Biomarker Analysis of the PULSAR Study: An Ongoing Phase 2, Double-Blind, Placebo-Controlled, Randomized Study to Compare the Efficacy and Safety of Sotatercept (ACE-011) Versus Placebo When Added to Standard of Care for the Treatment of Pulmonary Arterial Hypertension (PAH)

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Introduction

• Pulmonary arterial hypertension (PAH) is characterized by pulmonary vascular remodeling leading to increased pulmonary arterial pressure and right ventricular dysfunction.¹

• Mutations in the bone morphogenetic protein (BMP) receptor type II gene (BMPR2) are a major factor underlying familial PAH cases; however, the relevance of the BMPR-II pathway extends beyond familial PAH.¹

• Disruptions in vascular homeostasis due to dysregulated BMP signaling are associated with the development and progression of PAH.¹²

• By studying genetic variations and associated biomarker profiles, we can better assess the efficacy of sotatercept for the treatment of PAH in different genetic backgrounds.

• Here we describe 1) the effect of sotatercept on select PAH biomarkers and 2) whether the efficacy of sotatercept is affected by BMPR2 genetic variation.

BMP: bone morphogenetic protein; BMPR2: bone morphogenetic protein receptor type II; PAH: pulmonary arterial hypertension.
Sotatercept is an investigational product that is not approved for any use in any country.

ACTRIIA/B: activin receptor type II A/B; ALK: activin receptor-like kinase; BMP: bone morphogenetic protein; BMPR-II: bone morphogenetic protein receptor type 2; Fc, fragment crystallizable; GDF: growth differentiation factor; PAH: pulmonary arterial hypertension; pSmad: phosphorylated Smad.

**Sotatercept: Mechanism of action**

- Sotatercept is a novel, first-in-class investigational fusion protein comprising the extracellular domain of human activin receptor type IIA linked to the Fc domain of human immunoglobulin G1
- Sotatercept is proposed to act by rebalancing signaling between pro- and anti-proliferative pathways, thereby reversing the characteristic vascular remodeling seen in PAH
A Phase 2, randomized, double-blind, placebo-controlled study to compare the safety and efficacy of sotatercept versus placebo when added to standard of care (SOC) for the treatment of PAH in 106 patients at 43 sites across eight countries.

Inclusion criteria
- WHO Group 1 PAH
- WHO functional class II or III
- Baseline right-heart catheterization with PVR ≥5 Wood units
- Baseline 6-minute walk distance (6MWD) 150–550 m
- Stable treatment with SOC therapies including mono-, double, and triple therapies:
  - An endothelin receptor antagonist, a phosphodiesterase 5 inhibitor, a soluble guanylate cyclase stimulator, and/or a prostacyclin (including IV)

Primary treatment period (24 weeks)
- Placebo + SOC (n=32)
- Sotatercept 0.3 mg/kg + SOC (n=32)
- Sotatercept 0.7 mg/kg + SOC (n=42)

Extension period (ongoing)
- Sotatercept 0.3 mg/kg + SOC
- Sotatercept 0.7 mg/kg + SOC

End of placebo-controlled treatment period

Trial currently in open-label extension phase

6MWD: 6-minute walk distance; ACVRL1: activin A receptor type like 1; BMPR2: bone morphogenetic protein receptor type II; CAV1: caveolin 1; EIF2AK4: eukaryotic translation initiation factor 2 alpha kinase 4; ENG: endoglin; FC: functional class; GDF15: growth differentiation factor 15; KCNA5: potassium voltage-gated channel subfamily A member 5; KCNK3: potassium channel subfamily K member 3; NT-proBNP: N-terminal pro-brain natriuretic peptide; PAH: pulmonary arterial hypertension; PVR: pulmonary vascular resistance; SOC: standard of care; TAPSE: tricuspid annular plane systolic excursion; TGF-β1: transforming growth factor beta 1; VEGFR1: vascular endothelial growth factor receptor type 1; WHO: World Health Organization.

A Phase 2, randomized, double-blind, placebo-controlled study to compare the safety and efficacy of sotatercept versus placebo when added to standard of care (SOC) for the treatment of PAH in 106 patients at 43 sites across eight countries.

**Primary endpoint**
- Change in PVR from baseline to week 24

**Secondary endpoints**
- Change from baseline to week 24 in 6MWD, NT-proBNP, and TAPSE

**Exploratory endpoints**
- Change from baseline to week 24 in TGF-β ligands and other PAH biomarkers, and ECHO parameters

**Topline results**
Results presented at the American Thoracic Society 2020 Congress demonstrated improvements in:
- PVR (34% overall reduction)
- 6MWD
- NT-proBNP
- Pulmonary arterial pressure

Sotatercept was generally well tolerated and safety findings were consistent with other patient populations.

**Biomarker strategy**

**At baseline:**
- Sequence variation and/or mutation in ACVRL1, BMPR2, CAV1, EIF2AK4, ENG, KCNA5, KCNK3, SMAD9

**At baseline and every 3–4 visits:**
- NT-proBNP, activin A, GDF15, TGF-β1, VEGFR1
- BMPR2 mRNA expression

Trial currently in open-label extension phase

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6MWD: 6-minute walk distance; ACVRL1: activin A receptor type I; BMPR2: bone morphogenetic protein receptor type II; CAV1: caveolin 1; EIF2AK4: eukaryotic translation initiation factor 2 alpha kinase 4; ENG: endoglin; FC: functional class; GDF15: growth differentiation factor 15; KCNA5: potassium voltage-gated channel subfamily A member 5; KCNK3: potassium channel subfamily K member 3; NT-proBNP: N-terminal pro-brain natriuretic peptide; PAH: pulmonary arterial hypertension; PVR: pulmonary vascular resistance; SOC: standard of care; TAPSE: tricuspid annular plane systolic excursion; TGF-β1: transforming growth factor beta 1; VEGFR1: vascular endothelial growth factor receptor type 1; WHO: World Health Organization.

## PULSAR Study: Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo n=32</th>
<th>Sotatercept 0.3 mg/kg n=32</th>
<th>Sotatercept 0.7 mg/kg n=42</th>
<th>Total N=106</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>26 (81)</td>
<td>29 (91)</td>
<td>37 (88)</td>
<td>92 (87)</td>
</tr>
<tr>
<td>Age, median (range), years</td>
<td>46 (21–71)</td>
<td>49 (23–80)</td>
<td>49 (19–77)</td>
<td>48 (19–80)</td>
</tr>
<tr>
<td>Time since diagnosis, median (range), years</td>
<td>7 (0.3–22)</td>
<td>8 (0.7–26)</td>
<td>6 (0.8–24)</td>
<td>7 (0.3–26)</td>
</tr>
<tr>
<td>PAH classification, n (%)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>19 (59)</td>
<td>13 (41)</td>
<td>29 (69)</td>
<td>61 (58)</td>
</tr>
<tr>
<td>Heritable</td>
<td>7 (22)</td>
<td>5 (16)</td>
<td>5 (12)</td>
<td>17 (16)</td>
</tr>
<tr>
<td>Associated with connective-tissue disease</td>
<td>3 (9)</td>
<td>9 (28)</td>
<td>6 (14)</td>
<td>18 (17)</td>
</tr>
<tr>
<td>Drug or toxin-induced</td>
<td>1 (3)</td>
<td>4 (13)</td>
<td>2 (5)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Associated with corrected congenital shunts</td>
<td>2 (6)</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>WHO functional class, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>17 (53)</td>
<td>15 (47)</td>
<td>24 (57)</td>
<td>56 (53)</td>
</tr>
<tr>
<td>III</td>
<td>15 (47)</td>
<td>17 (53)</td>
<td>18 (43)</td>
<td>50 (47)</td>
</tr>
<tr>
<td>Standard-of-care PAH therapy, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parenteral prostacyclin</td>
<td>10 (31)</td>
<td>11 (34)</td>
<td>18 (43)</td>
<td>39 (37)</td>
</tr>
<tr>
<td>Monotherapy</td>
<td>3 (9)</td>
<td>3 (9)</td>
<td>4 (10)</td>
<td>10 (9)</td>
</tr>
<tr>
<td>Double therapy</td>
<td>12 (38)</td>
<td>11 (34)</td>
<td>14 (33)</td>
<td>37 (35)</td>
</tr>
<tr>
<td>Triple therapy</td>
<td>17 (53)</td>
<td>18 (56)</td>
<td>24 (57)</td>
<td>59 (56)</td>
</tr>
</tbody>
</table>

Primary analysis data cut-off date 14 January 2020.
Mean ± SE unless otherwise specified.

*Idiopathic and heritable PAH classifications were self-reported or physician-reported. Genetic testing was not required to be classified as heritable.

Summary of genetic variations

- Of 106 subjects enrolled, 94 had reportable genetic testing results, and 12 subjects had no reportable results.*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of patients with variations, n (%)</th>
<th>Type of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMPR2</td>
<td>25 (27)</td>
<td>2 with BMPR2/SMAD9 (single heterozygous variations in each gene)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23 with a single heterozygous variation in BMPR2</td>
</tr>
<tr>
<td>ACVRL1</td>
<td>2 (2)</td>
<td>Single heterozygous</td>
</tr>
<tr>
<td>EIF2AK4</td>
<td>2 (2)</td>
<td>1 with two unique heterozygous variations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 with a single homozygous (biallelic) variation</td>
</tr>
<tr>
<td>KCNA5</td>
<td>1 (1)</td>
<td>Single heterozygous</td>
</tr>
<tr>
<td>CAV1</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>ENG</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>KCNK3</td>
<td>0</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Primary analysis data cut-off date 14 January 2020.

*The genetic sequencing panel used was commercially available and not customized (ARUP laboratories: PAH panel, sequencing and deletion/duplication, Multigene 2009345).

ACVRL1: activin A receptor-like type 1; BMPR2: bone morphogenetic protein receptor type II; CAV1: caveolin 1; EIF2AK4: eukaryotic translation initiation factor 2 alpha kinase 4; ENG: endoglin; KCNA5: potassium voltage-gated channel subfamily A member 5; KCNK3: potassium channel subfamily K member 3.
Baseline characteristics based on *BMPR2* status

<table>
<thead>
<tr>
<th></th>
<th><em>BMPR2</em> variant cohort* (n=25)</th>
<th>Non-variant cohort* (n=69)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean age, years (SE)</strong></td>
<td>40 (3)</td>
<td>51 (2)</td>
<td>0.0021</td>
</tr>
<tr>
<td><strong>Mean time since diagnosis, years</strong></td>
<td>10 (1)</td>
<td>7 (1)</td>
<td>0.0581</td>
</tr>
<tr>
<td><strong>WHO group classification, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug or toxin-induced PAH</td>
<td>1 (4)</td>
<td>5 (7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Heritable PAH</td>
<td>12 (48)</td>
<td>4 (6)</td>
<td></td>
</tr>
<tr>
<td>Idiopathic PAH</td>
<td>12 (48)</td>
<td>40 (58)</td>
<td></td>
</tr>
<tr>
<td>PAH associated with CTD</td>
<td>0 (0)</td>
<td>18 (26)</td>
<td></td>
</tr>
<tr>
<td>PAH associated corrected congenital shunts</td>
<td>0 (0)</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td><strong>WHO functional class, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>16 (64)</td>
<td>32 (46)</td>
<td>0.1638</td>
</tr>
<tr>
<td>III</td>
<td>9 (36)</td>
<td>37 (54)</td>
<td></td>
</tr>
<tr>
<td><strong>Standard-of-care therapy, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monotherapy</td>
<td>0 (0)</td>
<td>7 (10)</td>
<td>0.1236</td>
</tr>
<tr>
<td>Double therapy</td>
<td>6 (24)</td>
<td>22 (32)</td>
<td></td>
</tr>
<tr>
<td>Triple therapy</td>
<td>17 (68)</td>
<td>33 (48)</td>
<td></td>
</tr>
<tr>
<td><strong>Prostacyclin infusion, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (40)</td>
<td>23 (33.3)</td>
<td>0.6271</td>
</tr>
<tr>
<td>No</td>
<td>15 (60)</td>
<td>46 (67)</td>
<td></td>
</tr>
<tr>
<td><strong>Pulmonary vascular resistance, dyn·s/cm²</strong></td>
<td>831 ± 61</td>
<td>799 ± 46</td>
<td></td>
</tr>
<tr>
<td><strong>6-minute walk distance, m</strong></td>
<td>438 ± 10</td>
<td>387 ± 10</td>
<td></td>
</tr>
<tr>
<td><strong>NT-proBNP, pg/mL</strong></td>
<td>508 ± 131</td>
<td>1188 ± 195†</td>
<td></td>
</tr>
</tbody>
</table>

Primary analysis data cut-off date 14 January 2020.
Data is shown as mean ± SE unless otherwise specified.
*BMPR2* variant is defined as any patient with detected pathogenic variants of *BMPR2*; *BMPR2* non-variant is defined as any patient with no detectable pathogenic variation in *BMPR2*.†n=68.
BMPR2: bone morphogenetic protein receptor type II; CTD: connective tissue disease; PAH: pulmonary arterial hypertension; NT-proBNP: N-terminal pro-brain natriuretic peptide; SE: standard error; WHO: World Health Organization.
Change from baseline in clinical characteristics by *BMPR2* variant status*

- Change from baseline in clinical characteristics did not differ between *BMPR2* variants and non-variants

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*BMPR2* variant is defined as any patient with detected pathogenic variants of *BMPR2*. *BMPR2* non-variant is defined as any patient with no detectable pathogenic variation in *BMPR2*.

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**PVR**

- **Subgroup**
  - Sotatercept 0.3 mg/kg + SOC
    - *BMPR2* variant
    - *BMPR2* non-variant
  - Sotatercept 0.7 mg/kg + SOC
    - *BMPR2* variant
    - *BMPR2* non-variant

**6MWD**

- **Subgroup**
  - Sotatercept 0.3 mg/kg + SOC
    - *BMPR2* variant
    - *BMPR2* non-variant
  - Sotatercept 0.7 mg/kg + SOC
    - *BMPR2* variant
    - *BMPR2* non-variant

**NT-proBNP**

- **Subgroup**
  - Sotatercept 0.3 mg/kg + SOC
    - *BMPR2* variant
    - *BMPR2* non-variant
  - Sotatercept 0.7 mg/kg + SOC
    - *BMPR2* variant
    - *BMPR2* non-variant

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Primary analysis data cut-off date 14 January 2020.

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**6MWD**: 6-minute walk distance; **BMPR2**: bone morphogenetic protein receptor type II; **CI**: confidence interval; **NT-proBNP**: N-terminal pro-brain natriuretic peptide; **PVR**: pulmonary vascular resistance; **SE**: standard error; **SOC**: standard of care.
**BMPR2 mRNA expression in PBMCs was assessed using a custom qRT-PCR expression assay**

**BMPR2 expression at baseline was similar across all dose groups and PAH subtypes**

**There was no significant difference in BMPR2 expression at week 24 compared with baseline in any treatment group or PAH subtype**

Primary analysis data cut-off date 14 January 2020.

*Includes drug or toxin-induced PAH and PAH associated with simple, congenital systemic-to-pulmonary shunts at least 1-year following shunt repair

†At baseline: n=25 for placebo; n=29 for sotatercept 0.3 mg/kg; n=33 for sotatercept 0.7 mg/kg. At week 24 n=29 for placebo; n=29 for sotatercept 0.3mg/kg; n=33 for sotatercept 0.7 mg/kg

‡At baseline: n=47 for idiopathic; n=15 for heritable; n=17 for PAH with CTD; n=8 for other. At week 24 n=48 for idiopathic; n=15 for heritable; n=18 for PAH with CTD; n=9 for other.

BMPR2: bone morphogenetic protein receptor type 2; Ct: cycle threshold; CTD: connective tissue disease; PAH pulmonary arterial hypertension; PBMC: peripheral blood mononuclear cell; qRT-PCR: quantitative reverse transcription polymerase chain reaction
BMPR2 expression was not significantly affected by treatment with sotatercept, regardless of BMPR2 gene status at baseline.

- Primary analysis data cut-off date 14 January 2020.
  n=32 for placebo; n=32 for sotatercept 0.3 mg/kg; n=42 for sotatercept 0.7 mg/kg.
- *BMPR2 variant is defined as any patient with detected pathogenic variants of BMPR2; BMPR2 non-variant is defined as any patient with no detectable pathogenic variation in BMPR2.
- †Data is shown as mean ± SE for baseline and weeks 3, 12, 21, and 24.

BMPR2: bone morphogenetic protein receptor type II; Ct: cycle threshold; mRNA: messenger RNA; SE: standard error; SOC: standard of care.
Biomarker analysis: NT-proBNP

Levels of NT-proBNP decreased from baseline to week 24 in both sotatercept treatment groups; no change from baseline was observed in the placebo group.
Levels of activin A decreased from baseline to week 24 in both sotatercept treatment groups; no change was observed in the placebo group.

No significant changes were seen between groups or from baseline in other biomarkers tested.

Activin A levels from baseline to end of primary treatment period (week 24) in the full analysis set:

- Levels of activin A decreased from baseline to week 24 in both sotatercept treatment groups; no change was observed in the placebo group.
- No significant changes were seen between groups or from baseline in other biomarkers tested.

Primary analysis data cut-off date 14 January 2020.
Quantitative ELISA used to detect activin A in human serum. LLOQ = 31.3 pg/mL.
n=32 for placebo; n=32 for sotatercept 0.3 mg/kg; n=42 for sotatercept 0.7 mg/kg.
*Data is shown as mean ± SE for baseline and weeks 3, 12, 21, and 24.
†Including growth differentiation factor 15 (GDF15), transforming growth factor beta 1 (TGF-β1), vascular endothelial growth factor receptor type 1 (VEGFR1)
Sotatercept was generally well tolerated, and safety findings were consistent with other patient populations.

<table>
<thead>
<tr>
<th>Treatment-emergent adverse events (TEAE), n (%)</th>
<th>Placebo n=32</th>
<th>Sotatercept 0.3 mg/kg + SOC n=32</th>
<th>Sotatercept 0.7 mg/kg + SOC n=42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serious TEAEs</td>
<td>3 (9)</td>
<td>2 (6)</td>
<td>10 (24)*</td>
</tr>
<tr>
<td>Serious related TEAEs</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>TEAEs leading to treatment discontinuation</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>TEAEs leading to death</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (2)*</td>
</tr>
<tr>
<td>AE of special interest‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia, n (%)§</td>
<td>0 (0)</td>
<td>2 (6)</td>
<td>5 (12)</td>
</tr>
<tr>
<td>Hemoglobin increase, n (%)</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td>7 (17)</td>
</tr>
</tbody>
</table>

* These 10 patients experienced SAEs of: leukopenia, neutropenia, pericardial effusion, tachycardia, chorioretinopathy, peripheral edema, pyrexia, bronchitis, influenza, respiratory tract infection, femur fracture, hypotension, device breakage, syncope, RBC increase.
† This patient died due to a cardiac arrest deemed unrelated to study treatment and had many pre-existing risk factors.
‡ These events identified as events of special interest at health authority request from the previous sotatercept studies in 350 patients.
§ Most patients had existing thrombocytopenia at study start and all were on concomitant prostacyclin infusion therapy; no patients had grade 3 or associated bleeding events.

Conclusions

• The treatment effect of sotatercept was observed independent of BMPR2 status, and sotatercept treatment did not affect BMPR2 mRNA expression in PBMCs

• Sotatercept reduces NT-proBNP expression, consistent with its observed clinical efficacy in PULSAR

• Sotatercept reduces activin A, consistent with its proposed mechanism of action

• These results provide further evidence of sotatercept’s potential broader role in the treatment of PAH, regardless of BMPR2 status
Acknowledgements

- We thank all the patients, their families, and all the PULSAR study investigators who participated in the trial
- The study was sponsored by Acceleron Pharma, Cambridge, MA, USA
- The authors received editorial assistance from InterComm LTD, supported by Acceleron Pharma
Thank you for listening

Questions?