Targeting the TGF-β superfamily in Myelodysplastic Syndromes (MDS)

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Bronx, NY
Reduced Survival Is an Inherent Feature of MDS: Even for low risk subgroups

Life expectancy is shorter for US patients with MDS than those with lung cancer*†[1,2]

Need for therapeutic approaches that increase blood counts

*Adjusted for age (lung cancer, median 66 yrs; MDS, median 69 yrs) and risk/stage.[1,2]
†All histological subtypes.[1,2]

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Ineffective Hematopoiesis is seen in MDS

Hypercellular marrows with decreased peripheral blood counts

↑ Increased Cell death (Apoptosis/Cell cycle arrest)
Decreased Proliferation / Differentiation

↑ Pro Apoptotic / Myelosuppresive/
Inflammatory cytokine signaling

**Tumor necrosis factor α**
- ↑ TNFα mRNA in MDS BMs
- ↑ TNF production by MDS Macrophages

Pro-apoptotic Type 1 receptors increased in low grade MDS and decreased in high grade cases and AML

Anti-TNF therapies (Remicaid & Enbrel) show efficacy

Thalidomide can degrade TNF mRNA

**Transforming growth factor β**
- ↑ membrane bound TGF β on progenitors
- ↑ conc. in serum

**Vascular Endothelial growth factor (VEGF)**
Secreted by malignant clone and ALIP cells
Expression in BM correlates with disease severity
Higher expression of high affinity VEGFR1

**Other TGF-β family members**
GDF11, GDF15

**Interferon γ (IFN γ)**
Interleukin 1β
Fibroblast Growth factor (FGF)
Hepatocyte growth factor (HGF)
Macrophage Inhibitory Protein (MIPα)
TGF-beta family members regulate hematopoiesis

Activin receptor ligands, GDFs

TGF-β

Transphosphorylated TβR complex

ALK4

ALK5

SMAD2

SMAD3

SMAD4

Phosphorylated SMAD2/3 complex

TIF γ

Stem cell proliferation and quiescence

Altered erythroid differentiation

SMAD6/7

ALK, activin-like kinase receptor; GDF, growth differentiation factor; TGF-β, transforming growth factor β.
Activated SMAD2/3 seen in MDS BM samples

BM, bone marrow; IHC, immunohistochemistry.

Inhibition of SMAD2 activation can stimulate MDS hematopoiesis in vitro

**Table: Colonies**

<table>
<thead>
<tr>
<th></th>
<th>Scr shRNA</th>
<th>anti-TBRI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDS 1</strong> BFU-E</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td><strong>MDS 1</strong> CFU-GM</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td><strong>MDS 2</strong> BFU-E</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td><strong>MDS 2</strong> CFU-GM</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td><strong>MDS 3</strong> BFU-E</td>
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</tr>
<tr>
<td><strong>MDS 3</strong> CFU-GM</td>
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<td>10</td>
</tr>
<tr>
<td><strong>MDS 4</strong> BFU-E</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td><strong>MDS 4</strong> CFU-GM</td>
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<td>15</td>
</tr>
<tr>
<td><strong>MDS 5</strong> BFU-E</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td><strong>MDS 5</strong> CFU-GM</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td>25</td>
<td>50</td>
</tr>
</tbody>
</table>

*P* = 0.007

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Alb/TGF+ transgenic mice develop anemia and mimic human bone marrow failure

- Megakaryocytes
- Erythroid progenitors

**Figure A**

- Alb/TGF+ 8wks old
- H&E Staining
- WT 8wks old

- Reticulin staining

- Fibrosis
Small molecule inhibitor of ALK5 (TGF-b receptor I), SD-208, can improve hematopoiesis in TGF overexpressing mice and can raise their hematocrit.

Why is SMAD2/3 signaling activated in MDS?

Negative regulator Smad7 is reduced

- **Controls** (N = 17)
- RA (N = 55)
- RARS (N = 48)
- RAEB (N = 80)
- 5q− (N = 16)

Log 2 (SMAD7 gene expression)

- Controls
- RA
- RARS
- RAEB
- 5q−

Log 2 (SMAD2 gene expression)

- Controls
- RA
- RARS
- RAEB
- 5q−

Lowest expression

SMAD2

SMAD7

RA, refractory anemia; RAEB; RA with excess blasts; RARS, RA with ringed sideroblasts.

SMAD7 is a negative regulator of ALK4 and ALK5

ALK, activin-like kinase receptor; GDF, growth differentiation factor; TGF-β, transforming growth factor β.

Inhibition of proliferation

Altered erythroid differentiation

**SMAD7**

**ALK4**

**ALK5**

**SMAD2**

**SMAD3**

**SMAD4**

**SMAD6/7**

**TGF-β**

**Transphosphorylated TβR complex (ALK5)**

**Activin receptor ligands, GDFs**

**Phosphorylated SMAD2/3 complex**

**Inhibition of proliferation**

**Altered erythroid differentiation**

ALK, activin-like kinase receptor; GDF, growth differentiation factor; TGF-β, transforming growth factor β.
**SMAD7 is reduced in MDS**

- **SMAD7 IHC**
- **Controls** vs. **MDS**
- Staining for SMAD7 (%)
  - **Strong staining**
  - **Weak or no staining**

Reduced SMAD7 leads to increased sensitivity to TGF-β

....That can be reversed by inhibition of TGF-b receptor kinase
Why is SMAD7 decreased in MDS?

miR-21 is increased in MDS and has a putative binding site on the SMAD7 3' UTR

**Gene**

Human SMAD7 SNM_005904 3’ UTR Length: 1518

**Conserved sites for miRNA families conserved in human, mouse, rat, dog, and chicken**

- miR-15/16/195/424/497
- miR-21
- miR-216
- miR-25/32/92/363/367
- miR-17-5p/20/93.mr/10
- miR-181

**Key**

- Sites conserved in human, mouse, rat, dog, and chicken
- Less conserved sites

**Human SMAD7 3’ UTR**

Mean intensity

<table>
<thead>
<tr>
<th>miR-21 (Log 2)</th>
<th>Controls</th>
<th>MDS</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td></td>
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</table>

Normalized miR-21 expression

<table>
<thead>
<tr>
<th>miR-21 expression</th>
<th>Control</th>
<th>All MDS</th>
<th>LR MDS</th>
<th>HR MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
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<td>4</td>
<td>6</td>
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<td>12</td>
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<tr>
<td></td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

**HR MDS**, high-risk MDS; **LR MDS**, low-risk MDS; miR-21, microribonucleic acid 21; UTR, untranslated region.

Parallel Transcriptional Analysis reveals miR-21 overexpression in MDS/AML Stem and Progenitor cells

Barreyro et al. Blood 2012
miR-21 binds to SMAD7 3’UTR and leads to reduced levels of SMAD7

A

5’ ...UGUUUAGAAUUUAACAUAGCUA... Wild-type mouse Smad7 3’UTR

3’ AGUUGUAGUCAGACUAUUCGAU miR-21

5’ UGUUUAGAAUUUAACAUAGAU CGA mutat mouse Smad7 3’UTR

B

Fold Change (mir21/control)

WT Smad7 UTR Mut Smad7 UTR

**
Inhibition of miR-21 can abrogate the effects of TGF–β on hematopoietic cells

Bhagat et al, Blood, 2013
Treatment with mir21 inhibitor leads to increase in red blood cells in TGF transgenic mice:

Inhibition of miR-21 stimulates erythropoiesis in MDS.
LY-2157299 (Galunisertib) is an oral ALK5 inhibitor

LY-215 is effective in vitro and in vivo in MDS

A Study of LY2157299 in Participants With Low/Int-1 Myelodysplastic Syndromes; ClinicalTrials.gov Identifier: NCT02008318

TGF transgenic mice treated for 2 weeks

Phase 2 Study of Monotherapy Galunisertib (LY2157299 Monohydrate) in Very Low-, Low-, and Intermediate-risk Patients with Myelodysplastic Syndromes

David Valcarcel¹, Amit Verma², Uwe Platzbecker³, Valeria Santini⁴, Aristoteles Giagounidis⁵, Maria Diez-Campelo⁶, Jan Janssen⁷, Richard F Schlenk⁸, Gianluca Gaidano⁹, Jaime Perez de Oteyza¹⁰, Ann L Cleverly¹¹, Alan Y Chiang¹², Michael M Lahn¹², Durisala Desaiah¹², Susan C Guba¹², Alan List¹³, Rami Komrokji¹³

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57th American Society of Hematology Annual Meeting; December 5-8, 2015; Orlando, Fl., USA
### Hematological Improvement in Patients Treated with Galunisertib, 150 mg BID

<table>
<thead>
<tr>
<th>Baseline Transfusion Need</th>
<th>Number of Patients (N)</th>
<th>Median number of transfused units at baseline</th>
<th>Post Treatment (N)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TI During any 8 Weeks</td>
</tr>
<tr>
<td>≥ 4 Units</td>
<td>24</td>
<td>7.5</td>
<td>4</td>
</tr>
<tr>
<td>1-3 Units</td>
<td>4</td>
<td>2.5</td>
<td>2</td>
</tr>
<tr>
<td>0 Units</td>
<td>10</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td></td>
<td></td>
</tr>
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</table>

*14 patients could not decrease their transfusion requirement by 4 units/8 weeks per IWG 2006 criteria. If only the ≥4 units at baseline patients are included, then the response rate is 9/24 or 37.5%

TI, transfusion independence; HI, hematological improvement; TR, transfusion reduction; HGB, hemoglobin
Heatmap of Biomarkers vs. Response Demonstrated no Correlation with mutations
Stem Cell Differentiation Block (CD34+CD38-Lin-CD33-) is associated with response to TGF-β inhibitor.
Response to TGF-β inhibition associated with increased progenitor differentiation

Pre-treatment

Viable, single cells
Lin-CD33- cells
CD123-CD45Rα- cells
CD34+CD38+ cells

Increased %age of CD34+/CD38+ progenitors
Decreased %age of Aberrant (IL1RAP) HSCs

Cycle 6

Viable, single cells
Lin-CD33- cells
CD123-CD45Rα- cells
CD34+CD38+ cells

Increased %age of CD34+/CD38+ progenitors
Decreased %age of Aberrant (IL1RAP) HSCs
Sotatercept and Luspatercept (ACE-536) act as novel ligand traps for TGF-β superfamily ligands.

**Sotatercept** (ACE-011)
- Extracellular domain of ActRIIA
- Fc domain of human IgG₁ antibody

**Luspatercept**
- Modified extracellular domain of ActRIIB
- Fc domain of human IgG₁ antibody

Amino acid homology between ECD of sotatercept and ACE-536 is ~ 60%

The murine orthologues of these molecules are RAP-011 and RAP-536; extracellular domains are identical, but linked to murine IgG2a Fc domain.
ACE-536 correct anemia by promoting late stage erythropoiesis

Inhibits SMAD2/3 signaling

Studies using RAP-536, murine analogue of luspatercept

TBS, tris-buffered saline; M:E, myeloid:erythroid.

ACE-536 increases hemoglobin and reduces transfusion burden in patients with low or intermediate-1 risk myelodysplastic syndromes (MDS): Final results from a phase 2 study

Uwe Platzbecker, MD1, Ulrich Germing, MD*,2, Aristoteles Giagounidis, MD PhD3, Katharina Götze, MD*,4, Philipp Kiewe, MD*,5, Karin Tina Mayer, MD*,6, Oliver Ottmann, MD7, Markus Radsak, MD*,8, Thomas Wolff, MD9, Detlef Haase, MD*,10, Monty Hankin*,11, Dawn Wilson*,11, Xiaosha Zhang*,11, Abderrahmane Laadem, MD12, Matthew L. Sherman, MD11, and Kenneth M. Attie, MD

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Study supported by Acceleron and Celgene

HI-E response rate by ring-sideroblast morphology, SF3B1 mutation

Response rate at higher dose levels (0.75–1.75 mg/kg)

<table>
<thead>
<tr>
<th>Patient Subgroup</th>
<th>IWG HI-E Response Rate (0.75-1.75 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS positive¹</td>
<td>19/35 (54%)</td>
</tr>
<tr>
<td>EPO &lt; 200 U/L</td>
<td>14/23 (61%)</td>
</tr>
<tr>
<td>EPO ≥ 200 U/L</td>
<td>5/12 (42%)</td>
</tr>
<tr>
<td>RS negative¹</td>
<td>0/4 (0%)</td>
</tr>
<tr>
<td>SF mutation² present</td>
<td>18/30 (60%)</td>
</tr>
<tr>
<td>SF mutation² absent</td>
<td>1/9 (11%)</td>
</tr>
</tbody>
</table>

Data as of 03 October 2014.
An open-label, phase 2, dose-finding study of sotatercept (ACE-011) in patients with low or intermediate-1 (Int-1)-risk myelodysplastic syndromes (MDS) or non-proliferative chronic myelomonocytic leukemia (CMML) and anemia requiring transfusion

Rami Komrokji,1 Guillermo Garcia-Manero,2 Lionel Ades,3 Abderrahmane Laadem,4 Bond Vo,4 Thomas Prebet,5 Aspasia Stamatoullas,6 Thomas Boyd, MD,7 Jacques Delaunay,8 David P. Steensma,9 Mikkael A. Sekeres,10 Odile Beyne-Rauzy11, Jun Zou4, Kenneth Attie12, Matthew L. Sherman12, Pierre Fenaux13, and Alan F. List14

1Moffitt Cancer Center, Tampa, FL; 2University of Texas M.D. Anderson Cancer Center, Houston, TX; 3Hôpital St Louis, Paris, France; 4Celgene Corporation, Summit, NJ; 5Institut Paoli Calmettes, Marseille, France; 6Centre Henri Becquerel, Rouen, France; 7North Star Lodge Cancer Center, Yakima, WA; 8CHU de Nantes – Hôtel Dieu, Nantes, France; 9Dana Farber Cancer Institute, Boston, MA; 10Leukemia Program, Cleveland Clinic, Cleveland, OH; 11Centre Hospitalier Universitaire Purpan Pavillon de Médecines, Toulouse, France; 12Acceleron Pharma, Cambridge, MA; 13Service d'Hématologie Séniors, Hôpital St Louis, Université Paris 7, Paris, France; 14Malignant Hematology, Moffitt Cancer Center, Tampa, FL

## Results: efficacy in HTB patients

<table>
<thead>
<tr>
<th>Sotatercept dose cohort</th>
<th>Overall (N = 45)</th>
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<tbody>
<tr>
<td>0.1 mg/kg (n = 7)</td>
<td>0</td>
</tr>
<tr>
<td>0.3 mg/kg (n = 6)</td>
<td>4 (66.7)</td>
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<tr>
<td>0.5 mg/kg (n = 17)</td>
<td>7 (41.2)</td>
</tr>
<tr>
<td>1.0 mg/kg (n = 14)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td><strong>Total (N = 45)</strong></td>
<td><strong>19 (42)</strong></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Transfusion burden reduction ≥ 4 RBC units/8 weeks, n (%)</th>
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<tbody>
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<td>0.1 mg/kg (n = 7)</td>
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<tr>
<td>0.3 mg/kg (n = 6)</td>
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<tr>
<td><strong>Total (N = 45)</strong></td>
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<table>
<thead>
<tr>
<th>Duration of longest response, median (range), days</th>
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<td>0.1 mg/kg (n = 7)</td>
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<td>0.3 mg/kg (n = 6)</td>
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<td>0.5 mg/kg (n = 17)</td>
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<tr>
<td><strong>Total (N = 45)</strong></td>
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</table>

<table>
<thead>
<tr>
<th>RBC-TI ≥ 56 days, n (%)</th>
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<tr>
<td>0.3 mg/kg (n = 6)</td>
</tr>
<tr>
<td>0.5 mg/kg (n = 17)</td>
</tr>
<tr>
<td>1.0 mg/kg (n = 14)</td>
</tr>
<tr>
<td><strong>Total (N = 45)</strong></td>
</tr>
</tbody>
</table>
Conclusions: Pathogenesis of ineffective hematopoiesis

- TGF β / Activin Ligands
- Transphosphorylated TBR complex
- Lustanercept, Sotatercept
- LY-2157299
- LY-2157299

- Smad 7
- Smad2
- Smad3
- Ineffective hematopoiesis
- Low Blood Counts

miR-21
Conclusions

- SMAD2/3 pathway is overactivated in MDS HSPCs
- SMAD7 is a negative regulator of ALK4/5 and is decreased in MDS
- Luspatercept and Sotatercept show promising evidence of clinical activity in a cohort of lower-risk MDS patients who were anaemic and refractory to ESAs
- Lustanercept is being evaluated in a multicenter Phase III trial in RARS
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Andrea Pellagatti

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John Greally

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Amittha Wickrema

Luspatercept study
U. Platzbecker et al.

Sotatercept study
Rami Komrokji,
Guillermo Garcia-Manero,
Alan List et al.

Acceleron
Ravi Kumar

Eli Lilly
Susan Guba