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

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BONE MARROW FAILURE SYNDROMES

AML
MDS
hypocellular

AA
DKC
SDS
PNH
LGL
AA/PNH

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

APLASTIC ANEMIA = LOW BLOOD COUNTS

Hemoglobin – variable, around 7-8 g/dL (>13 g/dL)
  weakness, fatigue, shortness of breath, lack of energy
Neutrophils – under 500/uL (>1500/uL)
  infections, mouth ulcers
Platelets – under 10,000/uL (>150,000/uL)
  bleeding (gums, nose, skin)

CLINICAL MANIFESTATIONS OF BONE MARROW FAILURE

anemia, bleeding, infection

AGE AT DIAGNOSIS
Aplastic Anemia Admissions to NIH Clinical Center

*NATURAL HISTORY* OF APLASTIC ANEMIA

Severity Criteria (two of three):
  platelets <20k/uL
  reticulocytes <1% (60k/uL)
  ANC <500/uL
  Very severe: ANC <200/uL
Pathophysiology of Aplastic Anemia

Immune attack

Stem cells

Hematopoietic progenitors

Circulating blood cells

Causes of Aplastic Anemia

Most of the cases of Aplastic Anemia have no identifiable cause

There are inherited (genetic) causes of aplastic anemia/bone marrow failure

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

Immunosuppressive therapy

Bone marrow transplantation

Supportive care

Novel agents

SAA treatment algorithm

1. Diagnose SAA
2. Start immunosuppressive therapy
3. Consider HSCT
4. Supportive care
5. Consider other treatments
6. Long-term follow-up
Immunosuppressive therapy

- Anti-thymocyte globulin (ATG)
  - Horse
  - Rabbit
- Cyclosporine (CsA)
  - Campath
  - Others (MMF, sirolimus, tacrolimus, cyclophosphamide)

Immunosuppressive therapy

- First line of treatment (vs. transplant)
- Salvage for treatment-refractory patients
- Treatment for relapsed disease

Anti-thymocyte Globulin (ATG) Production

Immunization with human thymocytes

Anti-human thymocytes antibodies

Purification of sera

Cytotoxicity assay

RESPONSE OF SEVERE APLASTIC ANEMIA TO INTENSIVE IMMUNOSUPPRESSION

ATG AND CSA FOR SEVERE APLASTIC ANEMIA

OVERALL SURVIVAL

60% response rate
A Randomized Trial of H-ATG vs. R-ATG in SAA

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

Scheinberg et al. NEJM 2011

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>
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Scheinberg et al. NEJM 2011

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

Blood Count Recovery in Responders

For new diagnosed SAA recommended/preferred immunosuppression regimen is horse ATG/CSA
Immunosuppressive therapy for refractory SAA

- **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

- For refractory SAA salvage immunosuppression with either rabbit ATG or Campath rescues 1/3 of patients

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

  - Survival rate: 1/3 Response Rate
  - Percent survival:
    - 0 25 50 75 100
  - Time in days:
    - 0 250 500 750 1000

- **SECOND IMMUNOSUPPRESSION FOR REFRACTORY SAA**

  Treatment arm (N=54) | Overall response
  rabbit ATG (N=27) | 9 (35%)
  alemtuzumab (N=27) | 10 (37%)

- **Immunosuppressive therapy for relapsed SAA**

  - cyclosporine
  - rabbit ATG
  - alemtuzumab
**ATG AND CSA FOR SEVERE APLASTIC ANEMIA RELAPSE**

**RELAPSE AFTER ATG + CSA**

<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 CaA</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>(86%)</td>
<td>(91%)</td>
<td>(78%)</td>
<td>(65%)</td>
<td>(64%)</td>
<td>(64%)</td>
<td>(57%)</td>
<td></td>
</tr>
</tbody>
</table>

Retreatment with rabbit ATG + CaA 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>
</tr>
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<tr>
<td>Campath (N=25)</td>
<td>14 (56%)</td>
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</table>

Scheinberg et. al, Blood 2012

**For relapsed SAA treatment with either rabbit ATG or Campath rescues 2/3 of patients**

**Conclusion/Take home points**

SAA is a rare disease
SAA is not cancer
Treatments are effective but do not work in 100% of patients

**Conclusion/Take home points**

Immunosuppression works in 2/3 of patients and is less toxic than stem cell transplant but it is not curative (disease can relapse)
Better immunosuppression treatments might be coming soon
Conclusion/Take home points

If you relapse after treatment or if you do not respond to immunosuppression there are treatment options

Thank you!

New Directions in Aplastic Anemia: What's on the Horizon

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Pathophysiology of Aplastic Anemia

HEMATOPOIETIC GROWTH FACTORS AS THERAPY FOR SAA

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

THROMBOPOIETIN

- Principal endogenous regulator of platelet production.
- Binds to megakaryocyte promotes production of platelets and mature megakaryocytes.
- Stimulatory effects on multilineage progenitors in vitro and in animal models.
- Clinically maybe beneficial to treat patients with thrombocytopenia (post-chemo, ITP)
Clinical development of THROMBOPOIETIN

- 1994- cloned by 5 independent groups
- 1996- clinical trials with mammalian cell-line produced or e. coli-produced full length thrombopoietin
  - Clearly stimulated platelet production in laboratory animals, normal volunteers given single doses
  - Not particularly effective to speed platelet recovery post-chemotherapy
- 1999-2000 Serious adverse events reported in platelet donors
  - Normal platelet donors given repeated subcutaneous doses developed profound, long-lasting thrombocytopenia
  - Due to stimulation of cross-reacting autoantibodies against endogenous TPO (Li et al. BLOOD, 2001)
  - Ended clinical development of recombinant thrombopoietin

2nd generation TPO agonists

- Romiplostim (Nplate): IV/SC, chronic refractory ITP
- Eltrombopag (Promacta): oral, chronic refractory ITP

ELTROMBOPAG FOR REFRACTORY SEVERE APLASTIC ANEMIA

- SAA with plt < 30K/uL
- Refractory to ATG/CSA
- Hematologic response at 3 months
- Responders followed monthly, on drug

Hematologic Response Criteria

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

PATIENTS CHARACTERISTICS

Age: median, range
Male: number, (%) 44, (18-77) 14, (54%)
Transfusion Dependent Number, (%):
Platelets 26, (100%)
Red blood cells 23, (88%)
Baseline Parameters Median, range:
Platelets (K/uL) 9, (5-15)
Neutrophils (K/uL) 5.8, (0.07-2.8)
Hemoglobin (g/dL) 8.8, (6.0-13.8)
Months from last IST Median, range: 12, (6-55)

REFRACTORY SAA ELTROMBOPAG STUDY RESULTS

Median follow up 13 months
26 patients enrolled
13 responders (64%)
9 platelet responses
2 hemoglobin responses
2 additional 4 at 16wks
4 neutrophil responses
2 additional 3 at 16wks
1 non-responders
10 stable disease
2 died of progression
2 clonal evolution to MDS
1 died
1 HSCT

Censure date 11/1/2011
25 evaluable patients
1 patient ineligible, not treated

PLATELET RESPONSES
Hemoglobin increases after eltrombopag

Median hemoglobin increase 3.6 g/dL (range 1.5-8.2 g/dL)

Neutrophil increases after eltrombopag

Median ANC increase 590/dL (460-990/UL)

Bone marrow cellularity at one year

Response summary of expanded cohort

Median follow up 9 months (range 3-47 months)

44 patients enrolled
1 patient ineligible, not treated

43 evaluable patients

17 responders (40%)

• 11 platelet responses
• 4 erythroid responses
  + additional 7 at >16wks
• 8 neutrophil responses
  + additional 3 at >16wks

26 non-responders

• 2 responded >16 weeks
• 1 died of progression
• 3 deaths from sepsis
• 6 clonal evolution

Lineage characteristics of responses

12 Weeks - Primary Endpoint
Best Response at Follow-up

Platelets
Neutrophils
Hemoglobin

Insights into SAA pathophysiology from eltrombopag responsiveness

HSC Growth Factor (+)

-immune attack

GT (-)

↑ probability of failure

↑ probability of recovery

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

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-naïve SAA

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

ANDROGEN THERAPY FOR APLASTIC ANEMIA

May/90
Feb/92

16%
48%

38 y/o liver transplant
26 y/o AA

47 y/o macrocytosis
21 y/o macrocytosis

71 y/o thyroid disease
44 y/o androgen-responsive AA

died age 33 yr MDS/AML
26 y/o dairy farmer

4 y/o died age 65 yr "blood disease" with pallor

"SHANK'S DISEASE" IN A MENNONITE FAMILY

26 y/o dairy farmer
progressive pancytopenia
no response to CSA, hormones
HCT from sister
minimal GVHD, full recovery

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 3' end to prevent recognition as a DNA "break" by non-homologous end joining machinery
- TTAGGG loss with proliferation: "end replication problem"

TELOMERE LENGTH IN TERT MUTATION LEUCOCYTES

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 novel Val329Gly mutation in DKC1

Telomere disease spectrum

- Moderate to severe aplastic anemia (low platelets only)
- Liver cirrhosis
- Lung fibrosis/IPF
- Skin/nail changes
- Skin/oral cancers
- Leukemia
SEX HORMONES INCREASE TELOMERASE ACTIVITY IN CULTURED HUMAN LYMPHOCYTES

Androgens for telomeropathy

Telomere elongation and clinical response to androgen treatment in a patient with aplastic anemia and a heterozygous hTERT gene mutation

Androgens for telomeropathy

DANAZOL FOR TELOMEROPATHIES

11-H-0268: “Danazol for Genetic Bone Marrow and Lung Disorders”
Danazol, 800 mg/d x 2 yrs for patients with short telomeres +/- mutations
Phase I/II design, N=31
Primary biologic end point: telomere maintenance/elongation
Secondary clinical end points: toxicity; efficacy (blood counts/PFT and CT)
Protocol opened in August 2011; 27 patients enrolled to date
Modest drug toxicity (minimal LFTs, mild headaches, muscle cramps)

ClinicalTrials.gov identifier: NCT01441037

Conclusion/Take home points

Eltrombopag works as a stem cell booster
Addition of Eltrombopag to standard hATG+Csa will likely improve response rate and decrease long-term complications

10% of aplastic anemia might be inherited (genetic cause)

Important to diagnose:
- Treatment implications
- Family/genetic counseling
Thank you!

Severe Aplastic Anemia (SAA) Treatment-Naive

NCT01623167 "Eltrombopag with Standard Immunosuppression for Aplastic Anemia"

Indication: SAA
Exclusions: Prior Treatment (ATG, high-dose cyclophosphamide, alemtuzumab), Fanconi Anemia, liver cirrhosis

Inclusions:
• > 2 yrs
• Bone marrow cellularity < 30%
• At least two of the following:
  - ANC < 500/mcL
  - Platelets < 20,000/mcL
  - ANC < 10,000/mcL

Purpose: To improve hemato logical response by adding eltrombopag (oral growth factor) to standard immunosuppressive therapy
Type: Inpatient and Outpatient, Non-Randomized
Duration: 10-14 days inpatient for horse ATG, CSA+ eltrombopag for 6 months, with 3 month, 6 month and annual follow-up visits

Severe Aplastic Anemia (SAA) Refractory

NCT01636795 "A Pilot Study of a Thrombopoietin-receptor Agonist (TPO-R agonist), Eltrombopag, in Aplastic Anemia Patients with Immunosuppressive-therapy Refractory Thrombocytopenia"

Indication: SAA with refractory thrombocytopenia following at least one treatment course of horse or rabbit ATG/cyclophosphamide.
Exclusions: Treatment with horse or rabbit ATG or Campath within 6 months of study entry, Fanconi Anemia, liver cirrhosis, PNH clone size greater than 50%.

Inclusions:
• Diagnosis of aplastic anemia, with refractory thrombocytopenia following at least one treatment course of horse or rabbit ATG/cyclophosphamide.
• Platelet count ≤ 30,000/mcL
• Age ≥ 18 years old

Purpose: To improve platelet counts with eltrombopag (oral growth factor) in patients with thrombocytopenia after immunosuppressive therapy
Type: Outpatient, Non-Randomized
Duration: 3 months of therapy. Monthly visits to NIH.

Moderate Aplastic Anemia (MAA)

NCT01328587 "Eltrombopag for Moderate Aplastic Anemia"

Indication: MAA with thrombocytopenia or anemia
Exclusions: Fanconi Anemia, liver cirrhosis, PNH clone size greater than 50%.

Inclusions:
• Current diagnosis of moderate aplastic anemia and
  • Age ≥ 18 years old
  • One or more of platelet count less than or equal to 30,000/mcL, Hemoglobin less than or equal to 8.5 g/dL OR platelet and/or red cell transfusion dependent

Purpose: To improve platelet counts with eltrombopag (oral growth factor).
Type: Outpatient, Non-Randomized.
Duration: 4 months of therapy. Monthly visits to NIH.

T-cell Large Granular Lymphocytosis (T-LGL)

NCT00345345 “Alemtuzumab to Treat T-Large Granular Lymphocytosis”

Indication: T-LGL, Neutropenia, Anemia

Inclusions:
1. T-LGL clone detectable by peripheral blood flow cytometry and TCR gene rearrangements
2. At least one cytopenia:
   • ANC < 500/mcL
   • Platelets < 20,000/mcL or < 50,000/mcL with bleeding
   • Hemoglobin < 9 g/dL or anemia requiring ≥ 2 units of RBCs/month transfusions

Purpose: To characterize response (improvement of blood counts) to immunosuppression (Campath) in patients with T-LGL.
Type: Inpatient and Outpatient, Non-Randomized
Duration: 11 days inpatient, with 3 month, 6 month and annual follow up visits

Myelodysplastic syndrome (MDS)

NCT00961064 “A Pilot Study of a Thrombopoietin-Receptor Agonist, Eltrombopag, in Patients With Low to Int-2 Risk MDS"

• Inclusion criteria:
  • Patients 18 years of age or older who have platelet count of less than 30 that has not responded to conventional treatment

• Outpatient study:
  • Treatment with eltrombopag once per day for 16 weeks
  • Evaluations at NIH every 4 weeks
  • If no response, then follow up every 3 months
  • If no response then follow up at 1 and 6 months off drug
Myelodysplastic syndrome (MDS)

Clofarabine followed by high dose lenalidomide for high risk MDS, CMMol and AML

NCT01629082

• Phase 1
  • Single course of IV clofarabine (2mg/m²/day) for cytoreduction
  • Oral lenalidomide consolidation with dose escalation from 25 mg daily for 21/28 days for 1 cycle in the first cohort up to 50 mg daily for 28/28 days for 2 cycles in the fourth cohort.
  • Oral lenalidomide maintenance, starting at a dose of 10 mg daily in 28 day cycles, with dose adjustments, for up to 12 cycles.

• Inclusion criteria:
  – MDS: Int-2 IPSS and higher
  – CMMol
  – AML
  – ECOG 0 to 2
  – Unresponsive to or unable to receive DNA hypomethylating agents or standard intensive induction chemotherapy (i.e. “7+3” cytarabine/anthracycline chemotherapy)

• Outpatient study:
  – Needs to stay locally for monthly revlimid prescription refills.