Aplastic Anemia: Understanding Your Disease and Treatment Options

Danielle Townsley, MD, MSc
Associate Director, Oncology
AztraZeneca Medimmune

Approximate Blood Cell Requirements

<table>
<thead>
<tr>
<th>cell type</th>
<th>total number</th>
<th>life span</th>
<th>daily production</th>
</tr>
</thead>
<tbody>
<tr>
<td>neutrophils</td>
<td>$2 \times 10^{10}$</td>
<td>1</td>
<td>$2 \times 10^{10}$</td>
</tr>
<tr>
<td>platelets</td>
<td>$1 \times 10^{12}$</td>
<td>5</td>
<td>$2 \times 10^{11}$</td>
</tr>
<tr>
<td>erythrocytes</td>
<td>$3 \times 10^{13}$</td>
<td>120</td>
<td>$2.5 \times 10^{11}$</td>
</tr>
</tbody>
</table>

*Hematopoietic Stem Cell*

Neutrophil Differentiation

- Myeloblast
- Promyelocyte
- Myelocyte
- Metamyelocyte
- Band
- Segmented neutrophil

Pathophysiology of Aplastic Anemia

- Immune attack (T lymphocytes)
- Hematopoietic Stem Cells
- Hematopoietic Progenitors
- Circulating blood cells
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 (Benzene, Chloramphenicol).

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

• 2010 → 90% survival in 1 year

Immunosuppressive therapy

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

• Cyclosporine (CsA)

Therapy terminology

• Treatment Naïve

• Refractory
  - Salvage Therapy

• Relapsed

• Term “remission” not used

RESPONSE OF SEVERE APLASTIC ANEMIA TO INTENSIVE IMMUNOSUPPRESSION

Immunization with human thymocytes

Xenogeneic polyclonal antibodies

Purification of sera

Cytotoxicity assay

ATG

IgG

ANC

Platelets
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%

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>1986-1989</td>
<td>84</td>
<td>32</td>
<td>65%</td>
<td>19%</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>11%</td>
<td>55%</td>
<td>7 yrs</td>
</tr>
<tr>
<td>EBMT</td>
<td>1991-1998</td>
<td>100</td>
<td>16</td>
<td>77%</td>
<td>11%</td>
<td>87%</td>
<td>5 yrs</td>
</tr>
<tr>
<td>Japan</td>
<td>1992-1997</td>
<td>119</td>
<td>9</td>
<td>68%</td>
<td>6%</td>
<td>88%</td>
<td>3 yrs</td>
</tr>
<tr>
<td>German/Austrian</td>
<td>1993-1997</td>
<td>114</td>
<td>9</td>
<td>77%</td>
<td>6%</td>
<td>87%</td>
<td>4 yrs</td>
</tr>
<tr>
<td>Japan</td>
<td>1995-2000</td>
<td>107</td>
<td>54</td>
<td>74%</td>
<td>9%</td>
<td>88%</td>
<td>4 yrs</td>
</tr>
<tr>
<td>NIH</td>
<td>1993-1998</td>
<td>104</td>
<td>50</td>
<td>64%</td>
<td>9%</td>
<td>60%</td>
<td>4 yrs</td>
</tr>
<tr>
<td>EBMT</td>
<td>2003-2008</td>
<td>192</td>
<td>46</td>
<td>78%</td>
<td>4%</td>
<td>78%</td>
<td>6 yrs</td>
</tr>
<tr>
<td>NIH</td>
<td>2003-2005</td>
<td>71</td>
<td>28</td>
<td>57%</td>
<td>10%</td>
<td>93%</td>
<td>3 yrs</td>
</tr>
<tr>
<td>NIH</td>
<td>2005-2010</td>
<td>120</td>
<td>28</td>
<td>68%</td>
<td>28%</td>
<td>96%</td>
<td>3 yrs</td>
</tr>
<tr>
<td>NIH</td>
<td>2003-2005</td>
<td>71</td>
<td>28</td>
<td>57%</td>
<td>10%</td>
<td>93%</td>
<td>3 yrs</td>
</tr>
<tr>
<td>NIH</td>
<td>2005-2010</td>
<td>120</td>
<td>28</td>
<td>68%</td>
<td>28%</td>
<td>96%</td>
<td>3 yrs</td>
</tr>
<tr>
<td>NIH</td>
<td>2003-2005</td>
<td>71</td>
<td>28</td>
<td>57%</td>
<td>10%</td>
<td>93%</td>
<td>3 yrs</td>
</tr>
<tr>
<td>NIH</td>
<td>2005-2010</td>
<td>120</td>
<td>28</td>
<td>68%</td>
<td>28%</td>
<td>96%</td>
<td>3 yrs</td>
</tr>
</tbody>
</table>

Young NS, Calado RT, Scheinberg P. Blood 2006.

Cytogenetics

Probability of response according to age

Survival Probability in Children

Overall Responders to IST

Survival in refractory SAA 1990s

Improved Survival Over Time

ATTEMPTS TO IMPROVE OUTCOMES OF IST FOR SAA

- Add to or replace ATG with megadose corticosteroids
  No increase in response; high toxicity (Marmont, Prog Clin Biol Res 1984)
- Replace ATG with high dose cyclophosphamide
  Toxicity (Toubl, Lancet 2001; Blood 2002)
- Replace ATG with moderate dose cyclophosphamide
  Excessive toxicity secondary to neuropenia (Scheinberg, Blood 2014)
- Add mycophenolate mofetil to ATG/CsA
  No improvement in response/survival (Scheinberg, Br J Haematol 2006)
- Add sirolimus to ATG/CsA
  No improvement in response/survival (Scheinberg, Haematologica 2009)
- Add G-CSF to ATG/CsA
  No improvement in response/survival (Locasciulli, Haematologica 2004)
- Prolonged CsA (2 years) to prevent relapse
  Delayed but ultimately equivalent rate (Scheinberg, Am J Hematol 2014)
- Replace horse with rabbit ATG, or alemtuzumab, frontline
  No improvement in response/survival (Scheinberg, NEJM 2010)
ELTROMBOPAG (EPAG)
A NON-PEPTIDE TPO RECEPTOR AGONIST

**Clinical Applications**
- Orally administered, $t_{1/2}$ 30 hrs.
- FDA accelerated approval for chronic ITP (2008).

**ELTROMBOPAG AND APLASTIC ANEMIA**
- 40% (17/43) hematologic response rate
- Durable tri- and bilineage responses
- Transfusion independence
- Well-tolerated

**ELTROMBOPAG FOR REFRACTORY SAA**
Lineage characteristics of responses

16 Weeks - Primary Endpoint

Best Response at Follow-up

![Diagram showing lineage characteristics of responses with Platelets, Neutrophils, and Hemoglobin](image)

**Initial Trial-EPAG for Refractory Severe Aplastic Anemia**
- 43 patients refractory to immunosuppressive therapy (median 2.5 cycles)
- Eltrombopag 50 mg daily
- Dose escalation every 2 weeks to 150 mg daily
- Primary endpoint: Hematologic response 12-16 weeks
- Responders continue EPAG until robust response or plateau

- Subset of non-responders had improvement in counts at 3 months and/or continued improvement in counts and decreased transfusion frequency after EPAG stopped
- Would extended treatment with EPAG improve response rate in refractory SAA?

**Extended Dosing with EPAG for Refractory SAA**
- 40 patients refractory to IST (median 1.5 cycles)
- Platelet count $\leq 30,000/\mu L$, ANC $<500/\mu L$, Hb $<9.0$ g/dl
- Eltrombopag 150 mg daily
- No dose escalation
- 3 month evaluation: Hematologic response
- 6 month evaluation: Primary Endpoint Hematologic response
- Responders continue EPAG until robust response or plateau

- 20 responders at 6 months (50%)
- 13 multi-lineage
- 5/20 responders were non-responders at 3 months

**CLONAL “CYTOGENETIC” EVOLUTION**
**HISTORIC COHORT**

![Graph showing clonal evolution](image)

- All evolution
- Evolution to monosomy 7
Pooled analysis of all 83 patients enrolled in both EPAG studies for rSAA

- 16 patients (18%) had clonal evolution
  - Detected early (3 mo), rarely with dysplasia
  - 6 patients-loss of chromosome 7 or 7q (5 nonresponders)
  - 9 patients-other cytogenetic abnormalities (normalized in 5)
  - 1 patient with AML (no metaphase growth at baseline)

ELTROMBOPAG FOR REFRACTORY SAA
Can EPAG be discontinued?

- 15 robust responders had EPAG stopped
  - 3 relapses, responded to EPAG
  - 12 (80%) with durable response, median f/u 3 years

Stopping criteria:
Robust response:
- Platelets >50,000/ul
- Hb >10 g/dL
- Neutrophils >1,000/ul
or
- stable counts x 6m

TPO AND HEMATOPOIETIC STEM CELLS

- TPO receptor (c-Mpl) expressed on HSCs and early progenitor cells

- TPO expands HSCs in vitro

- C-Mpl and Tpo “knockout” mice have reduced HSCs

- Multi-lineage marrow failure occurs in some congenital amegakaryocytic thrombocytopenia

ELTROMBOPAG FOR TREATMENT NAÏVE SAA

- Eltrombopag stimulation to expand HSC pool:
  - increased response rate?
  - accelerate count recovery?
  - prevent HSC depletion?
  - avoid clonal progression?

HEMATOLOGIC RESPONSE RATES

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1 N=30</th>
<th>Cohort 2 N=31</th>
<th>Cohort 3 N=31</th>
<th>All Cohorts N=92</th>
<th>Historic rates N=388*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>23 (77)</td>
<td>24 (77)</td>
<td>27 (87)</td>
<td>74 (80)</td>
<td>60%</td>
</tr>
<tr>
<td>CR</td>
<td>5 (17)</td>
<td>8 (26)</td>
<td>15 (48)</td>
<td>28 (30)</td>
<td>8%</td>
</tr>
<tr>
<td>6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>24 (80)</td>
<td>27 (87)</td>
<td>29 (94)</td>
<td>60 (87)</td>
<td>62%</td>
</tr>
<tr>
<td>CR</td>
<td>10 (33)</td>
<td>8 (26)</td>
<td>18 (58)</td>
<td>38 (58)</td>
<td>12%</td>
</tr>
</tbody>
</table>

ROBUST COUNT RECOVERY IN RESPONDERS
EPAG V. HISTORIC

- Neutrophils in very SAA
  - ANC>500/μL: 48 days
- Red cells
  - Transfusion independence: 39 days
- Platelets
  - Transfusion independence: 32 days

BONE MARROW ANALYSIS

- Neutrophils in very SAA
  - ANC>500/μL: 48 days
- Red cells
  - Transfusion independence: 39 days
- Platelets
  - Transfusion independence: 32 days

OVERALL SURVIVAL
MEDIAN FOLLOW-UP 23 MONTHS

97% at 2 years (95% CI, 94-100%)

- One (1) death on study: Thymoma with paraneoplastic encephalopathy
- Two (2) deaths after HSCT: MDS/AML; HSCT relapsed AML
  - Relapsed aplastic anemia: HSCT GVHD

ADVERSE EVENTS

<table>
<thead>
<tr>
<th>Event</th>
<th>Patients (N=92)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>ns (96%)</td>
<td></td>
</tr>
<tr>
<td>Maculopapular rash</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>Pruritus</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>Liver test abnormality</td>
<td>17 (18%)</td>
<td></td>
</tr>
<tr>
<td>Increased alanine aminotransferase level</td>
<td>9 (10%)</td>
<td></td>
</tr>
<tr>
<td>Increased aspartate aminotransferase level</td>
<td>3 (3%)</td>
<td></td>
</tr>
<tr>
<td>Blood bilirubin increased</td>
<td>12 (13%)</td>
<td></td>
</tr>
</tbody>
</table>
One-third with at least 1 mutation

SIMILAR PROPORTION OF PATIENTS WITH MUTATIONS AFTER IST + ELTROMBOPAG

20/90 (22%) patients
- 16 with one gene mutation

Chromosome Instability

CONSEQUENCES OF TELOMERE EROSION

- aneuploidy
- end-to-end fusion
- non-reciprocal translocation

Apoptosis (Hayflick phenomenon)

Values are given as number (%) of patients.

A SUBSET OF MUTATIONS CORRELATE WITH SURVIVAL - FREE FROM CLONAL EVOLUTION

Telomere attrition and candidate gene mutations preceding monosomy 7 in aplastic anemia

Telomere length of leukocytes at diagnosis of SAA predicts clonal evolution
TELOMERES AND CLONAL EVOLUTION

Predictors for response to EPAG + IST
- Longer Telomeres (>10th percentile)
- Younger Age


THE HIGH TPO PARADOX

Endogenous TPO levels are already markedly elevated in patients with severe aplastic anemia (AA)

Emmons R et al., Blood 87:4086 (1996)
Feng X et al., Haematologica 96:602 (2011)

HOW DOES EPAG IMPROVE HEMATOPOIESIS DESPITE HIGH TPO LEVELS?

Dr. Andre Larochelle

Heterodimerization of TPO and IFNγ Impairs Human Hematopoietic Stem/Progenitor Cell Signaling and Survival in Chronic Inflammation

59th ASH Annual Meeting, Plenary Session
Luigi J. Alvarado, Hai Cheng, Heather D. Huntsman, Alessio Andreoni, Danielle Townsley, Thomas Winkler, Xingmin Feng, Jay R. Knutson, Cynthia E. Dunbar, Neal S. Young, Andre Larochelle
National Heart, Lung, and Blood Institute (NHLBI)
National Institutes of Health (NIH)
December 10th, 2017

TELOMERES AND A SEX HORMONE

Danazol Treatment for Telomere Diseases

27 patients with telomere diseases, short telomeres ± mutations were enrolled, study closed early for efficacy (telomere elongation)


HOW DOES EPAG BIND TO c-MPL AT DISTINCT SITES

TPO and EPAG bind to c-MPL at distinct sites

Thrombopoietin Cytokine

Eltrombopag Small molecule

Signaling pathways

Hematopoietic stem/progenitor cell (HSPC)

Survival

Proliferation

Signaling pathways

Hematopoietic stem/progenitor cell (HSPC)
INFLAMMATORY CYTOKINES ARE ELEVATED IN APLASTIC ANEMIA

Inflammation plays a key role in the pathogenesis of aplastic anemia. Elevated levels of inflammatory cytokines, including interferon-γ (IFN-γ), have been observed in patients with aplastic anemia. This suggests a role for immune responses in the development of this disease.

Adapted from Young NS et al, Blood 108 (8): 2509 (2006)

EPAG MAINTAINS MORE CD34+ HSPCs THAN TPO IN THE PRESENCE OF IFNγ IN VITRO

Eltrombopag (EPAG) maintains a higher percentage of CD34+ hematopoietic stem and progenitor cells (HSPCs) compared to thrombopoietin (TPO) in the presence of interferon-γ (IFNγ). This indicates that EPAG may have a more effective role in promoting hematopoiesis under inflammatory conditions.

EPAG MAINTAINS STEM AND PROGENITOR CELLS IN THE PRESENCE OF IFNγ IN VITRO

Model of IFNγ-Mediated Bone Marrow Failure

Eltrombopag (EPAG) maintains stem and progenitor cells in the presence of IFNγ, indicating its potential role in inhibiting or mitigating the effects of IFNγ on hematopoiesis.

SUMMARY

- EPAG is an effective single agent for refractory severe AA, and in combination with IST associates with markedly higher response rates.
  - European (RACE) Trial ongoing ages ≥15
  - Global (SOAR) Trial – CSA/EPAG for severe AA
  - NIH Extension Trial - ongoing
- In tx naive SAA, clonal evolution rates similar to IST without EPAG, but longer follow up required to establish late events
- Somatic myeloid cancer mutations are common in adults with AA, but unclear clinical utility beyond standard cytogenetics
- Insights into mechanism suggest EPAG may ameliorate cytopenias in other inflammatory states (GVHD, chronic infections)

National Institutes of Health
- Neal S. Young
- Cynthia E. Dunbar
- Philip Scheinberg
- Thomas Winkler
- Rodrigo Calado
- Ronan Desmond
- Bogdan Dumitriu
- Katherine Calvo
- Fernanda Rodrigues
- Janet Valdez
- Feng Xingmin
- Andre Larochelle
- Kayvan Keyvanfar
- Stephanie Selvar
- Sachiko Kajigaya
- Marie Desierto
- Charles Bolan
- Olga Rios
- Barbara Weinstein
- Margaret Bevans

GSK, Novartis
- Connie Erickson-Miller
- Nicole Stone
- Krista McMarrach
- Brian Elliott
- Judith Horowitz
- Socorro Portela
- Kate McMarr
- Kelly Haines
- Neogenomics
- Maher Ablair
- Wandoong Mo
- Univ of Chicago
- Zayares Des
- Zejuan Li

ACKNOWLEDGEMENTS