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SPECIAL REPORT

Emerging insights and future prospects for therapeutic application of siRNA targeting angiotensinogen in hypertension

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ABSTRACT

Introduction: Hypertension is the main global risk factor for cardiovascular disease. Despite this, less than half of treated hypertensive patients are controlled. One reason for this is nonadherence, a major unmet need in hypertension pharmacotherapy. Small interfering RNA (small interfering ribonucleic acid) therapies inhibit protein translation, and, when linked to N-acetylgalactosamine, allow liver-specific targeting, and durability over several months. Targeted knockdown of hepatic angiotensinogen, the source of all angiotensins, offers a precision medicine approach.

Areas covered: This article describes the molecular basis for durability over months and the 24-h tonic target inhibition observed after one administration. We present an analysis of the published phase I trials using zilebesiran, a siRNA targeting hepatic angiotensinogen, which reduces blood pressure (BP) by up to 20 mmHg, lasting 24 weeks. Finally, we examine data evaluating reversibility of angiotensinogen knockdown and its relevance to the future clinical utility of zilebesiran.

Expert opinion: Further studies should assess safety, efficacy, and outcomes in larger, more broadly representative groups. An advantage of zilebesiran is the potential for bi-annual dosing, thereby reducing nonadherence and improving control rates. It may also reduce nighttime BP due to 24-h tonic control. The provision of adherence assessment services will maximize the clinical value of zilebesiran.

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1. Introduction: unmet needs in hypertension therapy

Hypertension is estimated to affect one in three adults worldwide, a prevalence which has doubled in the past 20 years and is expected to rise further [1]. It results in over 10 million deaths every year, and remains the leading global risk factor for cardiovascular disease (CVD) [2]. This costs the US economy over $50 billion annually [3]. Despite its consequences, hypertension remains under-treated, with less than half of treated patients exhibiting controlled blood pressure (BP) [1]. A major reason for this is non-adherence to medications (~45% of treated patients [4]), which is associated with tablet burden and side effects from the lifelong daily treatment of an asymptomatic condition [5–7]. Additionally, existing BP lowering therapeutics can lose efficacy with time due to compensatory pathways within the renin-angiotensin-system (RAS) that bring about a rise in renin and a restoration of angiotensin II (Ang II) signaling [8]; a form of treatment escape.

Accordingly, strategies to improve the treatment of hypertension could substantially reduce health and economic burdens globally. Indeed, for every 10 mmHg reduction in systolic BP (SBP), there are significant risk reductions for a range of major cardiovascular events (including stroke, heart attack, and heart failure), associated with a 13% risk reduction in all-cause mortality [9].

Taken together, there is an urgent unmet need for a therapy that tackles the problem of nonadherence and treatment escape, through improved durability and innovative targeting, respectively. Small interfering RNA (siRNA) targeting angiotensinogen (AGT), or AGT-siRNA, is now generating much interest as a durable therapy that targets the RAS at its source, generates sustained effects to reduce AGT and 24-h SBP, without worsening of kidney function, and may allow treatment at 6-monthly intervals [10]. In this review, we introduce siRNA therapeutics, describe the putative reasons for the durability of action of N-acetylgalactosamine (GalNAc)-siRNAs, summarize results of studies investigating AGT-siRNA in hypertension, and provide a focused analysis of recent translational research examining methods to reverse the effect of AGT-siRNA on BP.

2. Introduction to siRNA therapeutics: the molecular basis of longevity

2.1. Mechanism of action of siRNA

RNA interference, an evolutionarily conserved intracellular pathway, has translated to siRNA therapeutics capable of targeted knockdown of virtually any protein. These drugs are emerging from the treatment of rare diseases to that of common conditions, exemplified by the approval of inclisiran for hypercholesterolemia by the FDA in 2021 [11]. Inclisiran, which increases uptake of low

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Article highlights

- Using siRNA to suppress hepatic AGT is a novel weapon against nonadherence and treatment escape through improved durability and innovative targeting
- The unique molecular mechanisms of GalNAc-conjugated siRNA drugs (RISC recycling and endosomal storage) underpin the extended duration of action over months and 24-h tonic target inhibition
- In the phase I/first-in-humans (FIH) studies, the first-in-class AGT-targeting siRNA, zilebesiran, was well tolerated and dose dependently reduced serum AGT levels (reductions >90%), with sustained effects on BP (reduction in 24-h SBP >10 mmHg by week 8, and lasting 6 months at ≥200 mg), without worsening of renal function
- The future success of zilebesiran will depend on its safety and ability to reduce BP in larger, more representative hypertensive patient groups and its effective combination with other antihypertensive agents
- The utility of zilebesiran in uncontrolled hypertension will be enhanced by the availability of effective processes for assessment of adherence
- There is now preclinical evidence for effective use of licensed, well-known reversal agents in emergency situations, and reversal is unlikely to be a barrier to the clinical utility of zilebesiran

density lipoprotein (LDL) cholesterol by inhibiting translation of the proprotein convertase proprotein convertase subtilisin/kexin type 9 (PCSK9), the function of which is to bind LDL receptors in hepatocytes [12], results in effective and sustained reductions in LDL cholesterol by 50% lasting for 6 months after a single injection (with an onset of 2–3 weeks) [12,13].

The mechanism of action of siRNA at a cellular level explains the extraordinary durability of effect observed *in vivo* [14]. Synthetic siRNA drugs are composed of a short double stranded (RNA sequence, typically 20–24 base pairs. In brief, they enter the cell via endocytosis, escape from the endosome and then bind to the RNA-induced silencing complex (RISC) in the cytoplasm, which contains a functional core of endonucleases. The anti-sense strand is recognized as a 'guide strand,' and is retained, whilst the 'non guide' strand is released [15]. The RISC-complex cleaves the guide strand's complementary target mRNA, which silences its target gene [16]. Importantly, the same loaded guide strand can perform multiple rounds of mRNA slicing, and this 'RISC recycling' may contribute to tonic target inhibition, whilst storage of siRNA within hepatic endosomes (discussed below) may underpin the extended duration of action over months [17,18].

2.2. Intracellular delivery of siRNA drugs: a focus on endosomal escape

Translation of siRNA to the clinic has not been straightforward due to a multitude of challenges, including rapid renal clearance, degradation by ubiquitous RNAses, difficulties in delivery across the cell membrane, potential for off-target gene silencing, and immune-mediated toxicity. Successful solutions have evolved through a combination of chemical modifications (reviewed in detail elsewhere [14]) and the use of intelligent delivery strategies [11,19].

Conjugation to GalNAc allows an efficient and effective way for siRNAs to reach the liver and knock down liver proteins. GalNAc is a peptide ligand that binds exclusively to hepatocytes via the asialoglycoprotein receptor (ASGPR) [20,21]. Around 50–70% of GalNAc-siRNA conjugates are endocytosed by hepatocytes [22], whereas the majority of naked siRNA is cleared renally before this can occur [23]. ASGPR is uniquely suited for intracellular drug delivery due to its abundant expression (>10^6 copies/cell), rapid cycling resulting in endocytosis every 15 min, and its endogenous function: to distribute ASGPs into endosomes and traffic them to lysosomes for degradation [24]. As a result, ASGPR allows entry of 100-fold more siRNAs into hepatocytes than is possible for other cell surface receptors, which are less abundant (10^4–10^5) and have a slower rate of endocytosis (~90 min) [25]. As only ~2000 siRNAs are required for gene knockdown [25], and ASGPR is able to bind up to 10^6 siRNAs and carry them into the cell every 10–15 min, target knockdown is effective despite the low endosomal escape rate (~1%) of siRNA [26,27]. Zilebesiran (free acid molecular weight (MW) 17,334) is a novel first-in-class GalNAc-conjugated, chemically modified, siRNA which inhibits hepatic AGT synthesis. By our calculations, the maximum possible number of siRNA molecules delivered to a single hepatocyte at a dose of 100 mg sc (with full first pass drug delivery) is 2×10^7. This is enough to fill the maximal capacity of ASGPR, allowing the effective target knockdown observed with this drug.

A recent study showed that storage of GalNAc-siRNA in acidic intracellular compartments is essential for the extended duration of effect compared with siRNAs delivered in lipid nanoparticles, with endosomal release and loading into RISC occurring weeks after dosing [18]. Intriguingly, a slow leak of stable siRNA from intracellular compartments may act as a pharmacologic 'depot,' which continuously loads RISC over time, resulting in the slow onset and extended duration of effect observed clinically.

However, the mechanisms of endosomal escape are still poorly understood, and delivery across target cell membranes (other than hepatocytes) has been described as the main barrier to the future potential of siRNAs to treat a wider range of human diseases [28]. On balance, currently licensed GalNAc-siRNAs are effective, well tolerated, long-lasting, and enhancing the endosomal escape rate could adversely affect the pharmacokinetics and safety of these drugs.

3. Targeting angiotensinogen using GalNAc-siRNA: from bench to bedside

3.1. Targeting RAS at its source: preclinical proof-of-concept studies

An exciting novel target in hypertension is hepatic AGT, as it is the primary source of circulating AGT [8,29,], and the precursor to Ang II, which is responsible for vasoconstriction, fluid retention, inflammation, cardiac hypertrophy and fibrosis, and the secretion of aldosterone via the AT_1 (Ang II type 1) receptor [30–33]. Although angiotensin-I converting enzyme (ACE) inhibitors (ACEi) and AT_1 receptor blockers (ARB) are effective in treating hypertension and preventing end-organ damage [34], they require daily administration and, with long-term use, can result in a compensatory rise in renin, which brings about a rise in Ang II and a return toward baseline BP; so-called 'RAS escape' [8,35–37]. Suppressing hepatic AGT may prevent the rise in Ang II, due to depletion of its substrate, and ultimately improve long term BP control [8,36].

Although AGT-siRNA achieved almost complete depletion of circulating AGT in spontaneously hypertensive rats (SHRs) and

reduced BP to the same degree as daily dosing with valsartan, circulating Ang II levels were, surprisingly, unaffected [38] and this was also observed with zilebesiran (discussed below) [39]. Indeed, perhaps paradoxically, Ang II only declined when AGT-siRNA was combined with valsartan, which reduced BP and cardiac hypertrophy to the greatest extent. The authors postulated that this was due to high renin together with incomplete depletion of circulating AGT (97.9% for AGT siRNA alone vs 99.8% for AGT siRNA and valsartan). This is because AGT normally circulates in the micromolar range in contrast to Ang II, which circulates at around 20 picomolar, giving a ratio of 50,000:1. This means that, at 1%, AGT is still 10,000 fold higher than Ang II, which is clearly enough to maintain normal circulating Ang II [8]. However, AGT-siRNA alone did lower renal Ang II, suggesting BP lowering may occur at the level of the kidney rather than via circulating Ang II, although this remains unproven.

3.2. Reversibility of AGT siRNA: evidence from preclinical studies

The future success of long-term suppression of RAS using AGT-siRNA therapy may partly depend on effective and rapid reversal methods to restore BP in emergency settings, such as hypovolemia and hypotension (say linked to hemorrhage or sepsis), and acute kidney injury. A recent valuable preclinical study explored strategies to overcome the BP-lowering effects of siRNA mediated knockdown of AGT in SHRs. In brief, SHRs were dosed with AGT-siRNA (10 mg/kg fortnightly) for 4 weeks after being established on a low-salt diet (to potentiate BP-lowering), and their response to the vasopressors Ang II ($n = 11$, 0.05–5 µg/kg) and norepinephrine ($n = 1$, 0.1–10 µg/kg) was assessed at baseline and in the setting of siRNA-mediated AGT suppression [40]. During the final 2 weeks, SHRs were randomized to receive fludrocortisone ($n = 7$, 6 mg/kg daily), midodrine ($n = 6$, 4 mg/kg daily), or a high salt diet ($n = 6$, 4% NaCl) whilst evaluating BP using telemetry.

As expected, AGT-siRNA depleted AGT substantially (by >99%) and reduced mean arterial pressure (MAP) by 19 mmHg. Interestingly, Ang II rapidly and significantly raised MAP following siRNA-mediated AGT suppression compared with its effects at baseline. This difference may be due to suppression of the RAS, resulting in sensitization of AT1 receptors to Ang II. The alpha-adrenergic agonist norepinephrine increased MAP to a similar extent before and after AGT-siRNA. Gradual reversal to baseline MAP (increased by ~20 mmHg) was achieved with daily oral fludrocortisone and with high salt diet, but the alpha-adrenergic agonist midodrine had no effect on MAP. High salt diet was superior to fludrocortisone as it significantly increased MAP by 8 mmHg on the first day of administration, and restored MAP to baseline by day 4, whereas MAP was only significantly increased by day 5, and fully restored on day 7, with fludrocortisone.

Another possible reversal mechanism is to use an oligonucleotide complementary to the siRNA anti-sense strand (named 'REVERSIR™'), which binds to siRNA and reverses gene silencing, restoring normal levels of target protein within 4 days in mice [41]. However, using a newly developed investigational drug as a reversal agent for a newly licensed drug seems far less appealing than simply using NaCl or repurposing rigorously tested licensed drugs.

Taken together, these results suggest that vasopressors, norepinephrine (NE) and Ang II, which are already used to treat circulatory shock [42,43], may have pharmacological utility for urgent reversal of siRNA-mediated AGT suppression (likely in high dependency settings), whilst IV NaCl has potential in situations of urgency, but remains untested. In addition, oral NaCl or fludrocortisone could have potential utility in non-emergency settings, for instance in viral illness resulting in dehydration ('sick day rules'), when patients would usually be advised to withhold their medication.

3.3. Effects of zilebesiran in man

The first phase I randomized controlled trial [39] with zilebesiran assessed: safety, pharmacokinetics, and pharmacodynamics in four parts: seven single ascending doses (10, 25, 50, 100, 200, 400, or 800 mg, with 12 subjects per dose cohort and a 2:1 ratio of zilebesiran:placebo) in part A; efficacy and safety of a fixed dose in high and low salt diet (part B); and in combination with the ARB, irbesartan (part E). Part C, a multiple ascending dose study was planned for phase I but was removed as the pharmacokinetic and safety data gained from other parts were deemed sufficient to guide further studies using zilebesiran, whilst Part D, a study investigating the effects of zilebesiran in obese patients is, as yet, unpublished. Eligible patients included treated and untreated hypertensive adults (18–65 years) with mild to moderate hypertension (mean seated SBP of >130≤165 mmHg: Parts A and B; or > 135≤165 mmHg: Part E) and mean SBP ≥ 130 by 24-h ambulatory blood pressure monitoring (ABPM) following medication washout for ≥2 weeks. For all parts, patients exhibiting uncontrolled hypertension after week 8 of the study were started on add-on antihypertensive therapy, and patients treated with zilebesiran (but not placebo) entered extended follow up from week 12 until week 24. In total, 107 patients: 84 in part A, 12 in part B and 16 in part E (of whom 5 had previously participated in part A) were recruited. Of these, the proportion of patients experiencing adverse events (AEs) was similar between groups (72.5% and 87.5% for zilebesiran and placebo, respectively), and the majority of AEs were mild or moderate in severity, with no treatment-related serious AEs. Although there were injection-site reactions in 5 out of 80 patients receiving zilebesiran, these were mild and transient in nature. Importantly, there was no hypotension or deranged liver or kidney function in any participant.

In Part A, circulating AGT was reduced by >90% from week 3 to 12 in the groups given ≥100 mg zilebesiran. Impressively, this persisted up to week 24 at the top (800 mg) dose. In terms of exploratory endpoints, zilebesiran reduced mean 24-h SBP by >10 mmHg at week 8, and reductions were maintained to week 24 at doses ≥200 mg. At the top dose, it reduced SBP by 16.8 mmHg at 8 weeks and 22.5 mmHg at 24 weeks (although 2 out of 8 patients received add-on therapy). There were clinically significant and sustained reductions in SBP over a 24-h period (similar daytime and nighttime reductions). However, it is important to note that baseline BP data from zilebesiran-treated patients were used *in lieu* of placebo at the 24-week timepoint (at which the most impressive effects on BP were seen). In addition, there were 1–2 patients in each of the dose cohorts ≥200 mg who required an add-on drug for uncontrolled hypertension after week 8. Notably, the peak effect on circulating AGT was reached by

week 3, and on BP by week 8, likely due to the time required for sufficient cleavage of *Agt* mRNA to allow substantial depletion of circulating AGT.

In part B, the BP-lowering effects of a single fixed dose of 800 mg zilebesiran ($n = 12$, 2:1 ratio of zilebesiran:placebo) was assessed during a 14-day salt-controlled diet (7 days of low salt: 0.23 g/day and 7 days of high salt: 5.75 g/day) during days 43–56, when serum AGT was expected to reach a nadir in response to zilebesiran. Prior to the commencement of part B (from day −21 to −8), patients were exposed to high and low salt diet, which resulted in the expected rise and fall in BP respectively. Exposure to low salt diet amplified the effect of zilebesiran on 24-h SBP (−18.8 mmHg vs baseline) although no symptomatic hypotensive episodes were reported (patients were admitted for regular monitoring). Interestingly, increasing dietary NaCl for a week partially reversed the BP-lowering effects of zilebesiran, consistent with preclinical studies [40] and provides some basis for oral NaCl as a potential reversal agent.

In part E, 16 patients were administered 800 mg zilebesiran at day 1, and at day 41 (week 6). Interestingly, 6 of these achieved a level of SBP reduction that did not require add-on therapy (−21.8 mmHg), whilst 10 patients had persistent 24-h SBP ≥120 mmHg (−7.7 mmHg) and began a 14-day add-on treatment regimen with irbesartan (300 mg daily). In these patients, irbesartan reduced 24-h SBP by a further 6.3 mmHg, without altering serum creatinine or K^+ over the 12-week follow-up period. As peak effect on BP is not achieved until week 8 (see part A), and irbesartan was started at week 6, the size of the additive effect may be overestimated. Further, it is clear there is some variability in the response to zilebesiran. Nevertheless, this study revealed that ARB therapy can have a mild additive BP-lowering effect in patients treated with AGT-siRNA without affecting serum K^+ or GFR. This approach might have clinical utility in uncontrolled hypertension, although combining RAS inhibitors is currently not advised due to the risk of hyperkalemia [44].

4. Conclusion

Silencing of AGT using GalNAc-siRNA has potential as a novel pharmacological approach to hypertension. The phase I data [39] suggest that zilebesiran is acceptably well tolerated, without reducing kidney function (even when combined with an ARB), albeit in a small, select group of patients without high-risk comorbidities. The study demonstrated an impressive magnitude and durability of effect on BP (despite the study not being powered for this) with substantial, dose-dependent reductions in AGT (>90%) and 24-h SBP (>10 mmHg at doses ≥200 mg) lasting up to 6 months following a single sc injection of zilebesiran. The authors demonstrate convincing evidence for NaCl as a reversal agent over a week. The elegant preclinical reversal study by Uijl and colleagues provides evidence to underpin the clinical potential of using widely available vasopressors (Ang II and NE) for emergency reversal. Finally, there is preliminary evidence for use of an ARB for BP lowering in patients uncontrolled on AGT-siRNA alone. This merits further exploration in larger more broadly representative groups.

5. Expert opinion

siRNA drugs offer a transformative solution to targets previously 'undruggable' by the small molecule approach. Targeted knockdown of AGT in hepatocytes using GalNAc-siRNA capitalizes on the liver as the primary source of circulating AGT [29], and reduces the risk of off-target inhibition. Historically, safety data from siRNA drugs, which have hitherto been licensed for orphan conditions [11], were limited by smaller sample sizes. However, large-scale trials evaluating inclisiran for hypercholesterolemia have resulted in a wealth of data. Phase III trials (ORION 9, 10, & 11) included 3,660 patients, who received four 3-monthly injections of inclisiran and were followed for 540 days [13,45]. Apart from mild injection site reactions, the drug was well tolerated. Although zilebesiran appears similar in its safety profile, only larger population studies including high risk groups (chronic kidney disease, CKD; type 2 diabetes mellitus, T2DM; and heart failure) will reliably uncover adverse effects on kidney function, the major concern with RAS blockade. Another important area to address with long-term studies is whether there are any effects of chronic unopposed high renin. Although there is some theoretical evidence for abnormalities in renal vasculature [46], this has not been observed in clinical practice with RAS blockade (which also results in high renin), and would be unlikely to outweigh the impressive reductions in cardiovascular risk observed with RAS inhibition. The most likely clinical safety issue is the potential need to reverse the effects of this long-acting agent. Although the evidence for emergency reversal agents with noradrenaline and Ang II is encouraging [40,47], these will require clinical testing.

Due to its slow onset of action, zilebesiran will not be appropriate for accelerated hypertension (see Table 1 for summarized advantages and disadvantages). However, a slow onset may be advantageous in preventing hypotension associated with initiation of treatment as observed with other

Table 1. Potential advantages alongside possible limitations and challenges with using GalNAc-siRNA drugs targeting AGT. BP – blood pressure, FIH – first in humans, GalNAc- N-acetylgalactosamine, SBP – systolic blood pressure.

Advantages	Limitations and challenges
• siRNA drugs generally well-tolerated (c.f. inclisiran), apparent good tolerability of zilebesiran in FIH study • Impressive effect on BP (24-h SBP reduced by 20 mmHg with 800 mg zilebesiran) and tonic 24 h control • Durability over months – opportunity to improve adherence • Slow onset – may avoid first dose hypotension	• Need for effective reversal agents in certain situations • Requirement to reconfigure services to improve measurements of adherence • Slow onset – not for use in hypertensive emergencies

RAS inhibitors, particularly in comorbid patients [48,49]. Use in women of reproductive age is likely to be an absolute contraindication to drugs targeting AGT (unless contraception can be assured), given the known teratogenic effects of RAS inhibition and the physiological response to fluid shifts in pregnancy [8,50].

Given its acceptable tolerability (see Table 2), the substantial effects of zilebesiran on AGT and BP justify further development in primary hypertension. An exciting advantage of AGT-siRNA drugs is their 24-h duration of action, with similar effects on daytime and nighttime BP. A lack of normal dipping of SBP at night [51,52] independently predicts CVD, arterial stiffness, and end-organ damage [53]. Phase II studies might determine whether zilebesiran is able to restore or improve the physiological dipping pattern in patients, which may in turn improve long-term cardiovascular outcomes. Indeed, whether the efficacy of zilebesiran in BP-lowering will translate to improved long-term cardiovascular and mortality outcomes remains to be determined by larger studies.

Putative mechanisms for loss of efficacy include anti-drug antibodies (ADAs) or treatment escape. Phase III trials with inclisiran showed a very low incidence of ADAs, in <3% of samples in all three RCTs (3,660 patients). Reassuringly, the phenomenon of 'treatment-boosted ADA' (a rising antibody response to repeated dosing) did not occur [13,45]. In the zilebesiran study, transient, low-titer ADAs were observed in 2.5% of patients. As with all RAS inhibitors, treatment escape is possible, but was not observed over the extended follow-up period for zilebesiran. Preclinical studies have suggested full (~100%) depletion of AGT is required to suppress Ang II levels, due to the high circulating ratio of AGT to Ang II and the great physiological drive to upregulate renin [8]. However, reducing Ang II at the local tissue level may be enough to maintain efficacy in the face of incomplete depletion of circulating AGT (90% in the zilebesiran trial). Further studies should explore the mechanism of the impressive BP-lowering effect in man despite perhaps surprisingly normal circulating Ang II levels following siRNA-mediated AGT knockdown.

In making the most of this potentially very effective method to tackle adherence, the biggest problem in hypertension management, a lower threshold for considering nonadherence will be needed in treated patients with uncontrolled BP, ideally using robust measures of adherence assessment. Though not without limitations, recent studies show biochemical detection of drug levels to be the best method for determining patient adherence [54]. One might speculate that provision of drug assays will help identify problems with adherence, justify the use of zilebesiran, and help patients achieve better control and strengthen the case for cost-effectiveness. In this way, AGT-siRNA drugs could offer a precision medicine approach to tackling adherence, alongside radical reform of adherence services to maximize the utility of the drug.

On balance, the advantages of siRNA in treating hypertension, in terms of durability, are unique to this class of drug. Targeting AGT using siRNA has the potential to usher in a new era of hypertension pharmacotherapy. Confirmation of safety and efficacy in broader patient populations over the next few years could provide the basis for licensing zilebesiran. At present, two RCTs investigating zilebesiran (see Figures 1 and 2) are registered and expected to complete in 2024/25: a phase II, multi-center RCT, KARDIA-1 (NCT04936035) examining the

Table 2. Summary of key safety outcomes and changes in AGT and BP in the phase 1 RCT with zilebesiran. Key safety outcomes include AEs (AE and serious adverse events (SAE) and are expressed as number of subjects and % in brackets. Key AEs of interest included hypotension, hypokalemia, renal (acute renal failure), and drug-related hepatic disorders. One placebo patient had a transient alanine aminotransferase >3 times the upper limit of normal which was attributed to alcohol. Injection site reaction occurred in 6.3% of zilebesiran-treated patients as well as headache (19%), and upper respiratory infections (5%) [not shown]. Serum AGT was measured by enzyme linked immunosorbent assay and is expressed as mean % change from baseline. BP was measured by ABPM at weeks 6, 8, 12, and 24 in part a and during low salt and high salt intake prior to and at 6 weeks after zilebesiran dosing in part B. Patients with uncontrolled BP after week 8 of treatment required additional antihypertensive therapy (1–2 patients per dose cohort). In part E, 16 patients were dosed with zilebesiran and 10 of these patients still had 24-h SBP ≥120 mmHg at week 6. These patients were then dosed with irbesartan at week 6 and 24-h SBP measured again at week 8, to ascertain any incremental reduction in BP. ABPM – ambulatory blood pressure monitoring, AGT – angiotensinogen, BP – blood pressure, SBP – systolic blood pressure.

	Part A		Part B		Part E	
Key safety outcomes	Placebo ($n = 28$)	Zilebesiran ($n = 56$)	Placebo ($n = 4$)	Zilebesiran ($n = 8$)	Zilebesiran ($n = 6$)	Zilebesiran + irbesartan ($n = 10$)
AE	24 (85.7)	42 (75)	4 (100)	3 (37.5)	6 (100)	7 (70)
SAE	1 (3.6)	1 (1.8)	0	0	0	1 (10)
Hypotension	0	0	0	0	0	0
Hyperkalemia	0	0	0	0	0	0
Renal AE	0	0	0	0	0	0
Hepatic AE	0	1 (1.8)	1 (25)	0	0	0
Injection site reaction	0	5 (8.9)	0	0	0	0
AGT (% reduction)	>90% with doses ≥100 mg (week 3–12)		>90% (week 3–12)		>90% (week 3–12)	
	>90% with 800 mg (week 3–24)				No effect of irbesartan on AGT levels	
24 h SBP (change from baseline in mmHg)	≥-10 mmHg with ≥200 mg (week 8–24)		−9.1 after 1-week low Na diet (baseline)		−21.8 with 800 mg at week 6 and 8 ($n = 6$)	
	−22.5 with 800 mg (week 24)		−18.8 after 1-week low Na diet at week 6 after 800 mg		−7.7 with 800 mg at week 6 ($n = 10$)	
					−14 with dual therapy at week 8	
					= Incremental reduction of −6.3	

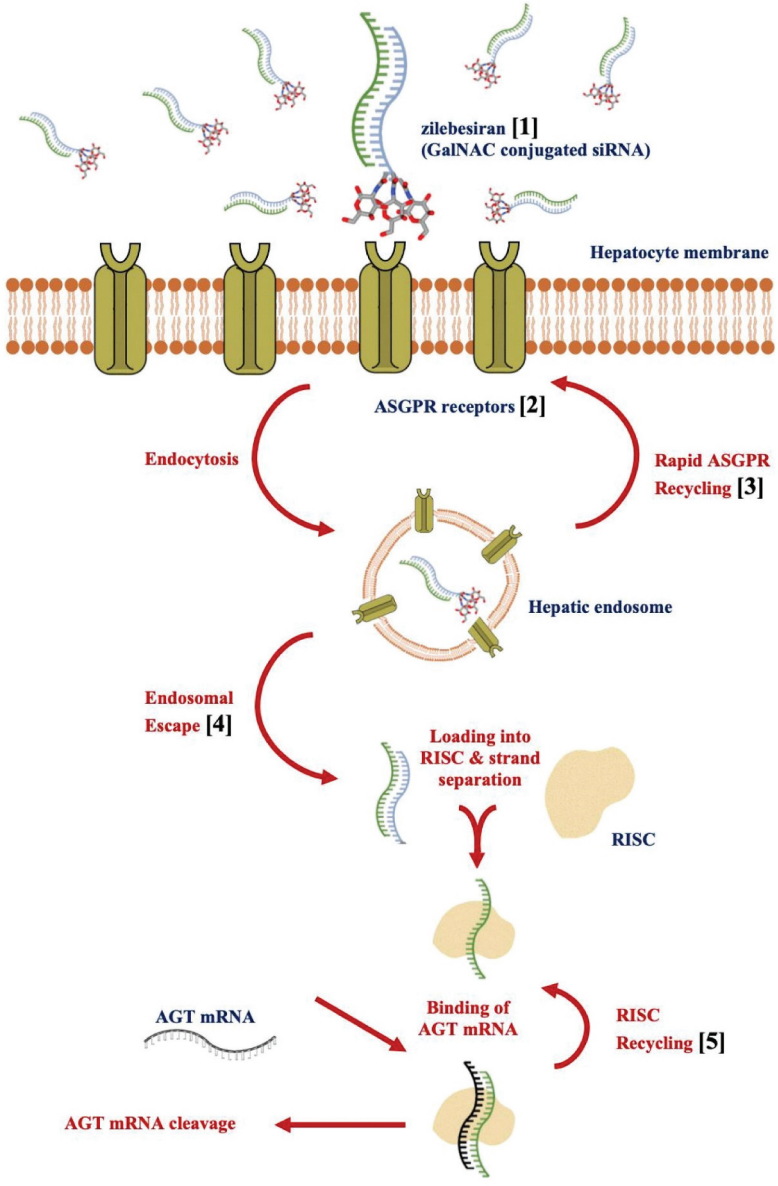

Figure 1. Schematic illustration of the putative mechanisms underlying the specificity and durability of AGT siRNA. AGT – angiotensinogen; ASGPR – Asialoglycoprotein; GalNAC – N-acetylgalactosamine; mRNA – messenger RNA; RISC – RNA-induced silencing complex; s.C. – subcutaneous; siRNA – small interfering RNA [1]; around 10^7 molecules of zilebesiran reach a single hepatocyte following s.C. administration of a 100 mg dose [2]; abundant expression in hepatocytes (>10^6 copies/cell) [3]; rapid cycling resulting in endocytosis every 15 min [4]; low endosomal escape rate (~1%) and the acidic environment in the endosome creates a repository for siRNA [5]; RISC recycling results in repeated knockdown of AGT mRNA.

efficacy and safety of 3- or 6-monthly sc injections of zilebesiran in patients ($n = 394$) with mild-to-moderate hypertension (untreated or following antihypertensive washout). Patients with comorbidities will be included but will (understandably) exclude patients with eGFR <30 mL/min/1.73 m^2 stage 3 or poorly controlled/newly diagnosed T2DM. The primary endpoint is change from baseline in 24-h mean SBP after 3 months of treatment, while other key endpoints will be additional measures of BP at 3 and 6 months, and safety. Another phase II RCT, KARDIA-2 (NCT05103332) will evaluate zilebesiran as an add-on therapy in 672 patients whose BP remains uncontrolled (≥130 to ≤160 mmHg) despite 4 weeks of run-in treatment with a specified ACEi (olmesartan), calcium channel blocker (amlodipine) or diuretic (indapamide). They will then receive a single sc injection of zilebesiran or placebo as an add-on therapy and will be evaluated at 3 and 6 months. The primary, secondary and exploratory endpoints will be similar to those for KARDIA-1. Data newly reported by Alnylam (September 2023) from a phase 2 study with zilebesiran (KARDIA-1) confirms the extent of the reduction of SBP and the durability of the response. In particular: (i) zilebesiran met its primary endpoint of >15 mmHg reduction of SBP at 3 months of treatment compared to placebo at the two highest single doses evaluated; (ii) zilebesiran met key secondary endpoints showing consistent and sustained reductions of SBP at 6 months, supporting quarterly or biannual dosing; and (iii) zilebesiran showed an encouraging profile of safety and tolerability in adults with mild-to-moderate hypertension [55].

Licensing of zilebesiran or other drugs of the same class could allow the management of hypertension to be revolutionized;

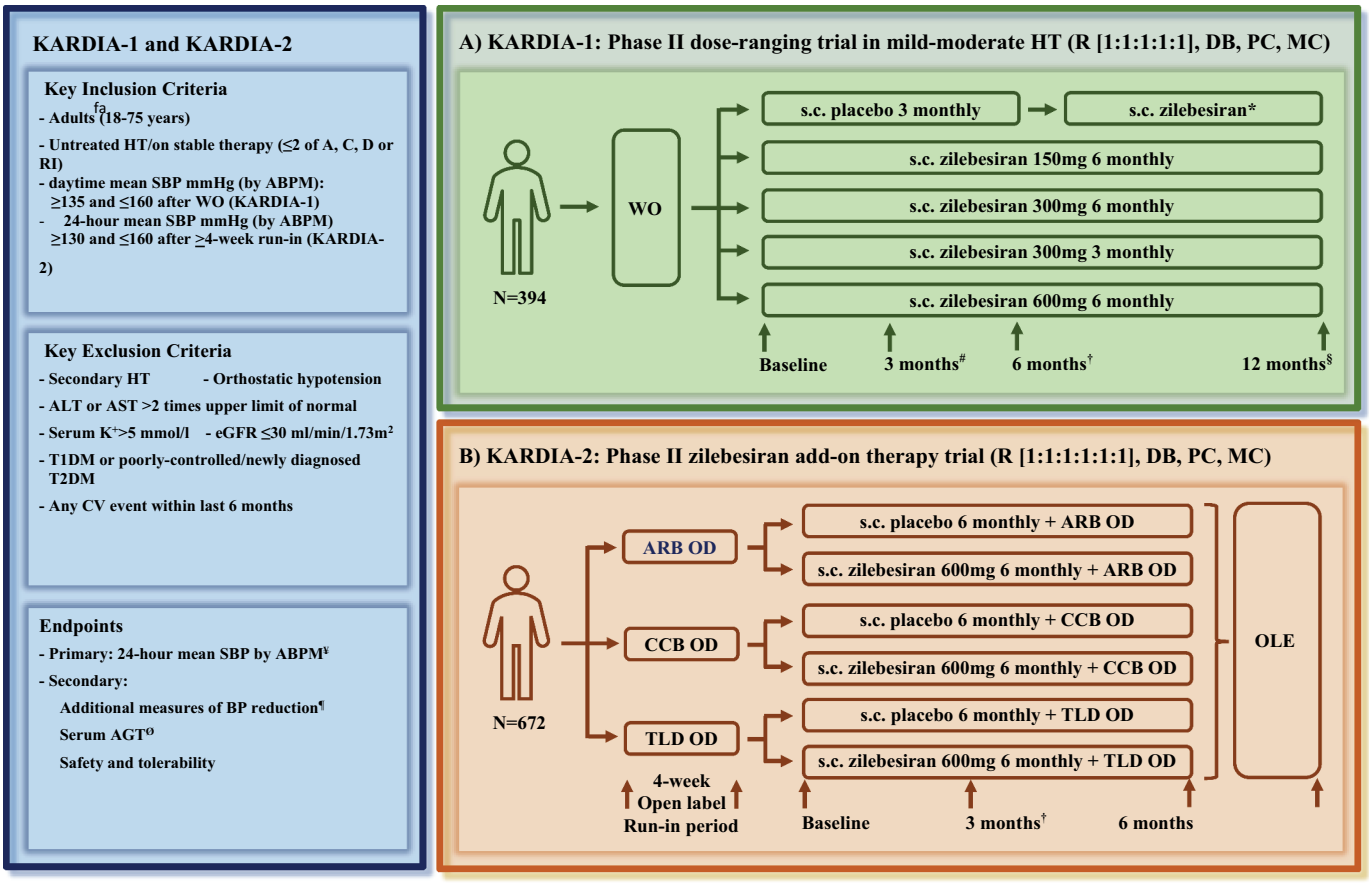

Figure 2. Study design of zilebesiran phase II clinical trials A) KARDIA-1 and B) KARDIA-2.

*Placebo group randomized across the initial four zilebesiran treatment regimens, participants randomized initially to zilebesiran regimens will remain on their originally assigned regimens through remainder of the study; # Primary endpoint; † Addition of oral anti-hypertensives as per investigator judgment up to end of extension period; § Double-blind extension period up to 12 months; ¥ Change from baseline at 3 months; ¶ a) Change from baseline at months 3 and 6 in Office SBP, b) change from baseline at month 6 in 24-h mean SBP and DBP (ABPM), c) proportion of patients at 6 months with 24-h mean SBP (ABPM) <130 mmHg and/or reduction ≥20 mmHg without additional medications, d) time-adjusted change in 24-h mean SBP (and DBP in Kardia-1) from baseline (ABPM) at 6 months, e) change from baseline in office SBP and DBP at 6 months, g) change from baseline in daytime and nighttime mean SBP and DBP (ABPM) at 6 months; Ø Change from baseline at 6 months; A – Angiotensin converting enzyme inhibitors or angiotensin II receptor blockers; ABPM – Ambulatory blood pressure monitoring; AGT – Angiotensinogen; ALT – Alanine aminotransferase; ARB – Angiotensin II receptor blocker (olmesartan); AST – Aspartate aminotransferase; BP – Blood pressure; C – Calcium channel blocker; CCB – Calcium channel blocker (amlodipine); CV – Cardiovascular; D – Thiazide or thiazide-like diuretics; DB – Double-blind; eGFR – estimated glomerular filtration rate; HT – Hypertension; K^+ – Potassium; MC – Multi-center; OD – Once daily; OLE – Open label extension period, s.c. zilebesiran 600 mg 6 monthly; PC – Placebo-controlled; R – Randomized; RI – Renin inhibitors; s.c. – Subcutaneous; SBP – Systolic blood pressure; T1DM – Type-1 diabetes mellitus; T2DM – Type-2 diabetes mellitus; TLD – Thiazide like diuretic (indapamide); WO – Wash out period (at least 4 weeks).

with durable and effective lowering of AGT and BP resulting from only twice-yearly administrations, which could even be performed in community settings. If the magnitude and duration of effect seen with zilebesiran is confirmed, the drug has the potential to give rise to major improvements in BP control and adherence respectively in the hypertensive population. Ultimately, and these are early days, if priced appropriately, zilebesiran could be groundbreaking in generating meaningful reductions in the global burden of cardiovascular and all-cause morbidity and mortality.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Collaboration NCDRF. Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. Lancet. [2021 Sep 11];398(10304):957–980. doi: 10.1016/S0140-6736(21)01330-1
2. WHO. Hypertension factsheet 2021. Available from: https://www.who.int/news-room/fact-sheets/detail/hypertension
3. Wang G, Grosse SD, Schooley MW. Conducting research on the economics of hypertension to improve cardiovascular health. Am J Prev Med. 2017 Dec;53(6 Suppl 2):S115–S117. doi: 10.1016/j.amepre.2017.08.005
4. Abegaz TM, Shehab A, Gebreyohannes EA, et al. Nonadherence to antihypertensive drugs: a systematic review and meta-analysis. Medicine (Baltimore). 2017 Jan;96(4):e5641. doi: 10.1097/MD.0000000000005641
5. Chang TE, Ritchey MD, Park S, et al. National rates of nonadherence to antihypertensive medications among insured adults with hypertension, 2015. Hypertension. 2019 Dec;74(6):1324–1332. doi: 10.1161/HYPERTENSIONAHA.119.13616
6. Burnier M, Egan BM. Adherence in hypertension. Circ Res. [2019 Mar 29];124(7):1124–1140. doi: 10.1161/CIRCRESAHA.118.313220
7. Lawson AJ, Hameed MA, Brown R, et al. Nonadherence to antihypertensive medications is related to pill burden in apparent treatment-resistant hypertensive individuals. J Hypertens. 2020 Jun;38(6):1165–1173. doi: 10.1097/HJH.0000000000002398
8. Cruz-Lopez EO, Ye D, Wu C, et al. Angiotensinogen suppression: a new tool to treat cardiovascular and renal disease. Hypertension. 2022 Jul;29: doi: 10.1161/HYPERTENSIONAHA12218731
   - **Discusses angiotensinogen suppression in hypertension, primarily from the perspective of preclinical studies.**
9. Ettehad D, Emdin CA, Kiran A, et al. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. Lancet. 2016 Mar 5;387(10022):957–967. doi: 10.1016/S0140-6736(15)01225-8
10. Desai AS, Webb DJ, Taubel J, et al. Zilebesiran, an RNA Interference Therapeutic Agent for Hypertension. N Engl J Med. [2023 Jul 20];389(3):228–238. doi: 10.1056/NEJMoa2208391
    - **Reports the key phase I trial data on zilebesiran, which did not show any major treatment-related safety signals and showed promise in generating a substantial and durable effect on blood pressure in patients with hypertension. These data justified the initiation of two phase 2 clinical trials (KARDIA-1 and KARDIA-2).**
11. Zhang MM, Bahal R, Rasmussen TP, et al. The growth of siRNA-based therapeutics: updated clinical studies. Biochem Pharmacol. 2021 Jul;189:114432.
12. Fitzgerald K, White S, Borodovsky A, et al. A highly durable RNAi therapeutic inhibitor of PCSK9. N Engl J Med. 2017 Jan 5;376(1):41–51. doi: 10.1056/NEJMoa1609243
13. Ray KK, Wright RS, Kallend D, et al. Two phase 3 trials of inclisiran in patients with elevated LDL cholesterol. N Engl J Med. [2020 Apr 16];382(16):1507–1519. doi: 10.1056/NEJMoa1912387
    - **Reports the phase III trial data on inclisiran for hypercholesterolaemia. These data supported the licensing of inclisiran, and provided a substantial body of safety and efficacy data underpinning the clinical use of siRNAs to address a common cardiovascular risk factor.**
14. Ranasinghe P, Addison M, Dear JW, et al. Small interfering RNA (siRNA): discovery, pharmacology and clinical development - an introductory review. Br J Pharmacol. 2022 Oct 17.
    - **Discusses the evolution of siRNA therapeutics from initial discovery, though refinement, to treatment of rare diseases and more recnrtly, common conditions. This paper highlights the potential for wider use of siRNA to address targets that are 'undruggable' with classical small-molecule approaches.**
15. Sheu-Gruttadauria J, MacRae IJ. Structural foundations of RNA silencing by argonaute. J Mol Biol. [2017 Aug 18];429(17):2619–2639. doi: 10.1016/j.jmb.2017.07.018
16. Setten RL, Rossi JJ, Han SP. The current state and future directions of RNAi-based therapeutics. Nat Rev Drug Discov. 2019 Jun;18(6):421–446. doi: 10.1038/s41573-019-0017-4
17. Jinek M, Doudna JA. A three-dimensional view of the molecular machinery of RNA interference. Nature. [2009 Jan 22];457(7228):405–412. doi: 10.1038/nature07755
18. Brown CR, Gupta S, Qin J, et al. Investigating the pharmacodynamic durability of GalNAc-siRNA conjugates. Nucleic Acids Res. 2020 Dec 2;48(21):11827–11844. doi: 10.1093/nar/gkaa670
19. Cheng CJ, Tietjen GT, Saucier-Sawyer JK, et al. A holistic approach to targeting disease with polymeric nanoparticles. Nat Rev Drug Discov. 2015 Apr;14(4):239–247. doi: 10.1038/nrd4503
20. Prakash TP, Graham MJ, Yu J, et al. Targeted delivery of antisense oligonucleotides to hepatocytes using triantennary N-acetyl galactosamine improves potency 10-fold in mice. Nucleic Acids Res. 2014 Jul;42(13):8796–8807. doi: 10.1093/nar/gku531
21. Cedillo I, Chreng D, Engle E, et al. Synthesis of 5′-GalNAc-conjugated oligonucleotides: a comparison of solid and solution-phase conjugation strategies. Molecules. 2017 Aug 15;22(8):1356. doi: 10.3390/molecules22081356
22. Nair JK, Willoughby JL, Chan A, et al. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. J Am Chem Soc. 2014 Dec 10;136(49):16958–16961. doi: 10.1021/ja505986a
23. Park J, Park J, Pei Y, et al. Pharmacokinetics and biodistribution of recently-developed siRNA nanomedicines. Adv Drug Deliv Rev. 2016 Sep 1;104:93–109. doi: 10.1016/j.addr.2015.12.004
24. Spiess M. The asialoglycoprotein receptor: a model for endocytic transport receptors. Biochemistry. [1990 Oct 30];29(43):10009–10018. doi: 10.1021/bi00495a001
25. Wittrup A, Ai A, Liu X, et al. Visualizing lipid-formulated siRNA release from endosomes and target gene knockdown. Nat Biotechnol. 2015 Aug;33(8):870–876. doi: 10.1038/nbt.3298
26. Dowdy SF. Overcoming cellular barriers for RNA therapeutics. Nat Biotechnol. 2017 Mar;35(3):222–229. doi: 10.1038/nbt.3802
27. Gilleron J, Querbes W, Zeigerer A, et al. Image-based analysis of lipid nanoparticle-mediated siRNA delivery, intracellular trafficking and endosomal escape. Nat Biotechnol. 2013 Jul;31(7):638–646. doi: 10.1038/nbt.2612
28. Dowdy SF, Setten RL, Cui XS, et al. Delivery of RNA therapeutics: the great endosomal escape! Nucleic Acid Ther. 2022 May 24;32(5):361–368. doi: 10.1089/nat.2022.0004
29. Stec DE, Davisson RL, Haskell RE, et al. Efficient liver-specific deletion of a floxed human angiotensinogen transgene by adenoviral delivery of cre recombinase in vivo. J Biol Chem. 1999 Jul 23;274(30):21285–21290. doi: 10.1074/jbc.274.30.21285
30. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol. 2007 Jan;292(1):C82–97. doi: 10.1152/ajpcell.00287.2006
31. Kumar U, Wettersten N, Garimella PS. Cardiorenal syndrome: pathophysiology. Cardiol Clin. 2019 Aug;37(3):251–265. doi: 10.1016/j.ccl.2019.04.001
32. Hunter PG, Chapman FA, Dhaun N. Hypertension: Current trends and future perspectives. Br J Clin Pharmacol. 2021 Oct;87(10):3721–3736. doi: 10.1111/bcp.14825
33. Sparks MA, Crowley SD, Gurley SB, et al. Classical renin-angiotensin system in kidney physiology. Compr Physiol. 2014 Jul;4(3):1201–1228.
34. Forrester SJ, Booz GW, Sigmund CD, et al. Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology. Physiol Rev. 2018 Jul 1;98(3):1627–1738. doi: 10.1152/physrev.00038.2017
35. Mooser V, Nussberger J, Juillerat L, et al. Reactive hyperreninemia is a major determinant of plasma angiotensin II during ACE inhibition. J Cardiovasc Pharmacol. 1990 Feb;15(2):276–282. doi: 10.1097/00005344-199002000-00015
36. Ren L, Colafella KMM, Bovee DM, et al. Targeting angiotensinogen with RNA-based therapeutics. Curr Opin Nephrol Hypertens. 2020 Mar;29(2):180–189. doi: 10.1097/MNH.0000000000000586

37. Sealey JE, Laragh JH. Aliskiren, the first renin inhibitor for treating hypertension: reactive renin secretion may limit its effectiveness. Am J Hypertens. 2007 May;20(5):587–597. doi: 10.1016/j.amjhyper.2007.04.001
38. Uijl E, Mirabito Colafella KM, Sun Y, et al. Strong and sustained antihypertensive effect of small interfering RNA targeting liver angiotensinogen. Hypertension. 2019 Jun;73(6):1249–1257. doi: 10.1161/HYPERTENSIONAHA.119.12703
39. Desai AS, Webb DJ, Taubel J, et al. Zilebesiran, an RNA interference therapeutic for hypertension. N Engl J Med. 2023;389(3). doi: 10.1056/NEJMoa2208391
40. Uijl E, Ye D, Ren L, et al. Conventional vasopressor and vasopressor-sparing strategies to counteract the blood pressure-lowering effect of small interfering RNA targeting angiotensinogen. J Am Heart Assoc. 2022 Aug 2;11(15):e026426. doi: 10.1161/JAHA.122.026426
41. Zlatev I, Castoreno A, Brown CR, et al. Reversal of siRNA-mediated gene silencing in vivo. Nat Biotechnol. 2018 Jul;36(6):509–511. doi: 10.1038/nbt.4136
42. Khanna A, English SW, Wang XS, et al. Angiotensin II for the treatment of vasodilatory shock. N Engl J Med. 2017 Aug 3;377(5):419–430. doi: 10.1056/NEJMoa1704154
43. De Backer D, Biston P, Devriendt J, et al. Comparison of dopamine and norepinephrine in the treatment of shock. N Engl J Med. 2010 Mar 4;362(9):779–789. doi: 10.1056/NEJMoa0907118
44. Makani H, Bangalore S, Desouza KA, et al. Efficacy and safety of dual blockade of the renin-angiotensin system: meta-analysis of randomised trials. BMJ. 2013 Jan 28;346(Jan28 1):f360. doi: 10.1136/bmj.f360
45. Raal FJ, Kallend D, Ray KK, et al. Inclisiran for the treatment of heterozygous familial hypercholesterolemia. N Engl J Med. 2020 Apr 16;382(16):1520–1530. doi: 10.1056/NEJMoa1913805
46. Watanabe H, Martini AG, Brown EA, et al. Inhibition of the renin-angiotensin system causes concentric hypertrophy of renal arterioles in mice and humans. JCI Insight. 2021 Dec 22;6(24). doi: 10.1172/jci.insight.154337
47. Ranasinghe P, Addison ML, Webb DJ. Small interfering RNA therapeutics in hypertension: a viewpoint on vasopressor and vasopressor-sparing strategies for counteracting blood pressure lowering by angiotensinogen–targeting small interfering RNA. J Am Heart Assoc. [2022 Oct 10];11(20):e027694. doi: 10.1161/JAHA.122.027694
48. Hill RD, Vaidya PN. Angiotensin II receptor blockers (ARB). Treasure Island (FL): StatPearls; 2022.
49. Hodsman GP, Isles CG, Murray GD, et al. Factors related to first dose hypotensive effect of captopril: prediction and treatment. Br Med J (Clin Res Ed). 1983 Mar 12;286(6368):832–834. doi: 10.1136/bmj.286.6368.832
50. Buawangpong N, Teekachunhatean S, Koonrungsesomboon N. Adverse pregnancy outcomes associated with first-trimester exposure to angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers: a systematic review and meta-analysis. Pharmacol Res Perspect. 2020 Oct;8(5):e00644. doi: 10.1002/prp2.644
51. Kario K. Nocturnal hypertension: new technology and evidence. Hypertension. 2018 Jun;71(6):997–1009. doi: 10.1161/HYPERTENSIONAHA.118.10971
52. Parati G, Stergiou G, O'Brien E, et al. European Society of hypertension practice guidelines for ambulatory blood pressure monitoring. J Hypertens. 2014 Jul;32(7):1359–1366. doi: 10.1097/HJH.0000000000000221
53. Ishikawa J, Hoshide S, Eguchi K, et al. Nighttime home blood pressure and the risk of hypertensive target organ damage. Hypertension. 2012 Oct;60(4):921–928. doi: 10.1161/HYPERTENSIONAHA.112.198101
54. Choudhry NK, Kronish IM, Vongpatanasin W, et al. Medication adherence and blood pressure control: a scientific statement from the American Heart Association. Hypertension. 2022 Jan;79(1):e1–e14. doi: 10.1161/HYP.0000000000000203
55. Alnylam Pharmaceuticals. Alnylam Reports Positive Topline Results from KARDIA-1 Phase 2 Dose-Ranging Study of Zilebesiran, an Investigational RNAi Therapeutic in Development to Treat Hypertension in Patients at High Cardiovascular Risk 2023 [Feb/7/2023]. Available from: https://investors.alnylam.com/press-release?id=27706