SUPPLEMENTARY APPENDIX

CONTENTS

METHODS

List of Institutional Review Boards/Independent Ethics Committees

1. Marshfield Clinic Research Institute
2. University of Tennessee Health Science Center IRB
3. Columbia University Medical Center Institutional Review Board
4. Research Ethics Committee of the Children's Hospital of Niño Jesús
5. University of Alberta Health Research Ethics Board
6. Baystate Medical Center IRB
7. Yorkshire & The Humber – Sheffield Research Ethics Committee
8. Comite De Protecion Des Personnes Ouest VI
9. Western Institutional Review Board

Complete List of Inclusion Criteria

1. BBS clinical diagnosis as per Beales criteria or Alström syndrome diagnosis as per Marshall criteria
    a. ≥90% of patients with BBS and 100% of patients with Alström syndrome were required to have a genetically confirmed diagnosis
2. Age ≥6 years at the time of randomization
3. Clinical obesity, defined as BMI ≥30 kg/m^2 (for those ≥16 years) or weight >97th percentile for age and sex on growth charts (for those aged 6 to 15 years)
4. Able to communicate with the investigator and provide informed consent
5. Female patients of child-bearing potential must be confirmed non-pregnant and use contraception during the trial
6. Male patients with female partners of child-bearing potential must use contraception and cannot donate sperm during the trial and for 90 days following the trial

Complete List of Exclusion Criteria

1. >2% weight loss from diet, exercise program, or both, with or without the use of weight loss agents, in prior 2 months
2. Use of any obesity medication within prior 3 months. GLP-1 receptor agonists may be used up to the dose approved for the treatment of diabetes mellitus as long as (1) the dose has been stable for ≥3 months prior to randomization and is planned to remain stable throughout the study, (2) it is not being prescribed for the treatment of obesity, and (3) the patient has not experienced weight loss during the previous 3 months
3. >10% durable weight loss from gastric bypass surgery
4. Diagnosis of psychiatric disorder that will interfere with study compliance
5. Patients without neurocognitive defects should not have a Patient Health Questionnaire-9 score ≥15, any suicidal ideation of type 4 or 5 on the Columbia-Suicide Severity Rating Scale, any lifetime history of a suicide attempt, or any suicidal behavior in prior month
6. Current pulmonary, cardiac, or oncologic disease considered severe enough to interfere with the study
7. Hemoglobin A1c >9·0%
8. History of significant liver disease or liver injury, or a current liver assessment due to abnormal liver tests for an etiology other than non-alcoholic fatty liver disease
9. Moderate-to-severe renal dysfunction defined as glomerular filtration rate <30 mL/min
10. History or close family history of melanoma or skin cancer, or patient history of ocular-cutaneous albinism
11. Significant dermatological findings relating to melanoma or pre-melanoma
12. Patient is not suitable to participate in the study per the opinion of the investigator
13. Participation in any drug or device clinical study in prior 3 months
14. Previous enrollment in a setmelanotide clinical trial or any prior exposure to setmelanotide
15. Significant hypersensitivity to any study drug excipient
16. Unable to comply with daily injections
17. First-degree relative currently enrolled in the double-blind portion of this study or enrolled in this study within prior 4 months

Daily Self-Assessment of Hunger

In patients ≥12 years of age without cognitive impairment, 3 aspects of hunger were self-reported by patients daily on a scale from 0 (not hungry at all) to 10 (hungriest possible). The questionnaire assessed average hunger ("In the last 24 hours, on average, how hungry did you feel?), maximal hunger ("In the last 24 hours, how hungry did you feel when you were the most hungry?"), and morning hunger ("This morning when you woke up for the day, how hungry did you feel?). Responses were based on a numerical Likert-type scale ranging from 0 to 10, with 0 = "not hungry at all" and 10 = "the hungriest possible." Each hunger aspect of the daily hunger questionnaire was scored separately; scores were averaged on a weekly basis. The investigator determined whether the patient had cognitive impairment (eg, could not assess their own hunger); a formal diagnosis was not required.

Randomization and Masking Details

Potential patients were identified by the investigators and their study staff, and candidates for enrollment were evaluated for eligibility by the investigator based on the inclusion and exclusion criteria. Randomization was conducted by an unblinded statistician at the vendor Advanced Clinical. Data were provided to Medidata for use in the Medidata RTSM (Randomization, Treatment Arm, Subject Randomized). All randomization events occurred within the RTSM and imported into Rave EDC. All study personnel except the unblinded statistician, RTSM Supply Manager, and RTSM Shipping manager remained blinded to participant treatment for the duration of the study. All study site staff remained blinded for the duration of the study. All study sponsor personnel except the Data Management Lead and unblinded programmer will remain blinded to efficacy measures for the duration of the trial or until planned unblinding.

The investigator, medical monitor, study site staff, and study site management team did not have access to the treatment assignment during the double-blind, placebo-controlled period; the blind was maintained through Week 52 (38 weeks of setmelanotide treatment for those randomized to placebo). During the double-blind, placebo-controlled period, setmelanotide and placebo were supplied in identical packaging.

Data Monitoring and Management

Data management was conducted by the vendor Advanced Clinical, who ensured data were complete, accurate, and logically consistent. The data management group processed and reviewed the data in the Medidata RAVE-X system and checked for omissions, inaccuracies, and inconsistencies.

COVID-19 Considerations and Risk Mitigation

Guidelines were developed and put in place by the study sponsor (Rhythm Pharmaceuticals) and data management vendor (Advanced Clinical) to ensure safe and secure handling of study data during the COVID-19 pandemic. To ensure protocol continuity and patient safety, some study visits or assessments were performed remotely at alternative facilities not involved in the clinical trial (eg, commercial clinical laboratories, private health practitioners, ambulatory health clinics). The guidelines were:

- Investigator or designee to contact alternative facility prior to remote visit to discuss visit requirements, assessments, and manner in which the results will be provided to the investigator and into the study records
- All attempts should be made to have the facility provide study results directly to the investigator in order to ensure secure and safe transfer of study data
- Secure and safe study transfer options were limited to:
    - Post-mailed if addressed to the investigator directly and marked as "Confidential"
    - Faxed if a secure protocol-dedicated fax transfer process can be verified and meets audit standards
    - Electronically transmitted if a secured process can be verified and meet audit standards
- The study data/results provided to the investigator were retained in the same manner as any other study source documents
- The name of the outside facility was clearly stated on the data/results document
- The printed name, signature, and date of the health care professional(s) that performed the assessments were clearly stated

As an alternative, in-home nursing services tailored to the specific study requirements and the patient were an option for some study sites

Statistical Analysis

Key secondary endpoints assessing mean change were tested using a 1-sample *t* test for each of the 100 multiple imputed data sets, which were combined using Rubin's rule, to compare with historical reference data of 0 at a 1-sided alpha level of 0·025; corresponding 2-sided 95% confidence intervals were provided. The key secondary endpoint assessing proportion of patients was tested as described for the primary hypothesis. Other secondary endpoints were tested using a 2-sample *t* test for each of the 100 multiple imputed data sets, which were combined using Rubin's rule, at a 1-sided alpha level of 0·025 with 95% confidence intervals. Adverse events were

summarized by frequency, severity, and relationship to treatment. Laboratory evaluations and vital signs were summarized by mean and standard deviation (SD) and compared with baseline values. Statistical analyses were assessed using SAS version 9·4. Independent data monitoring and auditing procedures were followed to comply with good clinical practice guidelines.

Summary of Amendments

The trial protocol was first signed August 14, 2018 (version 1·0), and subsequently underwent significant amendments in February 2019 (version 2·0) and August 2020 (version 3·0; Supplementary Table 1). The final version of the protocol (version 3·1) included a supplemental cohort of patients enrolled midcourse in the study. The pivotal cohort was defined as all enrolled patients at the time of the planned sixth Alström syndrome patient's enrollment. However, given the rarity of the syndromes, a supplemental cohort of patients was enrolled, randomized to placebo or setmelanotide in the 14-week double-blind period, and then treated with open-label setmelanotide following the same protocol that was used for the pivotal cohort (as outlined later), to gain more treatment experience.

RESULTS

Concomitant Medications in the SAS

All patients with BBS or Alström syndrome in the SAS (N=38) had ≥1 concomitant medication during the study. Common concomitant medications included vitamin D and analogues (65·8%), angiotensin-converting enzyme inhibitors (28·9%), anilides (28·9%), biguanides (26·3%), osmotically acting laxatives (23·7%), progestogens and estrogens (21·1%), and selective beta-2-adrenoreceptor agonists (21·1%).

Double-Blind, Placebo-Controlled Periods: BBS Cohort

After 14 weeks of treatment in the double-blind, placebo-controlled period, patients with BBS ≥18 years old who were treated with setmelanotide had an average 5·0 kg (95% CI, 9·0, 1·0; p=0·0079) or 3·6% (95% CI, 6·3%, 0·9%; p=0·0054) greater reduction in body weight from PCPB compared with those who received placebo (Supplementary Figure 2A). Similarly, patients <18 years old who were treated with setmelanotide had an average 4·5-kg (95% CI, 7·1, 1·9; p=0·0008) or 5·5% (95% CI, 8·8%, 2·2%; p=0·0013) greater reduction in body weight from PCPB compared with those who received placebo (Supplementary Figure 2B). Patients who received setmelanotide had an average 1·8-point (95% CI, 3·3, 0·3; p=0·0104) or 35·7% (95% CI, 77·4%, −6·1%; p=0·0446)

greater reduction in the weekly average of the daily average hunger score from PCPB compared with those who received placebo. Additionally, patients who were treated with setmelanotide had an average 0·9-point (95% CI, 2·2, −0·4; p=0·0840) or 14·4% (95% CI, 31·9%, −3·14%; p=0·051) greater reduction in the weekly average of the daily maximal hunger score and 0·8-point (95% CI, 1·8, −0·3; p=0·0725) or 12·2% (95% CI, 32·4%, −7·9%; p=0·1081) greater reduction in the weekly morning average hunger score from PCPB compared with those who received placebo. Following the double-blind period, hunger scores for patients who had initially received setmelanotide temporarily rebounded during dose re-titration (Weeks 15-16) before returning to previous levels when the target dose was re-attained (Supplementary Figure 2C). Patients who switched from placebo to setmelanotide demonstrated a catch-up effect with clear and rapid reduction in hunger scores after transitioning.

Description of Serious AEs

The serious AE of anaphylactic reaction was considered related to treatment by the investigator and occurred in a patient receiving placebo. The anaphylactic reaction resolved without sequelae following hospitalization and medication. The other serious AEs were considered not related to setmelanotide. The onset of the serious AE of blindness occurred while a patient was taking placebo. Neurological and orbital imaging/angiography were normal, as was pupillary response; there was a possible component of nonorganic functional loss in a setting of known history of hereditary retinal dystrophy due to BBS. The blindness did not resolve, and this patient subsequently had two serious AEs of suicidal ideation. The patient verbalized suicidal ideation to friends, administration of the Columbia-Suicide Severity Rating Scale revealed that the patient was having daily suicidal thoughts, and the patient was provided a crisis hotline number (which was not called); this serious AE of suicidal ideation was initially reported as resolved. Approximately 1 month later, the patient began having suicidal thoughts again, which were subsequently reported as resolved. Both serious AEs of suicidal ideation were considered unrelated to setmelanotide and resolved without change of study medication. The serious AE of anaphylactic reaction in a separate patient was considered related to treatment by the investigator and occurred in a patient receiving placebo. The reaction resolved without sequalae following hospitalization and medication.

SUPPLEMENTARY TABLES

Supplementary Table 1. Major Protocol Amendments

Amendment	Date	Study sites	Substantive changes
Version 2·0	**February 4, 2019**	**Global**	• The synopsis was clarified regarding ages of enrollment • Inclusion #2 was clarified regarding the ages of enrollment • Clarified in Exclusion #2 regarding allowable medications and the time frame • Exclusion #4 was rewritten to remove only DSM-V disorders • Exclusion # 7 was added to exclude patients with HbA1c >9·0 at screening • Added glomerular filtration rate • Exclusion #14 was added to exclude patients previously treated with setmelanotide • Exclusion #17 was added to exclude patients with first-degree relatives enrolled within the past 4 months • Text was added to clarify that the Investigator had direct access to the system to unblind the treatment in case of emergency • The prohibited medication section was revised • The Screening Period was further defined to be a minimum of 1 week with at least 4 days completed in the electronic hunger diary • Table 2, Schedule of Assessments was updated to add the HbA1c testing noted above • The list of biomarkers to be tested for was revised (some biomarkers were removed) • Extended the screening period • Text added to Table 2 footnote 8 to describe a minimum of 1 week of screening and to require hunger diary completion on 4 of 7 days before randomization • Text removed to clarify genetic testing samples would not be used for any other purpose • Text clarified to state Global Hunger was to be completed during visits and the Daily Hunger was to be completed electronically each day • Text was added to state the PWS-SEQ was to be used in place of the Global Hunger questions in patients with impaired cognitive function • Text added to state the neurocognition tests WAIS-IV or WISC-V could be waived after discussion with the sponsor • Clarified the collection of samples for ACTH and 24-hour urine • Added that archive samples would be discarded at the end of the study • Text added to further define unlikely AE relationship • Text added to describe options for analysis of incomplete data • Cockcroft Gault Equation was changed to the Modification of Diet in Renal Disease Study equation for patients ≥18 years or the Bedside Schwartz for patients

			<18 years
Version 3·0	**August 16, 2020**	**Global**	• Key secondary objectives were updated to reflect a mean percent change from baseline in the weekly average of the daily hunger score. Key Secondary Objectives #1 and #4 were removed (moved to Exploratory). Key Secondary Objective #2 was rewritten to specify baseline body weight. Other Secondary Objective #2 was removed (moved to Exploratory) • Key Secondary Endpoints #1 and #3 were removed (moved to Exploratory). Added Exploratory Endpoint describing the proportion of patients who achieve an improvement in daily hunger score • Updated to clarify the number of patients enrolled in the Pivotal and Supplemental cohorts • Updated Inclusion Criteria #2 to specify age at time of first dose • The Statistical Considerations section of the Synopsis and Section 8, Data Analysis/Statistical Procedures were revised • The Clinical Overview section was updated with current exposure numbers and entire Clinical Safety section replaced providing updated TEAEs and SAE incidence to align with the annual Investigator Brochure update • Text was added to allow Supplemental patients to roll into the extension study starting at Visit 8 or later • Flexibility added to visit schedule windows upon sponsor approval to accommodate difficulty in scheduling due to COVID-19, holidays, and other logistical issue • Table 2 Schedule of Assessments was updated to make WISC-V/WAIS-IV optional based on investigator discretion. (Already done for Version 2·2, United Kingdom only) • Table 2 Schedule of Assessments: removed Nutritional counselling and monitoring from V 2, 4, 6, 8, 10, and 12 (Already done for Version 2·1, Marshfield Clinic only) • Table 2 Schedule of Assessments bullet 11 was removed and 6.5.8 Measures of Insulin Sensitivity/Resistance OGTT was removed. (Already done for Version 2·1, Marshfield Clinic only) • Updated PK profile blood draw time point preferences to predose 4, 6, and 8 hours. Tubes provided in lab kits collect ~6 mL allowing for 2 aliquots. (Already done for Version 2·1, Marshfield Clinic only) • Clarified that study medication should be taken after obtaining vital signs

Supplementary Table 2. Dose Escalation Schedule

Study week	Setmelanotide dose, mg	
	Patients ≥16 years of age	Patients <16 years of age
1	2·0 (or placebo)	1·0 (or placebo)
2	2·0 (or placebo)	2·0 (or placebo)
3-14	3·0 (or placebo)	3·0 (or placebo)
15	2·0	1·0
16	2·0	2·0
17-66	3·0	3·0

Supplementary Table 3. Genes Identified With Variants in Pivotal and Supplemental Patients With Bardet-Biedl Syndrome

	Patients, n (%)
BBS1	12 (27·3)
BBS2	2 (4·5)
BBS3	1 (2·3)
BBS5	1 (2·3)
BBS6	1 (2·3)
BBS10	11 (25·0)
Not listed	11 (25·0)
Not confirmed	5 (11·4)

Supplementary Table 4. Baseline Characteristics of Patients in Double-Blind, Placebo-Controlled Period Analyses

	Pivotal patients with BBS and Alström syndrome (SAS)		Pivotal and supplemental patients with BBS	
Parameter	**Setmelanotide (n=19)**	**Placebo (n=19)**	**Setmelanotide (n=22)**	**Placebo (n=22)**
Age, mean (SD) [a]	19·3 (10·5)	20·3 (10·2)	18·5 (9·7)	21·5 (12·6)
Female, n (%)	10 (52·6)	13 (68·4)	9 (40·9)	15 (68·2)
Ethnicity, n (%)				
Hispanic or Latino	1 (5·3)	0	1 (4·5)	0
Not Hispanic or Latino	18 (94·7)	19 (100)	18 (81·8)	19 (86·4)
Not reported	0	0	1 (4·5)	2 (9·1)
Unknown	0	0	2 (9·1)	1 (4·5)
Race, n (%)				
White	14 (73·7)	17 (89·5)	15 (68·2)	19 (86·4)
Black or African American	1 (5·3)	2 (10·5)	1 (4·5)	1 (4·5)
Asian	1 (5·3)	0	0	1 (4·5)
Other	3 (15·8)	0	6 (27·3)	1 (4·5)
Cognitive impairment, n (%)	11 (57·9)	8 (42·1)	12 (54·5)	8 (36·4)
Weight, mean (SD), kg[a]	114·9 (34·3)	108·5 (26·5)	110·4 (35·8)	106·5 (31·8)
BMI, mean (SD)[a]	43·3 (13·3)	41·2 (8·4)	41·4 (10·0)	41·6 (10·1)
Maximal hunger score, mean (SD) [a,b]	7·6 (1·5) n=9	8·1 (1·3) n=13	6·3 (1·1) n=6	6·6 (2·1) n=12

BMI, body mass index; SAS, safety analysis set; SD, standard deviation. [a]At placebo-controlled period baseline. [b]In patients ≥12 years old without cognitive impairment; self-reported.

Supplementary Table 5. Changes in Anthropomorphic and Metabolic Parameters Compared With Baseline After 14 Weeks of Randomized, Double-Blind Treatment

Parameter	Pivotal patients with BBS and Alström syndrome (PCAS)		Pivotal and supplemental patients with BBS	
	Setmelanotide (n=16)	Placebo (n=17)	Setmelanotide (n=18)	Placebo (n=18)
	≥12 years old			
Mean (SD) change in body weight [95% CI], %	−2·4 (4·8) [−5·0, 0·1]	−0·3 (2·3) [−1·4, 0·8]	−3·7 (4·2) [−5·7, −1·6]	−0·2 (2·1) [−4·9, 3·2]
Difference [95% CI], %	−2·1 [−4·6, 0·4] p=0·052		−3·4 [−5·7, −1·2] p=0·0019	
Proportion achieving ≥25% reduction in maximal hunger score, estimated % [95% CI]	71·4 [29·0, 96·3][a,b]	20·2 [0, 45·2][a,c]	50·0 [11·8, 88·2][a,c]	33·3 [9·9, 65·1][a,d]
Difference [95% CI], %	51·2 [9·4, 93·0] p=0·0081		16·7 [−31·5, 61·5] p=0·63	
Mean (SD) change in maximal hunger score [95% CI], %	−26·7 (19·0) n=9	−14·8 (14·6) n=13	−30·1 (20·3) [−51·4, −8·8] n=6	−15·7 (14·5) [−24·9, −6·5] n=12
Difference [95% CI], %	−11·8 [−26·0, 2·3] p=0·051		−14·4 [−31·9, 3·1] p=0·051	

BBS, Bardet-Biedl syndrome; BMI, body mass index; PCAS, placebo-controlled analysis set; SD, standard deviation.

[a]Estimated proportion based on qualifying patients in the PCAS (ie, ≥12 years old without cognitive impairment). [a]n=7. [b]n=10.

[c]n=6. [d]n=12.

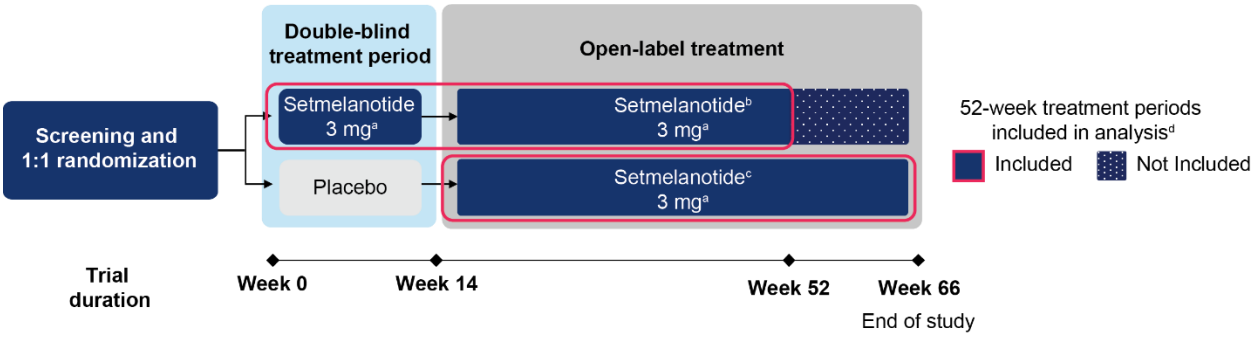
Supplementary Table 6. Treatment-Emergent Adverse Events in the Double-Blind, Placebo-Controlled Period

	Pivotal patients with BBS or Alström syndrome (SAS)			Pivotal and supplemental patients with BBS		
	Setmelanotide (N=19)	Placebo (N=19)	Overall (N=38)	Setmelanotide (N=22)	Placebo (N=22)	Overall (N=44)
Overall treatment-emergent AEs, n (%)	18 (94·7)	18 (94·7)	36 (94·7)	21 (95·5)	21 (95·5)	42 (95·5)
Treatment-emergent AEs occurring in ≥10% of the overall population, n (%)						
Injection site erythema	9 (47·4)	7 (36·8)	16 (42·1)	10 (45·5)	11 (50·0)	21 (47·7)
Injection site pruritus	6 (31·6)	5 (26·3)	11 (28·9)	7 (31·8)	9 (40·9)	16 (36·4)
Skin hyperpigmentation	11 (57·9)	0	11 (28·9)	13 (59·1)	0	13 (29·5)
Injection site bruising	3 (15·8)	6 (31·6)	9 (23·7)	6 (27·3)	9 (40·9)	15 (34·1)
Injection site pain	3 (15·8)	6 (31·6)	9 (23·7)	3 (13·6)	7 (31·8)	10 (22·7)
Nausea	4 (21·1)	5 (26·3)	9 (23·7)	5 (22·7)	6 (27·3)	11 (25·0)
Injection site induration	5 (26·3)	2 (10·5)	7 (18·4)	5 (22·7)	4 (18·2)	9 (20·5)
Headache	1 (5·3)	4 (21·1)	5 (13·2)	0	6 (27·3)	6 (13·6)
HDL cholesterol decrease	4 (21·1)	0	4 (10·5)	3 (13·6)	0	3 (6·8)
Vomiting	4 (21·1)	0	4 (10·5)	6 (27·3)	0	6 (13·6)
Injection site hemorrhage	1 (5·3)	0	1 (2·6)	3 (13·6)	2 (9·1)	5 (11·4)
Treatment-related AEs, n (%)	16 (84·2)	16 (84·2)	32 (84·2)	20 (90·9)	20 (90·9)	40 (90·9)
Serious AEs, n (%)	0	2 (10·5)	2 (5·3)	1 (4·5)	2 (9·1)	3 (6·8)
Serious treatment-related AEs, n (%)	0	1 (5·3)[a]	1 (2·6)	0	1 (4·5)[a]	1 (2·3)
AEs leading to study drug	1 (5·3)	3 (15·8)	4 (10·5)	0	2 (9·1)	2 (4·5)

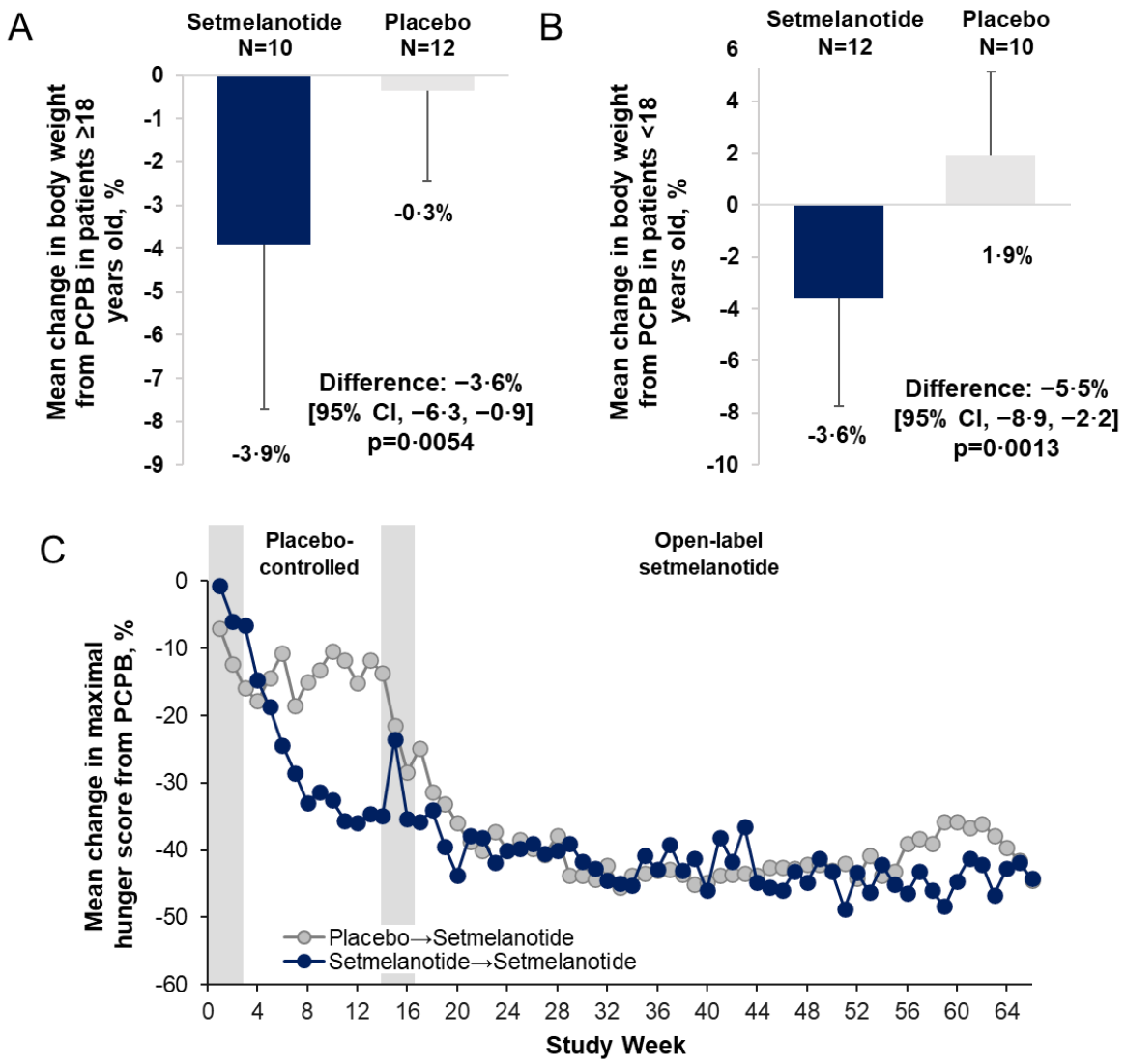
withdrawal, n (%)						
AEs leading to death, n (%)	0	0	0	0	0	0

AE, adverse event; BBS, Bardet-Biedl syndrome; HDL, high-density lipoprotein; SAS, safety analysis set. [a]Anaphylactic reaction to study drug (placebo).

SUPPLEMENTARY FIGURES



Supplementary Figure 1. Study design. [a]Dose escalation based on age up to 3·0 mg. [b]For patients who received >52 weeks of setmelanotide at the end of study, analysis is performed for 52 weeks of setmelanotide. [c]A multiple imputation model was used to impute data for patients who received <52 weeks of setmelanotide at time of primary analysis. [d]Efficacy outcomes were assessed at 52 weeks on active treatment for each study group (ie, Week 0 to 52 for the setmelanotide group and Week 14 to 66 for the group assigned to placebo during the double-blind treatment period).



Supplementary Figure 2. Weight changes in pivotal and supplemental patients **(A)** ≥18 years old (setmelanotide, n=10; placebo, n=12) and **(B)** <18 years old (setmelanotide, n=12; placebo, n=10) with BBS during the 14-week double-blind, placebo-controlled period. **(C)** Hunger score changes in pivotal patients with BBS. Grey bars indicate titration and re-titration periods. Hunger scores are reported in patients ≥12 years old without cognitive impairment. BBS, Bardet-Biedl syndrome; PCPB, placebo-controlled period baseline.