New Directions in Aplastic Anemia Treatment: What’s on the Horizon?

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National Institutes of Health

Today’s agenda

- Novel agents and active research
- Genetics of aplastic anemia
- Novel transplants for aplastic anemia

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
HEMATOPOIETIC GROWTH FACTORS AS THERAPY FOR SAA

Ganser A et al, Blood 1996; 76:1287: IL-3 pilot
Kojima S et al, Blood 2002;100:786: G-CSF vs monosomy 7
Tichelli A et al, Blood 2011; 117:4434: G-CSF shows no survival benefit

• 2nd generation small molecule thrombopoietin (TPO) agonist
• Orally administered non-peptide
• FDA accelerated approval in 2008 for treatment of chronic ITP

ELTROMBOPAG

• 44% (11/25) response rate
• Trilineage responses observed
• Transfusion independence
• Well-tolerated
CIRCULATING THROMBOPOIETIN LEVELS IN SAA

![Graph showing TPO levels in SAA and ITP](image)

Emmons R et al, Blood 1996; 87:4068
Fang X et al, Haematologica 2011; 96:802

ELTROMBOPAG FOR REFRACTIVE APLASTIC ANEMIA

- SAA with platelets < 30K/μL
- Refractory to IST

**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

**Eltrombopag**
- 50 mg daily
- Dose escalation every 2 weeks to 150 mg daily
- Hematologic response at 3-4 months

**Responders followed monthly, on drug (extension study)**


**Initial cohort n=25**
**Expanded cohort n=43**

**RESPONSE SUMMARY OF EXPANDED COHORT**

- Median follow up 9 months (range 3-47 months)
- 44 patients enrolled
- 43 evaluable patients
- 1 patient ineligible not treated
- 26 non-responders
  - 2 responded >16 weeks:
    - 1 died of progression
    - 3 deaths from sepsis
  - 6 clonal evolution

- 17 responders (40%)
  - 11 platelet responses
  - 4 erythroid responses
    - additional 7 at >16 weeks
  - 8 neutrophil responses
    - additional 3 at >16 weeks
LINEAGE CHARACTERISTICS OF RESPONSES

12 Weeks-Primary Endpoint  
Best Response at Follow-Up

- Platelets
- Neutrophils
- Hemoglobin

ROBUST RESPONDERS – CAN ELTROMBOPAG BE STOPPED?

- Platelets > 50,000/uL
- Hb > 10 g/dL
- Neutrophils > 1,000
- ≥ 8 weeks

Decrease dose by 50%

Counts remain > above limits for 8 weeks

Discontinue drug

4 patients tapered off drug after robust response attained

1 patient had drug stopped for cataract misdiagnosis

Median time off drug 13 months (range 1-15m)

No relapses or need to restart eltrombopag

CLONAL EVOLUTION IN REFRACTORY AA ON ELTROMBOPAG

<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>
</tr>
</thead>
<tbody>
<tr>
<td>7 (60)</td>
<td>NR</td>
<td>46XXy</td>
<td>-7[20]</td>
<td>3</td>
<td>N</td>
</tr>
<tr>
<td>8 (18)</td>
<td>NR</td>
<td>46XXy</td>
<td>+8[9]/46XX[11]</td>
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<td>N</td>
</tr>
<tr>
<td>13 (20)</td>
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<td>46XXy</td>
<td>-7[11]/46XX[12]</td>
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<td>N</td>
</tr>
<tr>
<td>26 (67)</td>
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<td>46XXy</td>
<td>+6[12]/46XX[13]</td>
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<td>Yes (mild)</td>
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<tr>
<td>31 (41)</td>
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<td>+21[3]/46XY[17]</td>
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<td>No</td>
</tr>
<tr>
<td>42 (66)</td>
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<td>46XXy</td>
<td>46XX[16]/46XY[12]</td>
<td>9</td>
<td>N</td>
</tr>
<tr>
<td>36 (23)</td>
<td>NR</td>
<td>46XXy</td>
<td>-7[8]/46XX[12]</td>
<td>3</td>
<td>N</td>
</tr>
<tr>
<td>42 (17)</td>
<td>NR</td>
<td>+1,der(1;7)</td>
<td>3</td>
<td>N</td>
<td>HSCT</td>
</tr>
</tbody>
</table>
DR(4) Includes those who lost their response as having a response, therefore 'best response'
Desmond, Ronan (NIH/NHLBI) [E], 5/23/2013

DR(3) Includes 2 NRs- Hoak who attained plt-T1 after coming off drug and Taylor-Fowler who had an erythroid response after coming off drug.
Desmond, Ronan (NIH/NHLBI) [E], 5/30/2013
### 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>
</tr>
</thead>
</table>


### ELTROMBOPAG FOR TREATMENT NAÏVE AA

- Eltrombopag stimulation may expand the HSC pool in humans
- Given early may allow for:
  - increased response rate
  - accelerated count recovery
  - prevent HSC depletion
  - avoid clonal progression

### ELTROMBOPAG ADDED TO IMMUNOSUPPRESSION

**12-H-0150, NCT01623167**

- **Eligibility**
  - SAA naïve to IST
  - Age >2
  - horse-ATG + CSA

- **Primary Endpoints**
  - quality of response
  - Toxicity

- **Phase III**
  - purpose is to determine whether eltrombopag improve the rate and quality of responses
RAPID HEMATOLOGIC RESPONSES IN PATIENTS AFTER IST WITH ELTROMBOPAG
N = 25

CD34+ ENUMERATION OF BONE MARROW ASPIRATES

ROBUST HEMATOLOGIC RESPONSES WITH ELTROMBOPAG COMPARED TO HISTORICAL IST
TRANSFUSION INDEPENDENCE IS ACHIEVED FASTER WITH ELTROMBOPAG

IST+ Eltrombopag (n=23)
IST(n=54)

ONGOING NHLBI ELTROMBOPAG TRIALS IN BONE MARROW FAILURE

• Newly diagnosed moderate and severe aplastic anemia
• Refractory aplastic anemia
• Moderate AA and unilineage bone marrow failure syndromes
• Low-int-1 risk MDS with any lineage cytopenias

Etiology of BM Failure

Acquired BM Failure
Inherited BM Failure
Inherited Bone Marrow Failure Syndromes

Usually associated with physical malformations from birth and bone marrow failure in childhood.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Phenotype</th>
<th>Pathway affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fanconi anemia</td>
<td>Pancytopenia</td>
<td>DNA repair</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>Pancytopenia</td>
<td>Monocytic leukemia</td>
</tr>
<tr>
<td>Shwachman-Diamond syndrome</td>
<td>Pancytopenia</td>
<td>Ribosomal dysgenesis</td>
</tr>
<tr>
<td>Severe combined immunodeficiency</td>
<td>Pancytopenia</td>
<td>Protein synthesis defect</td>
</tr>
<tr>
<td>Diamond-Blackfan anemia</td>
<td>Pancytopenia</td>
<td>Ribosomal dysfunction</td>
</tr>
<tr>
<td>Congenital amegakaryocytic thrombocytopenia</td>
<td>Pancytopenia</td>
<td>Growth factor receptor signaling</td>
</tr>
<tr>
<td>Diamond Blackfan anemia with predisposition to NHL</td>
<td>Pancytopenia</td>
<td>Translational regulation</td>
</tr>
<tr>
<td>Neutropenia with ataxia and cataracts</td>
<td>Pancytopenia</td>
<td>Translational regulation</td>
</tr>
</tbody>
</table>


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 REPAIR COMPLEX

TELOMERE REPAIR COMPLEX

Telomerase reverse transcriptase (TERT)

Template

3' 5'

Telomerase RNA (TERC)

Telomeres

3' 5'

3' 5'

TELOMERE REPAIR COMPLEX

Autosomal Dominant DKC

Mutations in TERC: RNA component of the telomerase complex, the template for telomere elongation

X-linked DKC

Mutations in DKC1: encodes dyskerin, a protein component of telomerase complex

Leukoplakia

Nail dystrophy

Leukoplakia

Courtesy by B. Alter, NCI

TELOMRES AND BONE MARROW FAILURE

DYKERATOSIS CONGENITA

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

Proband

Affected sister

Affected niece

Unaffected brother

Hematology

Normal peripheral blood counts

Mild anemia with macrocytosis

Mild thrombocytopenia

Severely hypoplastic

↓ CD34 number

↓ Colony formation

↑ Erythropoietin, thrombopoietin

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Hematology

Normal peripheral blood counts

Mild anemia with macrocytosis

Mild thrombocytopenia

Severely hypoplastic

↓ CD34 number

↓ Colony formation

↑ Erythropoietin, thrombopoietin

Proband

Affected sister

Affected niece

Unaffected brother
SHORT TELOMERS DUE TO TELOMERASE COMPLEX GENE MUTATIONS

- Normal
  - Self-renewal
  - Telomerase

- Germ-line telomere disease
  - Impaired genes
    - HGPS
    - SMC
    - POT1
    - POT7

- Telomere maintenance

SHORT TELOMERS OCCUR DUE TO ENVIRONMENTAL FACTORS – WITHOUT MUTATIONS

- Regenerative stress
  - Cell hyperproliferative activity
  - Telomere loss

- DNA damage
  - DNA replication or stress
  - Telomere loss

Suggestive personal/family history:
- AA, AML, MDS
- Thrombocytopenia, anemia
- Crytococcosis, MRH, portal hypertension
- Precocia
- Telomere disease: suggested
- Cryptic cirrhosis, NRH, portal hypertension
- Premature greying
- Mucocutaneous triad: leukoplakia, skin hypo/hyperpigmentation, nail dyskeratosis
- DEB or MMC chromosome breakage
- If BMT, genetic screen of family donors
- Flow-FISH or qPCR (PB)
- PB or BMC chromosome banding (FB)
- For strongly suggestive personal/family history...

Measure leukocyte telomere content
- > 1st percentile
- 1st-10th percentile
- < 1st percentile

Consider genetic screening for telomere maintenance genes (patient and family)
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)

RELAPSE RATE BY TELOMERE QUARTILES

Scheinberg et al. JAMA 2010
**Evolution Rate by Telomere Length**

Scheinberg et al. JAMA 2010

**Survival Probability by Telomere Length**

Scheinberg et al. JAMA 2010

**Sex Hormones Increase Telomerase Activity in Cultured Human Lymphocytes**

Calado RT et al, Blood 2009
DANAZOL FOR TELOMERE DISEASES

11-H-0209: “Danazol for Genetic Bone Marrow and Lung Disorders”
ClinicalTrials.gov identifier: NCT01441037

Eligibility:
1. evidence of a telomerase disease (mutation or very short telomeres), and
2. aplastic anemia and/or pulmonary fibrosis

26 patients enrolled
No significant toxicity, good hematologic responses thus far

GATA2 DEFICIENCY

Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia

GATA2 Mutations in Aplastic Anemia

Mutating GATA2 mutations identified in aplastic anemia
BONE MARROW FAILURE SYNDROMES

- AA/AA-PNH
- SDS
- PNH
- SSD
- SAA
- LGL
- MDS
- AML
- Hypocellular MDS
- AID: MS, IBD, uveitis, DM type 1, etc.

OPTIMIZING PBSC TRANSPLANTS FOR PATIENTS WITH ATG – REFRACTORY APLASTIC ANEMIA

• Keep the good parts of a PBSC allograft:
  - Higher CD34+ Stem Cell Numbers than a BM Transplant

• Modify graft to reduce C-GVHD risk by
  - Not using G-CSF cytokine polarized T-cells
  - Slow the speed of donor T-cell Engraftment
  - T-cell depleted G-CSF mobilized allograft combined with a Reduced dose (2 x 10^7/kg) of non-mobilized T-cells

Protocol 10-H-0154 For SAA

- Hypothesis: Transplanting an allograft with high doses of CD34+ selected cells combined with a BM equivalent dose of non-mobilized non-TH-2 polarized T-cells will reduce chronic GVHD by 50% while maintaining an engraftment rate of ≥ 90%.

Preparative Regimen

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cytoxan 60 mg/kg/d × 2 days</td>
</tr>
<tr>
<td>2</td>
<td>Fludarabine 25 mg/m²/d IV × 5 days</td>
</tr>
<tr>
<td>3</td>
<td>CD34+ selected G-CSF mobilized allograft + 2 x 10^7/kg non-mobilized T-cells</td>
</tr>
</tbody>
</table>

Post-Transplant

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>MTX</td>
</tr>
<tr>
<td>1</td>
<td>Cyclosporine</td>
</tr>
<tr>
<td>2</td>
<td>MTX</td>
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<tr>
<td>3</td>
<td>MTX</td>
</tr>
<tr>
<td>6</td>
<td>MTX</td>
</tr>
<tr>
<td>15</td>
<td>MTX</td>
</tr>
<tr>
<td>30</td>
<td>MTX</td>
</tr>
<tr>
<td>45</td>
<td>MTX</td>
</tr>
<tr>
<td>100</td>
<td>MTX</td>
</tr>
</tbody>
</table>

Hypothesis: Transplanting an allograft with high doses of CD34+ selected cells combined with a BM equivalent dose of non-mobilized non-TH-2 polarized T-cells will reduce chronic GVHD by 50% while maintaining an engraftment rate of ≥ 90%.

Protocol 10-H-0154 For SAA

- Etoposide 45 mg/m²/d × 3 days
- Fludarabine 25 mg/m²/d IV × 5 days
- CD34+ selected G-CSF mobilized allograft + 2 x 10^7/kg non-mobilized T-cells
- 20-fold lower T-cell dose than a PBSC allograft
- T-cells non-TH2 polarized
- Goal 8 x 10^6 CD34 cells/kg
Excellent Engraftment/Survival and Reduced cGVHD Using Partially T-Cell Depleted CD34 Selected PBSC Transplant

Demographics:
- N=13 patients transplanted
- All had failed prior ATG treatment
- 9/13 (69%) HLA Alloimmunized

Outcome:
- 12/13 engrafted with 11/13 patients surviving
- Significant reduction in risk of cGVHD compared to historical controls

R. Childs et al. unpublished data

Exploring Alternative Graft Sources for Patients Lacking an HLA Matched Related or Unrelated Donor

- Up to 40% of pts with SAA refractory to IST lack an HLA matched donor
- These patients may be candidates for an HLA mismatched allogeneic transplant using either a cord blood or haplo-identical stem cell transplant
- Umbilical Cord Blood (UCB) is an alternative graft source for patients with hematological malignancies that lack an HLA-matched donor who require a transplant
  - UCB transplantation has lower rates of graft versus host disease (GVHD) despite HLA mismatching
  - UCB is associated with delayed neutrophil and platelet engraftment and an increased risk of graft failure

Umbilical Cord Blood Transplantation (UCBT)

Umbilical Cord Blood (UCB) transplants are a transplant option for patients lacking an HLA identical donor:
1. Cord blood is a rich source of Hematopoietic progenitor cells more than human BM
2. Most cord transplants are mismatched for 1/6 or 2/6 HLA loci (HLA A, B, DR)
3. Less GVHD with MHC mismatching
About 40% of pts with SAA refractory to UIST lack an HLA matched donor

These pts may be candidates for a transplant from an HLA mismatched donor, using either cord blood stem cells or haplo-identical stem cells from a relative. Umbilical Cord Blood Transplantation is a useful alternative graft source in patients with hematologic disorders that lack an HLA-matched donor. The advantages of UCB are that these grafts cause lower rates of Graft versus host disease despite HLA mismatching. The primary disadvantage to the use of umbilical cord blood grafts in adults is that they contain about a log lower number of hematopoietic stem cells compared to a BM transplant, which results in delayed engraftment and increases the risk of graft failure.
UCB Transplantation for Aplastic Anemia: High Incidence of Graft Failure, Transplant Related Mortality, and Low Survival

Probability of Survival

Combined CD34+ Haploidentical and Cord Blood Transplantation for SAA

- Primary Investigator: Dr. Richard Childs (NHLBI)
- Hypothesis:
  - Co-transplantation of an UCB unit combined with CD34+ haplo-identical cells will
    - shorten time to neutrophil engraftment in patients with SAA
  - Transplanted haploidentical cells will provide a back up stem cell source if cord blood unit should fail to engraft

Conceptual Study Design

G-CSF 5ug/kg IV day +1 until engraftment (ANC>500 x 3 days)
Published data have shown that the outcome of UCB for SAA has thus far been extremely disappointing. Data from the NYBC has reported transplant related mortality rates of approximately 60%, largely as the consequence of a high incidence of graft failure in these pts who tend to be heavily transfused and HLA Ab allo-immunized.

The largest series reported to data last year from Europe recently reporting a 3 year probability of survival of only 38% in recipients of either single or dual cord transplant. Remarkably, pts receiving TNC numbers that are typical for a single cord unit in adults, <39 million TNCs/kg had abysmal survival of only 18% at 3 yrs.
Combined CD34+ Haploidentical and Cord Blood Transplantation for SAA

- Eligibility Criteria
  - Severe aplastic anemia between ages 4-55
  - ANC < 500 cells/ul
  - Failure to respond to standard immunosuppressive therapy
  - No available HLA matched donor (related or unrelated)
  - Availability of at least one ≥ 4/6 HLA-matched cord blood unit with TNC ≥ 1.5 x 10^7 cells/kg
  - Availability of at least one HLA-haploidentical family donor

NHLBI Protocol 08-H-0046: Combined Cord Blood and CD34+ Haploidentical Transplant for SAA

- Tacrolimus
- Fludarabine 25 mg/m² X 5
- Cyclophosphamide 60mg/kg X 2
- h-ATG (40mg/kg) x 4

OBJECTIVES

- Primary
  - Potential to achieve engraftment (ANC > 500) of cord unit and/or haplo donor in >80% patients by day 42

- Secondary
  - Achieve an ANC > 500 by day 10 in >80% of pts
  - Safety of novel transplant regimen
  - Day 100 and 200 TRM
  - Incidence and severity of acute and chronic graft-versus-host-disease (GVHD) following transplant
**Chimerism Patterns**

- Myeloid Chimerism
- T-Cell Chimerism

**Combined CD34+ Haploidentical and Cord Blood Transplantation for SAA**

- Co-infusion of allogeneic haplo-identical CD34+ cells with allogeneic UCB is a feasible transplant option for patients with SAA
  - Shortens the time to neutrophil recovery
  - Provides a backup stem cell source in the event of UCB graft failure
  - May improve the outcome of UCB transplantation in high-risk patients with SAA

- Primary Investigator: Dr. Richard Childs
- Research Nurse: Elena Cho, 301-594-8013

**HLA-haploidentical Bone Marrow Transplantation with Posttransplant Cyclophosphamide**


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Haploidentical BMT for Aplastic Anemia

HEMATOLOGY BRANCH, NHLBI

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