SUPPLEMENTAL MATERIAL

Efficacy and Safety of Tenapanor in Patients Receiving Maintenance Hemodialysis with Hyperphosphatemia: A Randomized Phase 3 Trial

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Key Protocol Amendments

The original protocol was dated November 24, 2015.

- The primary objective was to show the effect of tenapanor on the change in serum phosphate levels from baseline to the end of 8-weeks of treatment in hyperphosphatemic ESRD-HD subjects.
- The primary efficacy variable was serum phosphate measured as change from baseline to the last week of the 8-week randomized treatment period (RTP).

Protocol Edition No. 2 was dated March 3, 2016. The key changes are summarized below.

- The primary efficacy variable was modified to include “the difference in the change in serum phosphate from the end of the 8-week RTP to the end of the randomized withdrawal period (RWP) between treatment and placebo” (for the 4-week RWP), in addition to “serum phosphate measured as change from baseline to the last week of the 8-week RTP” (for the 8-week RTP).
- The efficacy analysis set was defined as follows: “All subjects who are randomized into the RWP and have at least one serum phosphate assessment will be members of this analysis set. The efficacy analysis set will be the primary analysis set for efficacy analysis of the 4-week RWP”.

Protocol Edition No. 3 was dated May 27, 2016. The key changes are summarized below.

- The primary objective was changed from “to show the effect of tenapanor on the change in serum phosphate levels from baseline to the end of 8-weeks of treatment in hyperphosphatemic ESRD-HD subjects” to “to compare the effect of tenapanor versus placebo by comparing the difference in the change in serum phosphate from the end of

the 8-week RTP to the end of the 4-week RWP or the end point visit for this period, between the pooled tenapanor treatments and placebo".

- The first secondary objective was changed from "to compare the effect of tenapanor versus placebo in phosphate-lowering treatment by comparing serum phosphate levels between groups from the end of the 8-week RTP to the end of the 4-week RWP" to "to show the effect of tenapanor on the change in serum phosphate levels from baseline to the end of 8 weeks of treatment".
- The number of sites was changed from 25 to 35 to 35 to 45.
- The sample size was changed from 150 male and female participants to 200 male and female participants, and the power calculation was updated accordingly.
- The primary efficacy variable was changed from "For the 8-week treatment period, the primary efficacy variables will be serum phosphate measured as change from baseline to the last week of the 8-week RTP. For the 4-week placebo-controlled RWP, the primary efficacy variable will be the difference in the change in serum phosphate from the end of the 8-week RTP to the end of the RWP between treatment and placebo" to "The primary efficacy variable will be the change in serum phosphate from the end of the 8-week RTP to the end of the 4-week RWP or the end point visit for this period. The primary efficacy analysis will be based on the difference between the pooled tenapanor treatment and placebo treatment groups".
- The efficacy analysis set was changed from "All subjects who are randomized into the RWP and have at least one serum phosphate assessment" to "All subjects who meet the study entry inclusion and exclusion criteria, complete the 8-week treatment period, and subjects who achieve at least a 1.2 mg/dL reduction in serum phosphate from baseline to the end of the 8-week RTP".

Study Outcome Measures

The primary objective of this study was to compare the effect of tenapanor versus placebo on serum phosphate by comparing the difference in the change in serum phosphate from the end of the 8-week randomized treatment period (RTP) to the end of the 4-week randomized withdrawal period (RWP), or the end point visit for this period, between the pooled tenapanor treatments and placebo.

The secondary objectives of this study were:

- to show the effect of tenapanor on the change in serum phosphate levels from baseline to the end of 8 weeks of treatment
- to compare the effect of different tenapanor dosing regimens on the number of participants reaching serum phosphate goal levels defined as <5.5 mg/dL during 8 weeks of treatment
- to evaluate the safety and tolerability of tenapanor as assessed by adverse event recording, stool form and frequency, vital signs, 12-lead electrocardiogram, physical examination, and clinical laboratory tests.

The exploratory objectives of this study included:

- to compare the effect of tenapanor on serum parathyroid hormone (PTH) levels during 8 weeks of treatment
- to compare the effect of tenapanor on intact serum fibroblast growth factor 23 (FGF23) levels during 8 weeks of treatment.

Study Assessments

Study drug exposure and adherence

Days of exposure to study drug were summarized with descriptive statistics by study period and treatment group for each of the analysis sets. Summary statistics were also presented for adherence to study drug in each treatment period by treatment group for each of the analysis sets. The percentage adherence to study drug was calculated as the total number of tablets dispensed minus the total number of tablets returned divided by two times the number of days during the treatment period, then multiplied by 100.

Serum intact FGF23 assay

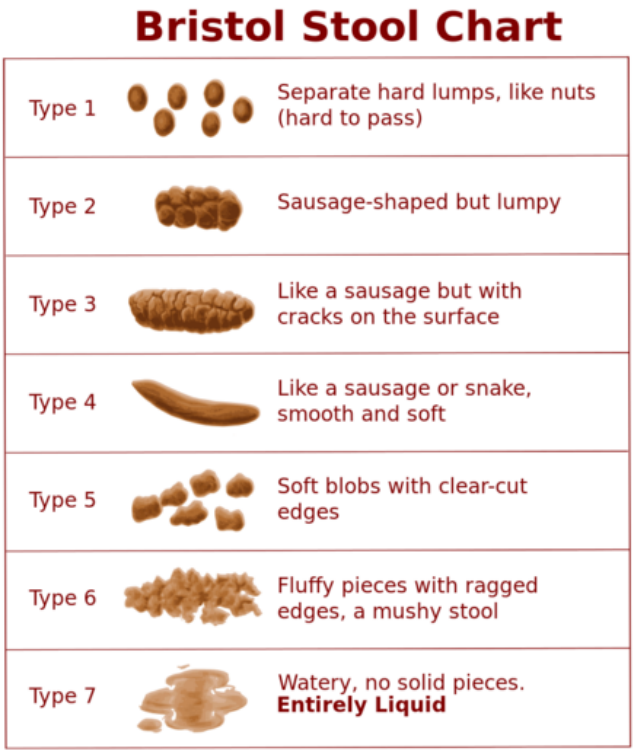
Intact FGF23 in serum was assessed using the Kainos Laboratories (Tokyo, Japan) FGF23 ELISA kit. This is a two-site enzyme-linked immunosorbent assay, with two specific murine monoclonal antibodies that bind to full-length FGF23. One antibody is immobilized onto a microtiter plate well for capture, and the other antibody is conjugated to horseradish peroxidase for detection. A sandwich complex is formed after the addition of the horseradish peroxidase-labelled antibody. Tetramethylbenzidine substrate is added to the wells and then measured on a Tecan Sunrise microplate reader at 450 nm. The enzymatic activity of the complex bound to the well is directly proportional to the amount of FGF23 in the sample.

Serum intact PTH assay

Intact PTH in serum was assessed using the Roche Diagnostics (Indianapolis, Indiana) Elecsys assay. The assay employs a sandwich test principle, in which a biotinylated monoclonal antibody reacts with the N-terminal fragment (1–37) of PTH and a monoclonal antibody labeled with a ruthenium complex reacts with the C-terminal fragment (38–84) of PTH.

Stool frequency and consistency

Participants called into a phone diary every day between 17:00 and 23:59 (local time) from the screening visit through to the last visit at the end of the study. They answered questions about stool form for each bowel movement, according to the Bristol Stool Form Scale (Lewis SJ, Heaton KW. *Scand J Gastroenterol* 32: 920–924, 1997) shown below and the number of bowel movements they have each day. Any increase in bowel movement frequency or loosening of the stool, regardless of the magnitude of the effect, was classified as an adverse event of 'diarrhea'.



Adverse event (AE) recording

Treatment-emergent AEs are presented. AEs were considered to be treatment-emergent during the RTP if the start date of the event was on or after the day of first dose of study drug through the completion of the RTP. Any AE considered drug-related regardless of the start date of the event, or any event that was present at baseline but worsened in severity or was subsequently

considered drug-related by the investigator, was also considered to be a treatment-emergent AE. AEs were considered treatment-emergent during the RWP if the start date of the event was on or after the day of first dose of study drug in the RWP through the final visit of the study. Any AE considered drug-related regardless of the start date of the event, or any event that was present at screening/washout/baseline and/or the 8-week RTP but worsened in severity (compared with both screening/washout/baseline and the 8-week RTP if applicable) in the RWP or was subsequently considered drug-related by the investigator, was also considered to be a treatment-emergent AE. If a participant had more than one occurrence of the same treatment-emergent AE, he/she was counted only once within the system organ class and preferred term.

Statistical Analyses

Sample size calculation

A sample size of 39 participants in the pooled tenapanor treatment and placebo groups would have 90% power to detect a difference in the change in mean serum phosphate from the end of the 8-week RTP to the end of the 4-week RWP with at least a 75% effect size; this effect size was based on a minimum 1.5 mg/dL difference between placebo and pooled tenapanor treatment with a standard deviation no greater than 2.0 mg/dl. A target enrollment of 200 participants allowed for a 20% dropout rate and a 50% responder rate (≥1.2 mg/dL serum phosphate reduction from baseline to end of RTP).

Other

The efficacy analyses utilized a patient's last study center visit as the endpoint visit. All statistical analyses were conducted using SAS (version 9.1.3 or higher; SAS institute, Inc, Cary, North Carolina).

Supplemental Table 1. Tenapanor dosing regimens

Tenapanor Regimen	Morning	Evening	Total Daily Dose
0 mg (RWP only)	0 + 0 mg	0 + 0 mg	0 mg
3 mg b.i.d.	3 + 0 mg	3 + 0 mg	6 mg
10 mg b.i.d.	10 + 0 mg	10 + 0 mg	20 mg
15 mg b.i.d.	15 + 0 mg	15 + 0 mg	30 mg
20 mg b.i.d.	10 + 10 mg	10 + 10 mg	40 mg
30 mg b.i.d.	30 + 0 mg	30 + 0 mg	60 mg

b.i.d., twice daily; RWP, randomized withdrawal period.

Supplemental Table 2. Proportion of patients with serum phosphate below 5.5 mg/dL during the randomized treatment period (RTP) (intention-to-treat analysis set)

	Tenapanor		
	3 mg b.i.d., *n* = 74	**10 mg b.i.d., *n* = 73**	**30 mg b.i.d. titration, *n* = 71**
Week 1			
Proportion	20/66	19/70	16/61
Percentage (%)	30.3	27.1	26.2
95% CI (%)	(19.6, 42.9)	(17.2, 39.1)	(15.8, 39.1)
Week 2			
Proportion	19/66	16/65	16/64
Percentage (%)	28.8	24.6	25.0
95% CI (%)	(18.3, 41.3)	(14.8, 36.9)	(15.0, 37.4)
Week 3			
Proportion	22/64	21/61	15/58
Percentage (%)	34.4	34.4	25.9
95% CI (%)	(22.9, 47.3)	(22.7, 47.7)	(15.3, 39.0)
Week 4			
Proportion	23/61	21/60	15/56
Percentage (%)	37.7	35.0	26.8
95% CI (%)	(25.6, 51.0)	(23.1, 48.4)	(15.8, 40.3)
Week 6			
Proportion	20/58	23/56	22/54
Percentage (%)	34.5	41.1	40.7
95% CI (%)	(22.5, 48.1)	(28.1, 55.0)	(27.6, 55.0)
Week 8			
Proportion	24/70	22/69	18/65
Percentage (%)	34.3	31.9	27.7
95% CI (%)	(23.3, 46.6)	(21.2, 44.2)	(17.3, 40.2)
End of RTP			
Proportion	24/74	23/72	20/69
Percentage (%)	32.4	31.9	29.0
95% CI (%)	(22.0, 44.3)	(21.4, 44.0)	(18.7, 41.2)

b.i.d., twice daily; CI, confidence interval; RTP, randomized treatment period.

Supplemental Table 3. Change in serum FGF23 from baseline to the end of the randomized treatment period (RTP) (intention-to-treat analysis set)

	Tenapanor		
	3 mg b.i.d., *n* = 74	**10 mg b.i.d., *n* = 73**	**30 mg b.i.d. titration, *n* = 71**
Baseline			
n	59	57	54
Mean ± SD	8137 ± 13 178	10 467 ± 22 682	10 994 ± 11 498
Geo. mean ± geo. CV	3455 ± 253	4112 ± 261	6089 ± 182
End of RTP			
n	57	57	54
Mean ± SD	6586 ± 11 245	9244 ± 13 883	8161 ± 8199
Geo. mean ± geo. CV	2489 ± 300	3682 ± 283	4558 ± 183
Change from baseline to end of RTP			
n	57	57	54
Mean ± SD	−102 ± 3890	−1223 ± 13 554	−2833 ± 8187
Ratio of geo. means (95% CI)	0.768 (0.656, 0.899)	0.887 (0.759, 1.037)	0.767 (0.652, 0.902)

FGF23 data are pg/mL.

Geo. means, geo. CVs, and 95% CIs are from an ANCOVA model with treatment and pooled investigator site as fixed factors, and baseline FGF23 (log-transformed) as a covariate.

ANCOVA, analysis of covariance; b.i.d., twice daily; CI, confidence interval; FGF23, fibroblast growth factor 23; geo. CV, geometric coefficient of variation (%); geo. mean, geometric mean; RTP, randomized treatment period.

Supplemental Table 4. AEs occurring in at least 2% of patients in any treatment group

Randomized treatment period			
	Tenapanor		
	3 mg b.i.d., *n* = 74	**10 mg b.i.d., *n* = 73**	**30 mg b.i.d. titration, *n* = 71**
Participants with any AE	39 (52.7)	51 (69.9)	49 (69.0)
Gastrointestinal disorders	24 (32.4)	35 (47.9)	40 (56.3)
Diarrhea	22 (29.7)	30 (41.1)	34 (47.9)
Vomiting	2 (2.7)	3 (4.1)	3 (4.2)
Flatulence	2 (2.7)	3 (4.1)	2 (2.8)
Abdominal discomfort	1 (1.4)	4 (5.5)	1 (1.4)
Abdominal distension	0 (0.0)	1 (1.4)	2 (2.8)
Abdominal pain	0 (0.0)	3 (4.1)	0 (0.0)
Abdominal pain upper	2 (2.7)	1 (1.4)	0 (0.0)
Frequent bowel movements	0 (0.0)	3 (4.1)	0 (0.0)
Nausea	2 (2.7)	1 (1.4)	0 (0.0)
Defecation urgency	0 (0.0)	2 (2.7)	0 (0.0)
Infections and infestations	11 (14.9)	5 (6.8)	8 (11.3)
Cellulitis	3 (4.1)	2 (2.7)	1 (1.4)
Nasopharyngitis	1 (1.4)	1 (1.4)	2 (2.8)
Pneumonia	2 (2.7)	1 (1.4)	0 (0.0)
Upper respiratory tract infection	1 (1.4)	0 (0.0)	2 (2.8)
Metabolism and nutrition disorders	4 (5.4)	10 (13.7)	9 (12.7)
Hyperphosphatemia	3 (4.1)	5 (6.8)	4 (5.6)
Fluid overload	1 (1.4)	1 (1.4)	2 (2.8)
Hypocalcemia	0 (0.0)	1 (1.4)	2 (2.8)
Injury, poisoning, and procedural complications	5 (6.8)	11 (15.1)	5 (7.0)
Arteriovenous fistula site complication	0 (0.0)	2 (2.7)	2 (2.8)
Vascular graft complication	0 (0.0)	3 (4.1)	1 (1.4)
Wound	1 (1.4)	2 (2.7)	0 (0.0)

Arteriovenous fistula thrombosis	2 (2.7)	0 (0.0)	0 (0.0)
General disorders and administration site conditions	7 (9.5)	5 (6.8)	3 (4.2)
Non-cardiac chest pain	2 (2.7)	0 (0.0)	0 (0.0)
Skin and subcutaneous tissue disorders	3 (4.1)	4 (5.5)	2 (2.8)
Pruritus	1 (1.4)	2 (2.7)	1 (1.4)
Cardiac disorders	3 (4.2)	2 (2.7)	3 (4.2)
Tachycardia	2 (2.7)	1 (1.4)	0 (0.0)

Randomized withdrawal period				
	Placebo, *n* = 82	**Tenapanor**		
		3 mg b.i.d., *n* = 25	**10 mg b.i.d., *n* = 23**	**30 mg b.i.d. titration, *n* = 34**
Participants with any AE	21 (25.6)	4 (16.0)	7 (30.4)	12 (35.3)
Metabolism and nutrition disorders	7 (8.5)	0 (0.0)	1 (4.3)	3 (8.8)
Hyperphosphatemia	3 (3.7)	0 (0.0)	1 (4.3)	0 (0.0)
Hyperkalemia	0 (0.0)	0 (0.0)	1 (4.3)	2 (5.9)
Fluid overload	2 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)
Hypermagnesemia	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Injury, poisoning, and procedural complications	4 (4.9)	2 (8.0)	0 (0.0)	2 (5.9)
Arteriovenous fistula site complication	1 (1.2)	1 (4.0)	0 (0.0)	0 (0.0)
Contusion	1 (1.2)	0 (0.0)	0 (0.0)	1 (2.9)
Arteriovenous fistula site hemorrhage	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)
Vascular graft thrombosis	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Infections and infestations	2 (2.4)	2 (8.0)	1 (4.3)	2 (5.9)
Sinusitis	0 (0.0)	1 (4.0)	0 (0.0)	1 (2.9)
Fungal skin infection	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Gastrointestinal viral infection	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)

Upper respiratory tract infection	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)
Gastrointestinal disorders	4 (4.9)	0 (0.0)	0 (0.0)	2 (5.9)
Diarrhea	2 (2.4)	0 (0.0)	0 (0.0)	1 (2.9)
Abdominal pain	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Food poisoning	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Investigations	2 (2.4)	1 (4.0)	0 (0.0)	2 (5.9)
Anticoagulation drug level above therapeutic	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Blood urea increased	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Venous pressure increased	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)
Respiratory, thoracic, and mediastinal disorders	1 (1.2)	1 (4.0)	3 (13.0)	0 (0.0)
Asthma	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)
Rales	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)
Rhinorrhea	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)
Throat irritation	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)

Data are number of patients experiencing AE (%) by system organ class and preferred term.

AE, adverse event; b.i.d., twice daily.

Supplemental Table 5. Treatment-related AEs occurring in at least 2% of patients in any treatment group

Randomized treatment period				
		Tenapanor		
	3 mg b.i.d., *n* = 74	**10 mg b.i.d., *n* = 73**	**30 mg b.i.d. titration, *n* = 71**	
Participants with any treatment-related AE	24 (32.4)	38 (52.1)	33 (46.5)	
Gastrointestinal disorders	21 (28.4)	34 (46.6)	31 (43.7)	
Diarrhea	19 (25.7)	30 (41.1)	28 (39.4)	
Flatulence	1 (1.4)	3 (4.1)	2 (2.8)	
Abdominal discomfort	0 (0.0)	3 (4.1)	1 (1.4)	
Abdominal distension	0 (0.0)	1 (1.4)	2 (2.8)	
Abdominal pain	0 (0.0)	3 (4.1)	0 (0.0)	
Frequent bowel movements	0 (0.0)	3 (4.1)	0 (0.0)	
Abdominal pain upper	2 (2.7)	0 (0.0)	0 (0.0)	
Defecation urgency	0 (0.0)	2 (2.7)	0 (0.0)	
Metabolism and nutrition disorders	1 (1.4)	6 (8.2)	2 (2.8)	
Hyperphosphatemia	1 (1.4)	4 (5.5)	1 (1.4)	
Randomized withdrawal period				
	Placebo, *n* = 82	**Tenapanor**		
		3 mg b.i.d., *n* = 25	**10 mg b.i.d., *n* = 23**	**30 mg b.i.d. titration, *n* = 34**
Participants with any treatment-related AE	5 (6.1)	0 (0.0)	1 (4.3)	0 (0.0)
Metabolism and nutrition disorders	3 (3.7)	0 (0.0)	1 (4.3)	0 (0.0)
Hyperphosphatemia	2 (2.4)	0 (0.0)	1 (4.3)	0 (0.0)

Data are number of patients experiencing AE (%) by system organ class and preferred term.

AE, adverse event; b.i.d., twice daily.

Supplemental Table 6. Serum chemistry and hematology values

Randomized Treatment Period			
		Tenapanor	
	3 mg b.i.d., *n* = 74	**10 mg b.i.d., *n* = 73**	**30 mg b.i.d. titration, *n* = 71**
Albumin, g/dl			
Baseline	3.90 ± 0.33	3.89 ± 0.27	3.94 ± 0.28
End of period	3.92 ± 0.34	3.84 ± 0.25	3.90 ± 0.27
Bicarbonate, mmol/L			
Baseline	24.5 ± 3.2	24.2 ± 3.0	23.9 ± 2.6
End of period	23.9 ± 3.2	24.0 ± 3.5	23.7 ± 2.8
Calcium, mg/dl			
Baseline	8.68 ± 0.90	8.69 ± 0.72	8.59 ± 0.77
End of period	8.77 ± 0.73	8.71 ± 0.74	8.57 ± 0.93
Chloride, mmol/L			
Baseline	96.6 ± 3.3	96.9 ± 3.5	96.8 ± 3.4
End of period	97.0 ± 3.3	96.9 ± 3.2	97.3 ± 3.5
Glucose, mg/dl			
Baseline	156.4 ± 80.3	154.2 ± 65.2	157.4 ± 109.7
End of period	150.2 ± 78.7	165.4 ± 70.1	153.2 ± 71.0
Hemoglobin, g/dl			
Baseline	11.11 ± 1.45	10.75 ± 1.37	10.77 ± 1.32
End of period	11.16 ± 1.60	10.96 ± 1.22	11.15 ± 1.26
Potassium, mmol/L			
Baseline	4.62 ± 0.65	4.72 ± 0.61	4.74 ± 0.69
End of period	4.72 ± 0.66	4.65 ± 0.67	4.82 ± 0.83
Sodium, mmol/L			
Baseline	136.1 ± 2.6	136.3 ± 2.8	136.6 ± 3.2
End of period	136.1 ± 2.3	135.8 ± 3.0	136.1 ± 2.5

Randomized Withdrawal Period				
	Placebo, *n* = 82	**Tenapanor**		
		3 mg b.i.d., *n* = 25	**10 mg b.i.d., *n* = 23**	**30 mg b.i.d. titration, *n* = 34**
Albumin, g/dl				
Baseline	3.91 ± 0.34	3.87 ± 0.32	3.92 ± 0.28	3.97 ± 0.22
End of period	3.88 ± 0.29	3.97 ± 0.34	3.89 ± 0.23	3.97 ± 0.28

Bicarbonate, mmol/L				
Baseline	24.5 ± 2.7	24.0 ± 3.2	23.6 ± 3.2	24.0 ± 3.0
End of period	24.0 ± 2.7	23.1 ± 2.2	23.3 ± 2.6	23.4 ± 3.0
Calcium, mg/dl				
Baseline	8.67 ± 0.78	8.68 ± 0.94	8.68 ± 0.81	8.47 ± 0.80
End of period	8.63 ± 0.73	8.65 ± 0.74	8.80 ± 0.64	8.74 ± 0.86
Chloride, mmol/L				
Baseline	97.2 ± 3.4	96.1 ± 3.2	97.2 ± 3.2	96.5 ± 3.1
End of period	97.3 ± 3.4	95.7 ± 3.9	97.8 ± 3.7	97.2 ± 3.5
Glucose, mg/dl				
Baseline	145.4 ± 58.4	189.2 ± 105.1	145.7 ± 60.9	159.8 ± 82.1
End of period	152.4 ± 84.7	164.3 ± 83.9	160.0 ± 89.2	155.5 ± 74.0
Hemoglobin, g/dl				
Baseline	11.00 ± 1.40	11.33 ± 1.59	10.56 ± 1.44	10.72 ± 1.35
End of period	10.96 ± 1.19	11.77 ± 1.90	10.73 ± 1.34	11.09 ± 1.57
Potassium, mmol/L				
Baseline	4.60 ± 0.56	4.66 ± 0.59	4.80 ± 0.67	4.85 ± 0.81
End of period	4.59 ± 0.68	4.54 ± 0.48	4.80 ± 0.69	4.90 ± 0.81
Sodium, mmol/L				
Baseline	136.4 ± 2.6	136.0 ± 2.6	136.6 ± 2.6	136.6 ± 3.1
End of period	136.3 ± 3.3	135.6 ± 2.5	136.3 ± 3.1	136.1 ± 2.8

Data are mean ± SD. Baseline is the pre-dose value on day 1.

b.i.d., twice daily.