable causes explain very few cases of AA.
• 1960’s → 10% survival in 1 year

• 2010 → 90% survival in 1 year

Immunosuppressive therapy

• Bone marrow transplantation

• Supportive care

• Novel agents

Immunosuppressive therapy

• Anti-thymocyte globulin (ATG)
  • Horse
  • Rabbit

• Cyclosporine (CsA)

• Campath

• Others
Immunosuppressive therapy

• First line of treatment in adults
• Salvage for treatment-refractory patients
• Treatment for relapsed disease

PROGRESS IN IMMUNOSUPPRESSIVE THERAPIES FOR SEVERE APLASTIC ANEMIA

<table>
<thead>
<tr>
<th>Era</th>
<th>Drug</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960s</td>
<td>corticosteroids</td>
<td>~10% (occasional)</td>
</tr>
<tr>
<td>1970s</td>
<td>ATGs</td>
<td>40-50%</td>
</tr>
<tr>
<td>1980s</td>
<td>ATG plus CSA</td>
<td>60-70%</td>
</tr>
</tbody>
</table>

• 1960’s → 10% survival in 1 year
• 2010 → 90% survival in 1 year
• Immunosuppressive therapy
• Bone marrow transplantation
• Supportive care
• Novel agents

Immunosuppressive therapy

• Anti-thymocyte globulin (ATG)
  • Horse
  • Rabbit
• Cyclosporine (CsA)
• Campath
• Others

Immunosuppressive therapy

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PROGRESS IN IMMUNOSUPPRESSIVE THERAPIES FOR SEVERE APLASTIC ANEMIA

- Era     Drug       Response
- 1960s   corticosteroids  ~10% (occasional)
- 1970s   ATGs          40-50%
- 1980s   ATG plus CSA   60-70%
A TG AND CSA FOR SEVERE APLASTIC ANEMIA
RESPONSE AT 3 MONTHS AND SURVIVAL

INTENSIVE IMMUNOSUPPRESSION FOR SAA
COMPARISON OF RESULTS

<table>
<thead>
<tr>
<th>Study</th>
<th>Years</th>
<th>N</th>
<th>Median Age (years)</th>
<th>Response</th>
<th>Relapse</th>
<th>Clonal Evolution</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>German</td>
<td>1985-1989</td>
<td>84</td>
<td>32</td>
<td>65%</td>
<td>10%</td>
<td>8%</td>
<td>58% at 11 yrs</td>
</tr>
<tr>
<td>NIH</td>
<td>1991-1998</td>
<td>122</td>
<td>35</td>
<td>61%</td>
<td>20%</td>
<td>11%</td>
<td>55% at 7 yrs</td>
</tr>
<tr>
<td>EGBMT</td>
<td>1991-1998</td>
<td>150</td>
<td>16</td>
<td>77%</td>
<td>12%</td>
<td>11%</td>
<td>87% at 5 yrs</td>
</tr>
<tr>
<td>Japan</td>
<td>1992-1997</td>
<td>119</td>
<td>9</td>
<td>68%</td>
<td>22%</td>
<td>6%</td>
<td>88% at 3 yrs</td>
</tr>
<tr>
<td>German/Austrian</td>
<td>1993-1997</td>
<td>114</td>
<td>9</td>
<td>77%</td>
<td>12%</td>
<td>6%</td>
<td>87% at 4 yrs</td>
</tr>
<tr>
<td>Japan</td>
<td>1996-2000</td>
<td>101</td>
<td>54</td>
<td>74%</td>
<td>42%</td>
<td>8%</td>
<td>88% at 4 yrs</td>
</tr>
<tr>
<td>NIH</td>
<td>1999-2003</td>
<td>104</td>
<td>30</td>
<td>82%</td>
<td>37%</td>
<td>6%</td>
<td>85% at 4 yrs</td>
</tr>
<tr>
<td>EGBMT</td>
<td>2002-2008</td>
<td>192</td>
<td>46</td>
<td>70%</td>
<td>33%</td>
<td>4%</td>
<td>76% at 6 yrs</td>
</tr>
<tr>
<td>NIH</td>
<td>2003-2005</td>
<td>77</td>
<td>26</td>
<td>57%</td>
<td>26%</td>
<td>10%</td>
<td>93% at 3 yrs</td>
</tr>
<tr>
<td>NIH</td>
<td>2005-2010</td>
<td>120</td>
<td>28</td>
<td>68%</td>
<td>26%</td>
<td>21%</td>
<td>96% at 3 yrs</td>
</tr>
</tbody>
</table>

NEW DIRECTIONS IN TREATMENT FOR APLASTIC ANEMIA

- Add to horse ATG + CsA platform
  - G-CSF (Neupogen)
  - Mycophenolate mofetil
  - Sirolimus
  - Long course immunosuppression
- Augment initial lymphocytotoxicity
  - Horse ATG
  - Rabbit ATG
  - Campath

Young NR, Calado RT, Scheinberg P. Blood 2006
Survival of refractory SAA following retreatment with rabbit ATG + CsA (salvage)

A Randomized Trial of H-ATG vs. R-ATG in SAA
Patients and Methods

• 120 consecutive patients (60 per arm)
• NIH Clinical Center
• 1:1 randomization
• Primary objective – response at 6 months

A Randomized Trial of H-ATG vs. R-ATG in SAA
Hematologic Responses at 3 and 6 months

<table>
<thead>
<tr>
<th></th>
<th>Horse ATG</th>
<th>Rabbit ATG</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>37/60 (62%)</td>
<td>20/60 (33%)</td>
<td>0.003</td>
</tr>
<tr>
<td>6 months</td>
<td>41/60 (68%)</td>
<td>22/60 (37%)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
A Randomized Trial of H-ATG vs. R-ATG in SAA
Blood Count Recovery in Responders

### Survival of refractory SAA following retreatment with rabbit ATG + CsA (salvage)

Survival of refractory SAA following retreatment with rabbit ATG + CsA (salvage)

<table>
<thead>
<tr>
<th>Time in days</th>
<th>Percent survival</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>250</td>
<td>75</td>
</tr>
<tr>
<td>500</td>
<td>50</td>
</tr>
<tr>
<td>750</td>
<td>25</td>
</tr>
<tr>
<td>1000</td>
<td>0</td>
</tr>
</tbody>
</table>

Scheinberg P, Nunez O, Young NS. Br J Haematol 2006

### Alemtuzumab (Campath-1H)

- **Anti-CD52 Antibody**
- Murine hypervariable regions fused into human IgG1
- CD52 expressed:
  - B and T cells
  - NK cells, dendritic cells
  - Monocytes, macrophages
  - Plasma cells, Eos
- No CD52 expression on:
  - RBCs, platelets
  - Hematopoietic stem cells

Ravandi and O’Brien, Cancer Invest. 2007 24: 718-725
Hernández-Campo, PMA, Cytometry © Clin Cytom. 2006 70:71
SECOND IMMUNOSUPPRESSION FOR REFRACTORY SAA

<table>
<thead>
<tr>
<th>Treatment arm (N=54)</th>
<th>Overall response</th>
</tr>
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<tbody>
<tr>
<td>rabbit ATG (N=27)</td>
<td>9 (35%)</td>
</tr>
<tr>
<td>alemtuzumab (N=27)</td>
<td>10 (37%)</td>
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ATG AND CSA FOR SEVERE APLASTIC ANEMIA RELAPSE

RELAPSE AFTER ATG + CSA

Cyclosporine dependence Post 1st relapse:

<table>
<thead>
<tr>
<th>Years post-relapse</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients on CSA</td>
<td>20/22</td>
<td>19/20</td>
<td>14/18</td>
<td>11/17</td>
<td>11/14</td>
<td>7/11</td>
<td>4/7</td>
</tr>
<tr>
<td></td>
<td>(86%)</td>
<td>(91%)</td>
<td>(78%)</td>
<td>(65%)</td>
<td>(79%)</td>
<td>(64%)</td>
<td>(57%)</td>
</tr>
</tbody>
</table>

Retreatment with rabbit ATG + CSA Post 1st relapse → 2/3 response

Rosenfeld S, Follmann D, Nunez O, Young NS. JAMA 2003
Scheinberg P, Nunez O, Young NS. Br J Haematol 2006
CAMPATH IMMUNOSUPPRESSION FOR RELAPSED SAA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Overall response</th>
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<tbody>
<tr>
<td>Campath (N=25)</td>
<td>14 (56%)</td>
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INITIAL BLOOD COUNTS PREDICT RESPONSE TO IMMUNOSUPPRESSION AND SURVIVAL


<table>
<thead>
<tr>
<th>Probability of response according to age</th>
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<tr>
<td>Number of patients (%)</td>
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<tr>
<td>------------------------</td>
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<td></td>
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<tr>
<td>HIV-ATG</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>&lt; 18</td>
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<tr>
<td>18 to 60</td>
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<tr>
<td>&gt; 60</td>
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</tbody>
</table>
Survival Probability in Children

Overall

Responders to IST

Survival in refractory SAA

1990s

No response

Survival probability

Improved Survival Over Time

All patients

N = 420

p<0.001

Time (years)
Improved Survival Over Time

Responders to IST
N = 246
p=0.54

Non-responders to IST
N = 174
p<0.001

HEMATOPOIETIC GROWTH FACTORS AS THERAPY FOR SAA

Ganser A et al, Blood 1990; 76;1287: IL-3 pilots
Kojima S et al, Blood 2002;100:786: G-CSF monosomy 7
Tichelli A et al, Blood 2011; 117:4434: G-CSF shows no survival benefit
**ELTROMBOPAG FOR REFRACTORY SEVERE APLASTIC ANEMIA**

- SAA with platelet counts < 30K/μL
- Refractory to ATG/CSA

**Eltrombopag**
- 50 mg daily
- Dose escalation every 2 weeks to 150 mg daily
- Hematologic response at 3 months
- Responders followed monthly on drug

**Hematologic Response Criteria**
- Platelets: >20K/μL increase, or transfusion independence
- RBCs: >1.5 g/dL increase in Hb, or transfusion independence
- ANC: >100% increase if severe neutropenia, or >500/μL increase

**NIH Protocol 09-N025A; ClinicalTrials.gov identifier: NCT00922883**

**REFRACTORY SAA ELTROMBOPAG STUDY RESULTS**

- Median follow up 13 months (range 4-28 months)
- 26 patients enrolled
- 1 patient ineligible, not treated
- 11 responders (44%)
  - 9 platelet responses
  - 2 hemoglobin responses
    - additional 4 at >16wks
  - 4 neutrophil responses
    - additional 3 at >16wks

- 25 evaluable patients
- 14 non-responders
  - 10 stable disease
  - 2 died of progression
  - 2 clonal evolution to MDS
  - 1 died
  - 1 HSCT

- Median follow up 13 months
- Censor date 11/1/2011

**BONE MARROW CELLULARITY AT ONE YEAR**

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
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</table>
MULTI-LINEAGE HEMATOLOGIC RESPONSES TO ELTROMBOPAG

Platelets

Hemoglobin

Neutrophils

Trilineage = 6
Bilineage = 7
Unilineage = 4

INSIGHTS INTO SAA PATHOPHYSIOLOGY FROM ELTROMBOPAG RESPONSIVENESS

stem cell number

correlation with blood counts, age, telomere length

SUMMARY

• Eltrombopag can promote tri-lineage hematopoiesis in SAA patients refractory to IST
  – 44% clinical response rate
  – Transfusion independence
  – Well-tolerated

• Eltrombopag stimulation may expand the HSC pool in humans

• Addition of Eltrombopag early in SAA may increase response rate, decrease time to response, prevent HSC depletion, and avoid clonal progression
ELTROMBOPAG FOR MODERATE AA
NHLBI 09-H-0154
clinicaltrials.gov NCT00922883

Eltrombopag, dose escalation to 150 mg QD by mouth
>18 years old; platelet count <30,000/uL
Assessment by blood counts and BM at 3 and 6 months

Horse ATG + CSA and ELTROMBOPAG
for treatment-naive SAA
NHLBI 12-H-xxxx

Add eltrombopag to existing horse ATG + CSA platform will increase overall response and decrease relapses

TELOMERES AND BONE MARROW FAILURE

TELOMERE STRUCTURE AND BIOLOGY

- Cap chromosome ends
- Tandem TTAGGG repeats
- Bound to array of proteins: telomerase complex
- Forms higher order chromatin T loop
- Shields T end to prevent recognition as a DNA "break" by non-homologous end joining machinery
- TTAGGG loss with proliferation: "end replication problem"
**TELOMRES AND BONE MARROW FAILURE**

**DYSKERATOSIS CONGENITA**

- Nail dystrophy
- Hyperpigmentation
- Leukoplakia

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**TELOMERE LENGTH IN TERT MUTATION LEUCOCYTES**

![Graph showing telomere length against age]

- Hsa 412 Tyr
- Val 694 Met
- Ala 202 Thr
- Cys 772 Tyr
- Val 1090 Met

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**SHORT TELOMERE LENGTH PREDICTS**

**RELAPSE AND EVOLUTION IN SEVERE APLASTIC ANEMIA**

- N = 168 consecutive patients on NIH IST protocols
- Mean age = 34 years (4-82 years)
- No relationship to response to treatment (PR, CR)
SHANK'S DISEASE™ IN A MENNONITE FAMILY

- 38 y/o liver transplant
- 71 y/o thyroid disease
- 47 y/o androgen-responsive
- 26 y/o AA
- 44 y/o macrocytosis
- 21 y/o macrocytosis
- 71 y/o thyroid disease
- 44 y/o androgen-responsive
- 26 y/o AA
- 24 y/o dairy farmer (progressive pancytopenia, no response to CSA, hormones, HSCT from sister, minimal GVHD, full recovery)
- 23 y/o macrocytosis
- 21 y/o macrocytosis
- 50 y/o AA
- 23 y/o AA

LATE PRESENTATION OF DYSKERATOSIS CONGENITA

- 37 y/o US Army officer in Afghanistan (tongue ulcer, diagnosed as squamous cell carcinoma)
- Single round of chemotherapy and radiation resulted in unexpected extreme, persistent pancytopenia
- Later, pulmonary metastases revealed Val295Gly mutation in DKC1

Peripheral Blood Telomere Length

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Telomere length (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>10</td>
<td>5.0</td>
</tr>
<tr>
<td>20</td>
<td>7.5</td>
</tr>
<tr>
<td>30</td>
<td>10.0</td>
</tr>
<tr>
<td>40</td>
<td>12.5</td>
</tr>
<tr>
<td>50</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Healthy subjects

- Thrombocytopenia, gray hair, very short telomere, TERT mutation
- 389 C/T (130 Ala/Val)

Early onset of graying (20's) and low platelets
**Sex Hormones Increase Telomerase Activity in Cultured Human Lymphocytes**

- Telomerase Activity (TPC units)

<table>
<thead>
<tr>
<th>Androgens</th>
<th>TPC (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyltrienolone</td>
<td>900</td>
</tr>
<tr>
<td>Nandrolone</td>
<td>600</td>
</tr>
<tr>
<td>β-Hydroxy-Testosterone</td>
<td>300</td>
</tr>
<tr>
<td>β-Estradiol</td>
<td>0</td>
</tr>
</tbody>
</table>

Calado RT et al, Blood 2009

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**Danazol for telomeropathy**

11-H-0298: “Danazol for Genetic Bone Marrow and Lung Disorders”

ClinicalTrials.gov identifier: NCT01441037

http://clinicaltrials.gov/ct2/show/NCT01441037?term=danazol+for+telomere&rank=1

- 15 patients enrolled in first 6 months.
- First patient enrolled on 08/19/2011
- First 6 months – no drug-related toxicities.

(Minimal elevation in LFTs in almost all patients and controllable headaches in 4 patients).