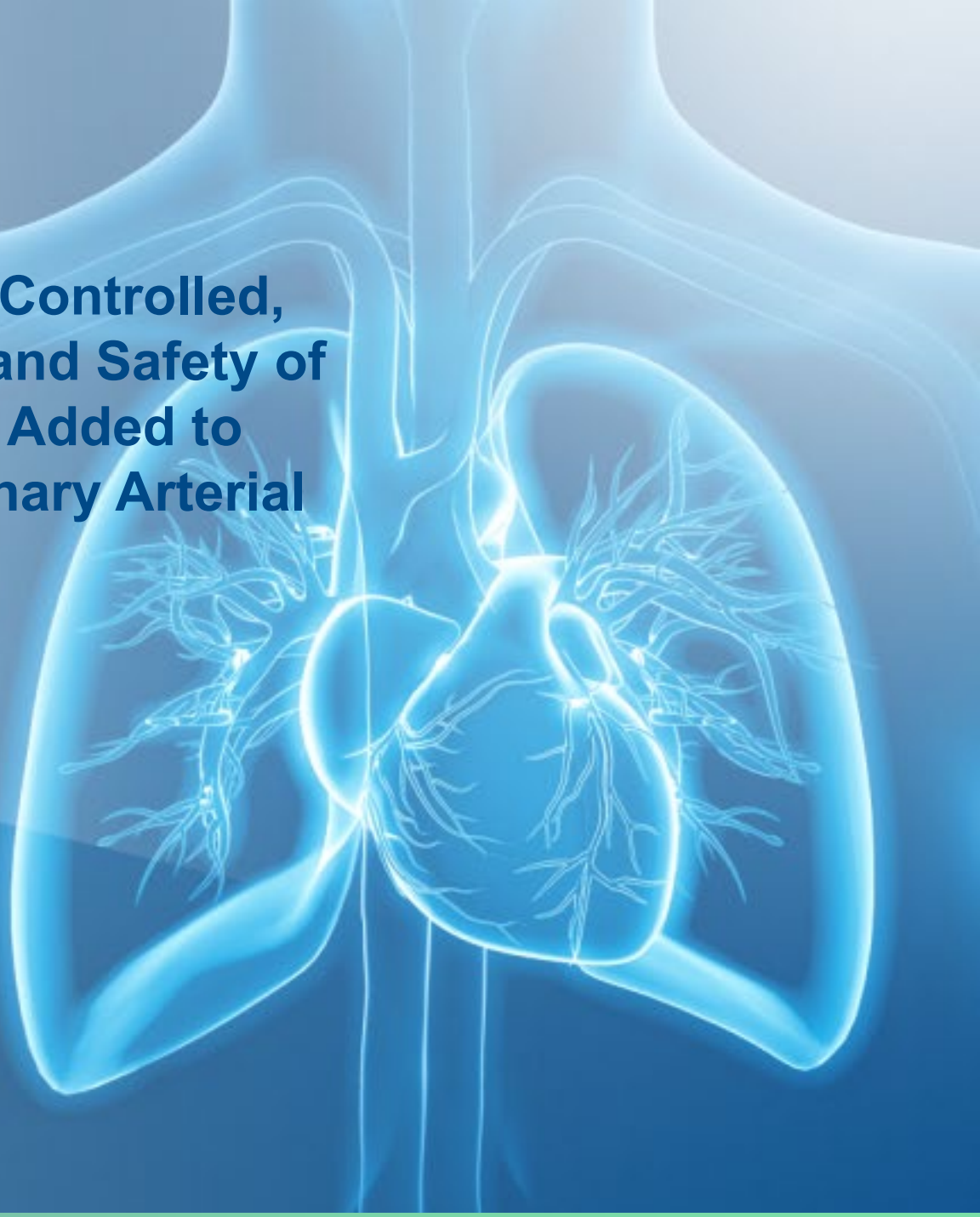
PVRI
Pulmonary Vascular Research Institute

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

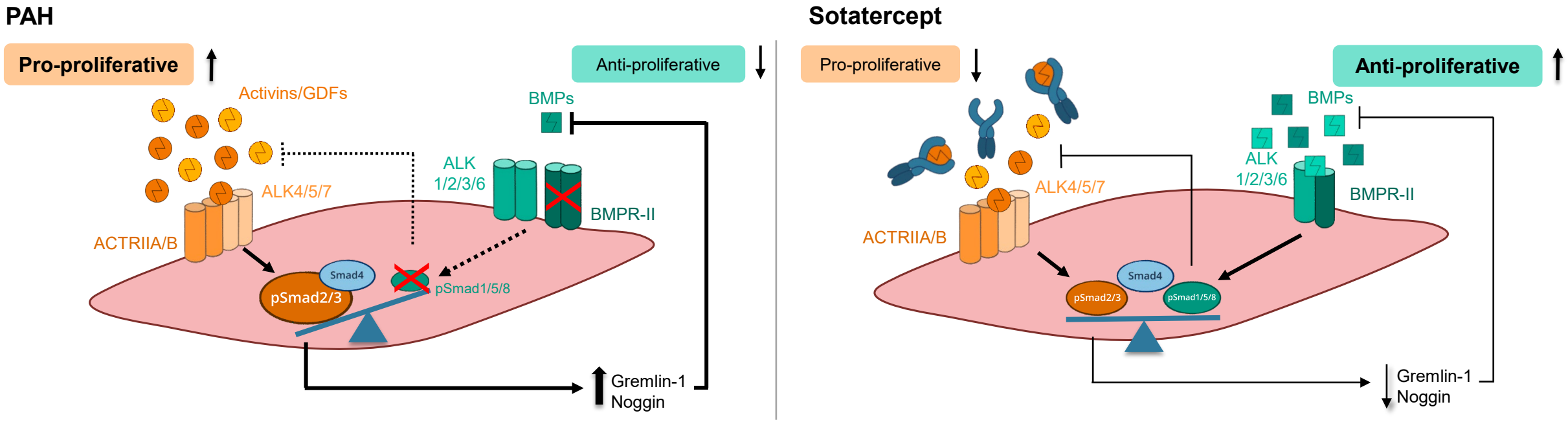
- Pulmonary arterial hypertension (PAH) is characterized by pulmonary vascular remodeling leading to increased pulmonary arterial pressure and right ventricular dysfunction[1]
- Mutations in the bone morphogenetic protein (BMP) receptor type II gene (*BMPR2*) are a major factor underlying familial PAH 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[1,2]
- 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.

1. Badesch, DB, et al. Sotatercept for the Treatment of Pulmonary Arterial Hypertension. Presentation at: ATS 2020 Virtual. American Thoracic Society International Conference; 24 June–10 November 2020.
2. Yung LM, *et al. Sci Transl Med* 2020; **12**: eaaz5660.

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



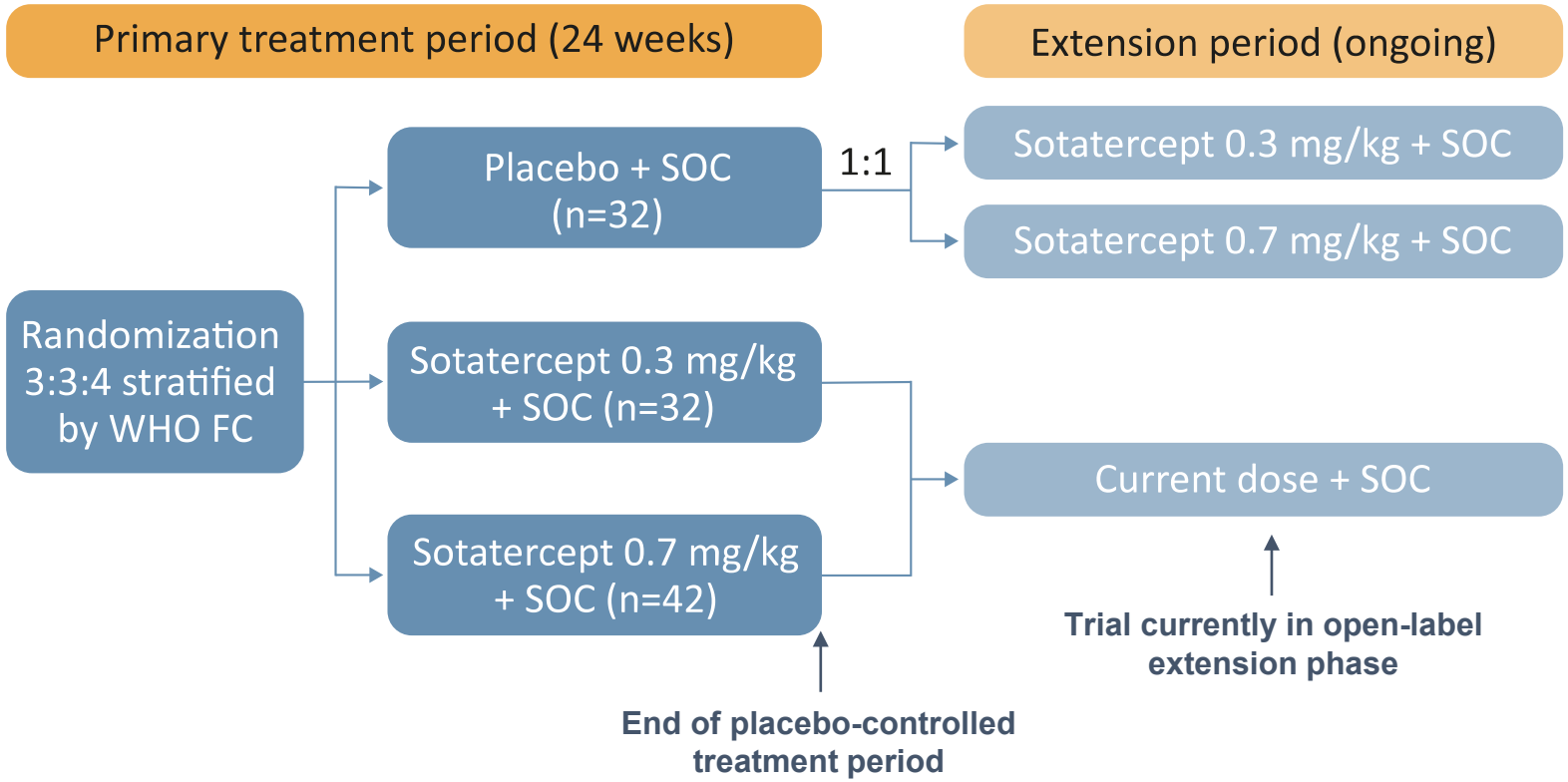
Sotatercept is an investigational product that is not approved for any use in any country.
ACTRIIA/B: activin receptor type 2A/B; ALK: activin receptor-like kinase; BMP: bone morphogenetic protein; BMPR-II: bone morphogenetic protein receptor type 2; Fc, fragment crystallizable; GDF: growth differentiation factor; PAH: pulmonary arterial hypertension; pSmad; phosphorylated Smad.

PULSAR study design

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)



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.

1. *Adapted from:* Badesch DB, et al. Sotatercept for the Treatment of Pulmonary Arterial Hypertension. Presentation at: ATS 2020 Virtual. American Thoracic Society International Conference; 24 June–10 November 2020.

PULSAR study design

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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[1]

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

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.

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PULSAR Study: Baseline characteristics

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

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.
PAH: pulmonary arterial hypertension; SE: standard error; WHO: World Health Organization.

1. *Adapted from:* Badesch DB, et al. Sotatercept for the Treatment of Pulmonary Arterial Hypertension. Presentation at: ATS 2020 Virtual. American Thoracic Society International Conference; 24 June–10 November 2020.

Summary of genetic variations

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

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

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;

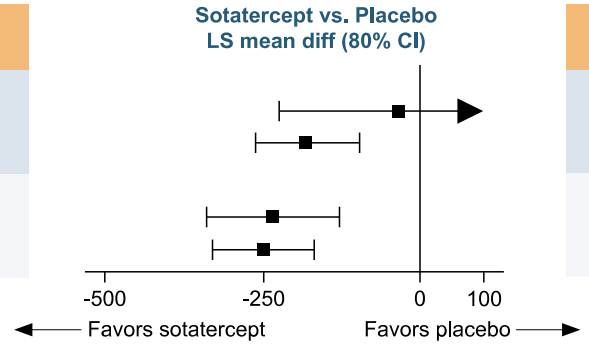
Baseline characteristics based on *BMPR2* status

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

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.

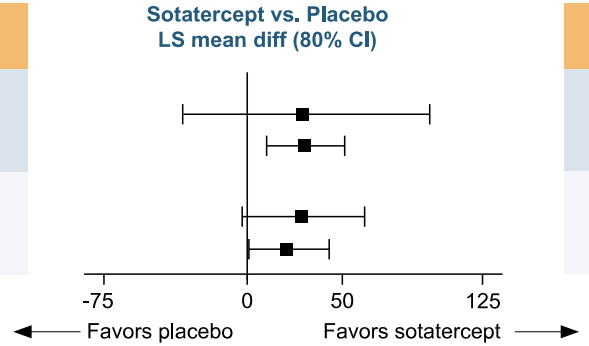
Change from baseline in clinical characteristics by *BMPR2* variant status*

PVR



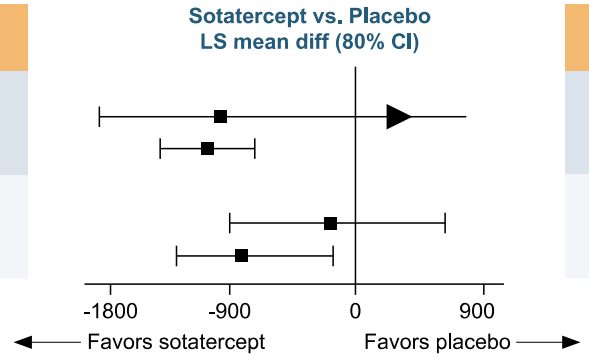
Subgroup	Sotatercept vs. Placebo LS mean diff (SE) (80% CI)	Placebo, n	Sotatercept, n
Sotatercept 0.3 mg/kg + SOC			
BMPR2 variant	-33 (150) (-226, 159)	9	2
BMPR2 non-variant	-179 (63) (-260, -98)	21	23
Sotatercept 0.7 mg/kg + SOC			
BMPR2 variant	-233 (82) (-377, -128)	9	14
BMPR2 non-variant	-248 (63) (-328, -167)	21	25

6MWD



Subgroup	Sotatercept vs. Placebo LS mean diff (SE) (80% CI)	Placebo, n	Sotatercept, n
Sotatercept 0.3 mg/kg + SOC			
BMPR2 variant	31 (50) (-33, 95)	9	2
BMPR2 non-variant	31 (16) (10, 52)	21	23
Sotatercept 0.7 mg/kg + SOC			
BMPR2 variant	29 (25) (-3, 61)	9	14
BMPR2 non-variant	21 (16) (0, 42)	21	25

NT-proBNP



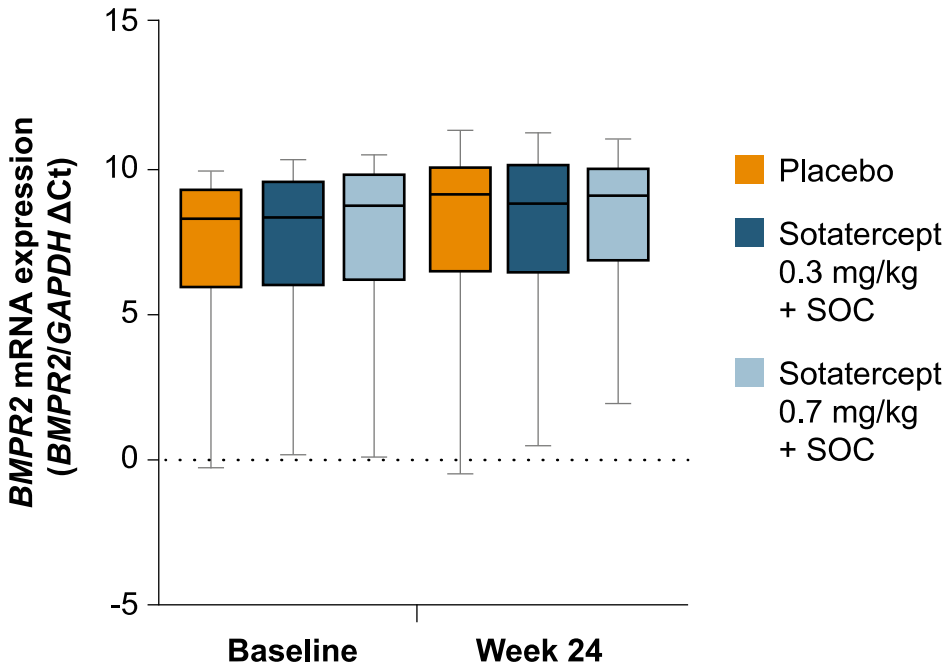
Subgroup	Sotatercept vs. Placebo LS mean diff (SE) (80% CI)	Placebo, n	Sotatercept, n
Sotatercept 0.3 mg/kg + SOC			
BMPR2 variant	-465 (1107) (-1884, 954)	9	2
BMPR2 non-variant	-1089 (271) (-1436, -742)	21	23
Sotatercept 0.7 mg/kg + SOC			
BMPR2 variant	-172 (585) (-921, 577)	9	14
BMPR2 non-variant	-814 (286) (-1180, -448)	21	25

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

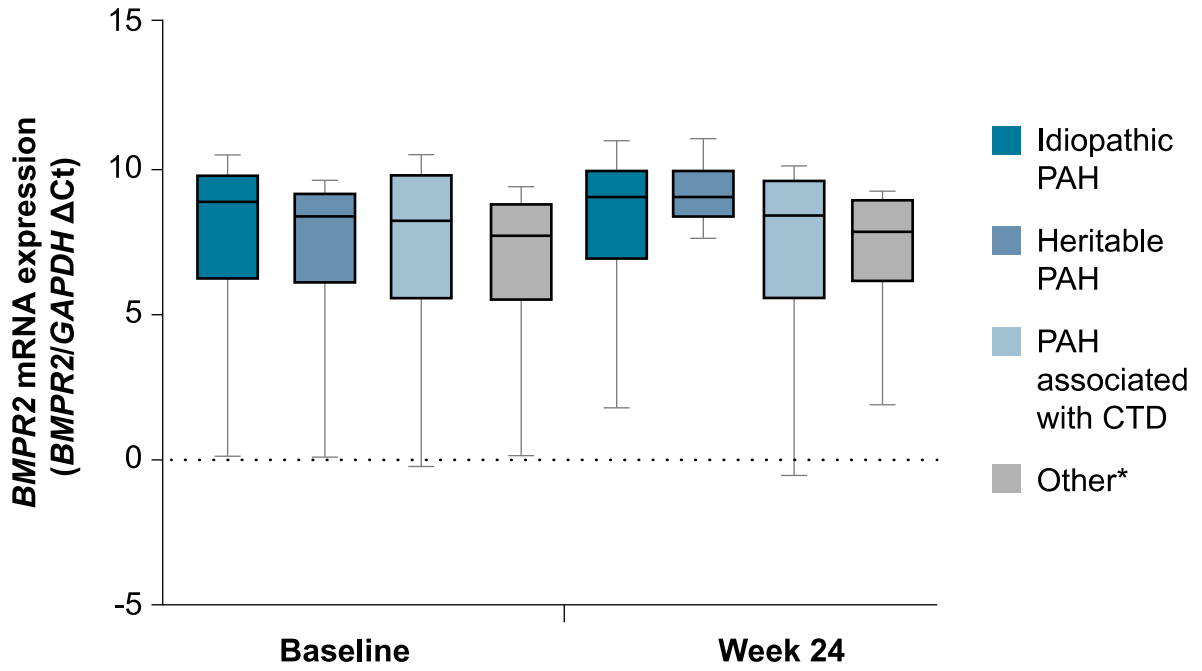
Primary analysis data cut-off date 14 January 2020.
**BMPR2* variant is defined as any patient with detected pathogenic variants of *BMPR2*; *BMPR2* non-variant is defined as any patient with no detectable pathogenic variation in *BMPR2*.
6MWD: 6-minute walk distance; BMPR2: bone morphogenetic protein 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.

Change in *BMPR2* mRNA expression

***BMPR2* expression at baseline and week 24 by dose group†**



***BMPR2* expression at baseline and week 24 by PAH subtype‡**

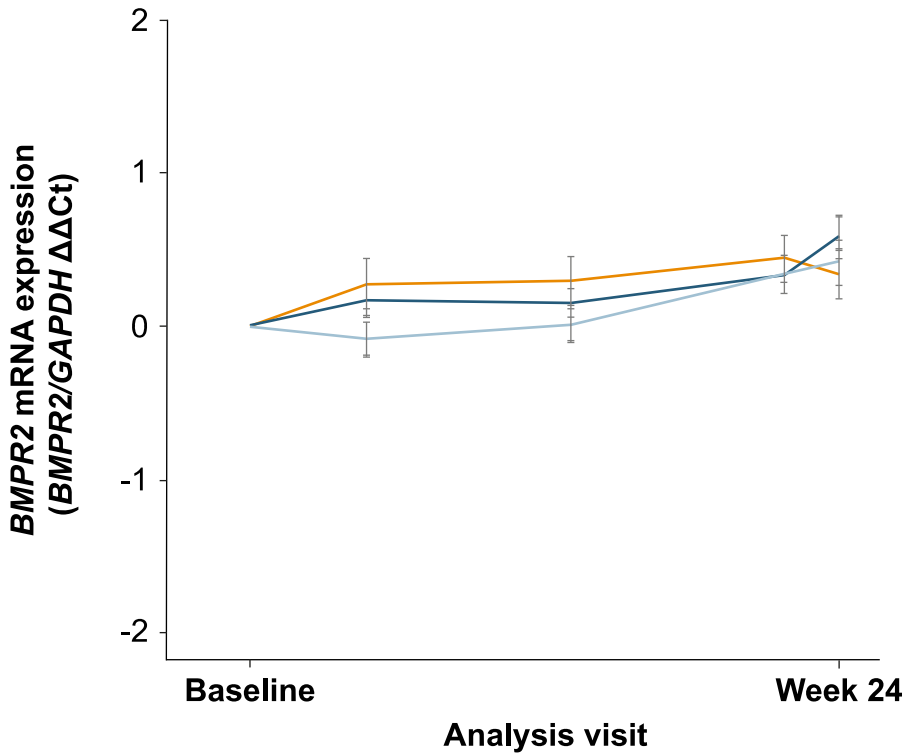


- *BMPR2* mRNA expression in PBMCs was assessed using a custom qRT-PCR expression assay
- *BMPR2* expression at baseline was similar across all dose groups and PAH subtypes
- There was no significant difference in *BMPR2* expression at week 24 compared with baseline in any treatment group or PAH subtype

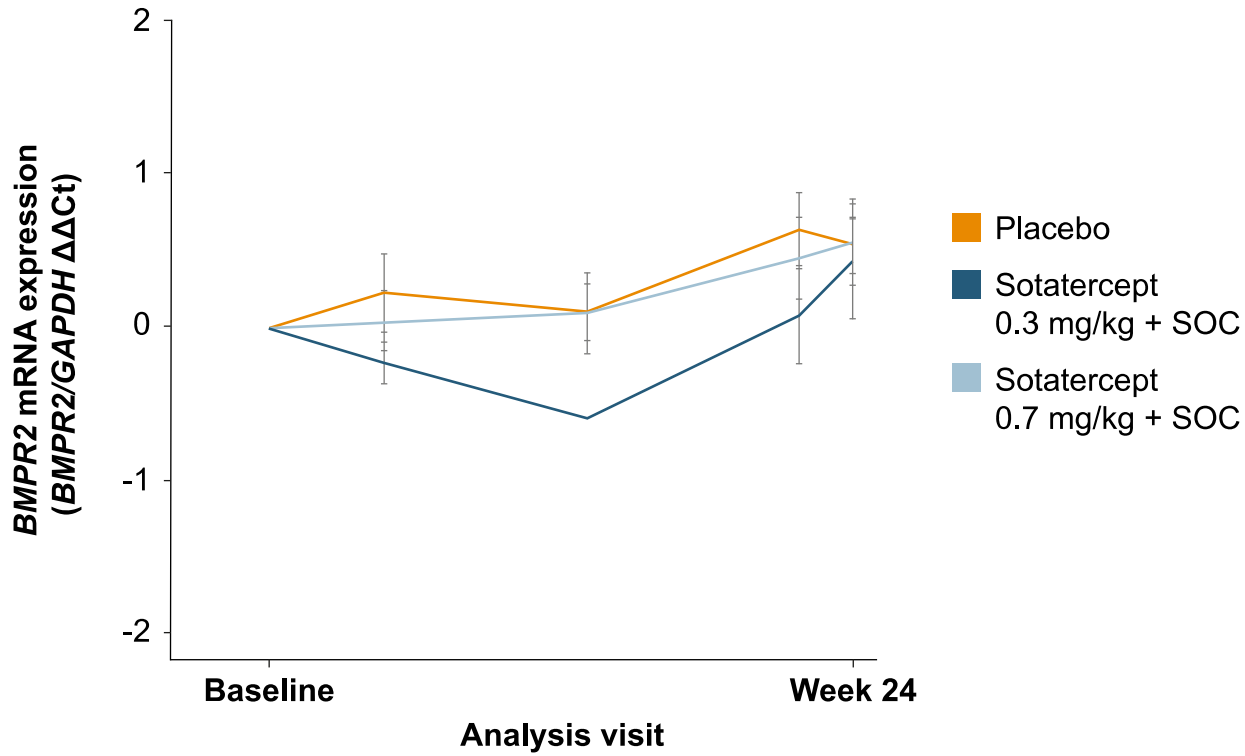
Primary analysis data cut-off date 14 January 2020.
*Includes drug or toxin-induced PAH and PAH associated with simple, congenital systemic-to-pulmonary shunts at least 1-year following shunt repair
†At baseline: n=25 for placebo; n=29 for sotatercept 0.3 mg/kg; n=33 for sotatercept 0.7 mg/kg. At week 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

Change in *BMPR2* mRNA expression

***BMPR2* expression in all cohorts by visit†**



***BMPR2* expression in *BMPR2* variants* by visit †**

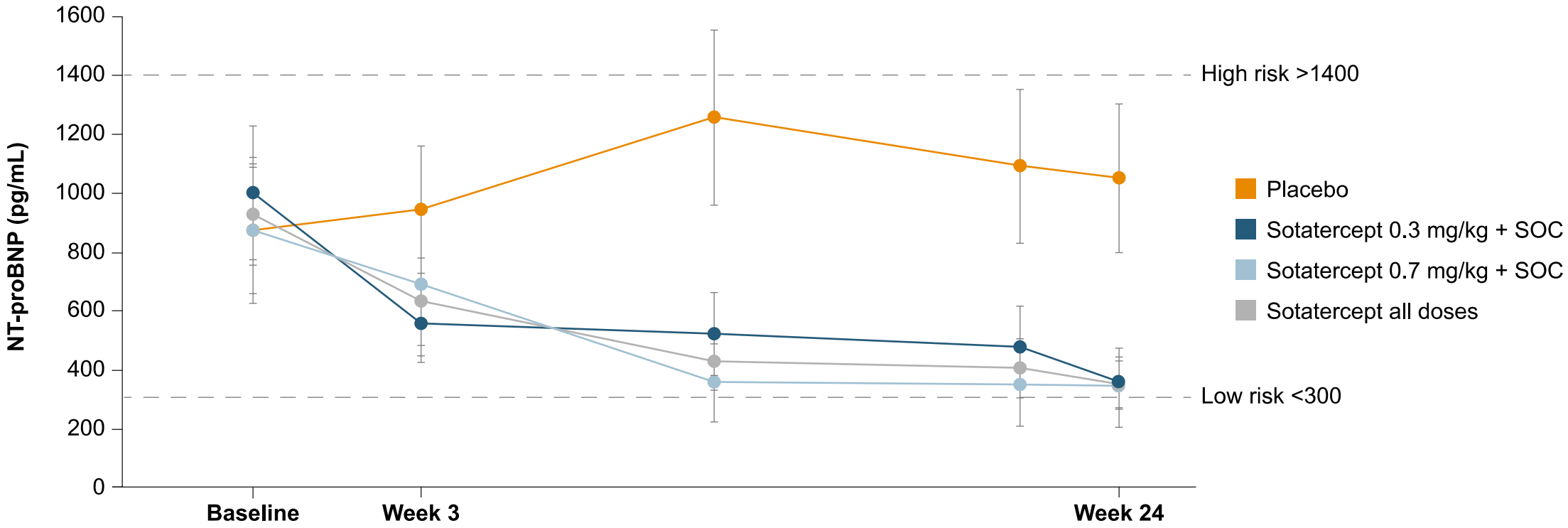


- *BMPR2* expression was not significantly affected by treatment with sotatercept, regardless of *BMPR2* gene status at baseline

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

NT-proBNP levels from baseline to end of primary treatment period (week 24) in the full analysis set*

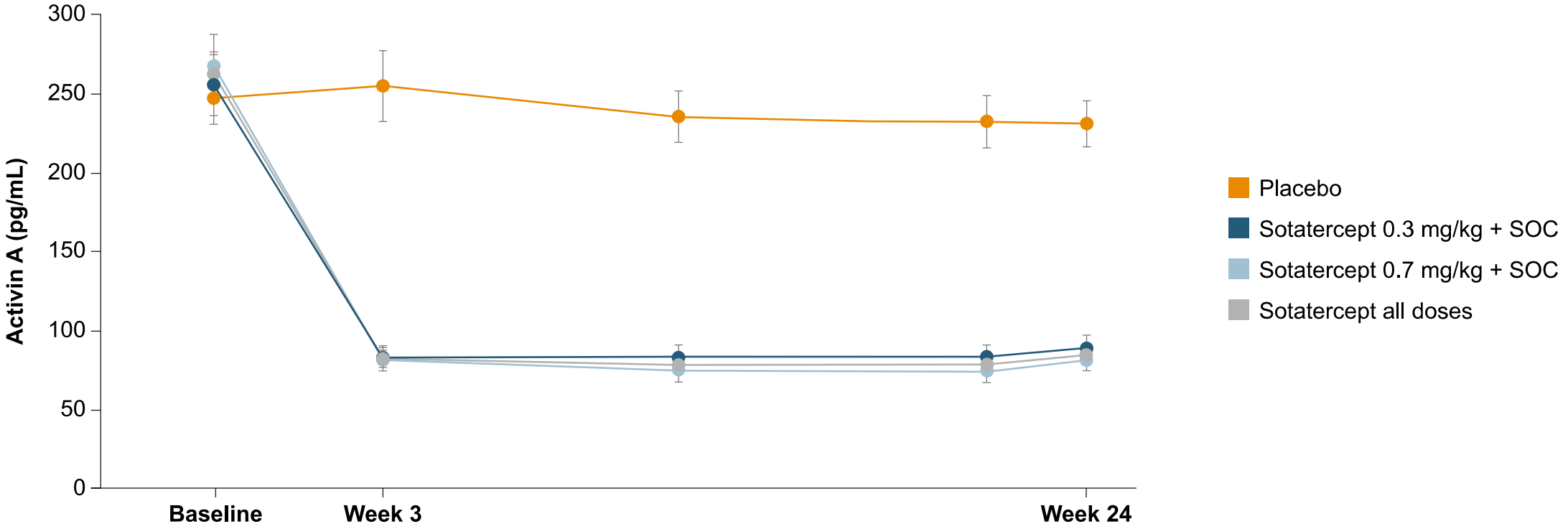


- Levels of NT-proBNP decreased from baseline to week 24 in both sotatercept treatment groups; no change from baseline was observed in the placebo group

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.
*Data is shown as mean ± SE for baseline and weeks 3, 12, 21 and 24.
NT-proBNP; N-terminal pro-brain natriuretic peptide; SE: standard error; SOC: standard of care.

Biomarker analysis: activin A

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)
ELISA: enzyme linked immunosorbent assay; LLOQ: lower limit of quantitation; SE: standard error; SOC: standard of care.

Safety

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

	Placebo n=32	Sotatercept 0.3 mg/kg + SOC n=32	Sotatercept 0.7 mg/kg + SOC n=42
Treatment-emergent adverse events (TEAE), n (%)	28 (88)	29 (91)	35 (83)
Serious TEAEs	3 (9)	2 (6)	10 (24)*
Serious related TEAEs	1 (3)	0 (0)	2 (5)
TEAEs leading to treatment discontinuation	1 (3)	1 (3)	3 (7)
TEAEs leading to death	0 (0)	0 (0)	1 (2)†
AE of special interest‡			
Thrombocytopenia, n (%)§	0 (0)	2 (6)	5 (12)
Hemoglobin increase, n (%)	0 (0)	1 (3)	7 (17)

* 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
AE: adverse event (based on Common Terminology Criteria for Adverse Events, CTCAE); RBC: red blood cell; SAE: serious adverse event; SOC; standard of care; TEAE: treatment-emergent adverse events
Adapted from: Badesch, DB, et al. Sotatercept for the Treatment of Pulmonary Arterial Hypertension. Presentation at: ATS 2020 Virtual. American Thoracic Society International Conference; 24 June–10 November 2020.

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

BMPR2: bone morphogenetic protein receptor type II; PAH: pulmonary arterial hypertension; NT-proBNP; N-terminal pro-brain natriuretic peptide; PBMC: peripheral blood mononuclear cell.

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Thank you for listening

Questions?