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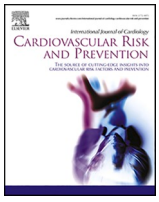

Gene editing of angiotensin for blood pressure management

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ABSTRACT

Arterial hypertension has remained the world's leading cause of morbidity and mortality for more than 20 years. While early Genome-Wide Association Studies raised the hypothesis that a precision medicine approach could be implemented in the treatment of hypertension, the large number of single nucleotide polymorphisms that were found to be associated with blood pressure and their limited impact on the blood pressure values have initially hampered these expectations. With the development and refinement of gene-editing and RNA-based approaches allowing selective and organ-specific modulation of critical systems involved in blood pressure regulation, a renewed interest in genetic treatments for hypertension has emerged. The CRISPR-Cas9 system, antisense oligonucleotides (ASO) and small interfering RNA (siRNA) have been used to specifically target the hepatic angiotensinogen (AGT) production, with the scope of safely but effectively reducing the activation of the renin-angiotensin system, ultimately leading to an effective reduction of the blood pressure with extremely simplified treatment regimens that involve weekly, monthly or even once-in-life injection of the drugs. Among the various approaches, siRNA and ASO that reduce hepatic AGT production are in advanced development, with phase I and II clinical trials showing their safety and effectiveness. In the current manuscript, we review the mode of action of these new approaches to hypertension treatment, discussing the results of the clinical trials and their potential to revolutionize the management of hypertension.

1. Epidemiology of arterial hypertension and the need for new approaches

Hypertension, defined as values of blood pressure ≥140 and/or 90 mm Hg of office blood pressure according to European guidelines [1], stands as a significant contributor to the global disease and mortality burden, impacting over 1 billion adults worldwide and exhibiting a notable increase in prevalence, with considerable regional variations [2]. Notably, the increasing availability of drugs effectively reducing blood pressure (BP) values has partially impacted the worldwide morbidity and mortality burden of arterial hypertension over the last 30 years, which, however, remains a major public health issue [3]. Challenges such as poor adherence to complex medication regimens and associated side effects from currently available treatment options, compounded by the inability of current treatment strategies to accommodate the diverse characteristics of hypertensive populations, have substantially limited the current ability to ensure adequate BP control in the general population [4,5]. Indeed, while the widely recognized advantages of lowering BP are notwithstanding, global hypertension control remains low, with less than one-fifth of patients achieving satisfactory management [6].

Existing antihypertensive medications target only specific mechanisms involved in BP regulation, leading to variable effectiveness across individuals and populations [7]. Although many systems and molecular pathways involved in BP regulation are known, there is limited capacity to translate this knowledge at the individual level, making it easier, more effective and rapid to obtain adequate BP control with the use of a combination of BP lowering medications, that counteract more than one mechanisms involved in BP regulation at a time [8]. Indeed, the use of combination therapy in the management of hypertension has been recommended by major international guidelines as a way forward to improve efficacy and reduce the risk of side effects related to antihypertensive treatments [1,9–11].

This is partially related to the evidence that monogenic forms of hypertension are rare, while in most cases, the biological architecture of the disease is extremely complex, resulting from the interaction between

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multiple genetic and environmental influences that might interact with each other in a summative or exponential fashion, ultimately resulting in the same final phenotype of elevated BP [12]. Based on this evidence, the National Institutes of Health has adapted the definition of precision medicine to hypertension (the "precision hypertension") as an approach to preventing and treating hypertension that considers the unique genetic makeup and environmental factors of each hypertensive patient [13]. This approach acknowledges that individuals with hypertension may respond differently to identical treatments due to genetic diversity, environmental influences, or other personal traits [14]. According to its definition, precision hypertension employs genomic analysis, along with other omics, molecular profiling, health data, environmental factors including lifestyle, and extensive data analysis, to gather and evaluate information about an individual's health [12,14]. Thus, precision hypertension aims to offer more precise diagnoses, predict disease outcomes more accurately, and develop targeted therapies that are more effective and associated with fewer side effects. By tailoring treatments to individual needs, precision hypertension has the potential to improve the management and treatment of hypertension substantially, thereby enhancing individual health outcomes and reducing overall costs related to disease management.

However, several obstacles hinder the widespread implementation of precision medicine in hypertension, including the slow adoption of genomic information in clinical practice and research, economic constraints, and the suspiciousness of patients and clinicians to novel genetic approaches to treatment. Nevertheless, more recent advancements in gene therapies and gene editing have partially addressed these challenges, opening the window to a precision hypertension management.

In this review, we summarize recent advancements in gene therapies for hypertension, and we discuss the potential advantages and challenges to their implementation in daily clinical practice.

2. From genome studies to gene therapy

Genomics has rapidly evolved since 2000's due to an increasing number of genome-wide association studies (GWASs) that have provided a fundamental stimulus to developing the precision medicine approach for different chronic diseases [15]. Genome-wide association studies represent a high throughput approach to explore the genomics bases of complex diseases, pinpointing the disease-linked single--nucleotide polymorphisms (SNPs) [16,17]. Identifying the unique SNP profile associated with a disease in each individual might reveal the underlying biological pathways providing the most significant contribution to the disease-specific phenotypes, ultimately enabling personalization of the therapy [17].

The GWASs approach has been extensively adopted to identify genetic variants causally associated with arterial hypertension in the last 20 years [18]. These studies have shown that, while certain rare instances of human hypertension originate from single gene mutations, the majority result from a combination of multiple genetic factors [17]. In cases of monogenic hypertension, where a single gene mutation follows Mendelian inheritance patterns, gene therapy or editing could offer a cure to the disease. In turn, in most cases of primary hypertension, the phenotype is influenced by multiple genes with minor effects on BP alongside environmental factors. While polygenic risk scores represent a promising development for disease prediction and they have shown utility in identifying individuals at risk of developing hypertension before its onset [19], the complex genetic architecture contributing to elevated BP and the relatively limited impact of each genetic variant on the BP values pose intricate challenges for the development of genetic intervention [13]. For example, one of the largest GWAS on hypertension has documented the contribution to the elevated BP values of gene variants that are shared with the genetic predisposition to different lifestyle exposures, including tea, coffee and alcohol intake, sedentary life, body mass index and waist circumference [20]. In the same GWAS, an ingenuity pathway analysis and upstream regulator assessment showed enrichment of canonical pathways implicated in the regulation of vascular biology and targeted by antihypertensive drugs, such as angiotensinogen, calcium channels, natriuretic peptide receptor, angiotensin-converting enzyme, angiotensin receptors and endothelin receptors. The assessment of tissue enrichment of BP loci identified a greater enrichment for the cardiovascular system, the heart, adrenal tissue and adipose tissues. These results emphasize that several genetic predispositions involved in influencing life-styles, vascular biology and its determinants might interact in a complex fashion, finally resulting in the acquisition of the hypertensive phenotype. Such challenges are further amplified by the evidence that, despite approximately 1500 SNPs with effects on BP have been identified [21], they only explain about 27 % of the estimated heritability of BP and 5.7 % of the variability in systolic BP (SBP) [17]. Furthermore, the evidence that many SNPs associated with elevated BP are either intergenic or located in genes lacking direct relevance to disease makes it challenging to pinpoint mechanisms underpinning the SNP-to-phenotype relationships [21]. Therefore, while GWAS hold promise in uncovering the biological pathways that in each individual could contribute to the elevation of BP, the translation of their finding into drug development and clinical benefits presents significant hurdles.

Despite these challenges, efforts to enhance GWAS utility are underway, including combining GWASs with other high-throughput technologies. Combinatorial omics approaches have proven beneficial for drug repurposing and identifying potential therapeutics [22,23]. Establishing clear, multilayer chains of molecular signals leading to the overactivation or downregulation of specific biological pathways involved in arterial hypertension makes it possible to identify different, putative therapeutic targets. The recent development of genetic approaches targeting the synthesis of angiotensinogen (AGT) at various levels in the cascade leading to the final protein synthesis represents a clear example of the potentials offered by a multi-level approach in the modulation of the biological pathways, whereby CRISPCas9 can provide the opportunity to modify directly the genome [24], while small-interfering RNAs (siRNAs) or antisense oligonucleotides (ASO) targeting the AGT mRNA might act downstream in the process leading to protein synthesis [25–27], however obtaining an effective inhibition of its expression.

3. The CRISPR-Cas9 system: a new frontier to gene editing

CRISPR-Cas9 is a genome editing tool that has introduced new frontiers in molecular biology and biotechnology [28]. Derived from the prokaryotic adaptive immune system [29], CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and its associated protein Cas9 enable precise and efficient manipulation of DNA sequences in various organisms, including humans. The system functions by utilizing guide a dual-guide RNA composed of a CRISPR RNA (crRNA)-trans-activating CRISPR RNA (tracrRNA) pair or a single-guide RNA (sgRNA) to direct the Cas9 protein to specific target sequences within the genome, where it induces double-stranded breaks (Fig. 1). Recognition of target DNA relies on both the complementarity with the spacer sequence of the guide RNA and the presence of a protospacer adjacent motif (PAM) located on the non-target strand (NTS) of the DNA. More specifically, upon initial recognition of the PAM, local unwinding of the target DNA occurs, followed by base-pairing of the sgRNA with the target strand (TS) of the DNA in a 5'-3' directional manner, starting from the PAM-proximal end of the target site [30]. This process triggers conformational changes in Cas9, leading to the activation of the nuclease domain [31]. Thus, by designing a sgRNA with a matching sequence to a specific DNA sequence, it is possible to target any specific genomic site with extreme precision. Subsequently, Cas9 cleaves the double-stranded DNA (dsDNA) substrate, generating double-strand breaks (DSBs) with either blunt ends or single-nucleotide 5' overhangs. These DSBs can then be repaired by end-joining pathways, which are typically error-prone, or by precise homology-directed repair (HDR) mechanisms [30] (Fig. 1).

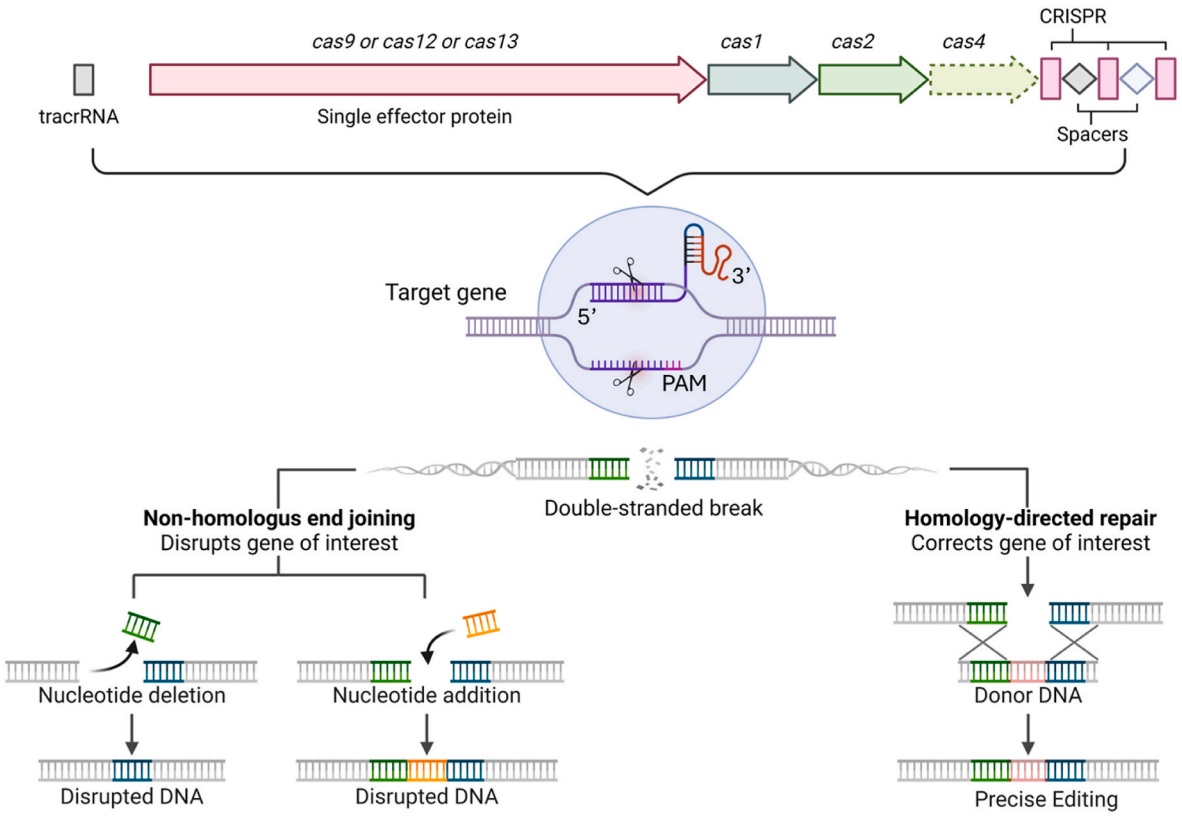

Fig. 1. Mode of action of CRISPR-Cas9 system. The CRISPR sequence is made up of a leader, repeats, and spacers. crRNA with a tracrRNA forms the single guide RNA (sgRNA) which, together with the presence of a protospacer adjacent motif (PAM) located on the non-target standard of the DNA, acts as a scaffold and recruit Cas9 to the target gene through RNA-DNA base pairing at the 5'-terminal specific binding sequence. This process triggers conformational changes in Cas9, leading to the activation of the nuclease domain and the generation of double strand breaks (DSBs) in the target DNA. These DSBs trigger non-homologous end joining (NHEJ) or homology-directed repair (HDR) mechanisms to repair mutations, with knock in or knock out specific genes, and modify genes.

End-joining serves as the primary mechanism of DNA repair in mammalian cells, relying on the direct rejoining of broken DNA ends through either the non-homologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ) pathways [32,33]. Before the rejoining occurs, processing of the exposed DNA ends may involve the addition or removal of nucleotides, leading to short insertions or deletions (referred to as indels) at the site of the DSBs [34]. It is believed that repeated cleavage of precisely repaired DSBs may accumulate indels, eventually preventing further cleavage [34]. This method is commonly utilized to selectively disrupt protein-coding gene sequences for gene knockouts or deletions by introducing two DSBs in close proximity. In contrast, HDR represents a precise pathway for repairing double-strand breaks (DSBs), contingent upon the presence of a homologous DNA molecule to direct the repair outcome [32]. By externally supplying an artificial homology repair template, HDR can be utilized to precisely introduce desired mutations, insertions, or deletions at the targeted genomic locus. The repair templates, administered either as double-stranded DNAs (typically through plasmids or viral vectors) or synthetic single-stranded DNA oligonucleotides (ssODNs), contain the desired mutation flanked by sequences homologous to regions on both sides of the DSB [32]. While this method theoretically enables editing with nucleotide-level precision, HDR is predominantly active in actively dividing cells, as it necessitates repair factors commonly expressed only during the S and G2 phases of the cell cycle [32].

Despite the numerous advantages of the CRISPR-Cas9 system for gene editing, it also presents limitations. The primary obstacle for most in vivo and ex vivo gene editing applications continues to be the targeted delivery of gene editors. In vivo, delivery of CRISPR-Cas9 into mammalian cells is typically accomplished using viral vectors [35] or non-viral approaches such as lipid-based nanoparticles [36,37]. The latter approach is increasingly preferred given that it is associated with reduced immunogenicity than viral-based vectors and the fact that adeno-associated viruses (representing the most commonly used viral vectors due to their high transduction efficiency and low immunogenicity) have a relatively small size to host the CRISPR-Cas9 system, making them difficult to use for this specific indication [35,38]. Another limitation is the restricted DNA-binding mechanism of Cas9 nucleases, limiting their targeting region to genomic sites flanked by a PAM sequence. This makes some genomic regions challenging to target. Although several naturally occurring Cas9 orthologs with alternative PAM specificities have been identified and utilized for genome editing, many of these have more stringent PAM requirements [39–41], enhancing targeting specificity and leading to suboptimal DNA cleavage and editing efficiencies. Various artificial Cas9 variants with modified or relaxed PAM specificities have been engineered to address this issue in recent years. While these variants significantly broaden the spectrum of targetable sites, relaxed PAM targeting might reduce targeting specificity [42]. A further limitation belongs to the Cas9 protein, which displays a certain tolerance towards mismatches between the sgRNA and the target DNA. This can result in the targeting and editing of partially complementary off-target sites elsewhere in the genome alongside the intended on-target locus [43–52]. The degree of nucleotide mismatches tolerance by Cas9 can range from single base mismatches to multiple consecutive mismatches, as well as nucleotide insertions or deletions [47–53]. Although most off-target sites are merely bound by Cas9 without undergoing DSBs/editing [31,48–53], and several studies have demonstrated a lower frequency of off-target cleavage events in vivo compared to isolated genomic DNA [49,54], the off-target activity of the CRISPR-Cas9 system has raised concerns regarding its therapeutic applications. Finally, there might be issues in controlling the subsequent

editing resulting from the CRISPR-Cas9 activity. Large deletions, chromosomal rearrangements and chromosome loss might limit editing precision at the intended genomic target site [55–57]. Furthermore, the choice of repair template and delivery might substantially influence the editing efficiency when using HDR [58,59].

4. Application of CRISPR-Cas9 to arterial hypertension research

The simplicity, versatility, and cost-effectiveness of CRISPR-Cas9 have substantially simplified genome editing, empowering researchers to investigate fundamental biological processes and develop novel therapeutic opportunities.

Given the mechanism used for gene editing, CRISPR-Cas9 is practical in understanding the impact of single-nucleotide polymorphisms or genes on specific diseases. CRISPR/Cas9 has been utilized in arterial hypertension to demonstrate the potential role of Rffl-lnc1, Gper1 and ARHGAP42 in BP control [60]. Particularly, Rffl-lnc1 is a novel long non-coding RNA within 5'-UTR intronic region of the "Ring finger and FYVE like domain containing E3 ubiquitin-protein ligase" (Rffl) gene, located on chromosome 17. Initially studied as a potential regulator of the QT interval, Chen et al. documented that the CRISPR/Cas9–mediated disruption of a 19bp sequence within the Rffl-lnc1 locus leads to increased BP. In turn, the G-protein-coupled estrogen receptor (Gper1), located on chromosome 7 is a G-protein coupled receptor which belongs to the rhodopsin-like receptor superfamily [61,62]. It represents a receptor for two ligands, estrogen [63–66] and aldosterone [67–69]. Due to the feature of *Gper1* being an estrogen receptor, *Gper1* was originally studied as a relevant gene involved in cancer risk. Several studies have shown that disturbances in *Gper1* expression were associated with the development of breast, endometrial and prostate cancer [69–71]. Waghulde et al. demonstrated that genomic excision of Gper1 obtained by a multiplexed CRISPR-Cas9 system reduced BP in genetically salt-sensitive hypertensive rats [72]. This was accompanied by altered microbiota. Microbiotal transplantation from hypertensive Gper1+/+ rats reversed the cardiovascular protective effect exerted by the genomic deletion of Gper1, suggesting a strong connection between microbiota and alteration in BP regulation in salt-sensitive hypertension mediated by Gper1. Finally, the Rho GTPase Activating Protein 42 (ARHGAP42) is coded by the *Arhgap42* gene on chromosome 11. The protein is a member of the GRAF (GAP for Rho-associated with focal adhesion kinase) family and is highly and selectively expressed in human and mouse smooth muscle tissues [73], controlling its contractility [74]. Based on this preliminary evidence, Bai et al. hypothesized that it could be involved in modulating vascular resistance, potentially explaining its association with variations in BP that emerged in previous GWAS. Starting from this hypothesis, the researchers showed that the CRISPR-Cas9 mediated deletion of the SNP rs604723 (C/T) in human smooth muscle cells markedly reduced the endogenous ARHGAP42 expression and, in mice treated with DOCA-salt, resulted in a faster acquisition and progression of arterial hypertension [75]. Finally, utilizing the CRISPR/Cas9 system Yang et al. obtained a mean BP reduction between 3 and 6 mm Hg by ablating the CPI-17 gene and preventing the activation of the CPI-17 (PKC-potentiated inhibitory protein of 17 kDa) protein. The reduction in BP was found to be associated with reduced blood vessel contractility [76]. These examples provide clear evidence that the CRISPR-Cas9 system can selectively silence specific genes potentially involved in arterial hypertension, providing an opportunity to assess their potential impact on BP regulation and the mechanisms behind such effects.

Therapeutically, CRISPR-Cas9 might be particularly effective in curing monogenic diseases, while its application to polygenic diseases, such as most forms of arterial hypertension, is more complex. Indeed, the multiple and different genetic variations that usually lead to the development of arterial hypertension in each subject make it difficult to hypothesize restoring a protective genetic background through multiple gene editing by the CRISPR/Cas9 technology. Yet, CRISPR-Cas9 can be used to induce specific mutations and thus modulate common pathways known to be upregulated in arterial hypertension, such as the renin-angiotensin system (RAS). This strategy has been used for the first time by Sun et al. in female and male spontaneously hypertensive rats (SHR), showing promising results in treating hypertension [24]. The group adopted the CRISPR-Cas9 system to selectively silent the AGT production in the liver by using the adeno-associated virus 8. Importantly, to avoid excessive reduction of the RAS function potentially compromising its homeostatic functions, they adjusted the viral dose to obtain a ≈50 % reduction in circulating concentrations of AGT and confirmed that the expression of adeno-associated virus 8 expression was detectable only in the hepatocytes and not in other tissues. Following CRIPR-Cas9-mediated ablation of the hepatic AGT, the authors documented a marked reduction of its AGT circulating levels, together with those of angiotensin I and angiotensin II. This was accompanied by a significant and sustained reduction of BP in rats that already developed hypertension or slowed down the development of hypertension in normotensive animals. Intriguingly, in a subset of SHR animals, a lower BP was still observed in gene-edited vs control animals after 1 year. A further important result was the documented capacity of gene-edited animals to maintain the capacity to respond to physiological challenges typically requiring the activation of the RAS. Indeed, in a subset of experiments, gene-edited and control SHR were challenged with a 10-day low-salt diet combined with furosemide treatment. Although gene-edited animals presented with a lower BP at the end of the dietetic challenge compared with their control peers, they did not reach critically low BP values.

Pathophysiological considerations support the selection of AGT as a potential target for gene-editing. Firstly, by reducing AGT expression, any potential RAS escape mechanism is prevented, given that it represents the precursor of all subsequent steps in the RAS activation. Secondly, previous animal studies documented significant reductions in BP obtained by silencing AGT expression in the whole body or at a tissue-specific level [77]. Furthermore, the overactivation of the RAS in patients with hypertension contributes to BP elevation but also to the progression of hypertension-mediated organ damage [1]. Thus, the total or partial silencing of the AGT expression might result in additional protective effects on the risk of adverse outcomes that can go beyond the BP reduction. Despite these advantages, this approach also raises several concerns. For example, the downregulated expression of the hepatic AGT might result in a reduced production of other RAS-derived peptides (including, for example, ACE2, Ang1-9, Ang1-7), some of which might have antihypertensive and cardiovascular protective effects. The effects of the reduced concentration of these peptides on vascular physiology remain completely unexplored. However, the first and probably most important concern in adopting gene-editing to modulate AGT expression is the irreversible genomic changes induced in the genetic information. Sun et al. showed that, within certain limits, it is possible to downregulate the AGT expression through gene-editing, while maintaining the capacity of the RAS to respond to physiological challenges [24]. However, the challenges adopted in their study were modest and of limited clinical relevance compared to severe conditions of hypotension and/or hypovolaemia that might develop following acute haemorrhagic events or during sepsis, where a more sustained activation of the RAS is normally required. In these conditions, a persistent and irreversible reduction of the RAS activity might profoundly impact patients' prognosis, as it might compromise the capacity to counteract severe decreases in tissue perfusion pressures. Therefore, novel and less permanent approaches for AGT silencing have been developed based on post-transcriptional silencing of mRNA signaling.

5. Small interference RNA or antisense RNA: alternative approaches to temporarily reduce the AGT expression in arterial hypertension

RNA molecules like antisense oligonucleotides (ASOs), small

interfering RNA (siRNAs), and microRNAs (miRNAs) can directly interact with mRNAs and noncoding RNAs (ncRNAs), promoting their degradation [78]. Consequently, the protein synthesis is reduced, similar to the know-out silencing obtained with CRISPR-Cas9, but acting downstream in the sequence of events leading to the protein synthesis. This holds several advantages, the first and most important is that changes in gene expression are reversible. Furthermore, these RNA-based approaches can theoretically target any gene of interest by identifying the appropriate nucleotide sequence on the target RNA. The final result of the ASO or siRNA-based gene expression modification is the same, consisting of the degradation of an mRNA that leads to a reduced or increased expression of a specific protein. However, the mechanisms by which these results are obtained differ slightly between ASO and siRNA.

ASOs are brief single-stranded (ss) oligonucleotides, typically comprising 12–24 nucleotides (nt), that pair up with specific RNA sequences and, through occupancy-mediated degradation and occupancy-only models can modify RNA and either decrease, restore, or adjust protein expression [79]. Conversely, siRNAs are short double-stranded RNAs that can be split into two strands within a specific protein complex once entered into the cell [80,81]. One of these strands, known as the "guide" strand, binds to a specific mRNA sequence, termed the "target" mRNA. This interaction leads to the cleavage and degradation of the target mRNA, thereby inhibiting translation and protein synthesis. The IONIS-AGT-LRx and zilebesiran are one ASO and one siRNA, respectively, that have been used to silence AGT hepatic expression in patients with arterial hypertension [25–27]. These RNAs' capacity to specifically target the hepatic AGT expression is ensured by their conjugation with GalNAc, a high-affinity ligand for the hepatocyte-specific asialoglycoprotein receptor (ASGPR) [82].

The IONIS-AGT-LRx was tested in a phase I trial in healthy volunteers and two phase II trials [25]. In the first phase II trial, the drug in monotherapy or the placebo was administered by weekly injections to patients with controlled hypertension. In the second phase II trial, patients with drug-resistant hypertension received weekly injections of IONIS-AGT-LRx or placebo in addition to two or three antihypertension medications. The trials lasted for 6 or 8 weeks and in all of them IONIS-AGT-LRx did not cause changes in potassium levels or liver and renal function or platelet count, while significantly reducing plasma AGT levels compared with placebo. A trend for BP reduction was also observed, although this was not considered the primary outcome of the trials, and thus, the studies were not powered for this endpoint.

More advanced is the development of zilebesiran, which represents the first in class siRNA therapeutic targeting and thus silencing the hepatic AGT mRNA expression. A phase I and two phase II clinical studies (KARDIA-1 and KARDIA-2) have proven the capacity of Zilebesiran to reduce BP and hepatic AGT expression, with a sustained effect lasting for 6 months [26,27]. In the phase I trial, a comprehensive assessment of zilebesiran's safety, pharmacokinetics, and pharmacodynamics was conducted in patients with mild-to-moderate hypertension [26]. The results indicated that Zilebesiran dose $\geq$100 mg induced >90 % decrease in serum AGT levels, sustained up to week 12 with the 100 mg dose and up to week 24 with the 800 mg dose. The drug was well tolerated and did not cause significant episodes of hypotension, hyperkalemia, or worsening renal function, even when an angiotensin-receptor blocker was added to the treatment. The only side effect recorded in 5/107 patients was a transient injection-site reaction. The extent of the BP reduction with the 200 mg injection was >10/5 mm Hg by week 8 and was consistent throughout the diurnal cycle and for up to 24 weeks following a single injection.

These findings were confirmed in the phase II KARDIA-1 study [27], including subjects with mild to moderate hypertension, defined as daytime mean ambulatory SBP (SBP) of 135–160 mm Hg. Patients underwent washout from antihypertensive treatment and were subsequently randomized to receive increasing doses of subcutaneous zilebesiran (150, 300, or 600 mg once every 6 months, or 300 mg once every 3 months) or placebo (once every 3 months) for 6 months. Once entered into the study and received the injection, no additional antihypertensive treatments were allowed until the third month, after which oral antihypertensive medications could be resumed up to month 5 when they were stopped again to assess the isolated effect of zilebesiran compared to placebo at month 6. Once the 6-month follow-up was completed, patients in the placebo group were re-randomized to one of the four zilebesiran groups for an extension phase of the study. The changes in 24-h mean ambulatory SBP from baseline to month 3 (the primary endpoint of the study) were: 7.3 mm Hg with zilebesiran 150 mg every 6 months, −10.0 mm Hg with zilebesiran 300 mg every 3 and 6 months combined, −8.9 with zilebesiran 600 mg every 6 months, and 6.8 mm Hg with placebo. The changes remained stable for up to 6 months. Only 0.9 % of patients receiving zilebesiran had adverse events (vs 0.7 % receiving placebo), and 3.6 % had serious AEs (vs 6.7 % receiving placebo). Drug-related adverse events were mild to moderate in severity, with hyperkalemia requiring treatment with potassium binders occurring in only 5.3 % of the recruited patients.

Finally, the KARDIA-2 study results presented at the ACC Annual Congress 2024 documented the capacity of Zilebesiran to effectively reduce BP in patients who had untreated SBP 155–180 mm Hg, or treated SBP 145–180 mm Hg. Following a run-in period with indapamide 2,5 mg, amlodipine 5 mg or Olmesartan 40 mg, 1500 patients underwent Zilebesiran injection and changes from baseline to 3 months in the 24-h mean ambulatory SBP (primary outcome) and office SBP (secondary outcome) were assessed. Again, Zilebesiran significantly reduced the values of ambulatory SBP from baseline, more significantly in the indapamide (−12.1 mm Hg) and amlodipine (−9.7 mm Hg) treated groups, and less significantly in the Olmesartan group (−4 mm Hg). Even more pronounced changes in the office SBP were recorded. No deaths or adverse events leading to study discontinuation were recorded in the trial.

While these results are extremely promising for the future development of RNA-therapeutics in hypertension, the use of ASO and siRNA technology largely repropose the concerns related to the use of CRISP-Cas9 system, with the potential increase in the risk of severe hypotension in patients with acute hypovolaemia or haemorrhages. Nevertheless, the shorter effect of IONIS-AGT-LRx and, to a lesser extent, of the Zilebesiran, enable greater versatility of the treatment, potentially making it adaptable to seasonal changes in the BP values. Furthermore, synthetic, high-affinity oligonucleotides complementary to the siRNA guide strand might reverse siRNA silencing activity, potentially providing a rescue treatment in emergency conditions in patients treated with Zilebesiran [83].

6. Future perspectives

Despite the availability of over 100 approved BP-lowering medications, patients with controlled BP represent a minority among those with a diagnosis of hypertension. This is primarily due to the complex architecture of hypertension and the limited patient adherence to the treatment. Gene-editing approaches targeting the synthesis of the heaptic AGT are effective in reducing the BP and substantially simply the treatment regimen, potentially improving medication adherence and persistence. The selective inhibition of the hepatic AGT synthesis limits off-target effects and, given that AGT is the unique precursor of the RAS, avoids escape phenomena that are common with currently available therapies. While promising, the efficacy and safety of these drugs require further clinical evaluation in larger patient populations with a more heterogeneous comorbidities burden. Beyond their potential impact in daily clinical practice, gene-editing therapies also offer new opportunities in hypertension research. Indeed, the capacity of these approaches to target, theoretically, any gene of interest by selecting the correct nucleotide sequence on the target RNA stimulate the identification of novel molecular pathways involved in BP regulation and the evolution/protection from hypertension-mediated organ damage.

CRediT authorship contribution statement

Stefano Masi: Writing – original draft, Data curation, Conceptualization. **Hermann Dalpiaz:** Writing – original draft, Validation, Data curation, Conceptualization. **Claudio Borghi:** Writing – original draft, Validation, Supervision, Data curation, Conceptualization.

Conflicts of interest

S.M. reports receiving honoraria for consulting, lecturing and editing activities from Servier and conducting international clinical trials from Vifor. C.B. has received research grant support from Menarini Corporate and Novartis Pharma; has served as a consultant for Novartis Pharma, Alfasigma, Grunenthal, Menarini Corporate, and Laboratoires Servier; and received lecturing fees from Laboratoires Servier, Takeda, Astellas, Teijin, Novartis Pharma, Berlin Chemie, and Sanofi.

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