Canadian Journal of Cardiology ■ (2023) 1–20

Review

Clinical Impact and Mechanisms of Nonatherosclerotic Vascular Aging: The New Kid to Be Blocked

Soroush Mohammadi Jouabadi, PharmD,[a,b] Ehsan Ataei Ataabadi, PharmD, PhD,[a] Keivan Golshiri, PharmD, PhD,[a] Daniel Bos, MD, PhD,[b,c] Bruno H.C. Stricker, MD, PhD,[b] A.H. Jan Danser, PharmD, PhD,[a] Francesco Mattace-Raso, MD, PhD,[d] and Anton J.M. Roks, PhD[a]

[a] *Division of Vascular Medicine and Pharmacology, Department of Internal Medicine, Erasmus MC University Medical Center, Rotterdam, The Netherlands*

[b] *Department of Epidemiology, Erasmus MC University Medical Center, Rotterdam, The Netherlands*

[c] *Department of Radiology and Nuclear Medicine, Erasmus MC University Medical Center, Rotterdam, The Netherlands*

[d] *Division of Geriatric Medicine, Department of Internal Medicine, Erasmus MC University Medical Center, Rotterdam, The Netherlands*

ABSTRACT

Ischemic cardiovascular disease and stroke remain the leading cause of global morbidity and mortality. During aging, protective mechanisms in the body gradually deteriorate, resulting in functional, structural, and morphologic changes that affect the vascular system. Because atherosclerotic plaques are not always present along with these alterations, we refer to this kind of vascular aging as nonatherosclerotic vascular aging (NAVA). To maintain proper vascular function during NAVA, it is important to preserve intracellular signalling, prevent inflammation, and block the development of senescent cells. Pharmacologic interventions targeting these components are potential therapeutic approaches for NAVA, with a particular emphasis on inflammation and senescence. This review provides an overview of the pathophysiology of vascular aging and explores potential pharmacotherapies that can improve the function of aged vasculature, focusing on NAVA.

RÉSUMÉ

La maladie cardiovasculaire ischémique et l'accident vasculaire cérébral (AVC) figurent en tête des causes de morbidité et de mortalité à l'échelle mondiale. Lorsqu'une personne prend de l'âge, les mécanismes protecteurs de l'organisme se détériorent graduellement, ce qui se solde par des changements fonctionnels, structuraux et morphologiques qui influent sur le système vasculaire. Étant donné que des plaques d'athérosclérose n'accompagnent pas toujours ces modifications, nous employons le terme « vieillissement vasculaire non artérioscléreux » (VVNA) lorsque nous parlons de ce type de vieillissement vasculaire. Afin de conserver une fonction vasculaire convenable durant le VVNA, il est important de préserver la signalisation intracellulaire, de prévenir l'inflammation et d'inhiber la sénescence cellulaire. Les interventions pharmacologiques qui ciblent ces phénomènes, en particulier l'inflammation et la sénescence, constituent des approches thérapeutiques possibles pour contrer le VVNA. Cette analyse présente un survol de la physiopathologie du vieillissement vasculaire et explore les pharmacothérapies qui pourraient améliorer la fonction du système vasculaire chez les personnes âgées, en mettant l'accent sur le VVNA.

Ischemic cardiovascular disease, characterised by myocardial infarction (MI) and angina pectoris, which can also lead to heart failure with decreased ejection fraction, along with ischemic stroke, remains a primary cause of morbidity and mortality worldwide.[1] While atherosclerosis and the derived arterial occlusion result in ischemia, it is important to acknowledge that nonocclusive arterial remodelling also contributes significantly to the development of ischemic conditions. This remodelling encompasses a range of factors, including endothelial dysfunction, microvascular disease, vasospasm, inflammation, and fibrosis, affecting both macro- and microvessels. Notably, these conditions can also occur in the absence of atherosclerotic plaques, and develop intrinsically with increasing age independently from extrinsically acting cardiovascular risk factors. We here apply the term nonatherosclerotic vascular aging (NAVA)—the new kid to be blocked—for this remodelling process.

Received for publication May 5, 2023. Accepted July 20, 2023.

Corresponding author: Dr Anton Roks, Division of Vascular Medicine and Pharmacology, 14th Floor Ee Building, Department of Internal Medicine, Erasmus MC University Medical Center, Dr Molewaterplein 40, Rotterdam, 3015GD, The Netherlands. Tel.: ±31-010-704-3547.

E-mail: a.roks@erasmusmc.nl

See page 14 for disclosure information.

https://doi.org/10.1016/j.cjca.2023.07.022

Advances in the pharmacotherapy of atherosclerotic disease have undeniably reduced the risk of premature disability and death resulting from MI and stroke. However, there has been a notable shift towards the increased prevalence of non-atherosclerotic diseases. This shift is evident from the increase in ischemia caused by coronary artery disease, (diastolic) heart failure, and vascular dementia without clinically relevant occlusion of the local vasculature. The likelihood of suffering from these nonatherosclerotic diseases is strongly age dependent, and classic risk factors, such as elevated blood pressure, smoking, and hyperlipidemia, play important auxiliary roles.[2] Unfortunately, the differentiation between atherosclerotic vascular aging and NAVA, as well as the recognition of common and distinct mechanisms that contribute to both conditions, remain inadequately understood, because effective NAVA models have been created only recently. As a consequence, there are currently no therapies for treating NAVA, highlighting a considerable clinical need. In this review, we present an overview of vascular aging pathophysiology and potential pharmacotherapies to improve the function of aged vasculature, with a focus on NAVA.

Vascular Aging in the Clinic

General definitions

The term "vascular aging" poorly represents a very complex process. For a working model, it might be sensible to divide vascular aging into atherosclerotic and nonatherosclerotic remodelling, which can take place independently but in reality interact. The first process is caused by the impact of the classic cardiovascular risk factors such as lifestyle, smoking, obesity, diabetes, etc that with time lead to cumulative damage and aberrant remodelling in the vascular system. Such vascular aging can be mimicked in an nonhuman animal model by exposure to these risk factors, without the need to age the animal (although aging has an impact!). Atherogenesis is such a process. Because of the external nature of the precipitating factors, we will refer to this process as extrinsic vascular aging for the remainder of this article. The second process involves an age-related inherent decline in the function and elasticity of the vascular system, referred to as intrinsic vascular aging. This process occurs independently from the external risk factors mentioned above and is a naturally occurring consequence of aging.

In its pure form, this can be observed only in aging animal models devoid of any risk factors. NAVA can largely take place on the basis of intrinsic vascular aging. In daily human life, however, intrinsic and extrinsic vascular aging aggravate each other, leading to premature NAVA and accelerated plaque formation (Fig. 1). This review will focus on NAVA, defining clinical variables, epidemiologic features, and the intrinsic and extrinsic mechanisms at play, the animal models used to investigate them, and future perspectives for treatment.

Hemodynamic features of NAVA

Stiffness and blood pressure. The principal function of the arterial system is to deliver an adequate supply of blood to tissues and organs. In performing this primary conduit function, the conductance arteries transform the pulsatile flow generated by ventricular contraction into a continuous flow of blood in the periphery.[3] This latter cushioning function is dependent on the viscoelastic properties of the tunica media of the arterial walls, mainly determined by elastin, which is very stretchable and is important to pulsatile behaviour, and collagen, which in contrast can resist stress. The biological aging of the vasculature is marked by endothelial dysfunction, major thinning and fracturing of the elastin fibres, and concomitant increased collagen deposition, resulting in an increased arterial stiffening, which is more pronounced in the central, predominantly elastic arteries, compared with the distal, predominantly muscular arteries.[4-6] These processes are mainly caused by alteration in the tunica media of the arteries and therefore caused by NAVA, although atherosclerosis can also play a role in the development and progression of arterial stiffness.[7] Longitudinal evidence of epidemiologic studies has shown the increase in vascular stiffness in aging as measured by changes in pulse-wave velocity (PWV). This evidence further revealed that PWV increment might commence in both sexes at as early as 10.4 years of age, with men living through a more pronounced progression than women.[5] Higher body mass index, higher blood pressure, impaired glycemic profile, chronic inflammation, end-stage renal disease, smoking, and alcohol consumption are important determinants of arterial stiffness, where blood pressure is of great interest owing to its potential bidirectional association with arterial stiffness.[5,8-12] Given the confounding effect of both intrinsic and extrinsic vascular aging, it might be challenging to directly translate vascular stiffness to NAVA, yet it serves as a proxy for deterioration in vascular physical function.

Stiffness and blood pressure–related morbidities. From a hemodynamic point of view, arterial stiffening influences the profile of blood pressure. The progressive stiffening of the central arteries speeds up the pulse wave, causing an early return of the pressure wave in late systole, with a consequent increase of systolic blood pressure, decrease of diastolic blood pressure, and therefore wider pulse pressure[13,14] and isolated systolic hypertension.[15] These abnormal changes have a negative effect on the heart, increasing LV afterload, decreasing coronary perfusion, and promoting left ventricle remodelling, dysfunction, and eventually failure. The changes in blood pressure profile and increased pulsatile pressure and flow load negatively affect the perfusion of several target organs, such as the brain and the kidneys, increasing the risk of cardiovascular morbidity and mortality.[16-20] Elevated arterial stiffness can also be responsible for acute cerebral events such as ischemic strokes, but also silent events due to cerebral small vessel disease causing cognitive decline and eventually disability. These events can be mostly due to high pulse pressure, which promotes arterial remodelling, increases arterial wall thickness, and induces the development and rupture of atherosclerotic plaques when those are present[21] (Fig. 2). The kidney and the brain receive approximately 20% of resting cardiac output and are low-resistance, high-flow end organs, which makes them vulnerable to pulsatile changes in the blood flow. This might suggest that systemic pulsatile pressure can cause vascular injury in both organs. A possible

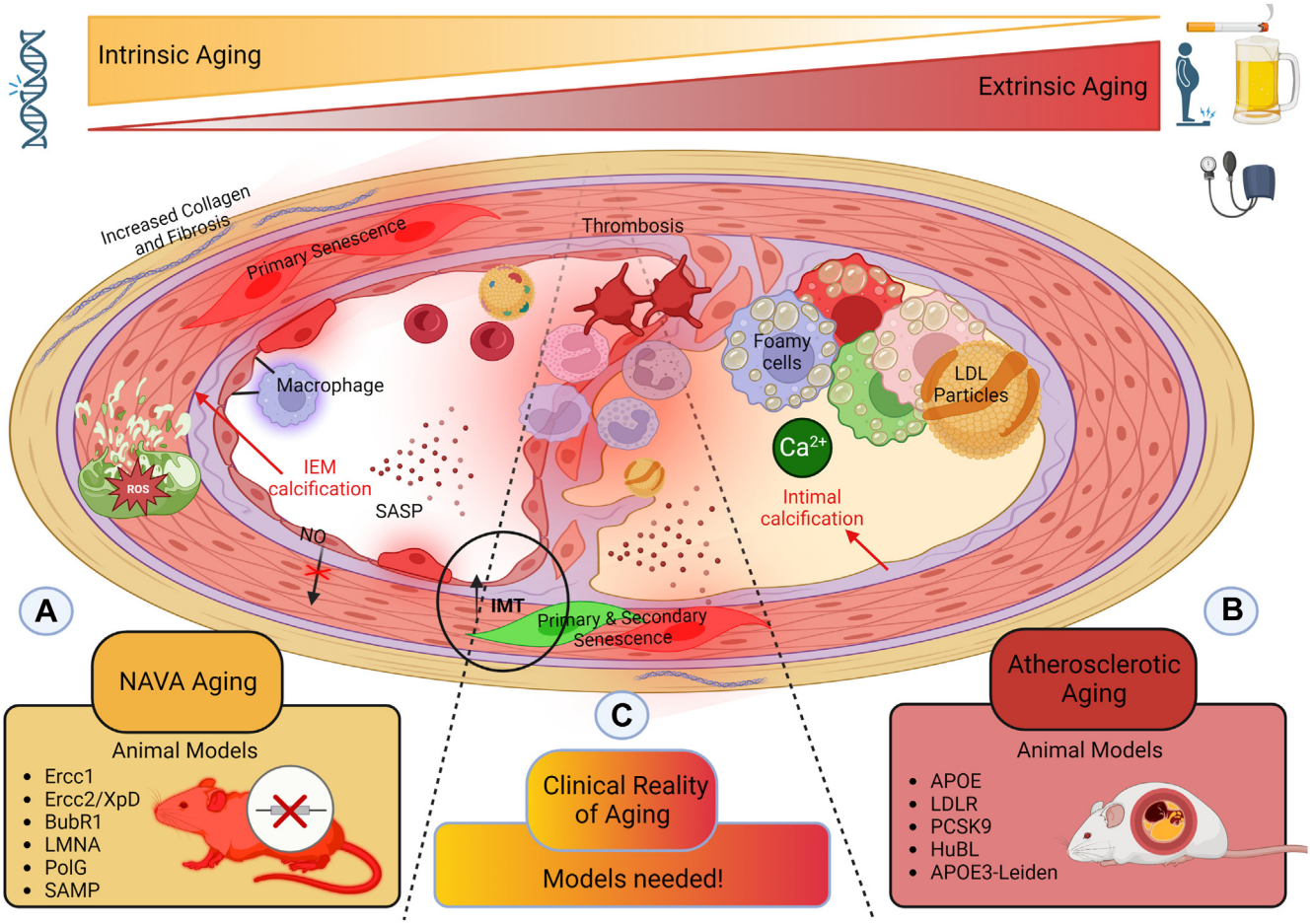

Figure 1. Placement of (**A**) nonatherosclerotic vascular aging (NAVA) vs (**B**) atherosclerotic vascular aging (AVA). NAVA is characterized by the intrinsic functional deterioration and stiffness of the vascular system, which occurs independently from the traditional risk factors. This condition is primarily highlighted by mitochondrial dysfunction and the presence of reactive oxygen species (ROS), as well as senescence of smooth muscle and endothelial cells and impairment of vasomotor function. Moreover, NAVA is accompanied by increased levels of collagen, fibrosis, and calcification in the internal elastic membrane (IEM). Animal models such as Ercc1 and senescence-accelerated prone mice (SAMPs) are available for studying NAVA. In AVA, the presence of plaque (calcified and lipid core) is predominantly characterized in the vascular system as a consequence of cumulative damage and abnormal remodelling that stem from the impact of extrinsic risk factors such as hypertension and lifestyle factors over time. (**C**) Clinical reality of aging: The clinical manifestation of vascular aging in the "real-world" is expected to arise from the interdependent interplay between NAVA and atherosclerosis. Unfortunately, there exists a paucity of animal models that can accurately exemplify this integrative aging process. IMT, intima-media thickness; LDL, low-density lipoprotein; NO, nitric oxide; SASP, senescence-associated secretory phenotype. Created with BioRender.com.

mechanism underlying kidney disease in subjects with elevated arterial stiffness is that arterial stiffness increases circumferential and shear stresses in the central large arteries, generating high pulsatile stress at the microvasculature level and inducing vasoconstriction within the kidney.[22] This hemodynamic stress on the kidney vasculature results in endothelial dysfunction and microvascular ischemia, leading to kidney injury.[23]

Clinical studies report discordant findings on the possible associations between arterial stiffness and bone and muscle loss.[24-26] Chronic inflammation, hormonal changes, and metabolic disorders could be common mechanisms for increasing the pace of arterial, bone, and muscle aging. Novel insights could give new preventive and therapeutic targets to slow down age-related degenerative processes and multiple complications in older adults. However, longitudinal studies with sequential simultaneous measurements are missing, and the answers to these questions remain unavailable.

Calcification of the internal elastic membrane

The internal elastic membrane (IEM; or lamina elastica interna) is a thin elastic layer that separates the tunica intima from the tunica media.[27] Despite the fact that this layer theoretically is present in any artery, the prevalence of IEM calcification seems to differ greatly across arteries. As such, this pattern of arterial calcification is much less recognised than 2 other main patterns of calcification: intimal calcification (as expression of atherosclerosis), and Mönckeberg's medial calcification.[28,29] IEM calcification has been mentioned as a potential NAVA trait that confers risk of stroke. In recent years, IEM calcification in the arteries supplying the brain has received

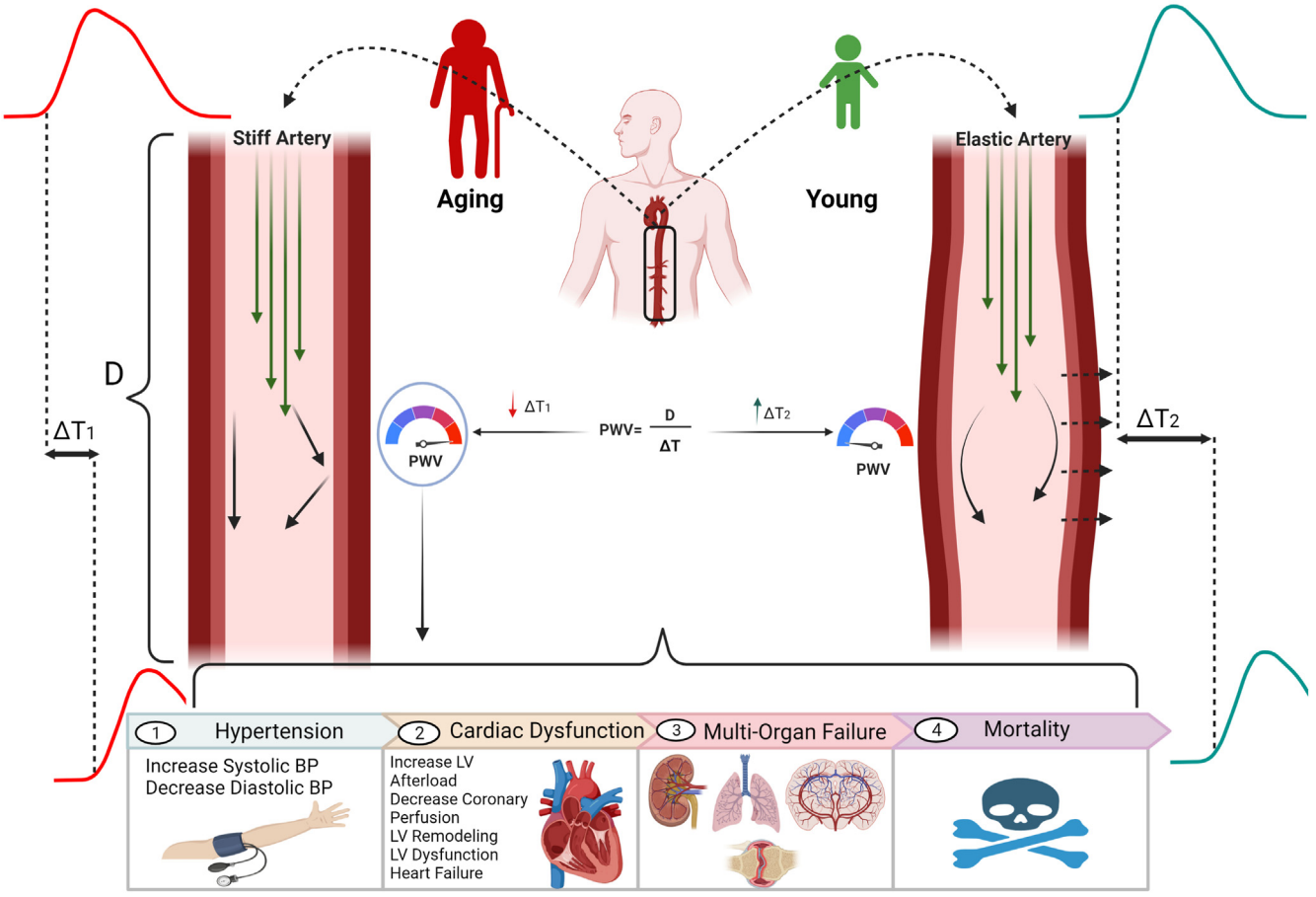

Figure 2. Arterial stiffness, nonatherosclerotic vascular aging, and related morbidities. Pulse-wave velocity (PWV) is a well established measure of arterial stiffness that can be computed by taking the ratio of the distance (D) between 2 sides of an artery to the transit time (ΔT) of the pressure wave between those sides.[14] With aging of the arteries, there is a shorter transition time ($\Delta T1 < \Delta T2$), which results in an increase in PWV. This indicates that aged arteries are stiffer than their younger counterparts, which can lead to hypertension in the early stages and ultimately multiorgan failure and mortality in the later stages of the disease. BP, blood pressure; LV, left ventricular. Created with BioRender.com.

attention because it can be readily visualised *in vivo* with the use of noncontrast computed tomography.[30,31] Its overall prevalence was found to be as high as 48% in community-dwelling persons aged 60 years and older, compared with a prevalence of 40% of intimal atherosclerotic calcification.[32] The prevalence of IEM calcification rapidly increases with age, affecting close to 80% of people aged 85 years and older. Also in terms of clinical consequences, it seems that making the distinction between patterns of calcification is relevant, because risks of subsequent clinical events, such as stroke, differ according to the pattern.[32] In addition, in patients undergoing treatment after stroke, the predominant pattern of calcification affects the prognosis of the patient; those who suffered a stroke who predominantly show IEM calcification in the intracranial arteries have more benefit from endovascular treatment than those with intimal calcification.[33] Most likely, this is due to the fact that those with predominantly IEM calcification patterns show a much less developed system of collateral blood vessels in the brain.[34]

Cell Biology of NAVA

Stiffening of the arterial tissue can be readily measured in patients with the use of ultrasound or tonometry. Blood pressure also, with pulse pressure as a measure best reflecting vascular aging, can be relatively easily measured.[35] Furthermore, intima-media thickness and calcification (both IEM and atherosclerotic) can be measured. Sometimes vascular function can be measured, for example, by quantifying reactive hyperemia or drug-induced dilation with the use of imaging. However, this is as far as clinical or epidemiologic studies go for the most part. In the cell biology field, focus shifts to aging at the cellular level of endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), and the impact thereof on intra- and extracellular matrix, vasomotor signalling, cell fates such as divisions, hypertrophy, apoptosis, and senescence, cytokines and cell activation, and cell metabolism.[36,37]

To bring order to the interrelationship of such cellular processes in the context of organismal aging, Lopez-Otin et al. introduced the hallmarks of biological aging. These hallmarks are basically the ontology of aging represented in a hierarchically structured network. High in this hierarchy is genomic instability, comprising accumulating genomic DNA damage and telomere attrition, epigenetic alteration, and loss of proteostasis. These hallmarks lead to mitochondrial dysfunction, cellular senescence, deregulated nutrient sensing, and

inflammation. This subsequently promotes the third category in the hierarchy: intercellular communication alteration and stem cell exhaustion.[38,39] The hallmarks of aging are well applicable on the fundamental cell biology that underlies NAVA and will be discussed accordingly.

Genomic instability

The accumulation of DNA damage is thought to set off the entire ontology of aging hallmarks.[40] Biological aging is led by response-induced DNA damage which is derived from the metabolic reorganisation of the organism, switching from a proliferation and repair condition to a state of maintenance, which in itself might be favourable for surviving the genetic damage. The metabolic change involves a coordinated remodelling of nutrient sensing, glycolysis, and mitochondrial respiration. Stem cell exhaustion, loss of cell-to-cell communication, introduction of cell fates such as apoptosis and senescence, and the proinflammatory stimulus aroused by the latter eventually lead to the progressive functional degradation that we know as aging.

The integrity of the genome might be compromised by various exogenous and endogenous risk factors.[40] In general, these risk factors are the processes that generate chemical adducts that transform the DNA structure, leading to breaks, crosslinks, and helix distortions. Free radicals, generated by chemical reactions or ionizing radiation, are the most prominent determinants. Exogenous risk factors include air pollution, smoking or other forms of chemicals intake, exposure to radiation, and excessive intake of lipids and carbohydrates. Endogenous risk factors involve the naturally occurring metabolic chemical processes that generate the chemical adducts, for example, mitochondrial and other redox reactions. Innate hypersensitivity to such genotoxic events, for example, because of relatively reduced genome maintenance or detoxification capacity or increased sensitivity to glucose and lipid oxidation, exists. Also, the DNA damage response or transcription stalling that results from the formation of DNA adducts displays interindividual and organ- and cell type–specific differences.[41,42] Thus, genomic instability is shaped by diverse factors, making it difficult to distinguish between those that are of external origin and those that are intrinsic in nature.

Vascular aging features can manifest as a result of genomic instability and are shaped through multiple locally and systemically acting pathways. We identified the accumulation of unrepaired DNA damage as one of the most important causal factors in vascular aging.[43,44] This is especially evident in patients with progeria syndrome, resulting from a specific mutation in the *LMNA* gene. This mutation causes the production of an atypical variant of the protein lamin A, which is essential for preserving the structural integrity of the cell nucleus.[45] These patients exhibit impaired endothelial function and massive loss of VSMCs, eventually leading to MI or cerebrovascular diseases.[46] Unlike the vascular aging phenotype observed in the general population, which is a combination of atherosclerotic and nonatherosclerotic vascular aging, progeria patients often do not exhibit atherosclerotic plaques.[47] The cardiovascular disease in progeria patients might be more similar to that of NAVA, which makes this condition an interesting basis for proxy models. Other previously summarised evidence supporting the role of genomic maintenance in vascular aging are the associations of genetic nucleotide polymorphisms of DNA repair and response genes with NAVA outcomes such as PWV and intima-media thickness and with increased risk of cardiovascular events.[43,48,49] Also, telomere length is associated with such NAVA outcomes.[50-52] Critically short telomeres lead to a DNA damage response similar to the one that drives aging. However, telomere length is measured cross-sectionally in circulating leucocytes, and not directly, longitudinally, in vascular tissue. Therefore the outcomes might be biased by inherited telomere length, which does not reflect attrition, and inflammatory events that are not related to NAVA. The meaningfulness of leucocyte telomere attrition, therefore, remains an open question.

Epigenetic alteration

As with accumulating DNA damage, life-long exposure to extrinsic factors can ultimately change the epigenetic patterns in vascular cells starting *in utero*.[53,54] This can primarily be mediated by perturbation in the activity of modulatory enzymes of DNA methylation, histone modification, noncoding RNAs, or chromatin remodelling. Consequently, there is a disruption in the regulation of gene expression and intercellular signalling pathways involved in the process of aging. This disruption, coupled with rare genetic mutations, leads to pathophysiologic changes.[55,56] The epigenetic changes are influenced by a variety of environmental factors that are quite similar to the aforementioned extrinsic factors, including smoking, microorganism infection, alcohol consumption, exposure to toxins, and heavy metal ions.[57] DNA methylation is one of the most important epigenetic determinants in vascular aging by regulating EC and VSMC function. It primarily influences proinflammatory gene function in ECs,[58,59] and proproliferation gene function in VSMCs.[60,61] Hypomethylation of the endothelial nitric oxide synthase (eNOS) gene promoter initiates an elevation in eNOS gene expression and boosts enzyme activity, leading to the generation of reactive oxygen species (ROS) and subsequent injury to the endothelial cells.[62] Consequently, this connection between epigenetic markers and the hallmarks of inflammation and cellular senescence further highlights their relevance in the aging process. The majority of studies on DNA methylation have focused on its role in atherosclerosis,[63,64] so the understanding of DNA methylation's specific involvement in NAVA is still limited.

Mitochondrial dysfunction

DNA damage is both cause and consequence of cellular metabolic dysfunction that involves mitochondrial respiration. Mitochondria are highly dynamic cytoplasmic organelles with a significant role in cell energy supply in the form of adenosine triphosphate (ATP). To form ATP, the double lipid bilayer mitochondrial membrane builds up an H^+ gradient between the inner and intermembrane spaces to drive H^+-ATPase. This requires a donation of electrons to the H^+ pumps of the so-called mitochondrial complexes. Oxygen is the final acceptor of these electrons. Metabolic events lead to free electrons or ROS that react with macromolecules, thus forming oxidised DNA nucleotides that lead to genomic

instability. In addition, mitochondrial DNA, lipids, and proteins also are damaged. Mitochondria therefore constantly undergo fusion and fission events to maintain their shape and function.[65] Any alteration in the control of mitochondrial dynamics can cause a malfunction in the mitochondria, which can have a significant impact on cellular signalling and metabolism.[66] This can set off a chain of events that can result in inflammation, apoptosis, proliferation stop, and inhibition of cell repair, such as by autophagy.[67-69] Mouse models with mitochondrial loss of function exhibit a reduction in mitochondrial respiration simultaneously with structural and functional changes associated with vascular aging. This suggests a strong correlation between the integrity of mitochondrial DNA and the onset and progression of vascular aging.[70-74] Besides its role in calcium homeostasis, cell survival, and apoptosis,[75] one of the mitochondria's leading functions is the generation of cellular energy.[76] Endothelium dysfunction and consequent vascular aging appear to be driven by shifts in cell energy spurred on by a decreased ATP synthesis rate and increased ROS.[77] Nevertheless, some evidence suggests that the core function of mitochondria in tissues with low metabolic activity is their signalling role in the vasculature, rather than energy shifts. This mitochondria-driven signalling regulation is responsible for cellular homeostasis, apoptosis, and inflammatory pathways.[72] In addition to EC aging, impaired mitochondrial biogenesis is responsible for VSMC aging. Biological aging in VSMCs may also produce higher levels of ROS, which can damage cellular structures and contribute to the formation of collagen in the arterial wall.[78] This excess collagen formation can eventually lead to stiffening of the arterial wall and contribute to the development of cardiovascular disease. However, comparing the effects of mitochondrial dysfunction between ECs and VSMCs requires caution owing to differences in their mitochondrial density and subcellular distribution.[72,79,80] Further research is needed to fully understand the role of mitochondrial dysfunction in vascular aging, particularly regarding mitochondria-induced cellular senescence.[72]

Cellular signalling alterations

Nitric oxide−cyclic guanosine monophosphate vasodilation. Mitochondrial dysfunction is at least partly an intermediate vascular aging hallmark owing to its impact on vascular signalling through excessive ROS production, in particular on nitric oxide (NO) signalling. NO is produced by eNOS or inducible NO synthase. NO binds soluble guanylyl cyclase (sGC), which subsequently augments its basal cyclic guanosine monophosphate (cGMP) production to release a burst of cGMP. cGMP relaxes VSMCs through dephosphorylation of actin-myosin filaments, a process mediated by protein kinase G.[36] Related to this, the loss of NO-cGMP−induced vasodilation during aging is well known.[36] This decrease can be caused by various mechanisms, such as loss of eNOS expression or its NO-producing activity, excessive presence of superoxides that react with NO to form peroxynitrite, and the presence of asymmetric dimethyl-L-arginine (ADMA), which inhibits the use of the NO substrate L-arginine and triggers endothelial aging.[81,82] The NO-inhibitory feature of intercellular ADMA further accelerates senescence process.[83]

Apart from mitochondrial ROS formation, superoxides are formed in various manners, such as uncoupling of eNOS from the cofactor tetrahydrobiopterin, increased activity of various oxidases, and inflammatory responses.[81] Loss of eNOS expression is not consistently found in aging, and reports vary as decreased, increased, or no change at all.[36] A possible explanation might be the progression of the intrinsic vascular aging process, at the beginning of which eNOS might still increase to compensate for the initial loss of dilator function, as caused, for example, by remodelling or inflammation, while in the end eNOS is decreased. Loss of eNOS activity can also be caused by phosphorylation of threonine 495, which involves protein kinase C.[84,85] *In vitro* proliferation- and stress-induced aging of human umbilical cord ECs and aging of rat aorta increase p-Thr495-eNOS.[86-88] The mechanisms driving these changes in aging are not known yet, but ROS production may play a key role. Apart from mitochondrial changes, aging-related ROS production importantly depends on production by nicotinamide adenine dinucleotide phosphate oxidase (NOX), as shown by various studies.[81] More recently, in mouse models of strongly advanced VSMC aging, NO-cGMP was found to be compromised by increased degradation by phosphodiesterase-1.[48,89] Another possible target is sGC, which can oxidise or be deactivated by nitrosothiols.[36] It is unknown how aging affects these mechanisms.

Renin-angiotensin system vasoconstriction and remodelling. Angiotensin II (Ang II) is the main bioactive regulator of the renin-angiotensin system (RAS) and plays an important role in stiffness, decreased vasodilation, and wall thickening potentially through inhibition of eNOS-NO-cGMP signalling.[90] This involves its type 1 (AT1) receptor. Although initially it was thought that Ang II is generated locally in the vascular wall from angiotensinogen synthesized in perivascular adipose tissue, recently obtained data in rodents after deletion of hepatic angiotensinogen with liver-targeted angiotensinogen small interfering RNA reveal that such vascular Ang II generation actually relies on hepatic angiotensinogen.[91-93] Thus, angiotensinogen, like renin, needs to be sequestered by the vascular wall to allow local angiotensin generation. Such uptake may occur via megalin.[94] Ang II additionally acts on Ang II type 2 (AT2) receptors, which have been proposed to counteract the effects of AT1 receptors. This implies that AT2 receptors induce vasodilation in an NO-dependent manner.[95] Yet, in apparent contrast with this concept, AT2 receptor agonists do not lower blood pressure.[96] One explanation for this observation is that AT2 receptors become AT1 receptor like under pathologic conditions,[97,98] and thus become vasoconstrictors as well, for example, because they occur on VSMCs rather than ECs.

In general, RAS activation in the vascular wall leads to a reduction in NO availability. This reduction occurs owing to an increase in the formation of peroxynitrite, which in turn activates the enzyme poly (ADP-ribose) polymerase 1 (PARP-1), an enzyme involved in DNA repair. This activation of PARP-1 contributes to cellular energy dysfunction and compromises the anti-aging pathways mainly mediated by sirtuins.[99,100] Furthermore, peroxynitrite can also directly scavenge NO and impair its signalling function.[101]

In addition to the reduced NO-cGMP signalling and increased Ang II constriction, increased constriction due to releases of constrictive prostaglandins has been observed in aged arteries.[102] Together, the reduced vasodilator and increased vasoconstrictor responses can contribute to an age-dependent increase in blood pressure and vascular stiffness. Moreover, Ang II has a profibrotic effect, potentially contributing to matrix stiffening, although no conclusive evidence exists regarding this mechanism.[103,104] Furthermore, increased RAS activity has been linked to VSMC senescence and vascular inflammation, and this is partly mediated through extracellular matrix signal−regulated kinase activation.[105]

Cytoskeleton and extracellular matrix signalling. Another important key player in stiffening and reduced vasodilation is the extracellular matrix in conjunction with the cytoskeleton. The cytoskeleton provides a dynamic framework for the spatial and temporal organisation and movement of signalling components. For example, transportation of these signalling components along the cytoskeleton enables its localisation where a downstream signalling pathway can be activated.[106] The role of the cytoskeleton can also be extended to some modulatory functions for intercellular signalling proteins such as kinases and phosphatases, but also for extracellular matrix. In the ECs, aging-induced disruption in the integrity of this framework leads to cell stiffness. On top of that, this disruption may alter the activity of kinases causing a reduction in ECs nitric oxide synthesis and endothelial dysfunction.[107] Cytoskeletal stiffness may further cause cellular senescence formation and accelerate aging.[108] Finally, cytoskeleton proteins mediate many inflammatory signalling pathways and regulate the inflammatory cell migration and adhesions via their actin filaments.[104,109] This once more underscores the highly interactive network of biological aging hallmarks.

Cellular senescence and senescence-associated secretory phenotype

DNA damage can lead to another important intermediate aging hallmark: cellular senescence. Cellular senescence is a G0 cell cycle phase arrest, which is accompanied by various dysfunctional cell features, such as cell enlargement with cytoskeletal changes, loss of the usual function of the affected cell type, and nutrient sensing and bioenergetic remodelling. Senescent cells (SnCs) display a proinflammatory secretory phenotype (senescence-associated secretory phenotype [SASP]), a rather elusive feature because it is cell type and condition dependent.[110-113] Central in the induction of these collective changes are cell cycle−dependent kinase-inhibiting pathways, notably P53/P21, P16, p27, and GATA4 pathways.[110,113] These molecules are often used as SnC markers, next to senescence-associated β-galactosidase (SA-β-Gal) staining and possible other markers, raising debate regarding the appropriate combination of such markers.[114,115] The accumulation of SnCs has been implicated in the biological aging process and disorders, because genetic or pharmacologic removal of p16-expressing, SA-β-Gal−positive, or p53/p21-increased cells from normal or accelerated aging mice was shown to increase life span and health features, among other mechanisms by improving vasodilator function.[116,117] It is important to note that the irreversibility of senescence and its exclusive involvement in aging is a matter of ongoing debate. Recent studies have proposed some mechanisms in which these cells can potentially be reactivated to reenter the cell cycle or be reprogrammed back into stem cells.[118-120] Moreover, senescence has been implicated in embryogenesis[121] and wound healing,[122-124] both features that decline during aging. Overall, no evidence exists whether cellular senescence plays a role in human vascular aging. However, the evidence that has accumulated over the years in vascular cell culture and animal models shows a reproducible association between the occurrence of SnCs and the aforementioned major vascular signalling problems. It is therefore a viable concept, although the mechanisms that connect senescence directly with NAVA *in vivo* remain to be found.

Chronic inflammation

Chronic low-grade inflammation is recognised as a distinct hallmark of both extrinsic and intrinsic vascular aging, but it is also closely intertwined with other aging features. One possible mechanism connecting the intermediate aging hallmark senescence with vascular aging is inflammation, evidently through SASP. Intriguingly, inflammation can itself trigger vascular senescence, and the formation of senescence and SASP can recruit immune cells, promote proinflammatory cytokine release, and trigger additional inflammation,[125] thus forming a loop of disease progression. This is often referred to as "inflammaging" a process that triggers NAVA.[126] Central in this loop is oxidative stress, triggering the activation of nuclear factor (NF) κB. Furthermore, NLRP3 inflammasome activation is involved in inflammaging.[127] This can be linked to decreased sirtuin-1, a main regulator of nutrient-sensing hallmark of aging. Decreased sirtuin-1 activity can be prompted by overt activation of the DNA damage response protein PARP-1, as it competitively consumes NAD+, which dually activates the competing proteins. Age-associated sirtuin-1 reduction in VSMCs links vascular senescence and inflammation to abdominal aortic aneurysm,[99,128,129] but also to decreased NO-mediated dilation via prostaglandin-induced loss of protein kinase G activity.[130,131] This couples DNA damage, the major causal aging hallmark, via senescence, the intermediate hallmark, with vascular aging.

Looking further into inflammaging, the NLRP3 inflammasome is responsible for interleukin (IL) 1β production. It is noteworthy that IL-1 occurs in 2 forms, membrane-bound IL-1α and soluble IL-1β, which both bind to the IL-1 receptor. The endocrine IL-1β is the prototype proinflammatory cytokine.[132] IL-1 is recognised for its role in causing plaque rupture, facilitating blood clot formation in the cardiovascular system, and reducing vasodilation in relation to NAVA.

IL-6, another important marker of inflammation and a downstream mediator of IL-1β,[133] has proinflammatory functions, acting in the acute-phase response, attracting monocytes and activating ECs.[134] IL-6 plasma levels increase with aging and are associated with increased cardiovascular disease risk, atherogenesis, and heart failure progression and mortality.[135] Signalling between IL-6 and CC chemokine ligand 2 (monocyte chemoattractant protein 1) stimulates the recruitment of VSMCs and monocytes to the vascular wall

and encourages thrombogenesis. This process is accompanied by heightened IL-6 levels, which are linked to endothelial dysfunction.[134,136] IL-1 and IL-6 also have effects on VSMCs, decreasing vasodilation due to NOX—driven superoxide production and increasing vasoconstriction via Rho A kinase, but paradoxically increased VSMC membrane hyperpolarisation has been reported.[137] The effects on human arteries and whether all 3 of these mechanisms are simultaneously acting locally in arterial tissue remain to be answered. Therefore, the acute interplay between NLPR3 components in coronary tissue and the effect of relevant blockers on vasomotor activity need to be explored.

Tumour necrosis factor α (TNFα) is a chief cytokine with pleiotropic inflammatory and proliferative effects, among others, on NAVA. Elevated TNFα levels, in particular, have been attributed to diminished EC-dependent dilation but also to increased VSMC proliferation and migration, as well as augmented oxidative stress.[138-141] When TNFα triggers sphingomyelinase activation, it results in a reduction of vasorelaxation mediated by NO. In addition, in the presence of high TNFα levels, there is an impact on the expression of eNOS mRNA and an increase in ROS production, leading to a decrease in NO bioavailability.[137] The impact of proinflammatory cytokines on intrinsic vascular aging was also highlighted by a transgenic mouse model with NF-κB malfunction, where inhibition of NF-κB resulted in delayed intrinsic vascular aging.[142]

In addition to its involvement in NO-cGMP signalling (as discussed previously), Ang II also plays a role in regulating inflammation. The binding of Ang II to its receptor on the cell surface initiates a series of intracellular signalling events that ultimately result in the activation of NOX. The resulting product, superoxide, interacts with various cellular components, including proteins and DNA, causing oxidative damage and promoting inflammation. This process is highly dependent on the proper functioning of the cytoskeleton system, which is critical for providing the necessary oxidase components for NADPH activation, as well as for regulating adhesion molecules for leukocyte invasion.[104,143,144] Collectively, these pieces of evidence indicate that inflammation plays a crucial role in the dysfunction of both ECs and VSMCs, and that it is closely intertwined with other important hallmarks such as senescence and SASP. This connection can be linked to the emergence of vascular disease, vascular stiffness, and NAVA.

Integrative network of NAVA hallmarks

Two key hypotheses have been inaugurated regarding the interplay of NAVA hallmarks with biological aging, namely, the geroscience hypothesis and the unitary theory. The latter proposes that because there are many interconnections within this hierarchy of the aging process, attacking one of the hallmarks might have an impact on others. For example, inhibition of inflammation may eventually prevent senescence formation, mitochondrial malfunction, etc, whereas the opposite is also true. The geroscience hypothesis broadens the effect of targeting aging hallmarks to diseases other than age-related disorders alone, for example, diabetes.[145,146] Within the context of this review, we have identified genomic instability as the main causal factor (primary hallmark) in vascular aging, followed by intermediate (antagonistic) hallmarks such as cellular senescence and inflammation, with intercellular communication alteration being further downstream (integrative hallmark). Although these hallmarks are hierarchically categorised, it is essential to note that their highly interactive network should not be disregarded. In some cases, one downstream hallmark can also initiate another hallmark upstream in a bidirectional manner, leading to complicated scenarios similar to a "hen and egg dilemma," such as intercellular communication alteration and inflammation/senescence, where it is difficult to determine which comes first. It is clear now that vascular aging hallmarks are not separate and distinct entities as they are often depicted, but instead function within a vast and intricately interconnected network (Fig. 3).

Vascular Aging Models: Challenges

The study of NAVA is always challenging considering the bottlenecks such as complexity of the phenotype, implementation costs, and predominantly abiding duration of studies. Before now, the complexity of NAVA could only be investigated *in vivo*. The interplay between the vasculature and blood, nervous system, inflammatory cells, kidney, heart, lung, and possibly other internal organs,[99] and even aging of the blood vessel in isolation, cannot be reproduced *in vitro*. Some features, such as cellular senescence, can be obtained in cell culture, either by passaging or exposure of vascular cells to stress stimuli, such as DNA damage. This can be exploited to unravel intracellular mechanisms of senescence features, endothelial leakage, or possibly cell-cell communication via adhesion molecules or extracellular vesicles. However, senescence is not synonymous with aging, and its consequences for cardiovascular disease appear to be ambivalent.[147] Animal models may only approximate human NAVA and require validation in a real-world setting. This also applies to pharmaceutical targeting, where effective intervention in animal models sometimes fails to be replicated in human clinical studies.[148] However, human studies are not often feasible owing to the unavailability of data, ethical issues, and the high cost of clinical trials. Therefore, practical, translational nonhuman animal models for NAVA are valuable tools to bypass such limitations.[149] Accordingly, mouse models have emerged as the most prominent intrinsic and extrinsic vascular aging models among other species.

Normal and accelerated aging animal models

There are 2 ways to simulate human NAVA in animal models: let the animal grow old (normal aging) or induce accelerated aging. For normal aging, rats or mice are commonly used. There are a plethora of studies that show reproduction of, for example, decreased vasodilator capacity and arteriosclerosis features.[99] In general, normal-aging mice and rats are robust models for human vascular aging, and a great advantage is ample characterisation. When combined with a metabolic or hypertensive challenge, it is now also possible to study cardiorenal disease, such as the currently untreatable heart failure with preserved ejection fraction.[150] A drawback, however, is the long experiment time that is required, often 18 months and preferably longer, and the loss of animals over that period.[151] Although the loss of

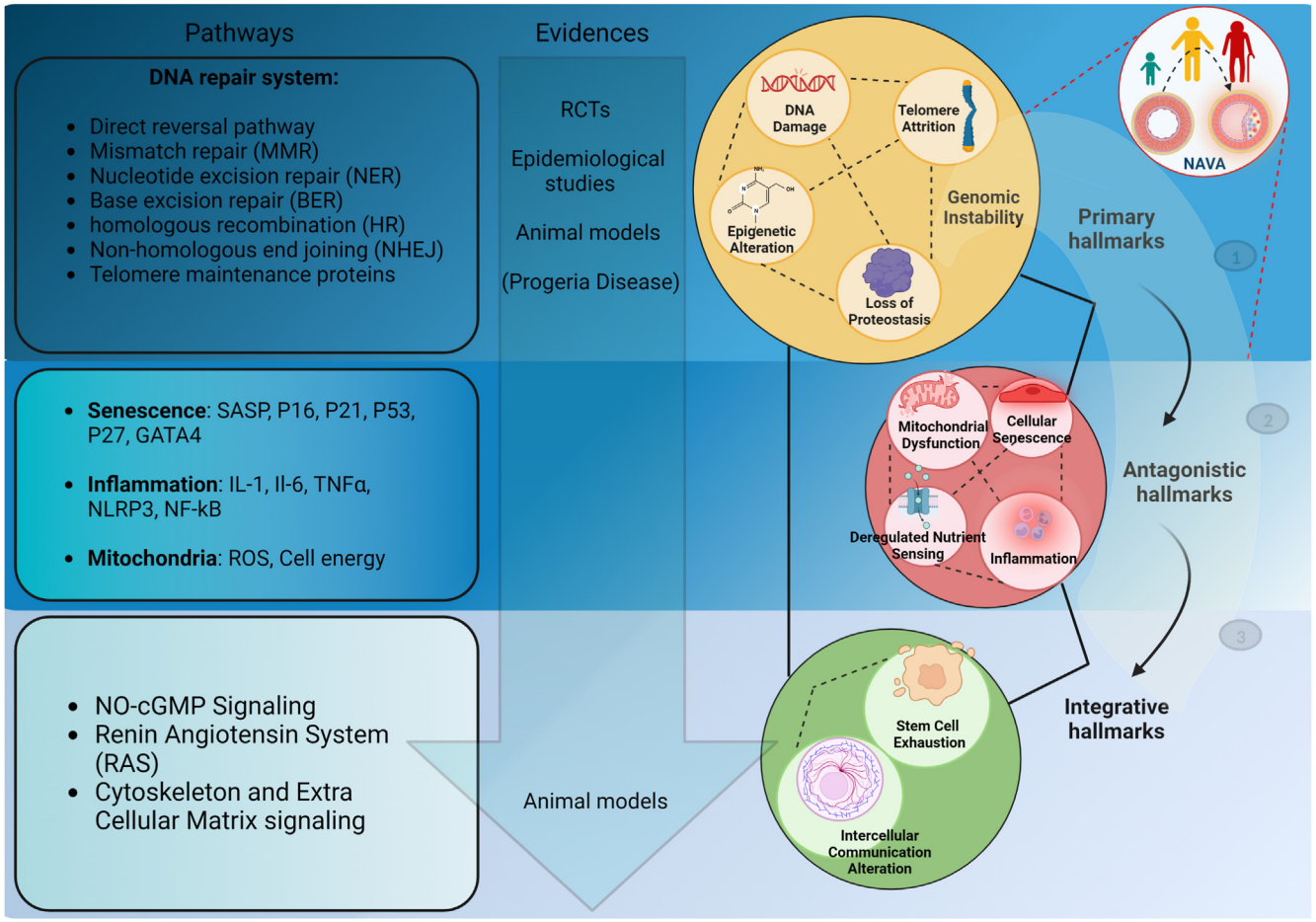

Figure 3. Hallmarks of nonatherosclerotic vascular aging (NAVA). The hallmarks of aging as outlined by Lopez et al.[38] are also applicable to NAVA aging. 1) Genomic instability is recognized as the principal causal factor (primary hallmark) in NAVA, followed by 2) intermediate (antagonistic) hallmarks such as cellular senescence and inflammation, with 3) intercellular communication alteration being further downstream (integrative hallmark). The left panel of the scheme illustrates the primary pathways involved in each stage 1) Disruption in DNA repair system such as nucleotide excision repair promotes genomic instability with progeria disease as its pronounced phenotype. There are multiple sources of evidence, ranging from patient-level to cell-level data, that substantiate the causal role of genomic instability in NAVA. 2) In addition, P16 and P21 pathways in cellular senescence hallmark, interleukin (IL) 6 and tumour necrosis factor (TNF) α in inflammation, and reactive oxygen species (ROS) in mitochondrial dysfunction, and 3) nitric oxide—cyclic guanosine monophosphate (NO-cGMP), renin-angiotensin system (RAS), and cytoskeleton system are important involved pathways of stage 2 and 3, respectively. NAVA hallmarks are not discrete and distinct entities, as is often assumed, but rather function within a vast and intricately interconnected network. This interactive hallmarks not only exist within a stage (**dashed lines**) but also each stage of this hierarchy is interacting with each other (**solid lines**). NF, nuclear factor; RCT, randomised controlled trial; SASP, senescence-associated secretory phenotype. Created with BioRender.com.

endothelium-dependent vasodilation is readily reproduced, endothelium-independent vasodilator dysfunction, such as occurs in humans[152] is seldom found. The time for the development of intrinsic vascular aging can be significantly reduced by using accelerated aging inbred strains, notably the senescence-accelerated prone mice (SAMPs).[151] SAMPs develop features of intrinsic vascular aging at 8 to 10 months of age. The model has been well characterised regarding endothelial dysfunction. However, to our knowledge, there are no accounts of vascular stiffness.

Induction of intrinsic vascular aging can be accomplished in various ways. Applicable in both mice and rats, infusion of vasoconstrictive compounds such as Ang II and L-NMMA, or metabolic challenges (eg, streptozotocin-induced diabetes) are well known ways to accelerate intrinsic vascular aging. However, this will of course be dependent on blood pressure or metabolic derailment, and not purely on intrinsic aging. A second way, applicable only in mice, is modulation of genes that affect aging, such as genes coding for nutrient-sensing mechanisms (sirtuin genes), klotho, and genome maintenance proteins. A detailed list is given elsewhere.[151] Many of the aging features in these models are linked to DNA damage response and are thus associated with senescence. Induction of accumulating DNA damage is a refined method to accelerate aging in a "pure" manner: DNA damage can account for a major part of the biology of aging, as discussed above.[40] As such, vascular senescence can be swiftly induced by undermining the DNA repair system, as was demonstrated in models making use of functional ablation of genome, telomere, and mitochondrial DNA maintenance genes, such as

BubR1, *Ercc*, *XpD*, *Lmna*, *Zmpste24*, *polG*, *Tert*, *Terc*, etc[151] (Fig. 1).

Special case: DNA repair mutants, *Ercc1*-based models

Of these models, *Ercc1* (excision repair cross-complement 1) mutant mice are one of the best characterised accelerated intrinsic vascular aging models, shown to be also suitable for testing pharmacotherapies. The first model tested in this respect is the $Ercc1^{\Delta/-}$ mouse, which harbours an allele for a total functional ERCC1 knockout (Δ: deletion of the 74-amino acid carboxy terminal) and an allele for a partial functional loss of $Ercc1^{\Delta/-}$ achieved by exon 10 truncation (Δ: deletion of the last 7 amino acids at the carboxy terminal).[153] ERCC1 is an endonuclease that is involved in at least 3 DNA repair systems, nucleotide excision repair, double-strand break repair, and cross-link repair. $Ercc1^{\Delta/-}$ mice and related models were instrumental in unraveling the biology of aging and showed that perpetuating DNA lesions can contribute to intrinsic aging by induction of a transition from a normal metabolic phenotype that supports growth and tissue repair to one that drives tissue maintenance and a proinflammatory status, resulting eventually in the functional loss that is observed during, and actually considered as, biological aging.[40,154] Recently, a novel potential contributing mechanism was supported by experiments in both wild-type and $Ercc1^{\Delta/-}$ mice, namely transcription stalling due to stochastic DNA damage.[155,156] Relevant to the present review, $Ercc1^{\Delta/-}$ mice model reproduced the main features of human vascular aging, that is, loss of vasodilation, increase of vascular stiffness, and increase of blood pressure.[48] A concern about this model is that the mice display widespread features of accelerated aging and might be biased by nonvascular causes.[99] To avoid such bias, vascular EC- and VSMC-targeted $Ercc1^{\Delta/-}$ models were generated, based on the Tie2(/TEK2)-cre and SM22alpha(/Tagln1)-cre cross bred with Ercc1-flox strains. Both models reproduced vascular aging features of $Ercc1^{\Delta/-}$ in segments that can be attributed to the respective cell types.[89,157] Therefore, the role of genomic DNA damage in vascular aging is at least partly due to cell-autonomous effects, validating the use of $Ercc1^{\Delta/-}$ and vascular cell−specific $Ercc1^{\Delta/-}$ models. Another possibility is XPG knockout, which is under development for the same purpose.[155,158]

Regarding the (cardio)vascular system, higher p21 and p16 mRNA expression, as measured by quantitative polymerase chain reaction, was observed in the aortas of $Ercc1^{\Delta/-}$ mice, but interestingly not in the heart tissue. In addition, the increase in senescence marker expression was more pronounced in male compared with female $Ercc1^{\Delta/-}$ mice, indicating a possible sex difference in the senescence/aging process. On top of that, SASP factors, including Il-1β, Il-6, Mcp1, and Tnfα, significantly increased in the liver and peripheral blood T cells of the $Ercc1^{\Delta/-}$ mice, suggesting the occurrence of secondary senescence formation.[159] A model in which Ercc1 has been selectively removed from VSMCs shows similar changes, suggesting the local impact of DNA damage in the vasculature.[89] Interestingly, senescence of VSMCs has been shown to promote the development of calcification markers.[160] In VSMC-specific accelerated aging mice, similar observations have been made, thus connecting the causal aging hallmark DNA damage to this phenotypic switch.[161] However, the relevance for IEM calcification still needs to be addressed.

Three deleterious mechanisms (see "Chronic Inflammation" section above) in vasomotor function mechanisms are also activated in $Ercc1^{\Delta/-}$ mutant mice. The paradoxical effects of IL-1 and IL-6 might explain why we observe lower Ca^{2+}-mediated contractions but a relatively higher RhoA contribution to these contractions in $Ercc1^{\Delta/-}$ mutant mice.[48,90] Altogether, the evidence supports the notion that accumulation of SnCs in the vasculature might contribute to vascular aging by creating a proinflammatory environment. An important question for future research examining this hypothesis is whether this contribution would be dependent on intrusion by inflammatory cells or take place through signalling directly evoked in vascular cells by the various cytokines.

Pharmacologic Targeting of Vascular Aging

Impediment to intrinsic and extrinsic vascular aging can be attempted with clinical feasibility with the use of dietary and pharmacologic tools. Rodents' life and health spans, including preservation of healthy vasomotor function, have been demonstrated to be extended by dietary restriction, which is notably manifold more potent in $Ercc1^{\Delta/-}$ mice than in wild-type mice, an effect that is attributable to genome protection.[90,155] Dietary restriction is, however, difficult to implement in daily human life, especially in economically developed countries. Despite this limitation, several clinical studies revealed the effectiveness of modifiable lifestyle risk factors such as reducing sedentary time and decreasing obesity and insulin resistance on reducing vascular stiffness.[162-164] In addition, structural and physiologic targeting of vascular function appears to be promising but unmapped.[5] NOS, RAS, cyclooxygenase (COX), proinflammatory, and telomerase pathways are currently known as drug targets for preventing or reversing intrinsic vascular aging.[165] In addition, some studies have proposed that IEM calcification could be a reversible characteristic of NAVA. However, the pathologic mechanism behind it must be fully determined before pharmacologic intervention can be taken.[28] In the context of this review, we focus on inflammatory, NO, and senescence pathways as potential drug targets in vascular aging pharmacotherapy (Fig. 4).

Targeting vascular inflammation

Inflammatory signalling pathways continue to be abundantly studied, granting multiple options for pharmacotherapy. Inhibition of pathways relevant to vascular aging can be accomplished through the use of non-selective drugs, such as colchicine, or by selectively inhibiting specific cytokines and inflammatory pathways, with the use of, for example, COX, IL-1, IL-6, or TNFα inhibitors. Selective inhibition of COX with salicylic acid and various types of nonsteroidal antiinflammatory drugs (NSAIDs) in the aorta of aging metabolic syndrome rats has been found to enhance endothelial function.[166,167] This has been corroborated in a human clinical trial involving salicylate therapy for a 4-week period, where direct evidence of NF-κB inhibition with salicylate was observed and endothelium-dependent dilation improved.[168]

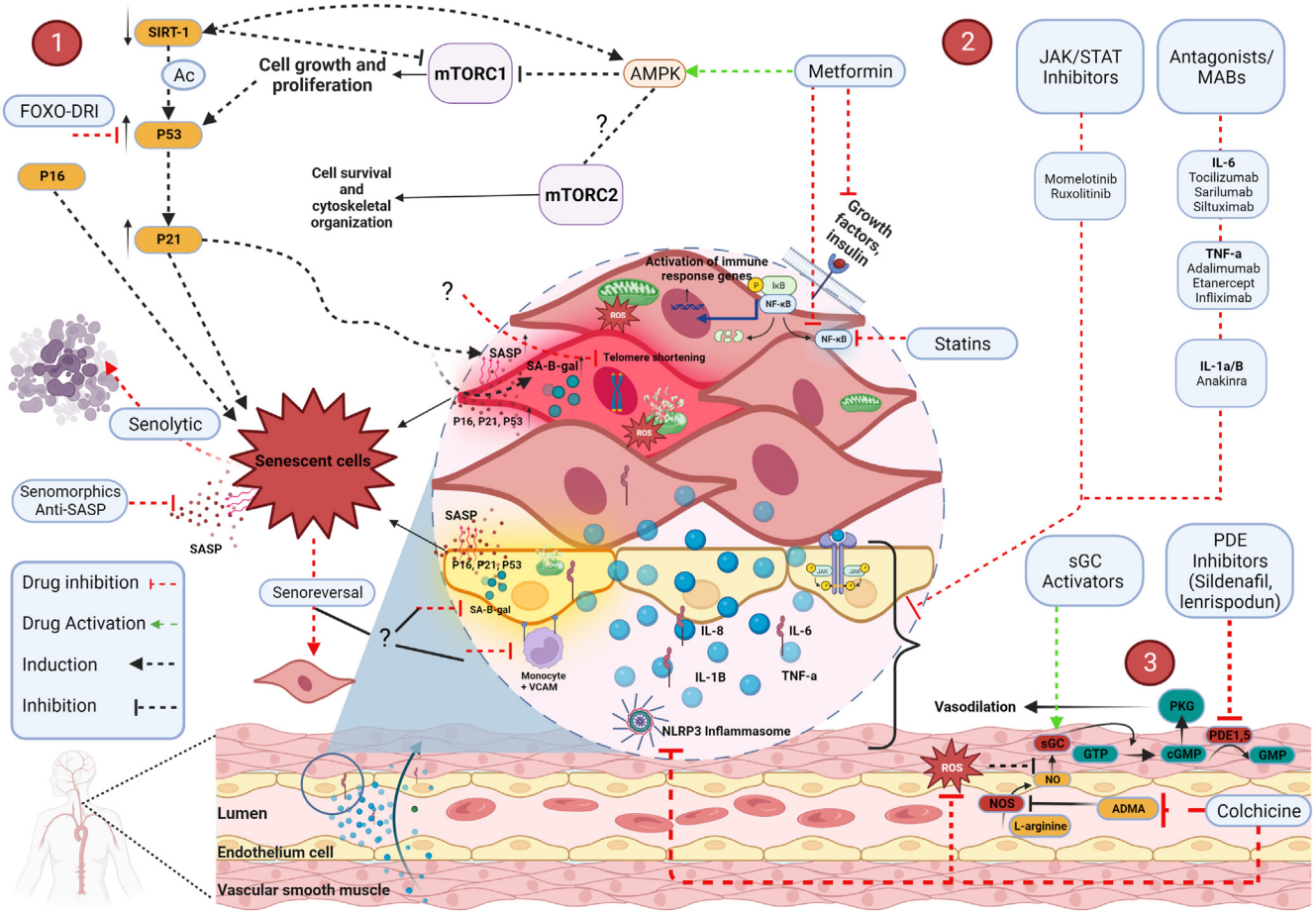

Figure 4. Pharmacologic targeting of nonatherosclerotic vascular aging (NAVA). The schematic diagram illustrates the potential pharmacologic targets for NAVA, specifically cellular senescence, chronic inflammation, and vasomotor dysfunction. 1) Anti-senescence therapies involve the clearance of senescent cells through targeting signalling pathways such as P53, AMPK, and mTORC1 (senolytics), targeting senescence phenotypes such as senescence-associated secretory phenotype (SASP) (senomorphics), and cellular reprogramming to restore functional cells (senoreverters). 2) The second approach involves the use of inhibitors of inflammatory markers such as interleukin (IL) 1 and tumour necrosis factor (TNF) α to reverse systematic inflammation and NAVA. 3) The third approach involves selective targeting of endothelial nitric oxide synthase (NOS), soluble guanylyl cyclase (sGC), and phosphodiesterase (PDE), which are the main regulatory pathways involved in vasomotor function, to enhance vasodilation and improve vascular function. In addition, some metabolic agents, such as metformin, may benefit NAVA by interacting with multiple targets simultaneously. Metformin indirectly enhances vasomotor function through AMPK-mediated endothelial NOS increase, inhibits inflammation by targeting nuclear factor (NF) κB, and inhibits cellular senescence through mTORC1 regulation. Limited or unknown evidence regarding pharmacologic efficacy is indicated with **question marks**, **black dashed lines** indicate physiologic inhibition/induction, and **coloured dashed lines** indicate drug inhibition/induction. ADMA, asymmetric dimethyl-L-arginine; GMP, guanosine monophosphate; GTP, guanosine triphosphate; NO, nitric oxide; PKG, protein kinase G; ROS, reactive oxygen species; VCAM, vascular cell adhesion molecule. Created with BioRender.com.

Furthermore, selective inhibition of IL-1 receptor signalling with the use of rilonacept improved vascular endothelium function in a clinical trial involving patients with chronic kidney disease. However, there were no changes in vascular stiffness as measured by PWV.[169] In diabetic rats, the use of anakinra, another IL-1 receptor antagonist, also demonstrated partial prevention of endothelium dysfunction.[170] Further comparison of acute vs chronic IL-1 inhibition revealed the efficacy of both on endothelium function, whereas neither had an effect on vascular stiffness.[171] Interestingly, IL-1 inhibition improved cardiac function and IL-6 inhibition was superior in the enhancement of vascular function, indicating their differential effectiveness.[172] In this context, blockade of the IL-6 pathway with tocilizumab, etanercept, or adalimumab[173] and of TNFα with infliximab[174] reduced stiffness and restored endothelial function in rheumatoid arthritis patients with pronounced endothelium dysfunction and arterial stiffness. Considering the complex and interactive nature of inflammatory pathways, an integrative approach that targets multiple pathways simultaneously may be effective in preventing vascular aging. Notably, a study by Lee et al. proposed that the reciprocal inhibitory interaction between TNFα and IL-6 mediates the deterioration of vascular function in type 2 diabetic mice.[175]

Regarding nonselective inhibition of inflammation, colchicine, an antigout agent with antiinflammatory properties, has shown to be effective in the inhibition of ROS, type II collagen, IL-1β, IL-6, and COX.[176] Short-term administration of low-dose colchicine did not improve endothelial function in patients with coronary artery disease but had

significant merits in patients with leukocyte activation.[177] An observational study showed that the colchicine effect on endothelium function is seen only in gout patients without established cardiovascular disease.[178] Although the efficacy of colchicine in the prevention of major cardiovascular disease is well established in the LoDoCo[179] and COLCOT[180] clinical trials, there is limited evidence regarding its efficacy in the prevention of vascular aging, stiffness, and endothelium function. Regarding the latter, cell culture studies suggested the protective effect of colchicine on endothelium dysfunction via reduction of ADMA, thrombomodulin, and osteoprotegerin levels.[181,182] In addition, colchicine inhibits VSMC proliferation and migration and decreases type II collagen, which may potentially be useful to prevent intima-media thickness and vascular stiffness.[182-184]

In addition to particular antiinflammatory drugs, drugs with an effect on metabolism, such as statins, Ang II receptor antagonists, and metformin, can target inflammation. It has emerged that the pleiotropic effect of these drugs is mediated by TNFα.[165] Among them, metformin, a commonly prescribed first-line medication for lowering blood glucose levels, has been proposed to have potential effects on vascular inflammation and aging through various other pathways, including NF-κB, Nrf2/GPx7, DICER1, MBNL1, STAT3, AMPK, SIRT1, insulin/IGF-1, and mTOR. However, the precise mechanisms involved in these effects are not yet fully understood.[185,186] Another example of metabolically challenged NAVA is the blockade of the RAS which is a well known strategy to reduce blood pressure, vascular hypertrophy, endothelial dysfunction, and vascular stiffening in models of extrinsic vascular aging. The effect on intrinsic vascular aging was studied in *Ercc1*$^{\Delta/-}$ mice. Surprisingly, the Ang II receptor antagonist losartan failed to reverse NAVA features, whereas dietary restriction was most effective.[90]

Targets in metabolic pathways

It is well known that metabolic disease, characterised by hyperglycemia and dyslipidemia, strongly accelerates extrinsic but also intrinsic vascular aging. Increases in ROS lead to macromolecular damage of, for example, advanced glycation end-products of matrix components and DNA. As already explained, DNA damage leads to a metabolic adaptation that, among others, involves suppression of Sirt1. This is at least partly driven by PARP-1—driven consumption of Sirt1 activator and oxidative phosphorylation driver nicotinamide adenine dinucleotide (NAD^+). Sirt1 elevates eNOS expression, protein levels, and activity, and decreases oxidative stress in ECs.[187] The Sirt1 activator SRT1720 can also achieve this effect and it thus reverses decreased endothelium-dependent relaxation in aged wild-type mice. Of note, the mechanism of the vasodilation-restoring effect of Sirt1 has been explored in *in vivo* aged mice. The effect involved prostaglandin-mediated improvement of sGC expression and activation instead of eNOS.[130] In addition, it has been discovered that Sirt1 reduces mitochondrial ROS in aging microvasculature, a mechanism that involves epigenetic regulation via $p66^{sch}$ and arginase II.[188]

Another popular intervention to counter intrinsic vascular aging in particular is NAD^+ supplementation. In this context, the supplementation of precursor nicotinamide mononucleotide (NMN) restored aortic endothelium-dependent relaxation and lower PWV in normally aged mice.[189] This was associated with the restoration of Sirt1 activity. Similar effects were observed for the neurovascular unit,[190] which could be mimicked by PARP-1 inhibition.[191] Nonatherosclerotic vascular aging is associated with heart failure with preserved ejection fraction, in mouse and rat models of which NMN was also shown to be effective.[192] As for clinical feasibility, NAD+ precursor treatments elevated NAD+ levels in human phase I studies,[193,194] and a potential but nonsignificant effect on blood pressure and vascular stiffness was reported. Based on this result, an additional randomised controlled trial to study the effect of 3-month interventions has been proposed.[195]

Targeting senescent cells

Strategies. The exponential growth in studies noting the association of SnC accumulation and many pathologic alterations urged the need for new drugs that selectively annihilate the extant SnCs or hinder their genesis.[39,196-198] Given the impaired apoptotic pathways of these cells,[198] there are 2 potential approaches for pharmacologic targeting. One approach involves using senolytics to manually eliminate these cells, and the other involves targeting the SASP expression with the use of senomorphics.

Senolytics may target a number of up-regulated enzymes implicated in prosurvival and antiapoptotic pathways, including P53, P21, B-cell lymphoma 2, protein kinase B, phosphoinositide 3-kinase, and Forkhead Box O4 (FOXO4).[199] Among these, p53 is considered to be the most important determinant of cellular senescence, because it is a tumour-suppressor protein that plays a critical role in regulating the cell cycle and preventing the formation of cancerous cells.[200] FOXO4-DRI,[201] UBX0101,[202] and P5091[203] are examples that have been shown to disrupt the P53 regulation pathway. On the other hand, increased lysosomal enzyme activity in SnCs is useful for directed senolysis but may have off-target effects in non-SnCs with high SA-β-Gal activity, such as macrophages. Currently, there are several studies exploring different approaches to address this issue, such as developing selective prodrugs, designing nanoparticles with SnC-specific coatings, using antibodies for targeted drug delivery, and using small molecules that selectively induce cell death in SnCs.[204-207] Furthermore, it has been demonstrated that the endogenous competitive NOS inhibitor ADMA[208] can lead to vascular senescence by hastening telomere shortening and decreasing telomerase activity in ECs.[209,210] Accordingly, colchicine's effects within ECs appear to result in reduced ADMA levels.[211] Similarly, high-dose atorvastatin therapy could reduce ADMA plasma levels, particularly in cardiac tissue, suggesting its potential senolytic activity.[212]

The transcription factor NF-κB, mTOR, JAK-STAT, and mitochondrial complex are among the mechanisms known to reduce the SASP.[213] Thus, inhibition of these pathways has been proposed as a promising strategy for developing senomorphics. NF-κB is a particularly important target for senomorphics, because it is a key mediator of cytokine release and inflammation. There are a variety of immunomodulatory agents, antiinflammatory agents, and metabolic regulatory agents that can target NK-κB and potentially reduce SASP,

for example, NSAIDs,[214] glucocorticoids, statins,[215,216] metformin,[217,218] and certain natural compounds such as curcumin and resveratrol.[219]

Effects on the cardiovascular system. Several studies have explored the effectiveness of senolytic therapy in vascular aging. Navitoclax, which inhibits B-cell lymphoma 2, an important mediator in apoptotic pathways, has been found to reduce plaque size, burden, and calcification, indicating its potential in inhibiting atherogenesis.[220] Another senolytic therapy, using dasatinib and quercetin, has been shown to improve vascular and endothelium function, specifically in terms of enhanced NO signalling and vascular relaxation. However, this therapy did not have a significant effect on VSMC contractile function.[221]

Consideration in treatment design. Both senolytic and senomorphic approaches have potential benefits and limitations, and it is currently unclear which one might be better, as they target different aspects of senescence. The "hit-and-run" strategy of senolytic administration involves delivering the drug intermittently to selectively eliminate SnCs while allowing healthy cells to recover between treatments. This approach may reduce the risk of side-effects associated with continuous drug exposure while still providing the therapeutic benefits of senolytic therapy. However, studies have shown that continuous treatment with SASP inhibitors may be necessary to maintain suppression of the SASP and prevent its harmful effects.[204,222] Previously, the majority of research efforts were directed toward eliminating SnCs because it was thought that they could not be reversed. But new research has revealed that specific cell types have the ability to be reprogrammed to exit senescence and resume the cell cycle.[116,204,223] This discovery generates an opportunity for a third approach, senoreverters, to target senescence. Ultimately, the choice between senolytics, senomorphics, and potentially senoreverters will depend on the specific disease being targeted and the individual patient's needs and medical history.

Identification of novel pharmacologic targets in the NO- cGMP pathway

The introduction of accelerated aging models based on DNA repair involving ERCC1 raises the need for having a tool to rapidly investigate markers and pharmacotherapies, especially in intrinsic vascular aging. The models confirmed the central role of NO-cGMP signalling, pointing to decreased eNOS, increased NOX-mediated NO depletion, and increased metabolism of cGMP by phosphodiesterases (PDEs).[48,89] Regarding the latter, it has been demonstrated that PDE1 replaces PDE5 as the primary cGMP-mediated vasodilation regulator in the arterial wall during intrinsic vascular aging and that PDE1 is up-regulated during the development of atherosclerosis and aneurysms as well.[224] Acute and chronic blockade of PDE1 alone or together with PDE5 in *Ercc1*$^{\Delta/-}$ models led to improvement of vasodilator responses independently from blood pressure, attenuated the proinflammatory status, and decreased expression of cellular senescence–related markers p16 and p21, but did not show effects on vascular stiffness.[225-227] Further underscoring the importance of cGMP, chronic treatment with the sGC activator BAY 54 had similar effects in *Ercc1*$^{\Delta/-}$ mice. It even increased survival, mimicking aspects of dietary restriction, the most efficient anti–vascular aging intervention identified thus far.[155,228] Of note, RAS blockade was not effective in *Ercc1*$^{\Delta/-}$ mice.[90] The results indicate that stimulation of the NO-cGMP vasodilatory mechanism is an alternative for the clinically used blockade of vasoconstriction, which apart from RAS inhibition can involve Ca^{2+} channel blockade, to protect against vascular aging features. A priority herein is the effect on senescence and inflammation, because these are the mechanisms that are the most identifiable with vascular aging.[40] Studies in Ercc1-based accelerated aging models and humans also showed NOX2, a superoxide producer, as a potential pharmacologic target. It was first demonstrated in the aortic rings of *Ercc1*$^{\Delta/-}$ mice that superoxide formation at the level of VSMCs partially explained the loss of relaxation during exposure to NO.[48] In a recent study in VSMC-targeted *Ercc1*$^{\Delta/-}$ this was pinpointed to at least involve NOX2.[89] Even more recently, it was shown that NOX2 is the main NOX to decrease vasodilation in isolated aged human skeletal muscle–feeding arteries. In contrast to mouse aorta, this was demonstrated only at the level of endothelium as NO-induced relaxation of VSMCs was not altered in these isolated human arteries from axillary and inguinal origins. After many years of investment in the development of NOX subtype–specific inhibitors, GSK2795039, a NOX2-specific drug for *in vivo* use, emerged,[229] but no clinical development has been reported. Further research would be necessary to better understand the potential implications of these observations and how they could potentially be used to develop new treatments or preventative measures for vascular senescence.

Conclusion and Future Perspectives

NAVA and senescence provoke the development of MI and heart failure, with physical and signalling alterations in the vasculature and endothelium function as their early indicators. The new direction of cardiovascular disease pathophysiology is shifting from extrinsic toward intrinsic vascular aging. This has created the need for models of accelerated vascular aging exemplifying features of NAVA, such as DNA repair–defective mice. Therefore, implementing strategies to prevent the onset of these aging features or to reverse existing dysfunction in their early stages may be effective in reducing the burden of cardiovascular diseases. Although these models simplify the understanding of disease and therapeutic mechanisms, the complexity of real-world vascular aging should be taken into consideration. Atherosclerosis and other extrinsic features are lacking in these models, but might be introduced by induction of dyslipidemia, inflammation, diabetes, calcifying conditions, or hypertension. As an example of why this is important, atherosclerosis can already start at a young age, and therefore in origin does not arise from intrinsic aging. Yet atherosclerosis is a process that requires time to develop into a state of disease, and thus it is associated with age. The combination of abundant exposure to classic cardiovascular risk factors that promote extrinsic vascular aging, and the increase of population age, which promotes both atherosclerotic and NAVA, intuitively predicts that both conditions will be at play simultaneously in the elderly. Moreover, because vascular aging results in chronic low-grade systemic inflammation, an interaction between

intrinsic and extrinsic vascular aging is to be expected. Therefore, an integrative approach for intervention would be ideal. The hallmarks of aging as previously defined can be a helpful tool to construct integrative research strategies.

Senolytics are another strategy that has been addressed in this review. Although there is a growing focus on identifying effective senolytics in preclinical studies and some translation in clinical studies, the detection and measurement of senescence in humans continue to be challenging tasks. This difficulty may be intensified if the focus and therapeutic targeting is specifically on VSMCs and ECs. Also, the identification of reliable markers is required to facilitate the discovery of senolytic agents by providing a clearer path forward,[204] but the question is whether a universal senescence marker is feasible, given the significant heterogeneity of markers between cell types. Targeted exploration of vascular-specific senescence markers is therefore warranted. One more topic of discussion revolves around the safety of new senolytic drugs, particularly owing to the negative outcomes that have been observed in cases of pulmonary hypertension.[147,230,231] Yet another question is whether senolytics should be attempted to treat or to prevent NAVA. Ideally, prevention is optimal, but momentarily NAVA is not considered as a disease, and no predictive clinical markers exist. Therefore, treatment for now needs to focus on reversal of preexisting NAVA in animal models. Considering the unrevealed safety profile of senolytics, FDA-approved clinically applicable drugs that reduce senescence or SASP as a pleiotropic effect might be the quickest way to the patient. Agents blocking the SASP inflammatory pathways or that reduce senescence, as was demonstrated for PDE1 inhibition and sGC activation in *Ercc1* mutant mouse models, might provide more certainty for clinical development. Although many drug repurposing trials are going on for different diseases, there is a scarcity of studies investigating potential senolytics or NAVA therapies. The COLCOT[180] and LoDoCo2[232] trials serve as successful examples of repurposing colchicine for the prevention of cardiovascular diseases and increasing longevity.

The current lack of possibilities to set up prospective studies to prevent NAVA might partly be solved by screening the impact of already available drugs in pharmacoepidemiologic studies embedded within long-term longitudinal cohorts. This might be preceded or accompanied by testing of repurposed drugs in a representative model of NAVA-like *Ercc1* mutant mice. This combination of human and other animal research may form an efficient platform to accelerate the development of effective, clinically applicable therapies.

Ethics Statement

This review does not contain original experiment results for which ethical approval is required.

Patient Consent

The authors confirm that patient consent is not applicable to this article.

Funding Sources

The authors have no funding sources to declare.

Disclosures

The authors have no conflicts of interest to disclose.

References

1. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart disease and stroke statistics—2015 update: a report from the American Heart Association. Circulation 2015;131:e29-322.
2. Knuuti J, Wijns W, Saraste A, et al. 2019 ESC guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J 2020;41:407-77.
3. Kyhl K, von Huth S, Bojer A, et al. Conductance artery stiffness impairs atrio-ventriculo-arterial coupling before manifestation of arterial hypertension or left ventricular hypertrophic remodelling. Sci Rep 2021;11:14467.
4. O'Rourke MF, Blazek JV, Morreels CL Jr, Krovetz LJ. Pressure wave transmission along the human aorta. Changes with age and in arterial degenerative disease. Circ Res 1968;23:567-79.
5. Kucharska-Newton AM, Stoner L, Meyer ML. Determinants of vascular age: an epidemiological perspective. Clin Chem 2019;65:108-18.
6. Benetos A, Adamopoulos C, Bureau J-M, et al. Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. Circulation 2002;105:1202-7.
7. Van Popele NM, Grobbee DE, Bots ML, et al. Association between arterial stiffness and atherosclerosis: the Rotterdam Study. Stroke 2001;32:454-60.
8. Roman MJ, Saba PS, Pini R, et al. Parallel cardiac and vascular adaptation in hypertension. Circulation 1992;86:1909-18.
9. Safar ME, Thomas F, Blacher J, et al. Metabolic syndrome and age-related progression of aortic stiffness. J Am Coll Cardiol 2006;47:72-5.
10. Mitchell GF, Parise H, Benjamin EJ, et al. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. Hypertension 2004;43:1239-45.
11. Reference Values for Arterial Stiffness Collaboration: Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: "establishing normal and reference values.". Eur Heart J 2010;31:2338-50.
12. Laucyte-Cibulskiene A, Chen C-H, Cockroft J, et al. Clusters of risk factors in metabolic syndrome and their influence on central blood pressure in a global study. Sci Rep 2022;12:14409.
13. Avolio AP, Deng F-Q, Li W-Q, et al. Effects of aging on arterial distensibility in populations with high and low prevalence of hypertension: comparison between urban and rural communities in China. Circulation 1985;71:202-10.
14. Cavalcante JL, Lima JA, Redheuil A, Al-Mallah MH. Aortic stiffness: current understanding and future directions. J Am Coll Cardiol 2011;57:1511-22.
15. Verwoert GC, Franco OH, Hoeks APG, et al. Arterial stiffness and hypertension in a large population of untreated individuals: the Rotterdam Study. J Hypertens 2014;32:1606-12.
16. Mattace-Raso FUS, van der Cammen TJM, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation 2006;113:657-63.
17. Sedaghat S, Mattace-Raso FUS, Hoorn EJ, et al. Arterial stiffness and decline in kidney function. Clin J Am Soc Nephrol 2015;10:2190-7.

18. Van Sloten TT, Sedaghat S, Laurent S, et al. Carotid stiffness is associated with incident stroke: a systematic review and individual participant data meta-analysis. J Am Coll Cardiol 2015;66:2116-25.

19. Poels MMF, Zaccai K, Verwoert GC, et al. Arterial stiffness and cerebral small vessel disease: the Rotterdam Scan Study. Stroke 2012;43:2637-42.

20. Scuteri A, Tesauro M, Appolloni S, et al. Arterial stiffness as an independent predictor of longitudinal changes in cognitive function in the older individual. J Hypertens 2007;25:1035-40.

21. Selwaness M, van den Bouwhuijsen Q, Mattace-Raso FUS, et al. Arterial stiffness is associated with carotid intraplaque hemorrhage in the general population: the Rotterdam study. Arterioscler Thromb Vasc Biol 2014;34:927-32.

22. Loutzenhiser R, Bidani A, Chilton L. Renal myogenic response: kinetic attributes and physiological role. Circ Res 2002;90:1316-24.

23. Safar ME, London GM, Plante GE. Arterial stiffness and kidney function. Hypertension 2004;43:163-8.

24. Li Xs, He H, Zhao Yl, et al. Bone mineral density is negatively associated with arterial stiffness in men with hypertension. J Clin Hypertens 2016;18:1106-11.

25. Kim TN, Park MS, Lim KI, et al. Skeletal muscle mass to visceral fat area ratio is associated with metabolic syndrome and arterial stiffness: the Korean Sarcopenic Obesity Study (KSOS). Diabetes Res Clin Pract 2011;93:285-91.

26. Kohara K, Okada Y, Ochi M, et al. Muscle mass decline, arterial stiffness, white matter hyperintensity, and cognitive impairment: Japan Shimanami Health Promoting Program study. J Cachexia Sarcopenia Muscle 2017;8:557-66.

27. Waller BF. Anatomy, histology, and pathology of the major epicardial coronary arteries relevant to echocardiographic imaging techniques. J Am Soc Echocardiogr 1989;2:232-52.

28. Vos A, van Hecke W, Spliet WG, et al. Predominance of nonatherosclerotic internal elastic lamina calcification in the intracranial internal carotid artery. Stroke 2016;47:221-3.

29. Amann K. Media calcification and intima calcification are distinct entities in chronic kidney disease. Clin J Am Soc Nephrol 2008;3:1599-605.

30. Kockelkoren R, Vos A, van Hecke W, et al. Computed tomographic distinction of intimal and medial calcification in the intracranial internal carotid artery. PLoS One 2017;12:e0168360.

31. Psychogios M, Brehm A, López-Cancio E, et al. European Stroke Organisation guidelines on treatment of patients with intracranial atherosclerotic disease. Eur Stroke J 2022;7:III-IV.

32. van den Beukel TC, van der Toorn JE, Vernooij MW, et al. Morphological subtypes of intracranial internal carotid artery arteriosclerosis and the risk of stroke. Stroke 2022;53:1339-47.

33. Compagne KCJ, Clephas PRD, Majoie C, et al. Intracranial carotid artery calcification and effect of endovascular stroke treatment. Stroke 2018;49:2961-8.

34. Luijten SPR, van der Donk SC, Compagne KCJ, et al. Intracranial carotid artery calcification subtype and collaterals in patients undergoing endovascular thrombectomy. Atherosclerosis 2021;337:1-6.

35. Steppan J, Barodka V, Berkowitz DE, Nyhan D. Vascular stiffness and increased pulse pressure in the aging cardiovascular system. Cardiol Res Pract 2011;2011:263585.

36. Ataei Ataabadi E, Golshiri K, Jüttner A, et al. Nitric oxide—cGMP signaling in hypertension: current and future options for pharmacotherapy. Hypertension 2020;76:1055-68.

37. Lacolley P, Regnault V, Avolio AP. Smooth muscle cell and arterial aging: basic and clinical aspects. Cardiovasc Res 2018;114:513-28.

38. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013;153:1194-217.

39. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: an expanding universe. Cell 2023;186:243-78.

40. Schumacher B, Pothof J, Vijg J, Hoeijmakers JHJ. The central role of DNA damage in the ageing process. Nature 2021;592:695-703.

41. Gyenis A, Chang J, Demmers J, et al. Genome-wide RNA polymerase stalling shapes the transcriptome during aging. Nat Genet 2023;55:268-79.

42. Vougioukalaki M, Demmers J, Vermeij WP, et al. Different responses to DNA damage determine ageing differences between organs. Aging Cell 2022;21:e13562.

43. Wu H, Roks AJM. Genomic instability and vascular aging: a focus on nucleotide excision repair. Trends Cardiovasc Med 2014;24:61-8.

44. Bautista-Niño PK, Portilla-Fernandez E, Vaughan DE, Danser AHJ, Roks AJM. DNA damage: a main determinant of vascular aging. Int J Mol Sci 2016;17:748.

45. Piekarowicz K, Machowska M, Dzianisava V, Rzepecki R. Hutchinson-Gilford progeria syndrome—current status and prospects for gene therapy treatment. Cells 2019;8:88.

46. Olive M, Harten I, Mitchell R, et al. Cardiovascular pathology in Hutchinson-Gilford progeria: correlation with the vascular pathology of aging. Arterioscler Thromb Vasc Biol 2010;30:2301-9.

47. Stehbens WE, Gilbert-Barness E, Olson RE, Ackerman J. Histological and ultrastructural features of atherosclerosis in progeria. Cardiovasc Pathol 1999;8:29-39.

48. Durik M, Kavousi M, van der Pluijm I, et al. Nucleotide excision DNA repair is associated with age-related vascular dysfunction. Circulation 2012;126:468-78.

49. Samani NJ, Schunkert H. Chromosome 9p21 and cardiovascular disease: the story unfolds. Circ Cardiovasc Genet 2008;1:81-4.

50. Benetos A, Okuda K, Lajemi M, et al. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. Hypertension 2001;37:381-5.

51. Panayiotou AG, Nicolaides AN, Griffin M, et al. Leukocyte telomere length is associated with measures of subclinical atherosclerosis. Atherosclerosis 2010;211:176-81.

52. Burnett-Hartman AN, Fitzpatrick AL, Kronmal RA, et al. Telomere-associated polymorphisms correlate with cardiovascular disease mortality in Caucasian women: the Cardiovascular Health Study. Mech Ageing Dev 2012;133:275-81.

53. Khalid M, Abdollahi M. Epigenetic modifications associated with pathophysiological effects of lead exposure. J Environ Sci Health C 2019;37:235-87.

54. Scherrer U, Rimoldi SF, Rexhaj E, et al. Systemic and pulmonary vascular dysfunction in children conceived by assisted reproductive technologies. Circulation 2012;125:1890-6.

55. Ding YN, Tang X, Chen HZ, Liu DP. Epigenetic regulation of vascular aging and age-related vascular diseases. Adv Exp Med Biol 2018;1086:55-75.

56. Herman AB, Occean JR, Sen P. Epigenetic dysregulation in cardiovascular aging and disease. J Cardiovasc Aging 2021;1:10.

57. Lin Z, Ding Q, Li X, et al. Targeting epigenetic mechanisms in vascular aging. Front Cardiovasc Med 2021;8:806988.

58. Kumar A, Kumar S, Vikram A, et al. Histone and DNA methylation-mediated epigenetic downregulation of endothelial Kruppel-like factor 2 by low-density lipoprotein cholesterol. Arterioscler Thromb Vasc Biol 2013;33:1936-42.

59. Wei L, Zhao S, Wang G, et al. SMAD7 methylation as a novel marker in atherosclerosis. Biochem Biophys Res Commun 2018;496:700-5.

60. Ma SC, Zhang HP, Jiao Y, et al. Homocysteine-induced proliferation of vascular smooth muscle cells occurs via PTEN hypermethylation and is mitigated by resveratrol. Mol Med Rep 2018;17:5312-9.

61. Xu L, Hao H, Hao Y, et al. Aberrant MFN2 transcription facilitates homocysteine-induced VSMCs proliferation via the increased binding of c-Myc to DNMT1 in atherosclerosis. J Cell Mol Med 2019;23:4611-26.

62. Challen GA, Sun D, Jeong M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet 2012;44:23-31.

63. Zaina S, Heyn H, Carmona FJ, et al. DNA methylation map of human atherosclerosis. Circ Cardiovasc Genet 2014;7:692-700.

64. Aavik E, Babu M, Ylä-Herttuala S. DNA methylation processes in atheosclerotic plaque. Atherosclerosis 2019;281:168-79.

65. Seungyoon BY, Pekkurnaz G. Mechanisms orchestrating mitochondrial dynamics for energy homeostasis. J Mol Biol 2018;430:3922-41.

66. Soubannier V, McBride HM. Positioning mitochondrial plasticity within cellular signaling cascades. Biochim Biophys Acta Mol Cell Res 2009;1793:154-70.

67. Simon HU, Haj-Yehia A, Levi-Schaffer F. Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis 2000;5:415-8.

68. Rambold AS, Lippincott-Schwartz J. Mechanisms of mitochondria and autophagy crosstalk. Cell Cycle 2011;10:4032-8.

69. Youn DH, Kim Y, Kim BJ, et al. Mitochondrial dysfunction associated with autophagy and mitophagy in cerebrospinal fluid cells of patients with delayed cerebral ischemia following subarachnoid hemorrhage. Sci Rep 2021;11:16512.

70. Foote K, Reinhold J, Yu EPK, et al. Restoring mitochondrial DNA copy number preserves mitochondrial function and delays vascular aging in mice. Aging Cell 2018;17:e12773.

71. Yu EPK, Reinhold J, Yu H, et al. Mitochondrial respiration is reduced in atherosclerosis, promoting necrotic core formation and reducing relative fibrous cap thickness. Arterioscler Thromb Vasc Biol 2017;37:2322-32.

72. Rossman MJ, Gioscia-Ryan RA, Clayton ZS, Murphy MP, Seals DR. Targeting mitochondrial fitness as a strategy for healthy vascular aging. Clin Sci 2020;134:1491-519.

73. Rossman MJ, Santos-Parker JR, Steward CAC, et al. Chronic supplementation with a mitochondrial antioxidant (MitoQ) improves vascular function in healthy older adults. Hypertension 2018;71:1056-63.

74. Park SH, Kwon OS, Park SY, et al. Vasodilatory and vascular mitochondrial respiratory function with advancing age: evidence of a free radically mediated link in the human vasculature. Am J Physiol Regul Integr Comp Physiol 2020;318:R701-11.

75. Chan SL, Liu D, Kyriazis GA, et al. Mitochondrial uncoupling protein-4 regulates calcium homeostasis and sensitivity to store depletion-induced apoptosis in neural cells. J Biol Chem 2006;281:37391-403.

76. Duchen MR. Roles of mitochondria in health and disease. Diabetes 2004;53:S96-102.

77. Tyrrell DJ, Blin MG, Song J, et al. Age-associated mitochondrial dysfunction accelerates atherogenesis. Circ Res 2020;126:298-314.

78. Schleicher E, Friess U. Oxidative stress, AGE, and atherosclerosis. Kidney Int Suppl 2007:S17-26.

79. Ungvari Z, Sonntag WE, Csiszar A. Mitochondria and aging in the vascular system. J Mol Med (Berl) 2010;88:1021-7.

80. Sell DR, Monnier VM. Molecular basis of arterial stiffening: role of glycation—a mini-review. Gerontology 2012;58:227-37.

81. Jüttner AA, Danser AHJ, Roks AJM. Pharmacological developments in antihypertensive treatment through nitric oxide—cGMP modulation. Adv Pharmacol 2022;94:57-94.

82. Scalera F, Martens-Lobenhoffer J, Täger M, et al. Effect of L-arginine on asymmetric dimethylarginine (ADMA) or homocysteine-accelerated endothelial cell aging. Biochem Biophys Res Commun 2006;345:1075-82.

83. Scalera F, Borlak J, Beckmann B, et al. Endogenous nitric oxide synthesis inhibitor asymmetric dimethyl l-arginine accelerates endothelial cell senescence. Arterioscler Thromb Vasc Biol 2004;24:1816-22.

84. Chen ZP, Mitchelhill KI, Michell BJ, et al. AMP-activated protein kinase phosphorylation of endothelial NO synthase. FEBS Lett 1999;443:285-9.

85. Fleming I, Fisslthaler B, Dimmeler S, Kemp BE, Busse R. Phosphorylation of Thr^{495} regulates Ca^{2+}/calmodulin-dependent endothelial nitric oxide synthase activity. Circ Res 2001;88:e68-75.

86. Yoon HJ, Cho SW, Ahn BW, Yang SY. Alterations in the activity and expression of endothelial NO synthase in aged human endothelial cells. Mech Ageing Dev 2010;131:119-23.

87. Liu DH, Chen YM, Liu Y, et al. Ginsenoside Rb1 reverses H_2O_2-induced senescence in human umbilical endothelial cells: involvement of eNOS pathway. J Cardiovasc Pharmacol 2012;59:222-30.

88. Jo-Watanabe A, Ohse T, Nishimatsu H, et al. Glyoxalase I reduces glycative and oxidative stress and prevents age-related endothelial dysfunction through modulation of endothelial nitric oxide synthase phosphorylation. Aging Cell 2014;13:519-28.

89. Ataei Ataabadi E, Golshiri K, van der Linden J, et al. Vascular ageing features caused by selective DNA damage in smooth muscle cell. Oxid Med Cell Longev 2021;2021:2308317.

90. Wu H, van Thiel BS, Bautista-Niño PK, et al. Dietary restriction but not angiotensin II type 1 receptor blockade improves DNA damage-related vasodilator dysfunction in rapidly aging $Ercc1^{\Delta/-}$ mice. Clin Sci (Lond) 2017;131:1941-53.

91. 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;11:e026426.

92. Uijl E, Ren L, Mirabito Colafella KM, et al. No evidence for brain renin-angiotensin system activation during DOCA-salt hypertension. Clin Sci (Lond) 2021;135:259-74.

93. Cruz-López EO, Uijl E, Danser AHJ. Perivascular adipose tissue in vascular function: does locally synthesized angiotensinogen play a role? J Cardiovasc Pharmacol 2021;78:S53-62.

94. Sun Y, Goes Martini A, Janssen MJ, et al. Megalin: a novel endocytic receptor for prorenin and renin. Hypertension 2020;75:1242-50.

95. Batenburg WW, Garrelds IM, Bernasconi CC, et al. Angiotensin II type 2 receptor—mediated vasodilation in human coronary microarteries. Circulation 2004;109:2296-301.

96. Verdonk K, Durik M, Abd-Alla N, et al. Compound 21 induces vasorelaxation via an endothelium— and angiotensin II type 2 receptor—independent mechanism. Hypertension 2012;60:722-9.

97. Verdonk K, Saleh L, Lankhorst S, et al. Association studies suggest a key role for endothelin-1 in the pathogenesis of preeclampsia and the accompanying renin-angiotensin-aldosterone system suppression. Hypertension 2015;65:1316-23.

98. Moltzer E, Verkuil AV, van Veghel R, Danser AH, van Esch JH. Effects of angiotensin metabolites in the coronary vascular bed of the spontaneously hypertensive rat: loss of angiotensin II type 2 receptor—mediated vasodilation. Hypertension 2010;55:516-22.

99. Ungvari Z, Tarantini S, Donato AJ, Galvan V, Csiszar A. Mechanisms of vascular aging. Circ Res 2018;123:849-67.

100. Yoon HE, Kim EN, Kim MY, et al. Age-associated changes in the vascular renin-angiotensin system in mice. Oxid Med Cell Longev 2016;2016:6731093.

101. Liaudet L, Vassalli G, Pacher P. Role of peroxynitrite in the redox regulation of cell signal transduction pathways. Front Biosci (Landmark Ed) 2009;14:4809-14.

102. Boulanger CM, Heymes C, Benessiano J, et al. Neuronal nitric oxide synthase is expressed in rat vascular smooth muscle cells: activation by angiotensin II in hypertension. Circ Res 1998;83:1271-8.

103. Kawano H, Do YS, Kawano Y, et al. Angiotensin II has multiple profibrotic effects in human cardiac fibroblasts. Circulation 2000;101:1130-7.

104. Thomas TH, Advani A. Inflammation in cardiovascular disease and regulation of the actin cytoskeleton in inflammatory cells: the actin cytoskeleton as a target. Cardiovasc Hematol Agents Med Chem 2006;4:165-82.

105. Minamino T, Yoshida T, Tateno K, et al. Ras induces vascular smooth muscle cell senescence and inflammation in human atherosclerosis. Circulation 2003;108:2264-9.

106. Cheng Y, Felix B, Othmer HG. The roles of signaling in cytoskeletal changes, random movement, direction-sensing and polarization of eukaryotic cells. Cells 2020;9.

107. Huveneers S, Daemen MJ, Hordijk PL. Between Rho(k) and a hard place: the relation between vessel wall stiffness, endothelial contractility, and cardiovascular disease. Circ Res 2015;116:895-908.

108. Mu X, Tseng C, Hambright WS, et al. Cytoskeleton stiffness regulates cellular senescence and innate immune response in Hutchinson-Gilford progeria syndrome. Aging Cell 2020;19:e13152.

109. Ivanov AI, Le HT, Naydenov NG, Rieder F. Novel functions of the septin cytoskeleton: shaping up tissue inflammation and fibrosis. Am J Pathol 2021;191:40-51.

110. Campisi J. Aging, cellular senescence, and cancer. Annu Rev Physiol 2013;75:685-705.

111. Niedernhofer LJ, Gurkar AU, Wang Y, et al. Nuclear genomic instability and aging. Annu Rev Biochem 2018;87:295-322.

112. Hernandez-Segura A, Nehme J, Demaria M. Hallmarks of cellular senescence. Trends Cell Biol 2018;28:436-53.

113. Childs BG, Baker DJ, Kirkland JL, Campisi J, van Deursen JM. Senescence and apoptosis: dueling or complementary cell fates? EMBO Rep 2014;15:1139-53.

114. Itahana K, Itahana Y, Dimri GP. Colorimetric detection of senescence-associated β galactosidase. Methods Mol Biol 2013;965:143-56.

115. Lee BY, Han JA, Im JS, et al. Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. Aging Cell 2006;5:187-95.

116. van Deursen JM. The role of senescent cells in ageing. Nature 2014;509:439-46.

117. Baker DJ, Childs BG, Durik M, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Nature 2016;530:184-9.

118. Kumari R, Jat P. Mechanisms of cellular senescence: cell cycle arrest and senescence associated secretory phenotype. Front Cell Dev Biol 2021;9:645593.

119. Saleh T, Tyutyunyk-Massey L, Gewirtz DA. Tumor cell escape from therapy-induced senescence as a model of disease recurrence after dormancy. Cancer Res 2019;79:1044-6.

120. Lapasset L, Milhavet O, Prieur A, et al. Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state. Genes Dev 2011;25:2248-53.

121. Kim SH, Rowe J, Fujii H, et al. Upregulation of chicken p15INK4b at senescence and in the developing brain. J Cell Sci 2006;119:2435-43.

122. Thanapaul R, Shvedova M, Shin GH, Roh DS. An insight into aging, senescence, and their impacts on wound healing. Adv Geriatr Med Res 2021;3.

123. Andrade AM, Sun M, Gasek NS, Hargis GR, Sharafieh R, Xu M. Role of senescent cells in cutaneous wound healing. Biology (Basel) 2022;11.

124. Huang W, Hickson LJ, Eirin A, Kirkland JL, Lerman LO. Cellular senescence: the good, the bad and the unknown. Nat Rev Nephrol 2022;18:611-27.

125. Freund A, Orjalo AV, Desprez PY, Campisi J. Inflammatory networks during cellular senescence: causes and consequences. Trends Mol Med 2010;16:238-46.

126. Franceschi C, Bonafè M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 2000;908:244-54.

127. Youm YH, Grant RW, McCabe LR, et al. Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. Cell Metab 2013;18:519-32.

128. Chen HZ, Wang F, Gao P, et al. Age-associated sirtuin 1 reduction in vascular smooth muscle links vascular senescence and inflammation to abdominal aortic aneurysm. Circ Res 2016;119:1076-88.

129. Ungvari Z, Orosz Z, Labinskyy N, et al. Increased mitochondrial H_2O_2 production promotes endothelial NF-κB activation in aged rat arteries. Am J Physiol Heart Circ Physiol 2007;293:H37-47.

130. Guo Y, Xu C, Man AWC, et al. Endothelial SIRT1 prevents age-induced impairment of vasodilator responses by enhancing the expression and activity of soluble guanylyl cyclase in smooth muscle cells. Cardiovasc Res 2019;115:678-90.

131. Wang Y, Liang Y, Vanhoutte PM. SIRT1 and AMPK in regulating mammalian senescence: a critical review and a working model. FEBS Lett 2011;585:986-94.

132. Ren K, Torres R. Role of interleukin-1β during pain and inflammation. Brain Res Rev 2009;60:57-64.

133. Abbate A, Trankle CR, Buckley LF, et al. Interleukin-1 blockade inhibits the acute inflammatory response in patients with ST-segment-elevation myocardial infarction. J Am Heart Assoc 2020;9:e014941.

134. Reiss AB, Siegart NM, de Leon J. Interleukin-6 in atherosclerosis: atherogenic or atheroprotective? Clin Lipidol 2017;12:14-23.

135. Markousis-Mavrogenis G, Tromp J, Ouwerkerk W, et al. The clinical significance of interleukin-6 in heart failure: results from the BIOSTAT-CHF study. Eur J Heart Fail 2019;21:965-73.

136. Esteve E, Castro A, López-Bermejo A, et al. Serum interleukin-6 correlates with endothelial dysfunction in healthy men independently of insulin sensitivity. Diabetes Care 2007;30:939-45.

137. Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. Biochem Pharmacol 2009;78:539-52.

138. Donato AJ, Henson GD, Morgan RG, et al. TNF-α impairs endothelial function in adipose tissue resistance arteries of mice with diet-induced obesity. Am J Physiol Heart Circ Physiol 2012;303:H672-679.

139. Goetze S, Xi XP, Kawano Y, et al. TNF-α—induced migration of vascular smooth muscle cells is MAPK dependent. Hypertension 1999;33:183-9.

140. Davis R, Pillai S, Lawrence N, Sebti S, Chellappan SP. TNF-α—mediated proliferation of vascular smooth muscle cells involves Raf-1—mediated inactivation of Rb and transcription of E2F1-regulated genes. Cell Cycle 2012;11:109-18.

141. Mistriotis P, Andreadis ST. Vascular aging: molecular mechanisms and potential treatments for vascular rejuvenation. Ageing Res Rev 2017;37:94-116.

142. Hasegawa Y, Saito T, Ogihara T, et al. Blockade of the nuclear factor-κB pathway in the endothelium prevents insulin resistance and prolongs life spans. Circulation 2012;125:1122-33.

143. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res 2000;86:494-501.

144. García-Martín A, Navarrete C, Garrido-Rodríguez M, et al. EHP-101 alleviates angiotensin II—induced fibrosis and inflammation in mice. Biomed Pharmacother 2021;142:112007.

145. Kirkland JL, Tchkonia T. Senolytic drugs: from discovery to translation. J Intern Med 2020;288:518-36.

146. Ungvari Z, Tarantini S, Sorond F, Merkely B, Csiszar A. Mechanisms of vascular aging, a geroscience perspective: JACC focus seminar. J Am Coll Cardiol 2020;75:931-41.

147. Rabinovitch M. Are senolytic agents guilty of overkill or inappropriate age discrimination? Circulation 2023;147:667-8.

148. Kennedy BK, Berger SL, Brunet A, et al. Geroscience: linking aging to chronic disease. Cell 2014;159:709-13.

149. Cai N, Wu Y, Huang Y. Induction of accelerated aging in a mouse model. Cells 2022;11:1418.

150. Withaar C, Lam CSP, Schiattarella GG, de Boer RA, Meems LMG. Heart failure with preserved ejection fraction in humans and mice: embracing clinical complexity in mouse models. Eur Heart J 2021;42:4420-30.

151. Barros PR, Costa TJ, Akamine EH, Tostes RC. Vascular aging in rodent models: contrasting mechanisms driving the female and male vascular senescence. Front Aging 2021;2:727604.

152. Newcomer SC, Leuenberger UA, Hogeman CS, Proctor DN. Heterogeneous vasodilator responses of human limbs: influence of age and habitual endurance training. Am J Physiol Heart Circ Physiol 2005;289:H308-315.

153. Weeda G, Donker I, de Wit J, et al. Disruption of mouse ERCC1 results in a novel repair syndrome with growth failure, nuclear abnormalities and senescence. Curr Biol 1997;7:427-39.

154. Vermeij WP, Hoeijmakers JH, Pothof J. Genome integrity in aging: human syndromes, mouse models, and therapeutic options. Annu Rev Pharmacol Toxicol 2016;56:427-45.

155. Vermeij WP, Dollé ME, Reiling E, et al. Restricted diet delays accelerated ageing and genomic stress in DNA-repair—deficient mice. Nature 2016;537:427-31.

156. Sims AA, Gurkar AU. DNA damage—induced stalling of transcription drives aging through gene expression imbalance. DNA Repair (Amst) 2023;125:103483.

157. Bautista-Niño PK, Portilla-Fernandez E, Rubio-Beltrán E, et al. Local endothelial DNA repair deficiency causes aging-resembling endothelial-specific dysfunction. Clin Sci (Lond) 2020;134:727-46.

158. de Boer M, te Lintel Hekkert M, Chang J, et al. DNA repair in cardiomyocytes is critical for maintaining cardiac function in mice. Aging Cell 2023;22:e13768.

159. Yousefzadeh MJ, Zhao J, Bukata C, et al. Tissue specificity of senescent cell accumulation during physiologic and accelerated aging of mice. Aging Cell 2020;19:e13094.

160. Nakano-Kurimoto R, Ikeda K, Uraoka M, et al. Replicative senescence of vascular smooth muscle cells enhances the calcification through initiating the osteoblastic transition. Am J Physiol Heart Circ Physiol 2009;297:H1673-84.

161. Liu Y, Drozdov I, Shroff R, Beltran LE, Shanahan CM. Prelamin A accelerates vascular calcification via activation of the DNA damage response and senescence-associated secretory phenotype in vascular smooth muscle cells. Circ Res 2013;112:e99-109.

162. Gomez-Sanchez M, Gomez-Sanchez L, Patino-Alonso MC, et al. Vascular aging and its relationship with lifestyles and other risk factors in the general Spanish population: Early Vascular Ageing Study. J Hypertens 2020;38:1110-22.

163. Lessiani G, Santilli F, Boccatonda A, et al. Arterial stiffness and sedentary lifestyle: role of oxidative stress. Vascul Pharmacol 2016;79:1-5.

164. Karimi L, Mattace-Raso FU, van Rosmalen J, et al. Effects of combined healthy lifestyle factors on functional vascular aging: the Rotterdam Study. J Hypertens 2016;34:853-9.

165. Ghebre YT, Yakubov E, Wong WT, et al. Vascular aging: implications for cardiovascular disease and therapy. Transl Med (Sunnyvale) 2016;6:183.

166. Rubio-Ruiz ME, Pérez-Torres I, Diaz-Diaz E, Pavón N, Guarner-Lans V. Non-steroidal anti-inflammatory drugs attenuate the vascular responses in aging metabolic syndrome rats. Acta Pharmacol Sin 2014;35:1364-74.

167. Lesniewski LA, Durrant JR, Connell ML, et al. Salicylate treatment improves age-associated vascular endothelial dysfunction: potential role of nuclear factor κB and forkhead box O phosphorylation. J Gerontol A Biol Sci Med Sci 2011;66:409-18.

168. Pierce GL, Lesniewski LA, Lawson BR, Beske SD, Seals DR. Nuclear factor-κB activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. Circulation 2009;119:1284-92.

169. Nowak KL, Chonchol M, Ikizler TA, et al. IL-1 inhibition and vascular function in CKD. J Am Soc Nephrol 2017;28:971-80.

170. Vallejo S, Palacios E, Romacho T, et al. The interleukin-1 receptor antagonist anakinra improves endothelial dysfunction in streptozotocin-induced diabetic rats. Cardiovasc Diabetol 2014;13:158.

171. Ikonomidis I, Lekakis JP, Nikolaou M, et al. Inhibition of interleukin-1 by anakinra improves vascular and left ventricular function in patients with rheumatoid arthritis. Circulation 2008;117:2662-9.

172. Ikonomidis I, Pavlidis G, Katsimbri P, et al. Differential effects of inhibition of interleukin 1 and 6 on myocardial, coronary and vascular function. Clin Res Cardiol 2019;108:1093-101.

173. Kume K, Amano K, Yamada S, et al. Tocilizumab monotherapy reduces arterial stiffness as effectively as etanercept or adalimumab monotherapy in rheumatoid arthritis: an open-label randomized controlled trial. J Rheumatol 2011;38:2169-71.

174. Bosello S, Santoliquido A, Zoli A, et al. TNF-α blockade induces a reversible but transient effect on endothelial dysfunction in patients with long-standing severe rheumatoid arthritis. Clin Rheumatol 2008;27:833-9.

175. Lee J, Lee S, Zhang H, Hill MA, Zhang C, Park Y. Interaction of IL-6 and TNF-α contributes to endothelial dysfunction in type 2 diabetic mouse hearts. PLoS One 2017;12:e0187189.

176. Elmazoglu Z, Aydın Bek Z, Sarıbaş SG, et al. S-Allylcysteine inhibits chondrocyte inflammation to reduce human osteoarthritis via targeting RAGE, TLR4, JNK, and Nrf2 signaling: comparison with colchicine. Biochem Cell Biol 2021;99:645-54.

177. Kajikawa M, Higashi Y, Tomiyama H, et al. Effect of short-term colchicine treatment on endothelial function in patients with coronary artery disease. Int J Cardiol 2019;281:35-9.

178. Toprover M, Shah B, Oh C, et al. Initiating guideline-concordant gout treatment improves arterial endothelial function and reduces intercritical inflammation: a prospective observational study. Arthritis Res Ther 2020;22:169.

179. Nidorf SM, Fiolet ATL, Eikelboom JW, et al. The effect of low-dose colchicine in patients with stable coronary artery disease: the LoDoCo2 trial rationale, design, and baseline characteristics. Am Heart J 2019;218:46-56.

180. Tardif JC, Kouz S, Waters DD, et al. Efficacy and safety of low-dose colchicine after myocardial infarction. N Engl J Med 2019;381:2497-505.

181. Cimmino G, Conte S, Morello A, et al. Colchicine inhibits the prothrombotic effects of oxLDL in human endothelial cells. Vascul Pharmacol 2021;137:106822.

182. Zhang FS, He QZ, Qin CH, et al. Therapeutic potential of colchicine in cardiovascular medicine: a pharmacological review. Acta Pharmacol Sin 2022;43:2173-90.

183. Yilmaz E, Akay KH. The efficacy of colchicine on carotid intima-media thickness: a prospective comparative study. J Stroke Cerebrovasc Dis 2021;30:105580.

184. Ozalper V, Kara M, Tanoglu A, et al. Evaluation of endothelial dysfunction in patients with familial Mediterranean fever: the relationship between the levels of asymmetric dimethylarginine and endocan with carotid intima-media thickness and endothelium-dependent vasodilation. Clin Rheumatol 2017;36:2071-7.

185. Cameron AR, Morrison VL, Levin D, et al. Anti-inflammatory effects of metformin irrespective of diabetes status. Circ Res 2016;119:652-65.

186. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia 2017;60:1577-85.

187. Man AWC, Li H, Xia N. The role of sirtuin1 in regulating endothelial function, arterial remodeling and vascular aging. Front Physiol 2019;10:1173.

188. Mengozzi A, Costantino S, Paneni F, et al. Targeting SIRT1 rescues age- and obesity-induced microvascular dysfunction in *ex vivo* human vessels. Circ Res 2022;131:476-91.

189. de Picciotto NE, Gano LB, Johnson LC, et al. Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. Aging Cell 2016;15:522-30.

190. Kiss T, Nyúl-Tóth Á, Balasubramanian P, et al. Nicotinamide mononucleotide (NMN) supplementation promotes neurovascular rejuvenation in aged mice: transcriptional footprint of SIRT1 activation, mitochondrial protection, anti-inflammatory, and anti-apoptotic effects. Geroscience 2020;42:527-46.

191. Tarantini S, Yabluchanskiy A, Csipo T, et al. Treatment with the poly(ADP-ribose) polymerase inhibitor PJ-34 improves cerebromicrovascular endothelial function, neurovascular coupling responses and cognitive performance in aged mice, supporting the NAD+ depletion hypothesis of neurovascular aging. Geroscience 2019;41:533-42.

192. Abdellatif M, Trummer-Herbst V, Koser F, et al. Nicotinamide for the treatment of heart failure with preserved ejection fraction. Sci Transl Med 2021;13:eabd7064.

193. Martens CR, Denman BA, Mazzo MR, et al. Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD^+ in healthy middle-aged and older adults. Nat Commun 2018;9:1286.

194. Katayoshi T, Uehata S, Nakashima N, et al. Nicotinamide adenine dinucleotide metabolism and arterial stiffness after long-term nicotinamide mononucleotide supplementation: a randomized, double-blind, placebo-controlled trial. Sci Rep 2023;13:2786.

195. Freeberg KA, Craighead DH, Martens CR, et al. Nicotinamide riboside supplementation for treating elevated systolic blood pressure and arterial stiffness in midlife and older adults. Front Cardiovasc Med 2022;9:881703.

196. Gasek NS, Kuchel GA, Kirkland JL, Xu M. Strategies for targeting senescent cells in human disease. Nat Aging 2021;1:870-9.

197. Zhang L, Pitcher LE, Prahalad V, Niedernhofer LJ, Robbins PD. Targeting cellular senescence with senotherapeutics: senolytics and senomorphics. FEBS J 2023;290:1362-83.

198. Hartman ML, Czyz M. BCL-w: apoptotic and non-apoptotic role in health and disease. Cell Death Dis 2020;11:260.

199. Robbins PD, Jurk D, Khosla S, et al. Senolytic drugs: reducing senescent cell viability to extend health span. Annu Rev Pharmacol Toxicol 2021;61:779-803.

200. Mijit M, Caracciolo V, Melillo A, Amicarelli F, Giordano A. Role of p53 in the regulation of cellular senescence. Biomolecules 2020;10.

201. Huang Y, He Y, Makarcyzk MJ, Lin H. Senolytic peptide FOXO4-DRI selectively removes senescent cells from *in vitro* expanded human chondrocytes. Front Bioeng Biotechnol 2021;9:677576.

202. Malaise O, Tachikart Y, Brondello J-M. Senolytic treatments applied to osteoarthritis: a step towards the end of orthopedic surgery. AME Medical Journal 2017;2:161.

203. An T, Gong Y, Li X, et al. USP7 inhibitor P5091 inhibits Wnt signaling and colorectal tumor growth. Biochem Pharmacol 2017;131:29-39.

204. Zhang L, Pitcher LE, Yousefzadeh MJ, Niedernhofer LJ, Robbins PD, Zhu Y. Cellular senescence: a key therapeutic target in aging and diseases. J Clin Invest 2022:132.

205. Baar MP, Brandt RMC, Putavet DA, et al. Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. Cell 2017;169:132-147.e116.

206. Demaria M, Ohtani N, Youssef SA, et al. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell 2014;31:722-33.

207. Yosef R, Pilpel N, Tokarsky-Amiel R, et al. Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. Nat Commun 2016;7:11190.

208. Böger RH. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "l-arginine paradox" and acts as a novel cardiovascular risk factor. J Nutr 2004;134:2842S-7S [discussion: 2853S].

209. Bode-Böger SM, Scalera F, Martens-Lobenhoffer J. Asymmetric dimethylarginine (ADMA) accelerates cell senescence. Vasc Med 2005;10(suppl 1):S65-71.

210. Scalera F, Bode-Böger SM. Nitric oxide—asymmetric dimethylarginine system in endothelial cell senescence. In: Ignarro LJ, ed. Nitric Oxide. 2nd ed. San Diego: Academic Press, 2010:483-511.

211. Cimmino G, Loffredo FS, de Rosa G, Cirillo P. Colchicine in atherothrombosis: molecular mechanisms and clinical evidence. Int J Mol Sci 2023:24.

212. Zheng D, Liang Q, Zeng F, et al. Atorvastatin protects endothelium by decreasing asymmetric dimethylarginine in dyslipidemia rats. Lipids Health Dis 2015;14:41.

213. Chaib S, Tchkonia T, Kirkland JL. Cellular senescence and senolytics: the path to the clinic. Nat Med 2022;28:1556-68.

214. Cai Z, Zhang Y, Liu S, Liu X. Celecoxib, beyond anti-inflammation, alleviates tendon-derived stem cell senescence in degenerative rotator cuff tendinopathy. Am J Sports Med 2022;50:2488-96.

215. Ota H, Eto M, Kano MR, et al. Induction of endothelial nitric oxide synthase, SIRT1, and catalase by statins inhibits endothelial senescence through the Akt pathway. Arterioscler Thromb Vasc Biol 2010;30:2205-11.

216. Liu S, Uppal H, Demaria M, et al. Simvastatin suppresses breast cancer cell proliferation induced by senescent cells. Sci Rep 2015;5:17895.

217. Moiseeva O, Deschênes-Simard X, St-Germain E, et al. Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF-κB activation. Aging Cell 2013;12:489-98.

218. Avogaro A, de Kreutzenberg SV, Federici M, Fadini GP. The endothelium abridges insulin resistance to premature aging. J Am Heart Assoc 2013;2:e000262.

219. Tilstra JS, Robinson AR, Wang J, et al. NF-κB inhibition delays DNA damage—induced senescence and aging in mice. J Clin Invest 2012;122:2601-12.

220. Childs BG, Baker DJ, Wijshake T, et al. Senescent intimal foam cells are deleterious at all stages of atherosclerosis. Science 2016;354:472-7.

221. Roos CM, Zhang B, Palmer AK, et al. Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. Aging Cell 2016;15:973-7.

222. Raffaele M, Vinciguerra M. The costs and benefits of senotherapeutics for human health. Lancet Healthy Longev 2022;3:e67-77.

223. An S, Cho SY, Kang J, et al. Inhibition of 3-phosphoinositide—dependent protein kinase 1 (PDK1) can revert cellular senescence in human dermal fibroblasts. Proc Natl Acad Sci U S A 2020;117:31535-46.

224. Golshiri K, Ataei Ataabadi E, Portilla Fernandez EC, Jan Danser AH, Roks AJM. The importance of the nitric oxide—cGMP pathway in age-related cardiovascular disease: focus on phosphodiesterase-1 and soluble guanylate cyclase. Basic Clin Pharmacol Toxicol 2020;127:67-80.

225. Golshiri K, Ataei Ataabadi E, Brandt R, et al. Chronic sildenafil treatment improves vasomotor function in a mouse model of accelerated aging. Int J Mol Sci 2020;21:4667.

226. Golshiri K, Ataei Ataabadi E, Rubio-Beltran E, et al. Selective phosphodiesterase 1 inhibition ameliorates vascular function, reduces inflammatory response, and lowers blood pressure in aging animals. J Pharmacol Exp Ther 2021;378:173-83.

227. Golshiri K, Ataabadi EA, Jüttner AA, et al. The effects of acute and chronic selective phosphodiesterase 1 inhibition on smooth muscle cell—associated aging features. Front Pharmacol 2021;12:818355.

228. Ataei Ataabadi E, Golshiri K, Jüttner AA, et al. Soluble guanylate cyclase activator BAY 54-6544 improves vasomotor function and survival in an accelerated ageing mouse model. Aging Cell 2022;21:e13683.

229. Hirano K, Chen WS, Chueng AL, et al. Discovery of GSK2795039, a novel small molecule NADPH oxidase 2 inhibitor. Antioxid Redox Signal 2015;23:358-74.

230. Snell TW, Johnston RK, Srinivasan B, et al. Repurposing FDA-approved drugs for anti-aging therapies. Biogerontology 2016;17:907-20.

231. Born E, Lipskaia L, Breau M, et al. Eliminating senescent cells can promote pulmonary hypertension development and progression. Circulation 2023;147:650-66.

232. Nidorf SM, Fiolet ATL, Mosterd A, et al. Colchicine in patients with chronic coronary disease. N Engl J Med 2020;383:1838-47.