Bone Marrow Failure

Neal S. Young, M.D.

Hematologist
DISCLOSURES

Off-Label Usage

• Cyclosporine (Many and Generic)
• Eltrombopag (GlaxoSmithKline)
• IV Ig (Many and Generic)

Interests

• None
BONE MARROW FAILURE FOR THE BOARDS

1. Aplastic anemia
2. Telomere diseases
3. PNH
4. PRCA and B19 parvovirus
5. Agranulocytosis
CLINICAL FEATURES OF APLASTIC ANEMIA
PHYSICAL MANIFESTATIONS OF PANCYTOPENIA

Anemia symptoms predominate: fatigue, exercise intolerance, dyspnea, angina, etc. Bleeding symptoms are usually alarming but bleeding is nuisance, not catastrophic. Fever, infection, sepsis very unusual at first presentation.
Pancytopenia may be detected incidentally on blood counts, without symptoms.

Physical examination may be normal or show pallor, petechiae, ecchymoses. Splenomegaly, adenopathy, cachexia all point to another diagnosis.
“NATURAL HISTORY” OF APLASTIC ANEMIA

“Camitta” criteria (two of three):
- platelets <20K/uL
- reticulocytes <1% (60K/uL)
- ANC <500/uL

Super-severe: ANC <200/uL

% Surviving

Years

Utah, total (n = 99)

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

Utah, extrapolated severe
AGE AT DIAGNOSIS OF SEVERE APLASTIC ANEMIA

Most patients are older children and young adults.
MAJOR PROSPECTIVE EPIDEMIOLOGIC STUDIES


Aplastic Anemia and the Public

Industrial Benzene Poisoning, 1920s

New Drug Development, 1990s

Drug etiology difficult to establish and may not influence treatment decisions.
SIMPLIFIED PATHOPHYSIOLOGY OF ACQUIRED APLASTIC ANEMIA

ONSET

inciting event

clinical presentation

immune response

RECOVERY

CR

PR

LATE DISEASE

abnormal clone

MDS, AML

Relapse of pancytopenia

HSC

PBC

Time
BONE MARROW FAILURE SYNDROMES

AID: MS, colitis, uveitis, DM type 1, etc.

BONE MARROW FAILURE SYNDROMES

AA/PNH

PNH

FA

AA

DKC

hypocellular MDS

MDS

AML

hypocellular MDS
DIFFERENTIAL DIAGNOSIS OF PANCYTOPENIA

• With hypocellular BM
  – Aplastic anemia
    • acquired versus constitutional (FA, DKC)
  – Myelodysplasia (20% cases)
  – Myelofibrosis (“dry tap”)
  – Rarely: ALL (children), AML (elderly) as aleukemic leukemia, lymphoma

• With normo- or hypercellular BM
  – Primary marrow disease
    • MDS
    • PNH
    • Myelofibrosis
    • Leukemias and lymphomas
    • Hairy cell leukemia
    • Large granular lymphocytosis
  – Secondary
    • Hypersplenism
    • Systemic lupus erythematosus
    • Vitamin deficiency (B12, folate)
    • Alcohol
    • Tuberculosis and other mycobacteria, brucellosis
    • Sarcoidosis
KEY POINTS IN INITIAL EVALUATION AND DIFFERENTIAL DIAGNOSIS

Younger patients:
Differentiate peripheral destruction from marrow failure (spleen, DAT, BM)
Distinguish acquired from congenital AA
Family history critical; physical examination can be normal
FA (chromosome x-linking) and DKC testing (telomere length) on blood

Older patients:
Major differential among bone marrow syndromes:
MDS, myelofibrosis (organomegaly, dry BM), metastatic cancer,
AML (aleukemic leukemia), lymphoma
Cytogenetics of BM aspirate for MDS
PATHOPHYSIOLOGY OF SAA

- Hematopoietic stem cell loss
- Immune destruction of marrow stem and progenitor cells
- Genetic susceptibility factors for both (HLA/telomerase genes)
APLASTIC BONE MARROW IN TWO AND THREE DIMENSIONS

green (BODIPY) = lipid
red = CD34
blue (DAPI) = cells

Markedly decreased:
- progenitor cell number (colony formation)
- stem cells (LTC-IC)
- CD34 cells

Normal or increased:
- cytokine plasma levels
- stroma cell function
RESPONSE OF SEVERE POST-HEPATITIS APLASTIC ANEMIA TO INTENSIVE IMMUNOSUPPRESSION

ANC

CSA
ATG

Platelets

Hct
Retic

*
OLIGOCLONAL T CELL EXPANSION IN A PATIENT WITH SEVERE AA:
TCR Vβ SUBFAMILY ANALYSIS BY FLOW CYTOMETRY
Vβ T CELL SUBFAMILY EXPANSION DURING IMMUNOSUPPRESSION

Pre-treatment

Remission

Relapse

*
MURINE MARROW APLASIA POST-LYMPHOCYTE INFUSION

day 7 post-LN cell infusion

day 17 post-LN cell infusion

green (BODIPY) = lipid
green = CD8
red = CD3
white (perlecan) = extracellular matrix
blue (DAPI) = cells
PATHOPHYSIOLOGY OF ACQUIRED APLASTIC ANEMIA

- T cell-mediated (CD8)
- Type 1 cytokines (Υ-IFN)
- Apoptosis (Fas-FasL)
- “Bystander” destruction
DEFINITIVE TREATMENTS OF APLASTIC ANEMIA

- Hematopoietic stem cell transplantation (BM/MUD/haplo)
- Immunosuppression (hATG + CSA, salvage regimens)
AGE AND OUTCOME OF BMT IN SAA
CENTER FOR INTERNATIONAL BLOOD AND MARROW TRANSPLANTATION RESEARCH

5 year overall survival
adjusted for PS, time to BMT, conditioning regimen

Mortality affected by:
prior GVHD
time to transplant (>3 months)
no prior IST, year of transplant

cGVHD risk equal for all >20 yrs old
half patients had extensive cGVHD
cGVH risk higher with poor PS

Gupta V et al, Haematologica 2010; 95:2119
LONG TERM OUTCOME AFTER BMT FOR SAA

N=61 consecutive patients
BMT 1991-2010; Cytoxan + ATG
Mean age=21; most adults

87% survival at 6 yrs

Low rate of complications
Mainly: osteonecrosis, endocrine dysfunction

Cumulative incidence grade II-IV cGVHD =23%

Konapacki J et al, Haematologica 2012; 97:710
# MATCHED UNRELATED TRANSPLANTS IN SAA

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>N</th>
<th>Design</th>
<th>Conditioning</th>
<th>Graft failure</th>
<th>Median age (years)</th>
<th>aGVHD grade II-IV</th>
<th>cGVHD</th>
<th>Survival</th>
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<tbody>
<tr>
<td>Kim</td>
<td>2007</td>
<td>40</td>
<td>Prospective</td>
<td>Cy/TBI</td>
<td>5%</td>
<td>27</td>
<td>30%</td>
<td>38%</td>
<td>75% at 3 yrs</td>
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<tr>
<td>Maury</td>
<td>2007</td>
<td>89</td>
<td>Retrospective</td>
<td>Various</td>
<td>14%</td>
<td>17</td>
<td>50%</td>
<td>28%</td>
<td>42% at 5 yrs</td>
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<tr>
<td>Viollier</td>
<td>2008</td>
<td>349</td>
<td>Retrospective</td>
<td>Various</td>
<td>11%</td>
<td>18</td>
<td>28%</td>
<td>22%</td>
<td>57% at 5 yrs</td>
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<tr>
<td>Kosaka</td>
<td>2008</td>
<td>31</td>
<td>Prospective</td>
<td>Cy/ATG/TBI</td>
<td>16%</td>
<td>8</td>
<td>13%</td>
<td>13%</td>
<td>93% at 3 yrs</td>
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<td>Perez-Albuerne</td>
<td>2008</td>
<td>195</td>
<td>Retrospective</td>
<td>Various</td>
<td>15%</td>
<td>10</td>
<td>43%</td>
<td>35%</td>
<td>51% at 5 yrs</td>
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<tr>
<td>Bacigalupo</td>
<td>2010</td>
<td>100</td>
<td>Retrospective</td>
<td>Flu/Cy/ATG/TBI</td>
<td>17%</td>
<td>20</td>
<td>18%</td>
<td>27% (no TBI)</td>
<td>50% (TBI group)</td>
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<tr>
<td>Kang</td>
<td>2010</td>
<td>28</td>
<td>Prospective</td>
<td>Flu/Cy/ATG</td>
<td>0%</td>
<td>13</td>
<td>46%</td>
<td>35%</td>
<td>68% at 3 yrs</td>
</tr>
<tr>
<td>Lee</td>
<td>2010</td>
<td>50</td>
<td>Prospective</td>
<td>Cy/TBI</td>
<td>0%</td>
<td>28</td>
<td>46%</td>
<td>50%</td>
<td>88% at 5 yrs</td>
</tr>
<tr>
<td>Yagasaki</td>
<td>2010</td>
<td>31</td>
<td>Retrospective</td>
<td>Various</td>
<td>3%</td>
<td>9</td>
<td>37%</td>
<td>27%</td>
<td>94% at 5 yrs</td>
</tr>
</tbody>
</table>

Scheinberg P, Young NS. Blood 2012; 120:1185
BONE MARROW BETTER THAN BLOOD AS A SOURCE OF STEM CELLS IN TRANSPLANT FOR APLASTIC ANEMIA

Bacigalupo A et al, Haematologica 2012; 97:1142
BMT FOR SAA FOR THE BOARDS

Treatment of choice for younger patients (<18 y/o)

Survival differences between children and adults
   But excellent results at least to 40 y/o

If transplant planned, perform soon after diagnosis
   HLA typing at presentation

Long-term complication rate low (≈6% standard, 12% MUD)

BM, not mobilized PB, as stem cell source (for sibling transplant)

Sibling transplant standard, but high resolution MUDs almost equal
   Start donor search early

Standard regimen yields best results
   CTX+ ATG conditioning, CSA/MTX for GVHD prophylaxis

Umbilical cord blood and haploidentical transplants promising but not standard
<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>
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<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>
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<tr>
<td>NIH</td>
<td>1991-1998</td>
<td>122</td>
<td>35</td>
<td>61%</td>
<td>35%</td>
<td>11%</td>
<td>55% at 7 yrs</td>
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<tr>
<td>EGMBT</td>
<td>1991-1998</td>
<td>100</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>
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<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>62%</td>
<td>37%</td>
<td>9%</td>
<td>80% at 4 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>
</tbody>
</table>
### A RANDOMIZED TRIAL OF H-ATG VS. R-ATG IN SAA HEMATOLOGIC RESPONSES AT 3 AND 6 MONTHS

Protocol 006-H-00034; 2006-2010; N = 120 at NIH Clinical Center
ATGAM (40 mg/kg/d x 4d vs Thymoglobulin (3.5 mg/kg/d x 5 d)

<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>
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</table>
INITIAL BLOOD COUNTS PREDICT RESPONSE TO IMMUNOSUPPRESSION AND SURVIVAL

Also predictive: “robust” hematologic responses to ATG; CRs; young age (measures of stem cell reserve?)
SURVIVAL IMPROVEMENT IN SAA TREATED WITH IST

Survival probability

Time (years)

Improved supportive care especially antifungal drugs
Second IST 30-40% response rate to rATG, Campath
High risk stem cell transplant MUD in children, alloBMT in older adults

Valdez JM et al, Clin Inf Dis 2011; 52:726
## SECOND IMMUNOSUPPRESSION IN REFRACTORY SAA

<table>
<thead>
<tr>
<th>Agent</th>
<th>RR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rATG/CSA</td>
<td>14/48 (29%)</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>10/27 (37%)</td>
</tr>
</tbody>
</table>

24/76 responders to either rATG or alemtuzumab
Censored for traffic accident (1)
Deaths due to fungal infection (1), evolution (1)

89% survival
Figure 1. Actuarial survival of 2479 patients with acquired severe aplastic anemia according to whether their first-line treatment BMT or immunosuppression: the 10-year survival is 73% in BMT recipients and 68% in those treated with immunosuppression (p=0.002).
SUMMARY OF OUTCOMES IN SAA AFTER IST

Hematologic recovery at 6 months (tx-ind, adequate ANC) 60-65%
Hematologic response to 2^{nd} IST 30-35%
Relapse (requirement for further IST) 30-40%
Evolution (MDS by cytos, morphology; AML) 15%

\approx 75-90\% \text{ overall response}

Failure due to:

- limiting stem cell reserve/regeneration
- non-immune pathophysiology
- immune therapy
Applicable to all ages

But children >> older adults in response, complications

Horse ATG + CSA first-line (65% response rate)
Rabbit ATG + CSA or Campath second-line (30% response)

About 1/3 of patients require CSA long-term or relapse

Major toxicities: allergic, serum sickness, volume overload
HSCT VS IST?

Children: HSCT from sibling donor 1st choice
   Excellent results with IST
   Move quickly to high resolution MUD/UC for refractory/recurrent relapse

Adults:  Upper age for HSCT from sibling donor increasing—50? 55?
   IST 1st rx for most older adults
   HSCT from sibling donor in older patients or MUD in younger if IST failure

3rd round of IST rarely successful!
No reason to transplant if response to IST!
ATTEMPTS TO IMPROVE ON STANDARD IST FOR SAA

Add androgens to ATG
  No increase in response rate (Champlin RE et al, Blood 1985; 66:184)

Add to or replace ATG with megadose corticosteroids
  No increase in response; high toxicity (Marmont AM et al, Prog Clin Biol Res 1984; 148:271)

Replace ATG with high dose cyclophosphamide
  Excessive toxicity secondary to neutropenia (Tisdale JF et al, Blood 2002; 137:549)

Replace ATG with “moderate” dose cyclophosphamide
  Excessive toxicity secondary to neutropenia (Scheinberg P; manuscript under review)

Add mycophenolate mofetil to ATG/CsA
  No improvement in response/survival (Scheinberg P et al, Br J Haematol 2006; 133:606)

Add sirolimus to ATG/CsA
  No improvement in response/survival (Scheinberg P et al, Haematologica 2009; 94:348)

Add G-CSF to ATG/CsA
  No improvement in response/survival (Locasciulli A et al, Haematologica 2004; 89:1054

Prolong CsA (2 years) to prevent relapse
  Delayed but ultimately equivalent outcome (Scheinberg P et al, under review)
PROLONGED CYCLOSPORINE DELAYS BUT DOES NOT PREVENT RELAPSE

ELTROMBOPAG (PROMACTA™)

- 2\textsuperscript{nd} generation thrombopoietin (TPO) agonist
- Synthetic mimetic molecule
- Oral, non-immunogenic
- FDA approval in 2008 for refractory chronic ITP
44% (11/25) response rate in previously refractory patients

Robust blood count increases (Hb 4 gr/dL, platelets 40,000/ul)

Tri- and bi-lineage responses most frequent

Durable after discontinuation of eltrombopag

Marrow cellularity restored

Transfusion independence

Well-tolerated by patients
**ELTROMBOPAG FOR REFRACTORY SAA EXTENSION STUDY**

<table>
<thead>
<tr>
<th>Age in years: median, range</th>
<th>44 (17-77)</th>
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<tr>
<td>Male: number, (%):</td>
<td>25 (58%)</td>
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<tr>
<td>Transfusion Dependent</td>
<td></td>
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<tr>
<td>Platelets</td>
<td>42 (98%)</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>40 (93%)</td>
</tr>
<tr>
<td>Baseline Parameters</td>
<td>Median, range:</td>
</tr>
<tr>
<td>Platelets (K/uL)</td>
<td>8 (2-22)</td>
</tr>
<tr>
<td>Neutrophils (K/uL)</td>
<td>0.57 (0.07-2.8)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>7.9 (6.0-13.3)</td>
</tr>
<tr>
<td>Months from last Immunosuppressive Therapy</td>
<td>Median, range:</td>
</tr>
<tr>
<td></td>
<td>9 (6-117)</td>
</tr>
<tr>
<td>Number of prior IST</td>
<td>2 (1-4)</td>
</tr>
</tbody>
</table>

≈40% response rate (protocol criteria)

[44% best response]

≈16% clonal evolution

Durable responses in 5/5 pts–after drug withdrawal (median follow-up 13 mos)

Minimal drug toxicity

Desmond R et al, Blood 2014; 123:1818
ELTROMBOPAG ADDED TO IMMUNOSUPPRESSION AS FIRST THERAPY

<table>
<thead>
<tr>
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<th>3 months</th>
<th>6 months</th>
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<tr>
<td><strong>N = 30</strong></td>
<td></td>
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<tr>
<td>Complete Response</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Partial Response</td>
<td>18</td>
<td>14</td>
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<tr>
<td>Off Study/Not evaluable</td>
<td>2</td>
<td>3</td>
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<tr>
<td>Too Early</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Overall RR (ITT)</td>
<td>77% (23/30)</td>
<td>80% (24/30)</td>
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<tr>
<td>ORR (completed)</td>
<td>82% (23/28)</td>
<td>89% (24/27)</td>
</tr>
<tr>
<td>Complete RR (ITT)</td>
<td>17% (5/30)</td>
<td>33% (10/30)</td>
</tr>
<tr>
<td>CRR (completed)</td>
<td>18% (5/28)</td>
<td>37% (10/27)</td>
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Censored 5/21/14
<table>
<thead>
<tr>
<th>Subject (Age)</th>
<th>Baseline</th>
<th>Clone</th>
<th>Time on eltrombopag (months)</th>
<th>Dysplasia</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>7 (60)</td>
<td>NR</td>
<td>46XY[20]</td>
<td>-7[20]</td>
<td>3</td>
<td>N</td>
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<tr>
<td>31 (41)</td>
<td>NR</td>
<td>46XY</td>
<td>+21(3)/46XY(17) -7[2]/46XY[19]</td>
<td>3 6</td>
<td>Yes (mild)</td>
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<tr>
<td>42 (17)</td>
<td>NR</td>
<td>No metaphases</td>
<td>+1,der(1;7)[4]/46XY[16]</td>
<td>3</td>
<td>N</td>
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SUPPORTIVE CARE IN APLASTIC ANEMIA
SUPPORTIVE CARE: ANEMIA

1. Transfuse to prevent symptoms
   - packed rbcs, usually 2 u every 2 wks
   - target Hb>7gr/dL (>9 gr/dL if cardiopulmonary disease)

2. Prevent secondary hemochromatosis
   - initiate iron chelation between 50-100 u transfusion
   - chelation: deferoxamine (subcu), deferasirox (oral)
   - phlebotomory in recoveryed patients (monitor ferritin)
   - PNH “treats” iron overload
SUPPORTIVE CARE: NEUTROPENIA

1. Infection remains major cause of death in AA

2. Aggressive, early and broad spectrum treatment
   
   fever and neutropenia (<500/uL)
   
   parenteral antimicrobials
   
   add vancomycin for suspected catheter infection

3. Continue therapy for 10-14 days regardless of culture results

4. Early introduction of antifungal drugs
   
   persistent fever
   
   evidence of sinus, lung infection

5. G-CSF-mobilized neutrophils
   
   in selected circumstances, such as bridge to HSCT
SUPPORTIVE CARE: THROMBOCYTOPENIA

1. Most bleeding in AA is minor: cutaneous, gingival, nasal
2. Use of single donor, leucocyte-depleted platelets
   ↓alloantigen exposure, alloimmunization, refractory state
3. For prophylaxis, maintain at >10,000/uL
4. Refractoriness to platelets usually due to HLA antibodies
   prophylaxis not indicated
   use best matched HLA donors for active bleeding
5. For major hemorrhage/surgical intervention
   maintain platelets at >50-80,000/uL
ATG TOXICITIES

1. Early allergic reactions (with infusion, worst on day 1)
   fever, rigors, urticaria
   hypotension, anaphylaxis

2. Serum sickness (about day 10)
   “flu-like” illness
   typical exanthem
   multisystem manifestations, especially serositis
   immune complex deposition
   bump corticosteroids and treat symptoms

3. Worse pancytopenia
   with infusions, especially thrombocytopenia
RECURRENT PANCYTOPENIA SECONDARY TO RAEB-T—10 YEARS AFTER ATG/CSA

BM blasts; 12/03

del 13q (q12q14); 7/97
>300 patients, treated since 2000 at NIH Clinical Center on horse ATG regimens

Median time to evolution = 613 days (IQR 208-977; range 171-2725)

Monosomy 7, complex cytogenetics, leukemia

EVOLUTION TO HIGH RISK MDS/AML IN SEVERE APLASTIC ANEMIA

≈12%
TELOMERES AND MARROW FAILURE
TELOMERASE ELONGATES THE 3’ END OF TELOMERES BY ADDING TTAGGG REPEATS
TELOMERE LENGTH MEASUREMENT
TELOMERE LENGTH IN TERT MUTATION LEUCOCYTES

![Graph showing telomere length in TERT mutation leucocytes. The graph plots telomere length (kb) against age (years). The x-axis represents age in years, ranging from 0 to 100, and the y-axis represents telomere length in kb, ranging from 0 to 16. The data points are color-coded: yellow for controls and red for patients. Key mutations highlighted include His 412 Tyr, Val 694 Met, Ala 202 Thr, Cys 772 Tyr, and Val 1090 Met.]
HEMATOLOGY/HEMATOPOIESIS IN “NORMAL” FAMILY MEMBERS WITH *TERC* MUTATIONS

**Hematology**
- normal peripheral blood counts
- mild anemia with macrocytosis
- mild thrombocytopenia

**Hematopoiesis**
- severely hypoplastic
- ↓CD34 number
- ↓colony formation
- ↑erythropoietin, thrombopoietin

<table>
<thead>
<tr>
<th>proband</th>
<th>unaffected brother</th>
<th>affected sister</th>
<th>affected niece</th>
</tr>
</thead>
</table>

[Images of hematopoietic tissue samples for each family member]
TELOMERE SHORTENING: TISSUE REPAIR AND REGENERATION

Telomere repair mutations:
TERT, TERC, etc.

telomere erosion

Environment:
immunity, toxins, infections, etc.

stem cell loss
Bone marrow failure

aberrant repair/regeneration
Pulmonary fibrosis Cirrhosis
TELOMERE LENGTH AND SAA OUTCOMES

A) EVOLUTION RATE

B) MONOSOMY 7 EVOLUTION

No. at risk

TL < 1st quartile

TL > 1st quartile

Follow-up, years

Log rank P=0.009

Log rank P=0.002

*
ACCELERATED TELOMERE LOSS PRECEDES CLONAL EVOLUTION

Stable SAA controls
Clonal evolution

Normalized telomere length vs. Months since SAA treatment

ACCELERATED TELOMERE LOSS PRECEDES CLONAL EVOLUTION
TELOMERES AND CLINICAL PRACTICE

History and physical examination

- *hematology-pulmonary-hepatic diseases linked in pedigrees*
- *subtle hair, nail, skin, mucous membrane findings*

Screening

- *telomere length of leukocytes for diagnosis*
- *stem cell transplant donors in pedigrees*

Prognosis

- *evolution of AA to MDS/AML*
- *transplant outcomes*
- *other organ involvement (cryptic cirrhosis, fibrosis)*

Treatment

- *avoidance of regenerative stress (smoke, alcohol, etc.)*
- *chemotherapy sensitivity*
- *sex hormones for TERT modulation*
TELOMERES, ANEUPLOIDY, AND CANCER

iatrogenic: marrow stress 2°
chemotherapy, HSCT

physiologic:
aging

constitutional: TERT, TERC,
SBDS, DDX11 mutations

telomere erosion

end-end fusion
unbalanced translocations
gain/loss of chromosomal DNA

+mutation/selection

malignant transformation
TELOMERE DISEASES

Disease Risk Factors
- EtOH
- smoking

DKC Complex
- Liver
- Lung
- Skin/mucosa
- BM

genetic penetrance

TELOMERE ATTRITION AND CANCER

TERT as general cancer risk: AML, GI, lung, GU, others

Inflammatory/Immune Diseases

AA: MDS/AML
IBD: adenocarcinoma
Barrett’s: esophageal cancer

DKC:
cancer of the tongue, AML

Specificity

Penetrance

Skin/mucosa
Liver
BM
Lung
TELOMERES AND TELOMERE DISEASES FOR THE BOARDS

• Telomere triad: marrow, lung, liver
  – AA, MDS, AML; moderate anemia thrombocytopenia
  – Pulmonary fibrosis
  – Cirrhosis, steatosis, liver failure
• Variable penetrance: minimal to severe organ involvement
• Telomeres can be measured in commercial laboratories
• Marrow failure patients can respond to IST but at risk of MDS
• Implications of diagnosis
  – risk avoidance (alcohol, cigarette smoke)
  – genetic testing
  – monitoring for cancers
  – prognosis in SAA (clonal evolution)
  – therapy (androgens)
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)
TRIAD OF CLINICAL FEATURES

Hemoglobinuria

Intravascular Haemolysis

• Disabling Symptoms
  - Abdominal pain
  - Dysphagia
  - Erectile failure
  - Severe lethargy

Budd-Chiari Syndrome

Thrombosis
  • Liver, cerebral
  • 50% of patients
  • Fat in 33%

Aplastic Anemia

Bone Marrow Failure
  • Often precedes PNH
  • Selects for PNH clone
**PATHOGENESIS OF PNH**

- **PIG-A gene**:
  - Extracellular space
  - Cytoplasm
  - Transmembrane
  - GPI-Anchored
  - GPI-anchored protein

- **Glycosylphosphoinositol (GPI) anchor**

- **Glycan Core**
  - Phosphatidylinositol
  - Phospho-ethanolamine
  - Glucosylceramide

- **Pathogenesis**

*Note:* The image contains detailed molecular structures and chemical bonds associated with the pathogenesis of PNH.
1. *somatic* mutation of PIG-A in a hematopoietic stem cell

2. aborted GPI anchor synthesis

3. absent cell surface GPI-anchored proteins

4. *clonal* expansion of GP-AP deficient cells = PNH

- hemolysis (CD59-)
- BM failure (?)
- thrombosis (?)
- C’ susceptibility
- clonal expansion
- rbc contents
GPI-AP-DEFICIENT CLONES IN AA, MDS, AND PNH

- Controls: N=73
- AA: N=236 [42%]
- MDS: N=120 [18%]
- PNH: N=21 [100%]
ECULIZUMAB (ANTI-C5 ANTIBODY) = SOLIRIS

Humanized monoclonal antibody
Blocks late in C’ cascade (after C3a)
Indicated for symptomatic anemia
Increases susceptibility to Gram- cocci
Given as infusion q 2 wks
Meningococcal vaccine/prophylaxis

Abolishes *intravascular* hemolysis
Appears to reduce thrombosis risk

Extremely expensive!
EFFECT OF ECULIZUMAB ON HEMOLYSIS – LDH

LDH (IU/L)

Placebo

Eculizumab

P<0.001

Normal

Study Week

-4 -2 0 2 4 6 8 10 12 14 16 18 20 22 24 26

Screening
EXTRAVASCULAR HEMOLYSIS IN PNH UNCOVERED BY ECULIZUMAB TREATMENT

Corticosteroids
50 → 20 mg

Pregnancy
Eculizumab

Hemoglobin

Reticulocytes

LDH

*
ECULIZUMAB REDUCES THROMBOSES IN PNH

- Combined data from TRIUMPH, SHEPHERD, UK cohort, and phase III extension study compared to pre-treatment events
- Pre-rx: 126 thrombotic events in 195 patients (103 on anticoagulation)
- TE events: $\text{pre-rx} = 7.5$ vs $\text{post-rx} = 1.22/100$ patient years (84% reduction)

ECULIZUMAB (SOLIRIS) IN PRACTICE

Advantages
Dramatically reduces intravascular hemolysis and related symptoms (anemia and NO-induced)
May be effective in preventing thrombosis without bleeding risk
Well tolerated

Disadvantages
Expensive (!)
Risk of specific infections with Gram- cocci
Life-long infusions
Unmasked extravascular hemolysis in some cases
BMT FOR PNH

N = 211 patients
French multicenter retrospective

AA = aplastic anemia
TE = thromboembolism
RHE = recurrent hemolytic anemia

Peffault de la Tour R et al 2012; Haematologica 97:1666
PNH FOR THE BOARDS

Diagnostic triad: hemolysis, thrombosis, marrow failure
any one may predominate
*hemoglobinuria*, not hematuria
venous clots in abdomen, head—may be first manifestation!
flow cytometry for GPI-anchored proteins/clone size
>50% *clone* predicts major hemolysis and thrombosis

Treatment: eculizumab, BMT or IST, anticoagulation
no rx for small clones
large clones with hemolysis, transfusion need: Soliris
when marrow failure predominates, treat with HSCT or ATG
consider anticoagulation or eculizumab for large clones
PURE RED CELL APLASIA
PURE RED CELL APLASIA

CLINICAL CHARACTERISTICS

rare
females>males
clinical associations:

- thymoma
- CLL
- neoplasia, collagen vascular disease
- medical drugs (anti-epileptics)
- viruses

immune mechanisms
- antibodies to erythroid precursors
- autoimmune cytotoxic T cells
DIFFERENTIAL DIAGNOSIS OF PRCA

- Self-limited
  - transient erythroblastopenia of childhood
  - transient aplastic crisis of hemolytic disease (B19)
- Fetal
  - nonimmune hydrops fetalis (B19)
- Constitutional
  - Diamond-Blackfan anemia
- Acquired
  - persistent B19 parvovirus infection
  - immune-mediated (including thymoma-associated)
  - MDS (5q-)

TREATMENT OF ACQUIRED IMMUNE PRCA

- corticosteroids
- azathioprine/cyclophosphamide (?)
- cyclosporine
- antithymocyte globulin
- plasmapheresis
- anti-CD20; anti-IL2R
- thymectomy (for tumor excision)
B19 Parovirus
<table>
<thead>
<tr>
<th>Disease</th>
<th>Infection Duration/Frequency</th>
<th>Host Character</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fifth Disease</td>
<td>Acute/Common</td>
<td>Normal children</td>
</tr>
<tr>
<td>B19 arthralgia/arthritis</td>
<td>Acute/Common</td>
<td>Normal adults</td>
</tr>
<tr>
<td>Transient aplastic crisis</td>
<td>Acute/Common</td>
<td>Hemolysis</td>
</tr>
<tr>
<td>Acquired red cell aplasia</td>
<td>Chronic/Rare</td>
<td>Immunodeficiency</td>
</tr>
<tr>
<td>Hydrops fetalis</td>
<td>Chronic/Rare</td>
<td>Mid-trimester fetus</td>
</tr>
<tr>
<td>Congenital anemias</td>
<td>Chronic/Rare</td>
<td>Fetus</td>
</tr>
</tbody>
</table>

- **B19 in serum**
- **Globoside (P antigen) on erythrocyte membrane is cell receptor**
- **BM giant pronormoblasts**
Viremia with flu sx
Antibody reponse with 5th disease sx

Antibody clears virus

Reticulocytopenia normal (rbc life span determines anemia)

Anderson MJ et al, J Inf Dis 152:257, 1985
PERSISTENT B19 PARVOVIRUS INFECTION
CLINICAL RISK FACTORS AND TREATMENT

• congenital immunodeficiency
  – Nezelof’s syndrome
• acquired immunodeficiency
  – AIDS
• iatrogenic immunodeficiency
  – immunosuppressive therapies
  – cytotoxic drugs
Pathophysiology

- Common virus in the community
- DNA single stranded—very stable, very contagious
- Infects and induces apoptosis in human erythroid progenitors
- P antigen (globoside) is receptor
- Humoral immune response needed to clear infection

B19 causes different diseases

- TAC, hydrops, PRCA are hematologic
- Fifth disease in family members!

Serologic testing

- IgG prevalent in normal population = past infection
- IgM = recent infection
- Maybe *no* antibody response in persistent disease

B19 DNA

- Low levels for months post-infection
- High levels = persistent infection
- (DNA blots better than pcr because less sensitive!)

Rx

- Ig for persistent infection (PRCA)
CLASSIC PRESENTATION OF AGRANULOCYTOSIS

Angina, tonsillar abscess, pharyngeal necrosis

May be asymptomatic and detected on blood count monitoring or incidentally. Sepsis can be overwhelming. As with neutropenic fever post-chemorx, treat with parenteral, broad-spectrum antibiotics immediately.
BONE MARROW MORPHOLOGY IN AGRANULOCYTOSIS

absent myeloid precursors

“maturation arrest”
**DRUGS ASSOCIATED WITH AGRANULOCYTOSIS IN THE INTERNATIONAL APLASTIC ANEMIA AND AGRANULOCYTOSIS STUDY**

<table>
<thead>
<tr>
<th>Drug</th>
<th>RR</th>
<th>Excess Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>cinepazide</td>
<td>∞</td>
<td>- -</td>
</tr>
<tr>
<td>sulfasalazine</td>
<td>∞</td>
<td>- -</td>
</tr>
<tr>
<td>antithyroid drugs</td>
<td>97</td>
<td>5.3</td>
</tr>
<tr>
<td>macrolides</td>
<td>54</td>
<td>6.7</td>
</tr>
<tr>
<td>procaineamide</td>
<td>~50</td>
<td>3.1</td>
</tr>
<tr>
<td>aprindine</td>
<td>~49</td>
<td>2.7</td>
</tr>
<tr>
<td>dipyrone</td>
<td>16</td>
<td>0.6</td>
</tr>
<tr>
<td>cotrimoxazole</td>
<td>16</td>
<td>1.7</td>
</tr>
<tr>
<td>thenalidine</td>
<td>~16</td>
<td>2.4</td>
</tr>
<tr>
<td>carbamazepine</td>
<td>11</td>
<td>0.6</td>
</tr>
<tr>
<td>digitalis glycosides</td>
<td>2.5-9.9</td>
<td>0.1-0.3</td>
</tr>
<tr>
<td>indomethacin</td>
<td>6.6</td>
<td>0.4</td>
</tr>
<tr>
<td>troxerutin</td>
<td>6.0</td>
<td>0.3</td>
</tr>
<tr>
<td>sulfonylureas</td>
<td>4.5</td>
<td>0.2</td>
</tr>
<tr>
<td>corticosteroids</td>
<td>4.1</td>
<td>- -</td>
</tr>
<tr>
<td>butazones</td>
<td>3.9</td>
<td>0.2</td>
</tr>
<tr>
<td>dipyridamole</td>
<td>3.8</td>
<td>0.2</td>
</tr>
<tr>
<td>beta-lactams</td>
<td>2.8</td>
<td>0.2</td>
</tr>
<tr>
<td>propanolol</td>
<td>2.5</td>
<td>0.1</td>
</tr>
<tr>
<td>salicylates</td>
<td>2.0</td>
<td>0.06</td>
</tr>
</tbody>
</table>

RR = multivariate relative risk estimate

Excess risk expressed as number of cases per $10^6$ users in one week

Age association: elderly are medical drug users!

*
# IMMUNE VS TOXIC AGNANULO.iOSYSIS

<table>
<thead>
<tr>
<th></th>
<th>Immune</th>
<th>Toxic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>paradigm drug</strong></td>
<td>aminopyrine</td>
<td>phenothiazone</td>
</tr>
<tr>
<td><strong>time to onset</strong></td>
<td>days to weeks</td>
<td>weeks to months</td>
</tr>
<tr>
<td><strong>clinical character</strong></td>
<td>acute/explosive</td>
<td>asymptomatic/insidious</td>
</tr>
<tr>
<td><strong>rechallenge</strong></td>
<td>prompt recurrence with small test dose</td>
<td>latent period and high doses required</td>
</tr>
<tr>
<td><strong>laboratory</strong></td>
<td>leucoagglutinins, other antibody tests positive</td>
<td>direct or metabolite-mediated cytotoxicity</td>
</tr>
</tbody>
</table>
PROGNOSIS IN AGRANULOCYTOSIS

<table>
<thead>
<tr>
<th>Study, date</th>
<th>Agents</th>
<th>Treatment</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kastlin, 1926</td>
<td>mainly amidopyrine</td>
<td>observation</td>
<td>93%</td>
</tr>
<tr>
<td>Jackson, 1939</td>
<td>diverse</td>
<td>miscellaneous</td>
<td>73%</td>
</tr>
<tr>
<td>Huguley, 1964</td>
<td>dipyrrone</td>
<td>penicilllin</td>
<td>37%</td>
</tr>
<tr>
<td>Pretty, 1964</td>
<td>dipyrrone</td>
<td>penicilllin</td>
<td>40%</td>
</tr>
<tr>
<td>European studies:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden, 1966-70</td>
<td>dipyrrone, NSAIDs,</td>
<td>antibiotics</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>thyrostatics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland, 1960-68</td>
<td>diverse</td>
<td></td>
<td>15%</td>
</tr>
<tr>
<td>Ulm, 1968-80</td>
<td>mainly dipyrrone</td>
<td></td>
<td>25%</td>
</tr>
<tr>
<td>Barcelona, 1970-89</td>
<td>unknown</td>
<td></td>
<td>17%</td>
</tr>
<tr>
<td>IAAAS, 1980-86</td>
<td>miscellaneous</td>
<td>antibiotics</td>
<td>9%</td>
</tr>
<tr>
<td>Otsuka, 1989-92</td>
<td>OPC-8212</td>
<td>monitoring, early</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>antibiotics+G-CSF</td>
<td></td>
</tr>
</tbody>
</table>

Markedly improved—but not 0.
AGRANULOCYTOSIS FOR THE BOARDS

Almost always associated with medical drug use (often polypharmacy)
Typically an elderly patient with co-morbidities
Self-limiting—but high fatality rate (10% at least)
Most important is early treatment of possible infection
parenteral broad spectrum antibiotics
No convincing studies support G-CSF—but used routinely!