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Add-On Treatment With Zilebesiran for Inadequately Controlled Hypertension
The KARDIA-2 Randomized Clinical Trial

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IMPORTANCE In prior monotherapy studies of patients with hypertension, single subcutaneous doses of zilebesiran, an investigational RNA interference therapeutic, reduced serum angiotensinogen levels and systolic blood pressure (SBP) at 3 and 6 months.

OBJECTIVE To evaluate the efficacy and safety of zilebesiran vs placebo when added to a standard antihypertensive medication.

DESIGN, SETTING, AND PARTICIPANTS This phase 2, randomized, prospective, double-blinded trial enrolled adults with uncontrolled hypertension from 150 sites across 8 countries between January 2022 and June 2023. The final follow-up date was December 11, 2023, and analyses were conducted on March 1, 2024.

INTERVENTIONS Eligible patients were initially randomized in cohorts to receive open-label run-in treatment for at least 4 weeks with indapamide 2.5 mg, amlodipine 5 mg, or olmesartan 40 mg (4:7:10 randomization), each administered once daily. Within cohorts, adherent patients with 24-hour mean ambulatory SBP of 130 mm Hg to 160 mm Hg were subsequently randomized (1:1) to additional blinded treatment to receive single subcutaneous doses of zilebesiran 600 mg or matching placebo.

MAIN OUTCOMES AND MEASURES The primary end point in each cohort was the difference between zilebesiran and placebo in change from baseline in 24-hour mean ambulatory SBP at 3 months.

RESULTS Of 1491 patients entering the run-in phase, 663 (130 receiving indapamide, 240 receiving amlodipine, and 293 receiving olmesartan) were randomized to receive zilebesiran (n = 332) or placebo (n = 331). The least-squares mean difference between zilebesiran and placebo in change from baseline to 3 months in 24-hour mean ambulatory SBP was −12.1 mm Hg (95% CI, −16.5 to −7.6; $P < .001$) for indapamide, −9.7 mm Hg (95% CI, −12.9 to −6.6; $P < .001$) for amlodipine, and −4.5 mm Hg (95% CI, −8.2 to −0.8; $P = .02$) for olmesartan. Across cohorts, more patients who received zilebesiran than placebo experienced hyperkalemia (18 [5.5%] vs 6 [1.8%]), hypotension (14 [4.3%] vs 7 [2.1%]), and acute kidney failure (16 [4.9%] vs 5 [1.5%]) events, but most episodes were mild and resolved without medical intervention.

CONCLUSIONS AND RELEVANCE In patients with uncontrolled hypertension despite treatment with indapamide, amlodipine, or olmesartan, the addition of single-dose zilebesiran resulted in significant SBP reductions compared with placebo at 3 months, with low rates of serious adverse events.

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Supplemental content

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Despite the availability of effective antihypertensive therapies, many patients treated for hypertension do not meet guideline-recommended blood pressure (BP) targets, leaving them at risk for cardiovascular events.[1] Even among treated patients, residual BP variability[2-5] over the diurnal cycle and between treatment encounters contributes to heightened cardiovascular risk. Among other factors, suboptimal BP control is heavily influenced by therapeutic inertia and poor patient adherence to daily oral antihypertensive medications.[6-9] In this context, there is an unmet need for strategies to improve BP control without enhancing treatment complexity.

Zilebesiran is an investigational, subcutaneously administered RNA interference therapeutic that targets hepatic synthesis of angiotensinogen, the most upstream precursor to angiotensin peptides. With its prolonged duration of action and continuous control of BP over the full 24-hour diurnal cycle, zilebesiran offers an alternative approach to hypertension treatment that may overcome key obstacles to achieving optimal BP control associated with currently available therapies. In prior phase 1 and 2 studies, treatment with single subcutaneous doses of zilebesiran was associated with dose-related reductions in serum angiotensinogen levels and clinically meaningful reductions in systolic BP (SBP) enduring up to 6 months, with few serious adverse events (AEs).[10,11]

In clinical practice, patients with hypertension commonly require combination treatment with more than 1 agent to achieve adequate BP control.[12] KARDIA-2 was designed to evaluate the efficacy and safety of zilebesiran as add-on therapy in patients with BP inadequately controlled with a first-line antihypertensive.

Key Points

Question Does adding the RNA interference therapeutic zilebesiran to a commonly used first-line antihypertensive drug produce additional blood pressure reductions in patients with inadequately controlled hypertension?

Findings In this phase 2 trial, a single subcutaneous dose of zilebesiran 600 mg added to indapamide, amlodipine, or olmesartan background therapy showed significant additional reductions in 24-hour mean ambulatory and office systolic blood pressure at 3 months, regardless of background treatment.

Meaning These data support the potential for use of subcutaneously administered zilebesiran as an effective and well-tolerated treatment for continuous control of blood pressure in combination with commonly used first-line oral antihypertensive medications.

Methods

Study Design and Participants

KARDIA-2 was a phase 2, prospective, randomized, placebo-controlled study conducted at 150 sites in Canada, Estonia, Germany, Latvia, Lithuania, Poland, the UK, and the US. The study protocol was approved by an institutional review board or ethics committee at each participating center before enrollment of the first patient. Patients provided written informed consent for study participation. The trial was conducted in accordance with International Council for Harmonization Good Clinical Practice Guidelines and the principles of the Declaration of Helsinki.

Eligible patients were aged 18 to 75 years with untreated hypertension (mean seated office SBP of at least 155 mm Hg but not higher than 180 mm Hg) or uncontrolled hypertension despite 1 or 2 antihypertensive agents (mean seated office SBP of at least 145 mm Hg but not higher than 180 mm Hg). Patients with known secondary hypertension; symptomatic orthostatic hypotension; serum potassium greater than 5.0 mmol/L; estimated glomerular filtration rate (eGFR) less than 30 mL/min/1.73 m^2 (by Modification of Diet in Renal Disease method[13]); symptomatic heart failure; or type 1, poorly controlled type 2, or newly diagnosed diabetes were excluded. Details of study design and eligibility criteria are provided in the study protocol in Supplement 1. This study followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline.

Study Procedures

The study included an open-label run-in period of at least 4 weeks and a 6-month double-blind treatment period (eFigure 1 in Supplement 2). Following discontinuation of antihypertensive therapies, eligible patients with screening eGFR less than 45 mL/min/1.73 m^2 or urine albumin:creatinine ratio of 300 mg/g or more were preferentially assigned to the olmesartan run-in cohort. Remaining patients were randomized in 4:7:10 ratio to receive open-label treatment with indapamide 2.5 mg once daily, amlodipine 5 mg once daily, or olmesartan 40 mg once daily (20 mg once daily for patients with eGFR ≤60 mL/min/1.73 m^2 enrolled outside the US, per local labeling), respectively, for at least 4 weeks. These background therapies were intended to represent antihypertensive drug classes and doses commonly used in clinical practice, with maximum-dose olmesartan selected to test efficacy and particularly safety of zilebesiran in combination with a potent renin-angiotensin system (RAS) inhibitor. After the run-in period, patients in each background therapy cohort with 24-hour mean ambulatory SBP between 130 mm Hg and 160 mm Hg and at least 80% adherence to protocol-specified background therapy were randomized in a 1:1 ratio to receive a single subcutaneous dose of zilebesiran 600 mg or matching placebo.

Automated seated office BP measurements[14] were conducted in triplicate and the mean was calculated at each study visit after a 5-minute rest period, based on the method used in the SPRINT trial.[15] Ambulatory BP assessments were conducted using an automated device[16,17] that collected measurements every 20 minutes during the day and every 30 minutes during the night for a 24-hour period. Prior to month 3, no changes to background antihypertensives were permitted outside of rescue treatment for severe or symptomatic hypertension. Subsequently, investigators were instructed to add rescue antihypertensives to achieve guideline-recommended BP

targets. Further methodological details are provided in the eMethods in Supplement 2.

Outcomes

The primary end point was the difference in the change from baseline to 3 months in 24-hour mean ambulatory SBP between zilebesiran and placebo for each background therapy cohort. Key secondary end points were tested hierarchically in each cohort in the following order: between-group difference in change from baseline at 3 months in office SBP, time-adjusted change from baseline through 6 months in 24-hour mean ambulatory SBP, time-adjusted change from baseline through 6 months in office SBP, and percentage of patients meeting the protocol-defined BP response criterion at 6 months (24-hour mean ambulatory SBP less than 130 mm Hg and/or reduction from baseline of at least 20 mm Hg without rescue antihypertensive medication). SBP assessments at month 6 were analyzed as time-adjusted changes from baseline to evaluate the consistency of zilebesiran treatment effect throughout the 6-month period. Serum angiotensinogen levels were measured at each visit in a central laboratory using enzyme-linked immunosorbent assays. Safety outcomes were rates of investigator-reported AEs (defined per Medical Dictionary for Regulatory Activities terminology) and occurrence of protocol-defined laboratory abnormalities.

Statistical Analysis

The sample size for each cohort was estimated assuming an SD of 16 mm Hg in the change from baseline in 24-hour mean ambulatory SBP at 3 months, a 15% dropout rate, and a 2-sided type I error rate of .05. A sample size of 630 patients (120 patients in the indapamide cohort, 210 patients in the amlodipine cohort, and 300 patients in the olmesartan cohort) was predicted to provide at least 80% power to detect differences in the change from baseline in 24-hour mean ambulatory SBP of 8 mm Hg, 6 mm Hg, and 5 mm Hg between zilebesiran and placebo in the indapamide, amlodipine, and olmesartan cohorts, respectively.

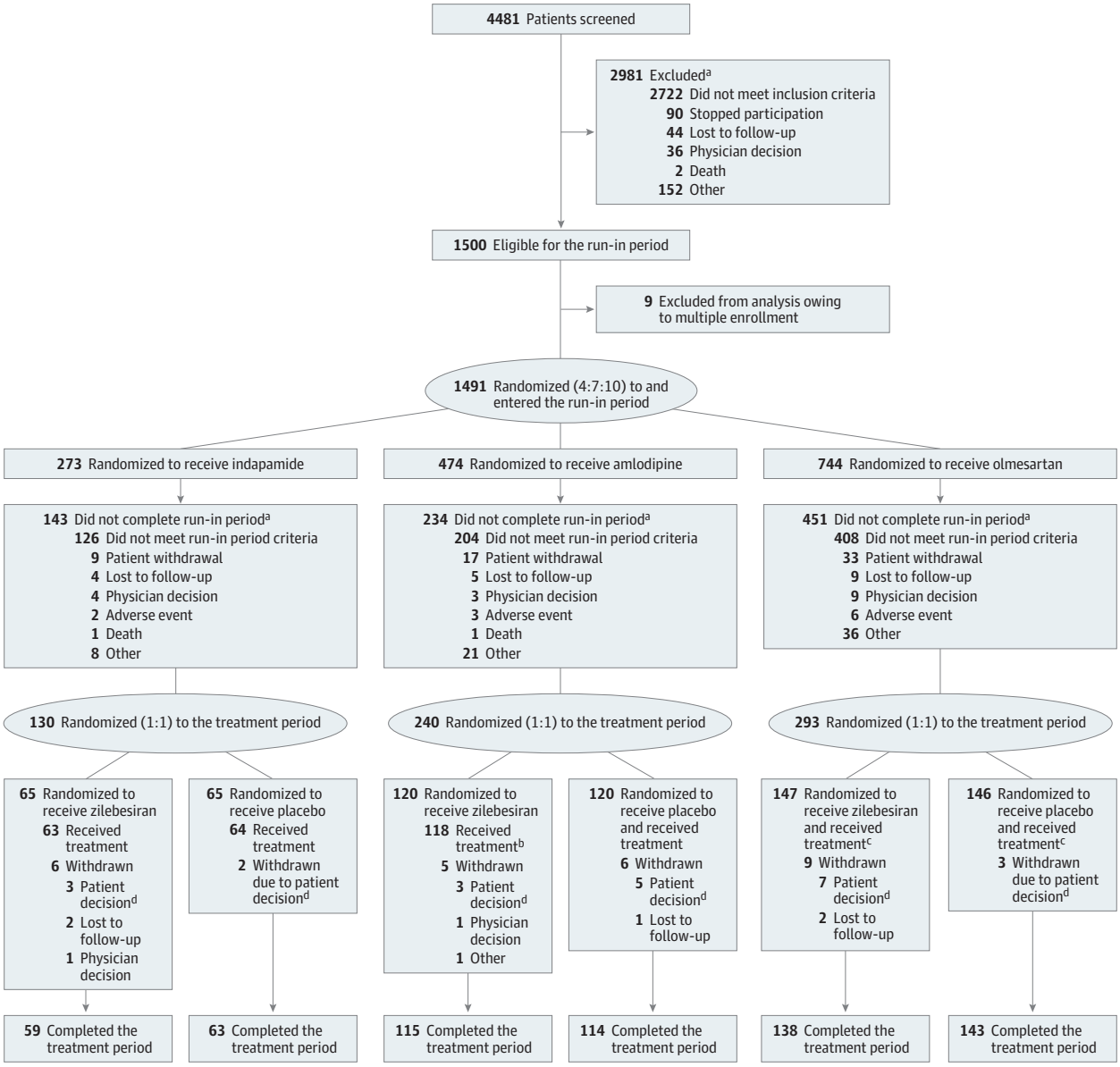
All analyses were performed separately within each of the 3 background therapy cohorts. To control the overall type I error at .05 in each cohort, the primary end point and the key secondary end points were tested in hierarchical order as outlined in the outcome section above. The primary end point and secondary end points of change from baseline in office SBP at 3 months and time-adjusted SBP through 6 months were evaluated as the least-squares mean difference (hereafter referred to as *difference*) in change from baseline in SBP between treatment groups using a mixed model for repeated measures. For the 3-month BP analyses, assessments from patients receiving rescue antihypertensive therapy within 2 weeks of an assessment were censored. Time-adjusted SBP was calculated as the weighted mean of change from baseline at each study visit. The proportion of patients meeting the prespecified BP response criterion was assessed as odds ratios (ORs) estimated from logistic regression models. The percentage change from baseline in serum angiotensinogen levels by visit and laboratory assessments and frequency of AEs by treatment group were summarized in each cohort using descriptive statistics. Further details of baseline assessments and statistical analyses are reported in the eMethods in Supplement 2 and the statistical analysis plan in Supplement 3. Analyses were conducted using SAS statistical software version 9.4 (SAS Institute).

Results

Patients were enrolled between January 2022 and June 2023. Owing to discovery of multiple patient enrollments after initial database lock, 4 patients accounting for 9 patient IDs were excluded from analyses. After these exclusions, 4472 patients were screened for enrollment and 1491 (33.3%) were randomized to receive background antihypertensive treatment in the open-label run-in period, including 273 receiving indapamide, 474 receiving amlodipine, and 744 receiving olmesartan. Of these, 663 patients (44.5%) (130 receiving indapamide, 240 receiving amlodipine, and 293 receiving olmesartan) met criteria for randomization to receive zilebesiran or placebo in the double-blinded period (**Figure 1**). Two patients with eGFR less than 45 mL/min/1.73 m^2, intended for randomization to the olmesartan cohort, were incorrectly randomized to the indapamide (n = 1) and amlodipine (n = 1) cohorts. Three patients assigned to the indapamide cohort and 1 patient assigned to the amlodipine cohort were randomized but not treated. One patient in the amlodipine cohort was excluded owing to a Good Clinical Practice violation. One patient assigned to the placebo group of the olmesartan cohort erroneously received a partial dose of zilebesiran (100 mg) (see eMethods in Supplement 2 for further details). Of the 293 patients in the olmesartan cohort, 11 (3.8%) received 20 mg once daily based on kidney function, per local guidelines. Six-month follow-up for all analyses was complete for 95% of patients.

At the end of the run-in period and before dosing of zilebesiran or placebo, mean (SD) changes in office SBP were −15.5 (15.6) mm Hg with indapamide, −14.7 (15.2) mm Hg with amlodipine, and −13.0 (18.0) mm Hg with olmesartan. Baseline characteristics of the 658 patients in the full analysis set are summarized by cohort in **Table 1**. For the overall population, the mean (SD) age was 58.5 (10.3) years, mean (SD) 24-hour ambulatory SBP was 143.4 (8.2) mm Hg, mean (SD) office SBP was 144.5 (12.2) mm Hg, 376 participants (57.1%) were male, 187 (28.4%) self-reported as Black or African American, 151 (22.9%) had diabetes, 398 (60.5%) had obesity (body mass index ≥30), 66 (10.0%) had eGFR less than 60 mL/min/1.73 m^2, and 77 (11.7%) were previously untreated for hypertension. Of those 581 participants previously treated for hypertension, 304 (46.2%) were treated with 1 antihypertensive treatment, 239 (36.3%) were treated with 2, and 38 (5.8%) were treated with more than 2. Slight differences in baseline characteristics across cohorts were noted in the context of preferential assignment of patients with eGFR less than 45 mL/min/1.73 m^2 (9 [3.1%]) or urine albumin:creatinine ratio greater than 300 mg/g (12 [4.1%]) to the olmesartan cohort, as well as differential dropout during the run-in period.

Figure 1. Patient Flow in the KARDIA-2 Study of Add-On Treatment With Zilebesiran for Hypertension



Data are shown for the full analysis set. Owing to multiple patient enrollments, which were discovered after initial database lock, 4 patients accounting for 9 patient IDs were excluded from all analyses.

[a]A patient could have multiple reasons for exclusion or run-in failure and was counted separately for each reason.

[b]One patient was excluded owing to Good Clinical Practice Guidelines violation.

[c]One patient assigned to the placebo group accidentally received a partial dose of zilebesiran (100 mg), and is therefore included in the placebo group of the full analysis set and the zilebesiran group of the safety analysis set and excluded from the pharmacodynamic analysis set.

[d]No patients who stopped participating did so because of an adverse event.

At 3 months, the difference in change from baseline in 24-hour mean ambulatory SBP between zilebesiran and placebo was −12.1 mm Hg (95% CI, −16.5 to −7.6) for the indapamide cohort, −9.7 mm Hg (95% CI, −12.9 to −6.6) for the amlodipine cohort, and −4.5 mm Hg (95% CI, −8.2 to −0.8) for the olmesartan cohort (**Figure 2**A). The difference in change from baseline in office SBP at 3 months was also greater for zilebesiran than placebo and similar to or greater than the change observed in ambulatory SBP, regardless of background therapy, with a difference of −18.5 mm Hg (95% CI, −22.8 to −14.2) for indapamide, −10.2 mm Hg (95% CI, −13.4 to −6.9) for amlodipine, and −6.7 mm Hg (95% CI, −10.2 to −3.3) for olmesartan (Figure 2B).

The longitudinal trajectory of change from baseline in office SBP by visit and treatment group, along with the percentage of patients in each treatment group receiving rescue antihypertensives between months 3 and 6, is shown for each background therapy cohort in eFigure 2 in Supplement 2.

Table 1. Baseline Characteristics by Background Therapy Cohort[a]

Characteristic	Background medication						Overall	
	Indapamide		Amlodipine		Olmesartan			
	Zilebesiran (n = 63)	Placebo (n = 64)	Zilebesiran (n = 118)	Placebo (n = 120)	Zilebesiran (n = 147)	Placebo (n = 146)	Zilebesiran (n = 328)	Placebo (n = 330)
Age, mean (SD), y	57.9 (10.7)	60.6 (10.2)	57.6 (10.2)	58.4 (9.8)	59.3 (10.4)	57.7 (10.6)	58.5 (10.4)	58.5 (10.3)
Male, No. (%)	33 (52.4)	39 (60.9)	65 (55.1)	70 (58.3)	87 (59.2)	82 (56.2)	185 (56.4)	191 (57.9)
Female, No. (%)	30 (47.6)	25 (39.1)	53 (44.9)	50 (41.7)	60 (40.8)	64 (43.8)	143 (43.6)	139 (42.1)
Country of enrollment, No. (%)								
US	55 (87.3)	50 (78.1)	97 (82.2)	94 (78.3)	119 (81.0)	116 (79.5)	271 (82.6)	260 (78.8)
Canada	1 (1.6)	5 (7.8)	7 (5.9)	7 (5.8)	7 (4.8)	12 (8.2)	15 (4.6)	24 (7.3)
UK	3 (4.8)	6 (9.4)	11 (9.3)	15 (12.5)	13 (8.8)	10 (6.8)	27 (8.2)	31 (9.4)
Other	4 (6.3)	3 (4.7)	3 (2.5)	4 (3.3)	8 (5.4)	8 (5.5)	15 (4.6)	15 (4.5)
Race, No. (%)[b]								
Asian	4 (6.3)	0	8 (6.8)	4 (3.3)	3 (2.0)	13 (8.9)	15 (4.6)	17 (5.2)
Black or African American	16 (25.4)	14 (21.9)	39 (33.1)	41 (34.2)	38 (25.9)	39 (26.7)	93 (28.4)	94 (28.5)
Multiracial	0	0	0	0	0	1 (0.7)	0	1 (0.3)
Native Hawaiian or Other Pacific Islander	1 (1.6)	0	0	0	0	0	1 (0.3)	0
White	41 (65.1)	48 (75.0)	71 (60.2)	74 (61.7)	106 (72.1)	93 (63.7)	218 (66.5)	215 (65.2)
Other	1 (1.6)	1 (1.6)	0	0	0	0	1 (0.3)	1 (0.3)
Not reported	0	1 (1.6)	0	1 (0.8)	0	0	0	2 (0.6)
24-h Ambulatory SBP, mean (SD), mm Hg	143.4 (8.5)	143.2 (8.4)	143.3 (7.8)	142.6 (8.2)	143.6 (8.2)	144.2 (8.3)	143.5 (8.1)	143.4 (8.3)
24-h mean Ambulatory SBP ≥145 mm Hg, No. (%)	31 (49.2)	28 (43.8)	46 (39.0)	48 (40.0)	67 (45.6)	69 (47.3)	144 (43.9)	145 (43.9)
Office SBP, mean (SD), mm Hg	143.9 (12.1)	145.4 (11.5)[c]	142.8 (11.5)	144.1 (11.5)	144.8 (12.2)	145.8 (13.6)[d]	143.9 (12.0)	145.1 (12.5)
BMI ≥30, No. (%)	46 (73.0)	39 (60.9)[c]	69 (58.5)	79 (65.8)	80.0 (54.4)[d]	85 (58.2)[c]	195 (59.5)	203 (61.5)
eGFR <60 mL/min/1.73 m^2, No. (%)[e]	10 (15.9)	10 (15.6)	6 (5.1)	7 (5.8)	17 (11.6)	16 (11.0)	33 (10.1)	33 (10.0)
Type 2 diabetes, No. (%)[f]	14 (22.2)	13 (20.3)	26 (22.0)	27 (22.5)	38 (25.9)	33 (22.6)	78 (23.8)	73 (22.1)
Any prior antihypertensive treatment, No. (%)[g]	56 (88.9)	61 (95.3)	98 (83.1)	102 (85.0)	132 (89.2)	132 (91.0)	286 (86.9)	295 (89.7)
No. of prior antihypertensives, No. (%)[h]								
0	7 (11.1)	3 (4.7)	20 (16.9)	18 (15.0)	16 (10.9)	13 (8.9)	47 (13.1)	34 (10.3)
1	33 (52.4)	33 (51.6)	57 (48.3)	55 (45.8)	63 (42.9)	63 (43.2)	153 (46.6)	151 (45.8)
2	19 (30.2)	25 (39.1)	37 (31.4)	41 (34.2)	61 (41.5)	56 (38.4)	117 (35.7)	122 (37.0)
>2	4 (6.3)	3 (4.7)	4 (3.4)	6 (5.0)	7 (4.8)	14 (9.6)	15 (4.6)	23 (7.0)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure.

[a] Analyses are presented for the full analysis set unless otherwise specified.

[b] Race was self-reported by study participants based on fixed categories. Race and ethnicity data were collected to assess the diversity and generalizability of the study and because there are well-recognized differences in response to antihypertensive medications by ethnicity.

[c] Assessment missing for 1 patient.

[d] Assessment missing for 2 patients.

[e] eGFR was calculated based on the Modification of Diet in Renal Disease equation. Patients with screening eGFR <45 mL/min/1.73 m^2 or urine albumin:creatinine ratio of ≥300 mg/g were preferentially assigned to receive olmesartan.

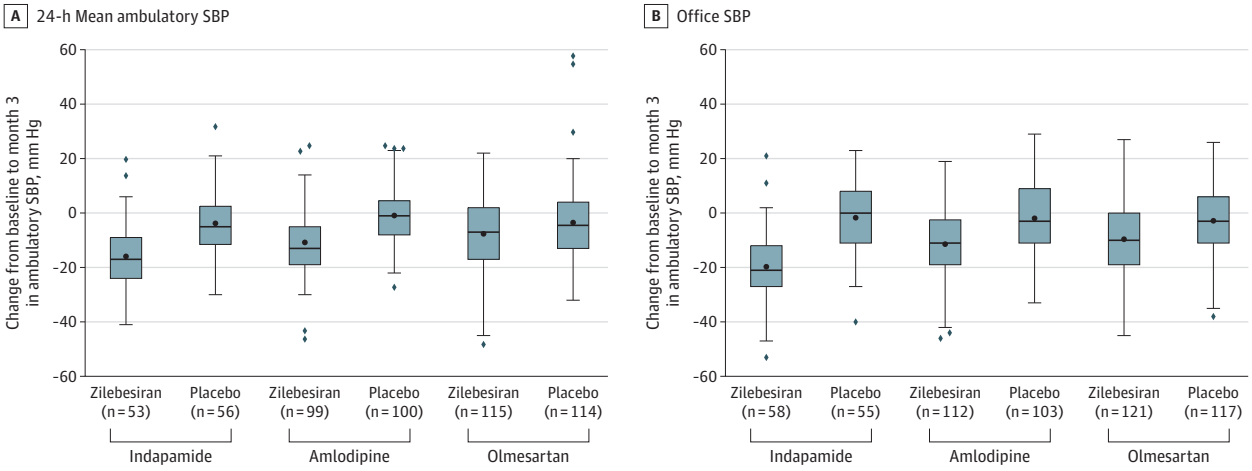
[f] Type 2 diabetes was defined as medical history of diabetes (excluding gestational diabetes) based on review of electronic medical chart data; patients with type 1 diabetes were excluded from participating.

[g] Analysis presented in the safety analysis set: indapamide + placebo, n = 64; indapamide + zilebesiran, n = 63; amlodipine + placebo, n = 120; amlodipine + zilebesiran, n = 118; olmesartan + placebo, n = 145; olmesartan + zilebesiran, n = 148.

[h] Prior antihypertensive medications are any medications taken prior to randomization to background medication.

In the indapamide cohort, more patients in the placebo group than in the zilebesiran group received rescue antihypertensive therapy at month 6 (25 [41.7%] vs 9 [15.5%]). Despite this, the between-group difference in the time-adjusted change from baseline in both ambulatory (difference, −11.0 mm Hg [95% CI, −14.7 to −7.3]) and office (difference, −13.6 mm Hg [95% CI, −16.9 to −10.3]) SBP remained statistically significant through 6 months (**Table 2**). Accordingly, a greater percentage of patients receiving zilebesiran (34 [64.2%]) than placebo (8 [14.0%]) met the prespecified

Figure 2. Change From Baseline at 3 Months in Systolic Blood Pressure (SBP) by Background Therapy Cohort and Treatment Group



LSM difference (95% CI)	
Indapamide	-12.1 (-16.5 to -7.6); *P* <.001
Amlodipine	-9.7 (-12.9 to -6.6); *P* <.001
Olmesartan	-4.5 (-8.2 to -0.8); *P* = .018
LSM change from baseline (95% CI)	
Indapamide	
Zilebesiran	-15.7 (-18.9 to -12.6)
Placebo	-3.7 (-6.7 to -0.6)
Amlodipine	
Zilebesiran	-10.5 (-12.7 to -8.2)
Placebo	-0.7 (-3.0 to 1.5)
Olmesartan	
Zilebesiran	-7.7 (-10.3 to -5.1)
Placebo	-3.2 (-5.9 to -0.6)

LSM difference (95% CI)	
Indapamide	-18.5 (-22.8 to -14.20); *P* <.001
Amlodipine	-10.2 (-13.4 to -6.90); *P* <.001
Olmesartan	-6.7 (-10.2 to -3.3); *P* <.001
LSM change from baseline (95% CI)	
Indapamide	
Zilebesiran	-19.3 (-22.3 to -16.2)
Placebo	-0.8 (-3.8 to 2.3)
Amlodipine	
Zilebesiran	-11.5 (-13.8 to -9.2)
Placebo	-1.4 (-3.7 to 1.0)
Olmesartan	
Zilebesiran	-9.3 (-11.8 to -6.9)
Placebo	-2.6 (-5.1 to -0.1)

Analyses are presented for the full analysis set. Box plots demonstrate median (horizontal line), mean (circle), IQR (box upper and lower boundary), highest and lowest values within 1.5 × the IQR (whiskers), and more extreme values (diamonds). Least-squares mean (LSM) values were derived from mixed model for repeated measures analysis including treatment, visit, treatment × visit, and race (Black; all other races) as fixed factors and corresponding baseline SBP and baseline estimated glomerular filtration rate as covariates; assessments from patients who received rescue therapy within 2 weeks of an assessment were censored.

BP response criterion at 6 months in this cohort (OR, 12.4 [95% CI, 4.6-33.3]; *P* < .001).

In the amlodipine cohort, more patients in the placebo group received rescue antihypertensives at month 6 than in the zilebesiran group (55 [48.7%] vs 28 [25.2%]). As with indapamide, there were significantly greater reductions in time-adjusted change from baseline through 6 months in both ambulatory (difference, −7.9 mm Hg [95% CI, −10.6 to −5.3]) and office (difference, −8.6 mm Hg [95% CI, −10.9 to −6.3]) SBP observed with zilebesiran compared with placebo (Table 2). A greater percentage of patients receiving zilebesiran (41 [39.8%]) than placebo (14 [13.7%]) achieved the BP response criterion (OR, 5.1 [95% CI, 2.4-10.6]; *P* < .001) at 6 months.

Among patients in the olmesartan cohort, a greater percentage of patients in the placebo group received rescue medication at month 6 than in the zilebesiran group (75 [54.0%] vs 57 [42.5%]). The between-group difference in the time-adjusted change from baseline in ambulatory SBP was not statistically significant through 6 months (Table 2). Accordingly, by the prespecified testing hierarchy, formal statistical comparisons of the between-group difference in time-adjusted change from baseline in office SBP and percentage of patients who met BP response criterion (30 [25.9%] for zilebesiran vs 22 [17.2%] for placebo; OR, 1.7 [95% CI, 0.9-3.2]) through 6 months are presented for descriptive purposes only.

Across all 3 cohorts, mean (SD) percent changes from baseline to week 2 in serum angiotensinogen levels ranged from −87.8% (36.0) to −92.8% (16.0) in patients receiving zilebesiran. Mean (SD) percent changes ranged from −88.2% (41.6) to −94.5% (9.4) at 6 months, with minimal difference in the magnitude of reduction across cohorts. No change in angiotensinogen levels was noted in patients receiving placebo (eFigure 3 in Supplement 2).

Across cohorts, a greater percentage of patients assigned to receive zilebesiran than placebo had at least 1 investigator-reported AE, although the rate of serious AEs

Table 2. Time-Adjusted Change From Baseline Through Month 6 in Systolic Blood Pressure (SBP)[a]

	Background medication					
	Indapamide		Amlodipine		Olmesartan	
	Zilebesiran (n = 63)	Placebo (n = 64)	Zilebesiran (n = 118)	Placebo (n = 120)	Zilebesiran (n = 147)	Placebo (n = 146)
24-h Ambulatory SBP						
LSM change from baseline (95% CI), mm Hg	−15.6 (−18.3 to −13.0)	−4.6 (−7.2 to −2.0)	−9.7 (−11.6 to −7.8)	−1.8 (−3.6 to 0.1)	−7.6 (−9.5 to −5.6)	−5.8 (−7.7 to −3.8)
LSM difference from baseline, zilebesiran vs placebo (95% CI), mm Hg	−11.0 (−14.7 to −7.3)		−7.9 (−10.6 to −5.3)		−1.8 (−4.6 to 1.0)	
P value	<.001		<.001		.21	
Office SBP						
LSM change from baseline (95% CI), mm Hg	−18.1 (−20.4 to −15.7)	−4.5 (−6.8 to −2.2)	−11.5 (−13.1 to −9.9)	−2.9 (−4.5 to −1.2)	−10.8 (−12.4 to −9.2)	−6.3 (−7.9 to −4.7)
LSM difference from baseline, zilebesiran vs placebo (95% CI), mm Hg	−13.6 (−16.9 to −10.3)		−8.6 (−10.9 to −6.3)		−4.5 (−6.8 to −2.3)[b]	
P value	<.001		<.001			

[a] Analyzed by mixed model for repeated measures including treatment, visit, treatment × visit, and race (Black; all other races) as fixed factors and corresponding baseline SBP and baseline estimated glomerular filtration rate as covariates. Unstructured covariance matrix was used. Time-adjusted change from baseline is the area under the curve divided by the duration of time between time points. All collected blood pressure measurements were analyzed through month 6 as predefined in the statistical analysis plan.

[b] Statistical comparison presented for descriptive purposes only in line with prespecified statistical testing hierarchy.

was the same in both groups (**Table 3**). There were no deaths in either group during the 6-month double-blind period. AEs of injection-site reactions, hypotension, acute kidney failure, and hyperkalemia occurred in a greater number of patients treated with zilebesiran than placebo, but the number of hepatic AEs was similar between the groups. Hypotension and hyperkalemia AEs were typically graded as mild in severity, transient, and commonly resolved without intervention. Although serum potassium levels greater than 5.5 mmol/L were more common in patients receiving zilebesiran than placebo, most resolved on repeat measurement. Decline in eGFR of at least 30% from baseline was more common in patients receiving zilebesiran than placebo, but the majority of cases spontaneously corrected on repeat measurement.

Discussion

In patients with uncontrolled hypertension despite treatment with a thiazide-like diuretic (indapamide), dihydropyridine calcium channel blocker (amlodipine), or maximum-dose angiotensin receptor blocker (olmesartan), addition of a single dose of subcutaneous zilebesiran 600 mg was associated with significant reductions in 24-hour mean ambulatory and office SBP at 3 months compared with placebo. Despite protocol guidance to intensify antihypertensive treatment to achieve guideline-directed BP targets after 3 months, significant BP reductions from baseline with zilebesiran persisted through 6 months in the indapamide and amlodipine cohorts, but were attenuated in the olmesartan cohort. Increased instances of mild hypotension, hyperkalemia, and eGFR decline were observed with zilebesiran compared with placebo, but most episodes were transient and resolved after repeat measurement without the need for medical intervention.

These data from KARDIA-2 amplify and extend the results from the dose-ranging KARDIA-1 study[11] by confirming sustained reductions in circulating angiotensinogen levels for 6 months as well as clinically meaningful reductions in ambulatory and office SBP after a single dose of zilebesiran 600 mg across a range of background antihypertensive treatments. Incremental, placebo-adjusted reductions in 24-hour mean ambulatory SBP at month 3 of 12.1 mm Hg in combination with indapamide and 9.7 mm Hg in combination with amlodipine are clinically meaningful and were sustained through 6 months. These data support the potential use of zilebesiran in combination with a thiazide diuretic or calcium channel blocker in clinical practice to achieve additive BP reductions in clinical practice, where most patients require combination treatment with 2 or more agents to achieve recommended BP treatment targets.[18] Although this trial was not designed to evaluate long-term cardiovascular outcomes or safety, previous data suggest that BP reductions of the magnitude observed here would be expected to translate into significant cardiovascular risk reduction.[19]

Table 3. Adverse Events (AEs) and Laboratory Assessments by Cohort and Treatment Assignment[a]

Outcome	No. (%)							
	Background medication							
	Indapamide		Amlodipine		Olmesartan		Overall	
	Zilebesiran (n = 63)	Placebo (n = 64)	Zilebesiran (n = 118)	Placebo (n = 120)	Zilebesiran (n = 148)	Placebo (n = 145)	Zilebesiran (n = 329)	Placebo (n = 329)
AEs								
At least 1 serious AE[b]	0	2 (3.1)	3 (2.5)	1 (0.8)	4 (2.7)	4 (2.8)	7 (2.1)	7 (2.1)
At least 1 AE	31 (49.2)	25 (39.1)	64 (54.2)	56 (46.7)	87 (58.8)	69 (47.6)	182 (55.3)	150 (45.6)
Injection-site reaction AE	4 (6.3)	0	2 (1.7)	0	4 (2.7)	1 (0.7)	10 (3.0)	1 (0.3)
Hypotension/orthostatic hypotension AE[c]	0	0	7 (5.9)	4 (3.3)	7 (4.7)	3 (2.1)	14 (4.3)	7 (2.1)
Hyperkalemia AE[d]	2 (3.2)	0	6 (5.1)	2 (1.7)	10 (6.8)	4 (2.8)	18 (5.5)	6 (1.8)
Laboratory parameters								
Potassium >5.5 mmol/L	2 (3.2)	0	8 (6.8)	1 (0.8)	10 (6.8)	3 (2.1)	20 (6.1)	4 (1.2)
Confirmed on repeat measure[e]	1 (1.6)	0	2 (1.7)	0	2 (1.4)	0	5 (1.5)	0
Hepatic AE[f]	0	3 (4.7)	6 (5.1)	1 (0.8)	5 (3.4)	3 (2.1)	11 (3.3)	7 (2.1)
ALT >3 × ULN	0[g]	0	3 (2.5)	1 (0.8)[g]	4 (2.7)[g]	1 (0.7)[g]	7 (2.1)	2 (0.6)
AST >3 × ULN	0[g]	1 (1.6)	2 (1.7)	1 (0.8)[g]	3 (2.0)[g]	3 (2.1)[g]	5 (1.5)	5 (1.5)
Acute kidney failure AE[h]	4 (6.3)	1 (1.6)	4 (3.4)	1 (0.8)	8 (5.4)	3 (2.1)	16 (4.9)	5 (1.5)
Decrease ≥30% from baseline in eGFR	8 (12.7)	1 (1.6)	10 (8.5)	5 (4.2)	10 (6.8)	4 (2.8)	28 (8.5)	10 (3.0)
Confirmed on repeat measure[e]	3 (4.8)	0	1 (0.8)	2 (1.7)	4 (2.7)	1 (0.7)	8 (2.4)	3 (0.9)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; FDA, US Food and Drug Administration; MedDRA, Medical Dictionary for Regulatory Activities; ULN, upper limit of normal.

[a] AEs were reported by investigators based on clinical judgment and defined according to MedDRA terminology. Laboratory assessments were evaluated at a central laboratory. Analyses are presented in the safety analysis set

[b] Serious AEs included AEs that were life-threatening, required hospitalization, prolonged existing hospitalization, or resulted in disability, birth defect, or death.

[c] Hypotension/orthostatic hypotension AEs include AEs mapped to the FDA MedDRA Query for hypotension (narrow terms).

[d] Hyperkalemia AEs include AEs mapped to the customized query of hyperkalemia, blood potassium increased, and blood potassium abnormal.

[e] Repeated typically within 1-2 weeks.

[f] Hepatic AEs include AEs mapped to the Standardized MedDRA Query for drug-related hepatic disorders (both narrow and broad terms).

[g] Assessment missing for 1 patient.

[h] Acute kidney failure AEs include AEs mapped to the Standardized MedDRA Query for acute kindey failure (both narrow and broad terms).

Because olmesartan is a potent RAS inhibitor, it was uncertain how much additional BP lowering would be achieved and how the safety profile would be impacted with the addition of angiotensinogen inhibition. The observed incremental reductions of 4.5 mm Hg in 24-hour mean ambulatory SBP and 6.7 mm Hg in office SBP at 3 months with zilebesiran in the olmesartan cohort complement the incremental BP reductions seen with the combination of zilebesiran and irbesartan in the phase 1 substudy[10] and suggest potential for more complete suppression of RAS activation with zilebesiran than is achievable with receptor-level blockade of angiotensin alone.[20,21] Use of combination therapy with currently available RAS inhibitors to treat hypertension is discouraged by guidelines[22] owing to concerns over heightened risks of hypotension, hyperkalemia, and worsening kidney function. In this context, it is notable that the rates of hyperkalemia and worsening kidney function, reflected in both investigator-reported AEs and laboratory observations, among zilebesiran-treated patients were low overall and were similar in the olmesartan and amlodipine cohorts. However, given less-incremental BP-lowering efficacy on top of olmesartan, additional studies of longer duration that enroll patients with higher risk for adverse effects are required to provide further insight into the balance of safety and efficacy of zilebesiran use in combination with oral RAS inhibitors.

The enduring antihypertensive effect of zilebesiran offers the potential for a biannual subcutaneous dosing approach that might help to overcome challenges with therapeutic inertia and poor patient adherence to daily antihypertensive treatments, which are key drivers of inadequate BP control.[6-9] Between-group treatment differences were generally reduced between 3 and 6 months and were no longer statistically significant at 6 months in the olmesartan cohort. However, this attenuated treatment effect may be in part due to protocol guidance to adjust antihypertensives to meet guideline-recommended targets, which led to greater use of additional antihypertensive therapy, mostly diuretics and calcium channel blockers, in patients receiving placebo in all cohorts. Nonetheless, the time-adjusted changes from baseline through month 6 in 24-hour mean ambulatory and office SBP in the indapamide and amlodipine cohorts remained greater for patients treated with zilebesiran than placebo. Although the time-adjusted change from baseline through month 6 in 24-hour mean ambulatory SBP was not different between zilebesiran- and placebo-assigned patients in the

olmesartan cohort, there was a difference in the time-adjusted change in office BP over this interval favoring zilebesiran. This discrepancy may in part reflect the greater opportunity to detect office SBP reductions due to greater frequency of office BP measurements (monthly) in comparison with ambulatory BP measurements (at 6 months only) after 3 months.

Limitations

Important limitations of this study include modest sample size and short study duration, which may have limited the power to identify rare AEs. Because the study systematically excluded patients with high cardiovascular risk and those with comorbidities that might heighten risk for AEs with angiotensinogen inhibition, these results may not be generalizable to the broader population of patients with hypertension. Further trials enrolling higher-risk populations including the ongoing KARDIA-3 study (NCT06272487) will shed further light on the balance of efficacy and safety of zilebesiran as an add-on therapy.

Conclusions

In this study, among patients with uncontrolled hypertension, the addition of single-dose zilebesiran to background treatment with indapamide, amlodipine, or maximum-dose olmesartan was associated with significant incremental reductions in 24-hour mean ambulatory and office SBP at 3 months relative to placebo. Persistence of antihypertensive effects through 6 months for many patients, even in the face of rescue antihypertensive treatment, as well as low rate of serious AEs support the potential for combining biannual subcutaneous dosing of zilebesiran with commonly used first-line antihypertensives to achieve additive BP reductions. Although further study is needed to establish the long-term safety profile of zilebesiran, these results add to the growing corpus of evidence supporting a role for RNA interference therapeutics targeting hepatic angiotensinogen as a novel strategy for managing hypertension in clinical practice.

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