ORIGINAL ARTICLE

Zilebesiran, an RNA Interference Therapeutic Agent for Hypertension

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ABSTRACT

BACKGROUND

Angiotensinogen is the sole precursor of angiotensin peptides and has a key role in the pathogenesis of hypertension. Zilebesiran, an investigational RNA interference therapeutic agent with a prolonged duration of action, inhibits hepatic angiotensinogen synthesis.

METHODS

In this phase 1 study, patients with hypertension were randomly assigned in a 2:1 ratio to receive either a single ascending subcutaneous dose of zilebesiran (10, 25, 50, 100, 200, 400, or 800 mg) or placebo and were followed for 24 weeks (Part A). Part B assessed the effect of the 800-mg dose of zilebesiran on blood pressure under low- or high-salt diet conditions, and Part E the effect of that dose when coadministered with irbesartan. End points included safety, pharmacokinetic and pharmacodynamic characteristics, and the change from baseline in systolic and diastolic blood pressure, as measured by 24-hour ambulatory blood-pressure monitoring.

RESULTS

Of 107 patients enrolled, 5 had mild, transient injection-site reactions. There were no reports of hypotension, hyperkalemia, or worsening of renal function resulting in medical intervention. In Part A, patients receiving zilebesiran had decreases in serum angiotensinogen levels that were correlated with the administered dose ($r=-0.56$ at week 8; 95% confidence interval, −0.69 to −0.39). Single doses of zilebesiran (≥200 mg) were associated with decreases in systolic blood pressure (>10 mm Hg) and diastolic blood pressure (>5 mm Hg) by week 8; these changes were consistent throughout the diurnal cycle and were sustained at 24 weeks. Results from Parts B and E were consistent with attenuation of the effect on blood pressure by a high-salt diet and with an augmented effect through coadministration with irbesartan, respectively.

CONCLUSIONS

Dose-dependent decreases in serum angiotensinogen levels and 24-hour ambulatory blood pressure were sustained for up to 24 weeks after a single subcutaneous dose of zilebesiran of 200 mg or more; mild injection-site reactions were observed. (Funded by Alnylam Pharmaceuticals; ClinicalTrials.gov number, NCT03934307; EudraCT number, 2019-000129-39.)

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Hypertension is a major risk factor for ischemic heart disease, stroke, and chronic kidney disease and is the leading preventable factor in death from cardiovascular causes worldwide.[1-4] Despite effective therapeutic options, nearly half the patients with hypertension do not reach guideline-recommended blood-pressure targets,[5] partly as a consequence of physician failure to initiate or intensify antihypertensive therapy and poor patient adherence to prescribed daily oral medications.[6-8] Even when blood pressure appears to be well managed on the basis of intermittent office measures, control may remain suboptimal owing to marked variability in blood pressure over the diurnal cycle and in the long term.[9-11]

The renin–angiotensin–aldosterone system (RAAS) plays a central role in blood-pressure regulation. Zilebesiran is an investigational RNA interference therapeutic agent (a small interfering RNA [siRNA] covalently linked to an *N*-acetylgalactosamine [GalNAc] ligand) that binds with high affinity to the hepatic asialoglycoprotein receptor. It is designed to achieve specific reduction in hepatic angiotensinogen messenger RNA (mRNA) levels, thereby reducing the production of angiotensinogen, a therapeutic target for hypertension.[12] Angiotensinogen is the sole precursor of all angiotensin peptides, so RAAS inhibition with this approach may theoretically limit compensatory angiotensin activation associated with angiotensin-converting–enzyme inhibition or angiotensin-receptor blockade.[12-14] Moreover, with hepatocyte-targeted delivery, extrahepatic angiotensinogen expression may be preserved, limiting off-target effects in the kidney and other tissues. Liver-specific effects of this approach are supported both by data from preclinical studies of a GalNAc-conjugated angiotensinogen siRNA, which suggests near-complete knockdown of hepatic angiotensinogen mRNA expression without affecting renal angiotensinogen mRNA,[13,15] and by early clinical experience with GalNAc-conjugated antisense oligonucleotides targeting angiotensinogen.[12,13] Consistent and prolonged pharmacodynamic effects of GalNAc–siRNAs offer the potential for sustained reduction of blood pressure over a 24-hour period and for months, with twice-yearly or quarterly subcutaneous administration.

In this phase 1 study, we assessed the safety, pharmacokinetic, and pharmacodynamic profiles of zilebesiran in patients with hypertension. We also explored the potential of the drug to modulate blood pressure, including under controlled conditions of dietary salt intake. We further assessed the effect of zilebesiran in combination with irbesartan on safety end points and blood pressure.

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METHODS

STUDY DESIGN AND OVERSIGHT

This was a four-part, multicenter, phase 1 study of zilebesiran designed to assess safety, pharmacokinetic and pharmacodynamic characteristics, and exploratory antihypertensive efficacy with 24-hour ambulatory blood-pressure monitoring in patients with hypertension. This report presents data from Parts A and B, which were double-blind, randomized, placebo-controlled studies of a single ascending dose (Part A) and a single fixed dose during low- and high-salt diet conditions (Part B), and from Part E, an open-label study of a single fixed dose of zilebesiran with daily irbesartan coadministration. Part D is ongoing; Part C was planned as a multidose phase but was removed during a protocol amendment (see the protocol, available with the full text of this article at NEJM.org).

The study was conducted at four sites in the United Kingdom (see the Supplementary Appendix, available at NEJM.org). The study was approved by the relevant research ethics committees within the Research Ethics Service of the U.K. Health Departments and was conducted in accordance with the Good Clinical Practice guidelines of the International Council for Harmonisation and the provisions of the Declaration of Helsinki. All the patients provided written informed consent. A safety review committee conducted periodic assessments.

The study was sponsored by Alnylam Pharmaceuticals and was designed by the sponsor in collaboration with the principal investigators. Data were collected by the study investigators and analyzed by the sponsor; interpretation of the data was performed by the sponsor, the first four authors, and the last author. The first draft of the manuscript was prepared by the first au-

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thor, who had access to all the study data, and all the authors participated in the decision to submit the manuscript for publication. OPEN Health provided editorial assistance, funded by Alnylam Pharmaceuticals. All the authors contributed to the interpretation of the data and critical revision of the manuscript and attest to the accuracy and completeness of the data and the fidelity of the study to the protocol. All the investigators, their institutions, and the sponsor were required to maintain data confidentiality during the study.

STUDY PARTICIPANTS

Eligible patients included adults 18 to 65 years of age with treated or untreated hypertension who had a mean seated systolic blood pressure as assessed by automated cuff of more than 130 to 165 mm Hg (Parts A and B) or more than 135 to 165 mm Hg (Part E) and a mean systolic blood pressure of 130 mm Hg or more as assessed by 24-hour ambulatory blood-pressure monitoring after washout of antihypertensive medications for at least 2 weeks. Among the key exclusion criteria were secondary hypertension, postural hypotension, diabetes, previous cardiovascular events, and current or anticipated treatment with β-adrenergic receptor–blocking drugs. Full inclusion and exclusion criteria are provided in the protocol.

STUDY DESIGN

In Part A, eligible patients (12 per cohort) were randomly assigned in a 2:1 ratio to receive a single subcutaneous dose of zilebesiran (10, 25, 50, 100, 200, 400, or 800 mg) or placebo (Fig. S1 in the Supplementary Appendix). Add-on antihypertensive therapy was permitted at the discretion of the investigator at 8 weeks for uncontrolled hypertension.

In Part B, after sequential administration of a low-salt diet (0.23 g per day) and a high-salt diet (5.75 g per day) from days –21 through –8, patients were randomly assigned in a 2:1 ratio to a single dose of 800-mg zilebesiran or placebo and rechallenged with the same dietary protocol from days 43 through 56 (corresponding to the timing of the expected peak effect of zilebesiran). The principal goal of Part B was to assess the potential for dietary salt intake to modulate the blood-pressure–lowering effects of zilebesiran.

In Part E, all the patients received a single 800-mg dose of zilebesiran. Patients with a systolic blood pressure of 120 mm Hg or more at week 6 as assessed by 24-hour ambulatory blood-pressure monitoring received additional treatment with irbesartan at a dose of 300 mg once daily for 2 weeks.

After the conclusion of the treatment period at week 12 for Parts A, B, and E, patients entered an extended safety follow-up period (see the Supplementary Appendix). All the patients were given guidance regarding moderation of alcohol intake, avoidance of supplements that might affect blood pressure, and dietary salt restriction to 2.0 g per day for the study duration, except as otherwise instructed in Part B.

END POINTS AND ASSESSMENTS

The primary end point was the frequency of adverse events. Secondary end points were the change from baseline in the serum angiotensinogen level and pharmacokinetic characteristics. Exploratory end points included changes from baseline in systolic and diastolic blood pressure as measured by 24-hour ambulatory blood-pressure monitoring at weeks 6, 8, 12, and 24 in Part A; during low- and high-salt intake before and after dose administration in Part B; and before and after daily irbesartan coadministration in Part E.

Safety assessments included monitoring of adverse events, laboratory assessments, and vital signs. Pharmacodynamic assessments included measurement of serum angiotensinogen levels (enzyme-linked immunosorbent assay; IBL-America) as well as plasma levels of renin–angiotensin system metabolites. Noncompartmental pharmacokinetic measurements were calculated from plasma and urine samples with the use of Phoenix WinNonLin, version 8.3 (Certara).

STATISTICAL ANALYSIS

Power calculations were not used to determine sample size, and data were analyzed primarily by means of descriptive statistics. No formal statistical hypothesis testing was performed, and the widths of the confidence intervals were not adjusted for multiplicity and should not be used for hypothesis testing. Data from patients who received placebo were combined across

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Table 1. Demographic and Clinical Characteristics of Enrolled Patients at Baseline.*

Characteristic	Part A		Part B		Part E†		All Patients (N=107)‡
	Placebo (N=28)	All Zilebesiran (N=56)	Placebo (N=4)	Zilebesiran (N=8)	Zilebesiran (N=6)	Zilebesiran + Irbesartan (N=10)	
Mean age (range) — yr	52.9 (36–64)	53.0 (35–65)	50.0 (35–62)	59.0 (49–64)	54.0 (44–58)	55.2 (42–64)	53.5 (35–65)
Male sex — no. (%)	16 (57)	35 (62)	3 (75)	6 (75)	5 (83)	3 (30)	66 (62)
Race — no. (%)§							
White	21 (75)	35 (62)	3 (75)	5 (62)	6 (100)	4 (40)	71 (66)
Black	6 (21)	16 (29)	1 (25)	2 (25)	0	3 (30)	27 (25)
Asian	0	3 (5)	0	1 (12)	0	1 (10)	5 (5)
Other	1 (4)	2 (4)	0	0	0	2 (20)	4 (4)
Blood pressure — mm Hg¶							
Systolic	140.6±8.3	139.2±9.4	148.5±2.6	139.0±7.4	133.0±6.6	147.0±7.5	140.3±9.0
Diastolic	87.9±7.9	85.8±6.8	99.0±2.8	86.4±6.3	85.8±8.0	89.0±6.3	87.1±7.3
Body-mass index‖	29.3±3.1	28.6±3.0	29.3±2.0	29.0±4.3	29.7±3.6	28.3±4.7	28.7±3.2

* Plus–minus values are means ±SD. Percentages may not total 100 because of rounding.
† Part E was a single-group study, with all the patients receiving a single 800-mg dose of zilebesiran. Patients with a systolic blood pressure of 120 mm Hg or more at week 6 as assessed by 24-hour ambulatory blood-pressure monitoring received additional treatment with irbesartan at a dose of 300 mg once daily for 2 weeks and are shown in the zilebesiran + irbesartan column.
‡ Five patients from Part A participated in Part E.
§ Race was reported by the patients.
¶ Shown are mean 24-hour blood-pressure levels as assessed by ambulatory blood-pressure monitoring.
‖ The body-mass index is the weight in kilograms divided by the square of the height in meters.

dose cohorts in Part A. The relationship between individual serum angiotensinogen level and 24-hour mean systolic blood pressure was explored by combining data from all zilebesiran dose groups and placebo in Part A. A log-linear model was used to describe the relationship between the decrease in serum angiotensinogen level and the decrease in 24-hour mean systolic blood pressure. All statistical analyses were performed with the use of SAS software, version 9.4 (SAS Institute). Further details of the statistical analyses are provided in the Supplementary Appendix.

RESULTS

STUDY POPULATION

From May 30, 2019, through January 26, 2022, a total of 107 patients were enrolled, including 84 patients in Part A (56 assigned to zilebesiran and 28 to placebo), 12 in Part B (8 assigned to zilebesiran and 4 to placebo), and 16 in Part E, 5 of whom had previously participated in Part A and received only placebo. The characteristics of the patients at baseline are summarized according to study part and study-group assignment in Table 1.

In the pooled population (107 patients), the mean age was 53.5 years (range, 35 to 65), 62% were men, 25% were Black, and the mean (±SD) 24-hour systolic blood pressure was 140.3±9.0 mm Hg. With regard to sex and race, the enrolled population was generally representative of the broader population of persons with hypertension in the United Kingdom. However, the study population was slightly younger because patients older than 65 years of age were excluded from participation (Table S1). Randomization,

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Table 2. Summary of Adverse Events.*

Event	Part A		Part B		Part E†		Pooled Parts A, B, and E	
	Placebo (N=28)	All Zilebesiran (N=56)	Placebo (N=4)	Zilebesiran (N=8)	Zilebesiran (N=6)	Zilebesiran + Irbesartan (N=10)	Placebo (N=32)	All Zilebesiran (N=80)
	number of patients (percent)							
Adverse event	24 (86)	42 (75)	4 (100)	3 (38)	6 (100)	7 (70)	28 (88)	58 (72)
Any serious adverse event‡	1 (4)	1 (2)	0	0	0	1 (10)	1 (3)	2 (2)
Any severe adverse event§	1 (4)	1 (2)	0	0	0	0	1 (3)	1 (1)
Any adverse event leading to withdrawal	0	0	0	0	0	0	0	0
Death	0	0	0	0	0	0	0	0
Adverse events occurring in ≥5% of patients								
Headache	13 (46)	10 (18)	1 (25)	0	2 (33)	3 (30)	14 (44)	15 (19)
Injection-site reaction	0	5 (9)	0	0	0	0	0	5 (6)
Upper respiratory tract infection	3 (11)	4 (7)	0	0	0	0	3 (9)	4 (5)
Adverse events of interest¶								
Hypotension	0	0	0	0	0	0	0	0
Hyperkalemia	0	0	0	0	0	0	0	0
Renal adverse event	0	0	0	0	0	0	0	0
Hepatic adverse event	0	1 (2)	1 (25)	0	0	0	1 (3)	1 (1)

* Shown are adverse events that occurred during the treatment period among patients receiving zilebesiran or placebo. "All zilebesiran" in the final column includes all the patients who received zilebesiran in Parts A, B, and E: Part A (56 patients), Part B with a low- or high-salt diet (8 patients), and Part E with and without irbesartan (16 patients). Patients who received zilebesiran entered a follow-up period; patients who received placebo were not required to complete a safety follow-up period after week 12.

† Part E was a single-group study, with all the patients receiving a single 800-mg dose of zilebesiran. Patients with a systolic blood pressure of 120 mm Hg or more at week 6 as assessed by 24-hour ambulatory blood-pressure monitoring received additional treatment with irbesartan at a dose of 300 mg once daily for 2 weeks and are shown in the zilebesiran+irbesartan column.

‡ Serious adverse events were defined as adverse events that resulted in death, were life-threatening, resulted in inpatient hospitalization or prolongation of existing hospitalization, resulted in persistent or clinically significant disability or incapacity, were a congenital anomaly or birth defect, or were important medical events as determined by the investigators. All adverse events (including serious adverse events) were graded for severity. Serious adverse events included optic ischemic neuropathy (grade 3 event, in a patient receiving placebo in Part A), prostate cancer (grade 3 event, in a patient receiving 200-mg zilebesiran in Part A) that was detected on biopsy during screening and resulted in surgery, and acute anemia (grade 1 event, in a patient receiving zilebesiran and irbesartan in Part E) due to a complication of esophagogastroduodenoscopy with biopsy performed during screening.

§ Severe events were adverse events for which more than minimal, local, or noninvasive intervention was received; which had a severe effect on limiting self-care activities of daily living; or which had the potential for life-threatening consequences or death.

¶ Hypotension adverse events included hypotension, orthostatic hypotension, and diastolic hypotension. Hyperkalemia adverse events included abnormal blood potassium, increased blood potassium, abnormal plasma potassium, increased plasma potassium, and hyperkalemia. Hepatic adverse events included all events selected according to the *Medical Dictionary for Regulatory Activities* (MedDRA) terms for drug-related hepatic disorders. One patient receiving placebo (in Part B) had a transient elevation in the alanine aminotransferase level greater than 3 times the upper limit of the normal range that was attributed to alcohol consumption; one patient receiving zilebesiran (25 mg, in Part A) had a transient elevation in the aspartate aminotransferase level of 2.2 times the upper limit of the normal range. Renal adverse events included all the events selected according to the MedDRA terms for acute renal failure.

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study-agent administration, and follow-up in Parts A, B, and E are summarized in Figure S2.

SAFETY AND ADVERSE EVENTS

A summary of adverse events that occurred during the treatment period is reported in aggregate in Table 2 and according to dose in Table S2. Overall, adverse events were reported for 58 patients receiving zilebesiran (72%) and 28 receiving placebo (88%). Adverse events that were reported in at least 5% of patients were headache, injection-site reaction, and upper respiratory tract infection; most of these events were mild or moderate in severity. Injection-site reaction was the only treatment-related adverse event reported in more than two patients. Three serious adverse events occurred: ischemic optic neuropathy (in a patient receiving placebo in Part A), prostate cancer that resulted in surgery (in a patient receiving 200 mg of zilebesiran in Part A), and acute anemia as a complication of a procedure conducted during screening before receipt of the study agent (in a patient receiving zilebesiran and irbesartan in Part E).

There were no deaths or unplanned hospitalizations, and no patients received interventions for hypotension, hyperkalemia, or worsening of renal function. No clinically significant changes in serum levels of potassium, sodium, or creatinine or in the estimated glomerular filtration rate were reported (Fig. S3 and Table S3). A transient elevation in the alanine aminotransferase level (>3 times the upper limit of the normal range), which was attributed to alcohol consumption, was noted in 1 patient receiving placebo (Part B). Transient, low-titer antidrug antibodies were detected in 2 of 80 patients (2%). There were no clinically significant changes in body weight observed over a period of 12 weeks in Part A (Table S4).

PHARMACOKINETIC AND PHARMACODYNAMIC MEASUREMENTS

The pharmacokinetic profile of zilebesiran was suggestive of dose-proportional characteristics (Fig. S4). The change in the serum angiotensinogen level was negatively correlated with the zilebesiran dose at week 8 in Part A ($r=-0.56$ at week 8; 95% confidence interval [CI], −0.69 to −0.39). Zilebesiran doses of 100 mg or more corresponded with mean decreases in serum angiotensinogen levels of more than 90% that were sustained from week 3 through week 12 (Part A). Patients in Part A who received 800 mg of zilebesiran maintained decreases in serum angiotensinogen levels of more than 90% through week 24 (Fig. 1A), with similar decreases seen at week 12 in Parts B and E (Fig. 1B and 1C). The results from Part E did not support an additional effect of irbesartan on angiotensinogen levels. At zilebesiran doses of 200 mg or more, small decreases in plasma renin activity and levels of aldosterone, angiotensin I, and angiotensin II were observed (Table S5).

EXPLORATORY END POINTS

In Part A, there was a negative correlation between the zilebesiran dose and the change from baseline in the mean 24-hour ambulatory systolic blood pressure ($r=-0.41$ at week 8; 95% CI, −0.58 to −0.21) (Fig. 2); the decrease in systolic blood pressure correlated with the degree of decrease in the serum angiotensinogen level ($r=0.52$; 95% CI, 0.42 to 0.61) (Fig. S5). Decreases in systolic blood pressure (>10 mm Hg) and diastolic blood pressure (>5 mm Hg) were seen after single doses of zilebesiran of 200 mg or more at week 8 (Fig. 2). The observed change in the mean (±SE) systolic and diastolic blood pressure at week 24 was −22.5±5.1 mm Hg and −10.8±2.7 mm Hg, respectively, among the eight patients who received 800 mg of zilebesiran (six of whom did not receive additional antihypertensive therapy). Data supporting a decrease in systolic blood pressure over the full diurnal cycle, through week 24, are provided in Figure S6. Data supporting a decrease in both daytime and nighttime systolic and diastolic blood pressure through week 24 are provided in Figure S7.

In Part B, before the administration of zilebesiran, the change in the mean (±SE) 24-hour systolic and diastolic blood pressure was −9.1±4.5 and −2.4±3.1 mm Hg, respectively, after 1 week of exposure to a low-salt diet, and blood-pressure levels returned to baseline after exposure to a high-salt diet. After administration of 800 mg of zilebesiran, the corresponding changes after a low-salt diet were −18.8±4.3 mm Hg and −8.4±2.5 mm Hg; blood-pressure levels again returned to baseline after exposure to a high-salt diet. The results are consistent with a greater decrease in blood pressure in patients

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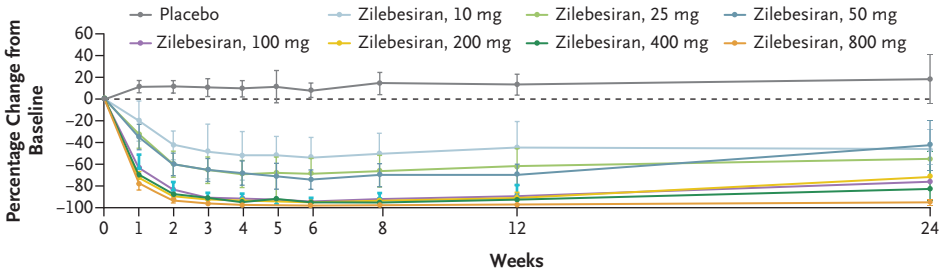
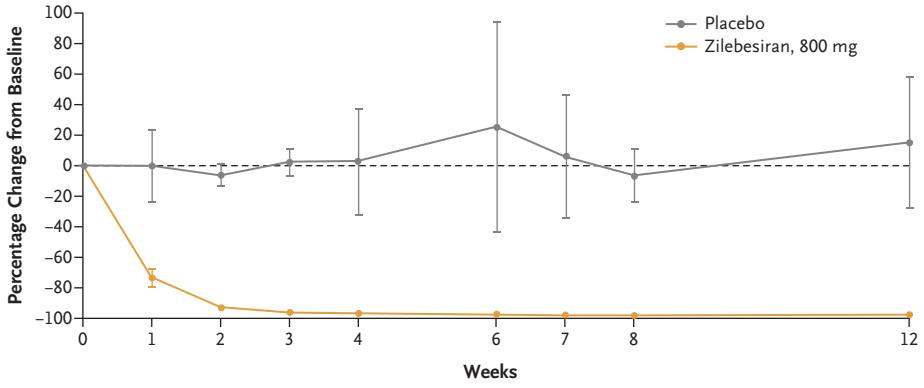
A Part A



No. of Patients

Placebo	28	28	28	28	27	17	26	27	28	12
Zilebesiran, 10 mg	8	8	8	8	8	8	8	8	8	5
Zilebesiran, 25 mg	8	8	8	8	8	8	8	8	8	8
Zilebesiran, 50 mg	8	8	8	8	8	8	8	7	7	7
Zilebesiran, 100 mg	8	7	8	8	8	8	8	8	7	7
Zilebesiran, 200 mg	8	8	8	7	6	2	3	7	8	8
Zilebesiran, 400 mg	8	8	8	8	8	0	8	8	8	8
Zilebesiran, 800 mg	8	8	8	8	8	0	8	8	8	8

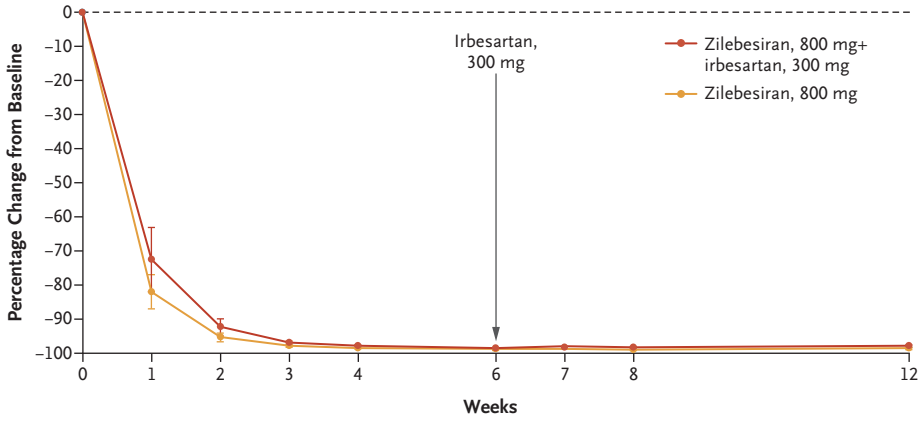
B Part B



No. of Patients

Placebo	4	3	4	4	4	4	4	4	4
Zilebesiran	8	8	8	8	8	8	8	8	8

C Part E



No. of Patients

Zilebesiran, 800 mg	6	6	6	6	6	6	0	6	6
Zilebesiran, 800 mg+ irbesartan, 300 mg	10	10	10	10	10	10	10	10	10

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Figure 1 (facing page). Change from Baseline in Mean Serum Angiotensinogen Level after Single Doses of Zilebesiran in Parts A, B, and E.

The graphs depict the mean percentage change from baseline in serum angiotensinogen levels after administration of single doses of zilebesiran in Parts A, B and E, over a period of 24, 12, and 12 weeks, respectively. In Part A, patients were randomly assigned to receive a single ascending subcutaneous dose of zilebesiran (10, 25, 50, 100, 200, 400, or 800 mg) or placebo (Panel A). In Part B, after sequential administration of low- and high-salt diets, patients were randomly assigned to a single dose of 800-mg zilebesiran or placebo and rechallenged with the same dietary protocol from days 43 through 56 (Panel B). Part E was a single-group study with all the patients receiving a single 800-mg dose of zilebesiran (Panel C). Patients with a 24-hour mean systolic blood pressure of 120 mm Hg or more as assessed by ambulatory blood-pressure monitoring at week 6 received additional treatment with irbesartan at a dose of 300 mg once daily for 2 weeks and are shown in the zilebesiran+irbesartan group. In all panels, the I bars represent 95% confidence intervals.

who followed a low-salt diet and received zilebesiran than in patients who followed a low-salt diet alone (Fig. S8).

In Part E, 6 patients had a mean (±SE) decrease from baseline in systolic blood pressure of 21.8±2.9 mm Hg at week 6 after administration of 800 mg of zilebesiran and did not receive add-on therapy. The 10 patients with persistent ambulatory systolic blood pressure of 120 mm Hg or more at week 6 who received additional treatment with irbesartan showed incremental changes in mean (±SE) systolic and diastolic blood pressure of –6.3±3.1 mm Hg and –3.0±1.9 mm Hg, respectively, at week 8 (Fig. S9).

DISCUSSION

In this phase 1 study involving patients with hypertension, we observed dose-related decreases in both serum angiotensinogen levels and blood pressure after single subcutaneous doses of zilebesiran that were sustained for up to 24 weeks. Among the treatment-related adverse events that were observed, the most common were mild, transient injection-site reactions. No hypotension, hyperkalemia, or worsening of renal function resulting in intervention was observed; however, the study was not large enough or of sufficient duration to assess uncommon serious adverse events. Angiotensinogen levels fell rapidly after the administration of single doses of zilebesiran and remained suppressed through week 24, findings consistent with the mechanism of action and preclinical models of zilebesiran.[15-17] These changes were accompanied by dose-dependent decreases in the mean systolic and diastolic blood pressure that were apparent by week 8 and were maintained to 24 weeks, findings that support a correlation between the extent of the decrease in systolic blood pressure and the magnitude of the decrease in serum angiotensinogen levels. Tonic blood-pressure control over the full 24-hour interval was achieved, with similar reductions in both daytime and nighttime blood pressure. Blood-pressure changes after zilebesiran treatment could be reversed through high dietary salt intake and were augmented by irbesartan coadministration.

Although RAAS antagonists are commonly used to treat hypertension, their efficacy may be blunted by compensatory angiotensin reactivation and aldosterone escape.[18] Strategies for more aggressive inhibition, such as the use of dual RAAS blockade, may carry a heightened risk of adverse effects, including hyperkalemia and worsening of renal function, potentially related to excessive renal RAAS inhibition.[19,20] Upstream RAAS inhibition through suppression of hepatic angiotensinogen, the precursor for all angiotensin metabolites, may overcome compensatory RAAS activation and enhance treatment efficacy.[12] Liver-specific silencing of angiotensinogen with zilebesiran may also help to limit the consequences of off-target renal RAAS inhibition.[15] Although renal RAAS activation was not directly measured in this study, no important changes in serum potassium or creatinine levels were noted after single-dose administration of zilebesiran in this population.

No drug-related hypotension resulting in medical intervention was observed during this study involving patients with hypertension who did not have serious coexisting conditions. Among patients typically seen in clinical practice, hypotension may develop as a consequence of unforeseen circumstances, including volume depletion, hemorrhage, infection or sepsis, myocardial injury, anaphylaxis or allergy, or coadministration of other agents that reduce blood pressure. The recovery of zilebesiran-associated decreases in

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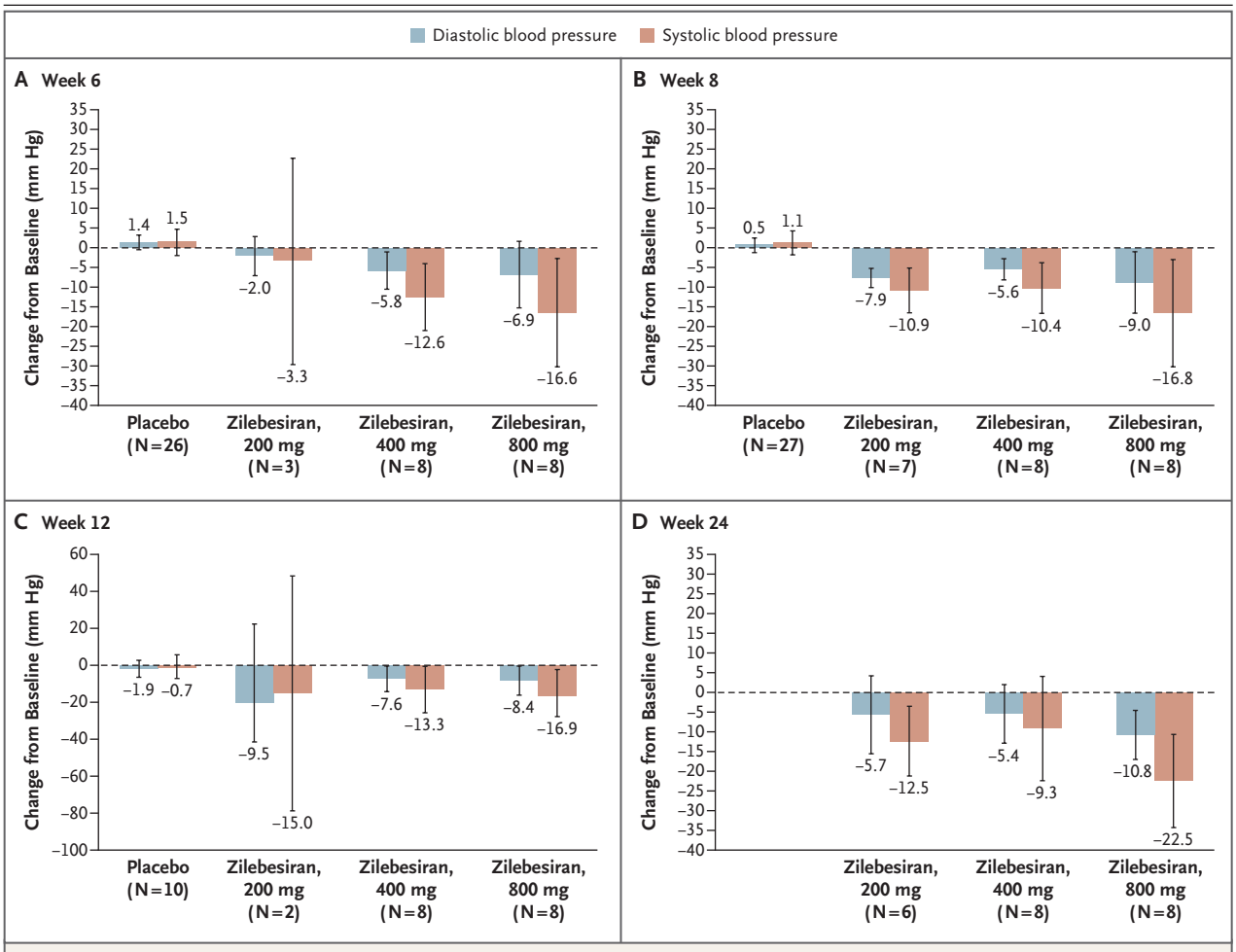

Figure 2. Decreases in Systolic and Diastolic Blood Pressure after Single Doses of Zilebesiran in Part A.

The charts depict the mean change from baseline in systolic and diastolic blood pressure, as assessed by ambulatory blood-pressure monitoring, at week 6 (Panel A), week 8 (Panel B), week 12 (Panel C), and week 24 (Panel D) after administration of single doses of zilebesiran in Part A. Patients were randomly assigned to receive a single ascending subcutaneous dose of zilebesiran (10, 25, 50, 100, 200, 400, or 800 mg) or placebo; data are shown for the 200-mg, 400-mg, and 800-mg doses of zilebesiran and placebo. In all panels, the I bars represent 95% confidence intervals. The mean baseline systolic blood pressure and diastolic blood pressure were as follows: placebo, 140.6 and 87.9 mm Hg; 200-mg zilebesiran, 138.4 and 83.3 mm Hg; 400-mg zilebesiran, 140.4 and 88.3 mm Hg; and 800-mg zilebesiran, 145.8 and 87.1 mm Hg. During the study, add-on antihypertensive therapy was permitted at the discretion of the investigator for severe hypertension (defined as systolic blood pressure >160 mm Hg and an increase of >10 mm Hg from the baseline mean systolic blood pressure that persists for ≥24 hours or that is accompanied by hypertensive symptoms). One patient in the 200-mg group received add-on antihypertensive therapy before week 8. After week 8, add-on antihypertensive therapy was permitted at the discretion of the investigator for uncontrolled hypertension (defined as systolic blood pressure ≥180 mm Hg). Patients receiving placebo were not required to enter the extended follow-up period (after week 12). Two patients in the 200-mg group, one patient in the 400-mg group, and two patients in the 800-mg group received add-on antihypertensive therapy.

blood pressure after exposure to high dietary salt intake suggests that the development of hypotension during zilebesiran treatment could be partially reversed by raising salt intake as an adjunct to standard interventions, including volume resuscitation and pressor support. Overall, these preliminary data regarding efficacy and safety support the potential for further study of quarterly or twice-yearly administration of zilebesiran as a treatment for patients with hypertension.

Lack of blood-pressure control over the diur-

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nal cycle, particularly nocturnal hypertension, is known to be associated with a heightened risk of cardiovascular events.[21] Similar risks have also been associated with a failure to suppress long-term variability in blood pressure.[22] Accordingly, there is interest in identifying treatment strategies that provide tonic control of blood pressure, including consistent and durable diurnal control over time. The results of this study are consistent with the maintenance of tonic blood-pressure control for up to 24 weeks after zilebesiran treatment in patients with hypertension.

This first-in-human, dose-escalation study should be viewed in the context of important limitations, including the small sample and relatively short duration of follow-up, which limit the potential to draw definitive conclusions about safety or efficacy. Although women of childbearing age were eligible for participation if using contraception, there are insufficient data to evaluate whether the teratogenic effects of other renin–angiotensin system inhibitors might also be seen with zilebesiran. However, preclinical studies evaluating angiotensinogen siRNA in rat models of preeclampsia did not detect any deleterious effects on the pups and showed that it did not cross the placenta.[17] Complete data regarding plasma and urinary renin–angiotensin system metabolites were not available to verify the liver-specific effects of zilebesiran seen in preclinical models. Furthermore, because enrollment was restricted to younger persons with stage 1–2 hypertension and limited medical coexisting conditions, the results may not be generalizable to the broader population of patients with hypertension. Further validation in larger, adequately powered randomized trials is warranted.

In this study, single-dose zilebesiran led to dose-dependent decreases in both serum angiotensinogen levels and 24-hour ambulatory blood pressure that were maintained for up to 24 weeks. Mild injection-site reactions were observed. Zilebesiran is being further evaluated as a potential treatment for hypertension in two phase 2 studies: KARDIA-1 (ClinicalTrials.gov number, NCT04936035) and KARDIA-2 (NCT05103332).

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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