NON-TRANSPLANT THERAPY OF BONE MARROW FAILURE

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Special Volunteer, Hematology Branch, NHLBI, NIH

ICKSH 2018
Seoul, Korea
Friday, March 30, 2018
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No conflicts to disclose from AZ Medimmune
SEVERE APLASTIC ANEMIA (SAA)

- Mortality 80-90% at 1-2 years
- Most patients <35 y/o

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

Super-severe: ANC <200/uL

Camitta et al, Blood 1979; 53:504
IMMUNOSUPPRESSIVE THERAPY (IST) FOR SAA

- **Standard IST =**
  - Horse antithymocyte globulin (hATG)
  - Cyclosporine (CsA)

- **Overall response rates 60-70%, worldwide**

- **Response correlates with long term survival**

Rosenfeld S, JAMA. 2003; Valdez JM, Clin Infect Dis. 2010; Scheinberg P, NEJM 2011
ATTEMPTS TO IMPROVE OUTCOMES OF IST FOR SAA

- **Add to or replace ATG with megadose corticosteroids**
  
  *No increase in response; high toxicity (Marmontl, Prog Clin Biol Res 1984)*

- **Replace ATG with high dose cyclophosphamide**
  
  *Toxicity (Tisdale, Lancet 2001; Blood 2002)*

- **Replace ATG with moderate dose cyclophosphamide**
  
  *Excessive toxicity secondary to neutropenia (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 2012)*
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 Improved Hematopoiesis in Refractory Aplastic Anemia

Matthew J. Olnes, M.D., Ph.D., Phillip Scheinberg, M.D., Katherine R. Calvo, Yong Tang, M.D., Ph.D., Bogdan Dumitriu, M.D., Ankur R. Parikh, M.D., Angelique Biancotto, Ph.D., Xingmin Feng, M.D., Ph.D., Jay Lozier, M.D., Neal S. Young, M.D., and Cynthia E. Dunbar, M.D.

• 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

Platelets
Neutrophils
Hemoglobin
Initial Trial-EPAG for Refractory Severe Aplastic Anemia

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

43 patients refractory to immunosuppressive therapy (median 2.5 cycles)

- 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?

Olnes et al. NEJM 2012
Desmond et al. Blood, 2014
Extended Dosing with EPAG for Refractory SAA

40 patients refractory to IST (median 1.5 cycles)
Platelet count ≤ 30,000/µL, ANC <500/µL, 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

N at risk
all evolution 122 62 28 6 0
mono 7 122 64 30 3 1

Scheinberg & Young. Blood 2012
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)
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

Desmond R et al. Blood 2012;123:12
Thomas Winkler, ASH 2017
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 stimulation to expand HSC pool:
  - increase response rate?
  - accelerate count recovery?
  - prevent HSC depletion?
  - avoid clonal progression?

Eltrombopag

![Eltrombopag molecule]

**Graph:**
- **Stem cell number**
- **Upward arrow** indicates probability of failure
- **Upward arrow** indicates probability of recovery

**Legend:**
- **IST (-)**
- **HSC growth factors (+)**
- **Immune attack**
ELTROMBOPAG ADDED TO STANDARD IST Treatment Naïve SAA

92 patients

## PATIENT CHARACTERISTICS

### Table 1. Characteristics of the Patients at Baseline.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cohort 1 (N = 30)</th>
<th>Cohort 2 (N = 31)</th>
<th>Cohort 3 (N = 31)</th>
<th>All Patients (N = 92)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age — yr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>39</td>
<td>28</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>Range</td>
<td>12–72</td>
<td>3–68</td>
<td>11–82</td>
<td>3–82</td>
</tr>
<tr>
<td>Age distribution — no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18 yr</td>
<td>5 (17)</td>
<td>6 (19)</td>
<td>8 (26)</td>
<td>19 (21)</td>
</tr>
<tr>
<td>18–64 yr</td>
<td>20 (67)</td>
<td>23 (74)</td>
<td>18 (58)</td>
<td>61 (66)</td>
</tr>
<tr>
<td>≥65 yr</td>
<td>5 (17)</td>
<td>2 (6)</td>
<td>5 (16)</td>
<td>12 (13)</td>
</tr>
<tr>
<td>Sex — no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16 (53)</td>
<td>17 (55)</td>
<td>17 (55)</td>
<td>50 (54)</td>
</tr>
<tr>
<td>Female</td>
<td>14 (47)</td>
<td>14 (45)</td>
<td>14 (45)</td>
<td>42 (46)</td>
</tr>
<tr>
<td>GPI-deficient neutrophils†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range — %</td>
<td>&lt;1–99</td>
<td>&lt;1–99</td>
<td>&lt;1–79</td>
<td>&lt;1–99</td>
</tr>
<tr>
<td>Distribution — no./total no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1%</td>
<td>20/28 (71)</td>
<td>18/30 (60)</td>
<td>15/26 (58)</td>
<td>53/84 (63)</td>
</tr>
<tr>
<td>≥1%</td>
<td>8/28 (29)</td>
<td>12/30 (40)</td>
<td>11/26 (42)</td>
<td>31/84 (37)</td>
</tr>
<tr>
<td>Neutrophil count — per cubic millimeter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>275</td>
<td>330</td>
<td>300</td>
<td>310</td>
</tr>
<tr>
<td>Range</td>
<td>0–1380</td>
<td>0–900</td>
<td>0–1810</td>
<td>0–1810</td>
</tr>
</tbody>
</table>

*3 patients east or southeast Asian.

## 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>3 months</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>
</tr>
<tr>
<td>CR</td>
<td>5 (17)</td>
<td>8 (26)</td>
<td>15 (48)</td>
<td>28 (30)</td>
</tr>
<tr>
<td>6 months</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>80 (87)</td>
</tr>
<tr>
<td>CR</td>
<td>10 (33)</td>
<td>8 (26)</td>
<td>18 (58)</td>
<td>36 (39)</td>
</tr>
</tbody>
</table>

### Historic rates N=388*

- OR: 60%
- CR: 8%
Robust count recovery in responders: EPAG v. historic

Platelets

Neutrophils

Baseline 3 months 6 months

IST + EPAG

Historic IST

Neutrophils in very SAA
- ANC>500/uL: 48 days

Red cells
- Transfusion independence: 39 days

Platelets
- Transfusion independence: 32 days

BONE MARROW ANALYSIS

# ADVERSE EVENTS

Table 3. Adverse Events of Grade 3 or Higher or Serious Adverse Events Attributed to Eltrombopag.*

<table>
<thead>
<tr>
<th>Event</th>
<th>Patients (N = 92)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. (%)</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
</tr>
<tr>
<td>Maculopapular rash†</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Liver test abnormality</td>
<td>17 (18)</td>
</tr>
<tr>
<td>Increased alanine aminotransferase level</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Increased aspartate aminotransferase level</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Blood bilirubin increased</td>
<td>12 (13)</td>
</tr>
</tbody>
</table>
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

## CLONAL “CYTOGENETIC” EVOLUTION AFTER IST+EPAG

7/92 (8%) AT 2 YEAR MEDIAN F/U

<table>
<thead>
<tr>
<th>Age</th>
<th>Response</th>
<th>Time to evolution</th>
<th>Cytogenetics</th>
<th>BM dysplasia</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>68yr</td>
<td>CR</td>
<td>3 months</td>
<td>46, XX, del(13)(q12q22)[cp3]/46,XX[17]</td>
<td>No</td>
<td>Cytogenetics normalized</td>
</tr>
<tr>
<td>39yr</td>
<td>CR</td>
<td>30 months</td>
<td>48, XX +6 +15 [2]/46,XX[18]</td>
<td>No</td>
<td>Stable</td>
</tr>
<tr>
<td>64yr</td>
<td>PR</td>
<td>3 months</td>
<td>45,XX,t(3;3)(q21;q26),-7[3]/46,XX[17]</td>
<td>Yes</td>
<td>AML/HSCT, death</td>
</tr>
<tr>
<td>72yr</td>
<td>PR/Relapse</td>
<td>30 months</td>
<td>45, XY, -7[20]</td>
<td>Yes</td>
<td>Stable</td>
</tr>
<tr>
<td>48yr</td>
<td>CR/Relapse</td>
<td>6m</td>
<td>46,XX,del(7)(p13p15)[3]/46,XX[19]</td>
<td>No</td>
<td>HSCT</td>
</tr>
<tr>
<td>61yr</td>
<td>PR</td>
<td>6m</td>
<td>45, XX,-7[7]/46,XX[16]</td>
<td>Yes</td>
<td>Proceeding to HSCT</td>
</tr>
<tr>
<td>16yr</td>
<td>NR</td>
<td>3m</td>
<td>45, XY,-7[6]/46,XY[14]</td>
<td>No</td>
<td>HSCT</td>
</tr>
</tbody>
</table>

Cumulative Incidence of Clonal Evolution by Competing Risk Analysis

**Eltrombopag, All Cohorts**

<table>
<thead>
<tr>
<th></th>
<th>1 yr</th>
<th>2 yr</th>
<th>3 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death, %</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>BMT, %</td>
<td>5.8</td>
<td>11.7</td>
<td>11.7</td>
</tr>
<tr>
<td>Evolution, %</td>
<td>5.4</td>
<td>7.7</td>
<td>11.9</td>
</tr>
</tbody>
</table>

**Historic Cohort**

<table>
<thead>
<tr>
<th></th>
<th>1 yr</th>
<th>2 yr</th>
<th>3 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death, %</td>
<td>2</td>
<td>3.9</td>
<td>6</td>
</tr>
<tr>
<td>BMT, %</td>
<td>7.8</td>
<td>10.9</td>
<td>11.8</td>
</tr>
<tr>
<td>Evolution, %</td>
<td>5.9</td>
<td>7.9</td>
<td>12.9</td>
</tr>
</tbody>
</table>

Townsley DM, et al. NEJM 2017; 376
Somatic Mutations and Clonal Hematopoiesis in Aplastic Anemia


Mutation Status in AA (n=256)

One-third with at least 1 mutation

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

SIMILAR PROPORTION OF PATIENTS WITH MUTATIONS AFTER IST+ELTROMBOPAG

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

IST + EPAG

Yoshizato, et al. NEJM

35% with mutations

Townsley DM, ASH 2016
### FREQUENCY OF MUTATIONS IN AA AND AFTER ELTROMBOPAG

#### Frequency of four commonly mutated genes in AA from two studies (without EPAG)\(^2,3\)

<table>
<thead>
<tr>
<th>Top four genes</th>
<th>Kulasekararaj <em>et al.</em></th>
<th>Yoshizato <em>et al.</em></th>
<th>+ Eltrombopag</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT3A</td>
<td>8.3%</td>
<td>8.4%</td>
<td>4%</td>
</tr>
<tr>
<td>ASXL1</td>
<td>8%</td>
<td>6.2%</td>
<td>8%</td>
</tr>
<tr>
<td>BCOR/BCORL1</td>
<td>4%</td>
<td>9.3%</td>
<td>7%</td>
</tr>
<tr>
<td>PIGA</td>
<td>N/A</td>
<td>7.5%</td>
<td>N/A</td>
</tr>
<tr>
<td>Median VAF</td>
<td>20%</td>
<td>9.3%</td>
<td>13%</td>
</tr>
</tbody>
</table>

Townsley DM, ASH 2016

Kulasekararaj AG *et al.* *Blood* 2014;124:2698–2704
**MUTATION STATUS AT TIME OF HEMATOLOGIC RESPONSE**

Eltrombopag added to IST

<table>
<thead>
<tr>
<th></th>
<th>Mutation Positive</th>
<th>Mutation Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>OR</td>
<td>20 (100)</td>
<td>60 (86)</td>
</tr>
<tr>
<td>CR</td>
<td>13 (65)</td>
<td>23 (33)</td>
</tr>
<tr>
<td>Evolution</td>
<td>3 (15)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Relapse</td>
<td>5 (25)</td>
<td>20 (29)</td>
</tr>
<tr>
<td>Deaths</td>
<td>-</td>
<td>3 (4)</td>
</tr>
</tbody>
</table>

CR proportion:
- **BCOR** 5/6
- **ASXL1** 5/7
- **DNMT3A** 4/4

*Townsley DM, ASH 2016*

*2 additional subjects were off study prior to response assessment; mutations were undetected at baseline*
AGE AND MUTATION STATUS

No mutations detected in pediatric

**Ages < 50 years**
19% (12/64) mutation positive → BCOR in half (6/12)

**Ages ≥ 50 years**
31% (8/26) mutation positive → ASXL1 in two-thirds (5/8)

Townsley DM, ASH 2016
DOES EPAG ALTER CLONAL DYNAMICS IN REFRACTORY AA?
(Winkler T, ASH 2017)

Similar gene profile detected in 19/33 (58%) patients at baseline, 16 post treatment.
No significant change in the number or size of somatic variants after EPAG.
No clear association with cytogenetic clonal evolution.

WES on BM (n=33) at baseline, time of evolution or response assessment:
- 12 patients with cytogenetic evolution on EPAG.
- 21 EPAG responders without evolution.
Telomere attrition and candidate gene mutations preceding monosomy 7 in aplastic anemia

Bogdan Dumitriu,1 Xingmin Feng,1 Danielle M. Townsley,1 Yasutaka Ueda,1 Tetsuichi Yoshizato,2 Rodrigo T. Calado,3 Yaqin Yang,4 Yoshiyuki Wakabayashi,4 Sachiko Kajigaya,1 Seishi Ogawa,2 Jun Zhu,4 and Neal S. Young1

1Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD; 2Department of Pathology and Tumor Biology, Kyoto University, Kyoto, Japan; 3Department of Internal Medicine, University of São Paulo at Ribeirão Preto Medical School, Ribeirão Preto, SP, Brazil; and 4DNA Sequencing and Computational Biology Core, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

2015 125: 706-709
doi:10.1182/blood-2014-10-607572 originally published online November 18, 2014

ARTICLE

Received 11 Apr 2016 | Accepted 5 Jul 2016 | Published 22 Aug 2016

Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults

Andrew L. Young1,2, Grant A. Challen3, Brenda M. Birmann4 & Todd E. Druley1,2
<table>
<thead>
<tr>
<th>Age</th>
<th>Response</th>
<th>Time to evolution</th>
<th>Cytogenetics</th>
<th>MDS/AML somatic gene mutations (VAF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>68yr</td>
<td>CR</td>
<td>3 months</td>
<td>46, XX, del(13)(q12q22)[cp3]/46,XX[17]</td>
<td>none detected</td>
</tr>
<tr>
<td>39yr</td>
<td>CR</td>
<td>30 months</td>
<td>48, XX +6 +15 [2]/ 46,XX[18]</td>
<td>DNMT3A (3%)</td>
</tr>
<tr>
<td>64yr</td>
<td>PR</td>
<td>3 months</td>
<td>45,XX,t(3;3)(q21;q26),-7[3]/ 46,XX[17]</td>
<td>none detected</td>
</tr>
<tr>
<td>72yr</td>
<td>PR/Relapse</td>
<td>30 months</td>
<td>45, XY, -7[20]</td>
<td>ASXL1 (24%) RUNX1 (12%)</td>
</tr>
<tr>
<td>48yr</td>
<td>CR/Relapse</td>
<td>6m</td>
<td>46,XX,del (7)(p13p15)[3]/46,XX[19]</td>
<td>DNMT3A (15%)</td>
</tr>
<tr>
<td>61yr</td>
<td>PR</td>
<td>6m</td>
<td>45, XX,-7[7]/46,XX[16]</td>
<td>none detected</td>
</tr>
<tr>
<td>16yr</td>
<td>NR</td>
<td>3m</td>
<td>45, XY,-7[6]/46,XY[14]</td>
<td>none detected</td>
</tr>
</tbody>
</table>

CONSEQUENCES OF TELOMERE EROSION

- p53
- Senescence/Apoptosis (Hayflick phenomenon)
- Chromosome Instability
- Aneuploidy
- End-to-end fusion
- Non-reciprocal translocation

Apoptosis (Hayflick phenomenon)
Telomere length of leucocytes at diagnosis of SAA predicts clonal evolution.

TELOMERES AND CLONAL EVOLUTION

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

Accelerated telomere loss precedes clonal evolution to 7-

Danazol Treatment for Telomere Diseases

Danielle M. Townsley, M.D., Bogdan Dumitriu, M.D., Delong Liu, Ph.D., Angélique Biancotto, Ph.D., Barbara Weinstein, R.N., Christina Chen, B.S., Nathan Hardy, B.S., Andrew D. Mihalek, M.D., Shilpa Lingala, M.D., Yun Ju Kim, M.D., Jianhua Yao, Ph.D., Elizabeth Jones, M.D., Bernadette R. Gochuico, M.D., Theo Heller, M.D., Colin O. Wu, Ph.D., Rodrigo T. Calado, M.D., Ph.D., Phillip Scheinberg, M.D., and Neal S. Young, M.D.

- 27 patients with telomere diseases, short telomeres ± mutations were enrolled, study closed early for efficacy (telomere elongation)
**THE HIGH TPO PARADOX**

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

---

Emmons R et al., Blood 87:4068 (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

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National Heart, Lung, and Blood Institute (NHLBI)
National Institutes of Health (NIH)
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TPO AND EPAG BIND TO c-MPL AT DISTINCT SITES

Thrombopoietin (TPO) and Eltrombopag are shown binding to c-MPL at distinct sites. Thrombopoietin is a cytokine that binds to the extracellular domain of c-MPL, leading to signaling pathways that involve hematopoietic stem/progenitor cells (HSPC). Eltrombopag, a small molecule, binds to the transmembrane domain of c-MPL, also activating signaling pathways and leading to survival, proliferation, and hematopoiesis.
INFLAMMATORY CYTOKINES ARE ELEVATED IN APLASTIC ANEMIA

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

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Maintenance of CD34+ HSPCs

TPO or eltrombopag

7-day ex vivo culture

+/− Interferon-γ

HSPC survival and function

Normal HSPC (CD34+)

CD34+ cell count

Without IFNγ

With IFNγ

n=26

***

CD34+ cell count

TPO

Eltrombopag

Without IFNγ

With IFNγ

89.2

89.9

28.8

49.9

SSC-A

CD34

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

Colony forming cell (CFC) assay

- **Without IFNγ**
- **With IFNγ**

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<thead>
<tr>
<th>Condition</th>
<th>TPO</th>
<th>Eltrombopag</th>
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<tbody>
<tr>
<td>Without IFNγ</td>
<td>250 ± 10</td>
<td>220 ± 5</td>
</tr>
<tr>
<td>With IFNγ</td>
<td>100 ± 5</td>
<td>150 ± 10</td>
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* *** p < 0.001, n.s. p > 0.05

Transplantation into NSG mice

- **Without IFNγ**
- **With IFNγ**

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<tr>
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<th>TPO</th>
<th>Eltrombopag</th>
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</thead>
<tbody>
<tr>
<td>Without IFNγ</td>
<td>10 ± 2</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>With IFNγ</td>
<td>15 ± 3</td>
<td>20 ± 5</td>
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* *** p < 0.001, n.s. p > 0.05

CD45+ = human cell engraftment (HSCs)
Model of IFNγ-Mediated Bone Marrow Failure Signaling Inhibition by TPO:IFNγ Heteromers in Human HSPCs

Thrombopoietin (TPO)

- TPO
- c-MPL
- Heteromers

HSPC survival/proliferation

IFNγ occludes TPO:c-MPL low-affinity site

Eltrombopag

- c-MPL
- Eltrombopag

HSPC survival/proliferation

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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 refractory AA, clonal evolution occurs early but may be transient, necessitating close monitoring

• 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)
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