Vascular cell types in progeria: victims or villains?

Ignacio Benedicto[1,2,#,*], Magda R. Hamczyk[3,4,#], Beatriz Dorado[2,3], Vicente Andrés[2,3,*]

1. Centro de Investigaciones Biológicas Margarita Salas (CIB), Consejo Superior de Investigaciones Científicas (CSIC), 28040 Madrid, Spain.
2. Centro Nacional de Investigaciones Cardiovasculares (CNIC), 28029 Madrid, Spain.
3. CIBER en Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain.
4. Aarhus Institute of Advanced Studies (AIAS), Aarhus University, 8000 Aarhus C, Denmark.

[#]Equal contribution.

*Correspondence: ignacio.benedicto@cib.csic.es (I. Benedicto); vandres@cnic.es (V. Andrés).

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ABSTRACT

Hutchinson-Gilford progeria syndrome (HGPS) is an ultrarare genetic disease caused by progerin, a broadly expressed mutant variant of lamin A protein that accelerates aging and leads to premature death typically in adolescence. Progerin affects many organs and reproduces many characteristics of physiological aging, with the main cause of death in HGPS being atherosclerotic cardiovascular disease. Due to the rarity of HGPS, advances in understanding the disease and progress toward new therapeutic approaches are crucially dependent on preclinical models. Here, we discuss recent research developments from a variety of HGPS experimental systems, with a special focus on *in vivo* studies of the role of vascular smooth muscle and endothelial cells, key players in atherosclerosis.

MAIN TEXT

Hutchinson-Gilford progeria syndrome (HGPS)

HGPS (see Glossary) is an ultrarare, devastating genetic disease characterized by accelerated aging and premature death (average life expectancy 14.6 years) (https://www.progeriaresearch.org/prf-by-the-numbers/). The disease affects approximately 1 in 20 million people, with no gender or ethnic bias. Fetal development appears normal, with newborns with HGPS typically asymptomatic; however, profound growth failure becomes evident during the first 2 years of life [1–3]. HGPS children have a disproportionately large head, with clearly visible veins and alopecia. Other craniofacial features include prominent eyes, a narrow nasal bridge, small mouth, thin lip vermilion, retrognathia, and micrognathia. HGPS children have an aged appearance, with lipodystrophy and sclerodermatous skin changes, and dental, ophthalmologic, and hearing problems are common [4–6]. Like physiological aging, HGPS is characterized by abnormalities affecting the musculoskeletal, endocrine, and cardiovascular systems [3,7–12]. However, HGPS does not feature increased incidences of cancer or

neurodegenerative diseases, and hence is considered a segmental accelerated aging syndrome.

Classical HGPS is caused by a heterozygous *de novo* mutation in the *LMNA* gene, which encodes A-type lamins, principally lamin A and lamin C, important components of the nuclear lamina [13,14]. Lamin A, the variant encoded by all 12 *LMNA* exons, is first produced as the precursor form prelamin A. Posttranslational processing at the C terminus includes farnesylation, cleavage of the last 3 residues, carboxymethylation, and cleavage of a 15-amino-acid peptide containing the farnesyl and carboxymethyl groups. In HGPS, a single-nucleotide substitution in exon 11 (most frequently c.1824C>T; p.G608G) causes abnormal usage of a cryptic splice site, resulting in aberrant *LMNA* mRNA splicing. The resulting protein thus lacks a 50 amino acid sequence that spans the cleavage site for ZMPSTE24, the metalloprotease that executes the final step in lamin A maturation. The resulting truncated lamin A variant, called progerin or prelamin A-Δ50, retains the C-terminal farnesyl and carboxymethyl moieties and remains permanently attached to the inner nuclear membrane. Progerin causes structural and functional alterations to the nucleus that progressively cause tissue damage and premature organismal aging. Low levels of progerin expression have also been detected in tissues from non-HGPS individuals [15–17], demonstrating that progerin can be expressed in a small subset of cells even in the absence of the HGPS-causing mutation. Interestingly, some of these studies have shown an age-related increase in progerin protein levels in the skin and coronary arteries [16,17], but its functional relevance during physiological aging remains to be determined.

The HGPS cardiovascular phenotype

Cardiovascular disease (CVD) complications are the main cause of death in HGPS, with more than 80% of patients dying of heart failure or myocardial infarction between the ages of 6 and 20 years [2]. Despite interindividual variability and a general absence of conventional risk factors like hypercholesterolemia, HGPS patients have generalized atherosclerosis [18]. The large arteries, coronary arteries, intramural cardiac arteries, and

aortic valves frequently contain atheroma plaques with varying degrees of calcification [1,17,19,20]. Progerin-induced vessel damage is characterized by massive loss of medial vascular smooth muscle cells (VSMCs) and fibrosis in the medial and adventitial layers [1,17,21]. The associated vascular stiffening and occlusive stenosis lead to reduced aortic, carotid, and coronary compliance and to peripheral vascular disease [1,11,17,22–24].

As they age, HGPS patients develop several electrocardiographic anomalies, including cardiac repolarization abnormalities [11,12,25,26]. The most prevalent echocardiographic abnormality in early disease stages (5−6 years of age) is left ventricular (LV) diastolic dysfunction, whereas severe disease in the second decade of life features LV hypertrophy, cardiac valve disease, systolic dysfunction, angina, dyspnea, and overt heart failure [2,11,12]. Longitudinal studies are needed to define the natural history of these cardiac alterations, which might be primary causes of disease or secondary consequences of chronic vascular stiffening, calcification, and stenosis and subsequent cardiac hypoperfusion.

HGPS treatment

Treatment options for HGPS patients are very limited (see Clinician's Corner). Although surgical interventions to relieve critical aortic stenosis have great potential to improve patients' quality of life and extend lifespan, these are high-risk procedures for these extremely frail individuals [27]. The only therapy approved for HGPS is lonafarnib, a farnesyltransferase inhibitor that ameliorates vascular stiffening, reduces bone and neurosensory alterations, and prolongs lifespan by 2.5−5 years [23,24,28–31]. Combined treatment with lonafarnib and the mTOR inhibitor everolimus is currently under clinical trial, although preclinical mouse studies revealed no added benefit versus lonafarnib alone [32]. Further research with *in vitro* and *in vivo* preclinical models is essential to advance understanding of HGPS and identify molecular targets that would enable the development of new therapies and clinical trials.

Progerin-induced alterations in vascular cell types

The cellular components of the blood vessel wall play key roles in cardiovascular pathophysiology (Box 1). Early studies of HGPS patient samples detected progerin in adventitial cells, VSMCs, endothelial cells (ECs), and cells within atherosclerotic plaques [17,33]. More recently, Barettino et al. performed a single-cell RNA-sequencing (scRNA-seq) analysis of aortas from progeroid *Lmna*$^{G609G/G609G}$ mice to obtain a detailed picture of progerin-induced molecular and functional alterations in the vessel wall [34]. This analysis revealed significant transcriptional alterations in different progeroid fibroblast populations, main components of the adventitia. All fibroblast subtypes from *Lmna*$^{G609G/G609G}$ mice showed increased expression of the senescence marker *Cdkn1a*, and some were specifically enriched in the expression of genes related to fibrosis, **extracellular matrix (ECM)** organization, and hypoxia. *Lmna*$^{G609G/G609G}$ mice were also enriched in a fibroblast subtype with a specific gene expression profile related to the immune response. The same study revealed increased abundance in progeroid mice of several types of aortic immune cells, including macrophages, T cells, natural killer cells, B cells, innate lymphoid cells, granulocytes, and dendritic cells. Two types of adventitial resident macrophages showed significantly upregulated expression of chemotaxis-related and pro-inflammatory genes in progeroid mice [34]. Notably, an earlier study reported that *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*LysMCre* mice, which express progerin specifically in myeloid cells, had normal body weight and lifespan and lacked HGPS-associated vascular features such as VSMC loss, adventitial thickening, and exacerbated atherosclerosis [35]. These findings suggest that any potential causal role of aortic macrophages in the HGPS vascular phenotype likely derives from environmental cues from progerin-expressing non-myeloid cell types that alter macrophage function, rather than from macrophage-intrinsic progerin expression.

Although scRNA-seq studies have begun to illuminate the heterogeneous fibroblast and immune-cell landscape of the HGPS aorta, further research is needed to precisely decipher the functional alterations of these and other cell types and their contribution to the development of the HGPS vascular phenotype. For example, the carotid arteries of

progeroid mice show accelerated injury-induced thrombus formation, potentially mediated by enhanced platelet reactivity, highlighting the need to study the role of circulating hematopoietic cells [36].

In vitro models for studying the roles of VSMCs and ECs in HGPS

Although VSMC dysfunction and death are well-established features of HGPS, the mechanisms mediating progerin-induced VSMC pathology are not fully understood. Given the central role of ECs in vascular homeostasis and the development of vascular disease, it is also important to study the effects of progerin expression in ECs. *In vitro* systems constitute useful platforms for dissecting the molecular drivers of such alterations and testing therapeutic strategies (Box 2), and multiple studies have used primary cultures and **induced pluripotent stem cell (iPSC)**-derived VSMCs and ECs to explore the effects of progerin expression in these cell types. Alterations identified in progerin-expressing VSMCs include nuclear morphological changes, reduced proliferation, abnormal histone acetylation, reduced expression of heterochromatin markers, replication stress, telomere fragility, genomic instability, increased oxidative stress, DNA damage, and elevated expression of pro-fibrotic, pro-inflammatory, senescence, and calcification markers [37–43]. The characteristics of progerin-expressing ECs include morphological and nuclear abnormalities, decreased proliferation, reduced expression of heterochromatin markers, increased oxidative stress, DNA damage, cellular senescence, a blunted response to mechanical stimuli, reduced uptake of acetylated low-density lipoprotein (LDL), impaired nitric oxide generation, defective angiogenesis, shortened telomeres, decreased expression of angiopoietin-2, and elevated IL-1β-stimulated neutrophil adhesion and expression of genes related to inflammation and **endothelial-to-mesenchymal transition (EndMT)** [39,44–53]. Previous studies of stem-cell–derived progerin-expressing ECs did not report alterations in proliferation, lipid uptake, nitric oxide production, angiogenesis, or genome stability [54,55].

VSMCs in mouse models of HGPS

VSMCs, principal components of the arterial tunica media, are particularly affected in HGPS, with severe loss of this cell type reported in the arteries of HGPS patients [17,21] and most animal models of HGPS with ubiquitous progerin expression, including hemizygous and homozygous *BACG608G* transgenic mice [56,57], *Lmna*$^{G609G/+}$ and *Lmna*$^{G609G/G609G}$ mice [58–60], *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice [35], *Ldlr*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice [61], *HGPSrev* mice [62], and heterozygous *LMNA* c.1824C > T Yucatan minipigs [63]. In these models, the severity of VSMC loss typically correlated with animal age and was aggravated by high-fat diet (HFD) feeding [35,64]. Most HGPS animal models show pathological changes in the medial layer similar to those observed in patients [17,21], with many HGPS models with ubiquitous progerin expression showing a progressive medial-layer accumulation of collagen and proteoglycans accompanied by elastin-layer straightening and breakage [35,56,59,65]. Hemizygous *BACG608G* mice, *Lmna*$^{G609G/+}$ mice, and *Lmna*$^{G609G/G609G}$ mice also showed medial aortic calcification [56,66–68].

Many studies have investigated the mechanisms underlying progerin-triggered VSMC alterations in mouse models with ubiquitous progerin expression. Transcriptomic changes related to contractility were observed in aortas of young *Lmna*$^{G609G/G609G}$ mice, and these changes were accompanied by below-normal aortic expression of *Myh11* mRNA and reduced expression of smooth-muscle–specific myosin heavy chain (SM-MHC) protein in the tunica media of the carotid arteries [69]. Transcriptomic analysis of the aortic media of *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice at 8 weeks of age—before manifest vascular disease—revealed changes in fibrosis-related pathways [70], and another study found that the medial layer of carotid arteries from 8-week-old *Lmna*$^{G609G/G609G}$ mice had increased protein expression of collagen III and the collagen-crosslinking enzyme lysyl oxidase [71]. The more severe disease of 13–15-week-old *Lmna*$^{G609G/G609G}$ mice featured enhanced aortic-media deposition of several collagens, including collagens III, IV, V, and XII [59]. Furthermore, 1-year-old *Lmna*$^{G609G/+}$ mice showed elevated aortic-media mRNA expression of *Col1a1*, *Col3a1*, and *Col8a1* [60]. Analysis of aortas from 30–32-week-old *Lmna*$^{G609G/+}$ mice, which show prominent medial calcification, revealed augmented mRNA expression of the osteogenic genes *Bmp2* and *Runx2,* together with elevated RUNX2 protein [66]. These findings are in line with *ex vivo* wire myography experiments showing

increased stiffening and reduced contractility in *Lmna*$^{G609G/G609G}$ aortas [59,72] and with accelerated vascular stiffening observed in HGPS patients [17,21,22].

Studies in 1-year-old *Lmna*$^{G609G/+}$ mice and 16-week-old *Lmna*$^{G609G/G609G}$ mice revealed greater loss of medial VSMCs along the inner curvature of the ascending aorta and at major branch points, which was attributed to differences in blood flow patterns and biomechanical forces [60]. Accordingly, VSMC loss in the aortic media was alleviated by disruption of the LINC (linker of the nucleoskeleton and cytoskeleton) complex in VSMCs upon ectopic KASH2 overexpression, indicating that reduced force transmission to the nucleus lessens the negative effects of progerin accumulation. Likewise, VSMC numbers in the aortic arch of *Lmna*$^{G609G/G609G}$ mice were increased by *in vivo* inhibition of metalloprotease 13, an important mediator of VSMC vulnerability to biomechanical forces [41]. Consistent with the mechanical stress hypothesis of VSMC death, VSMCs from *Lmna*$^{G609G/G609G}$ mice showed frequent nuclear membrane ruptures, detected by the cytoplasmic accumulation of a nuclear-targeted tdTomato reporter protein [73]. These nuclear membrane ruptures were apparent in 8-week-old *Lmna*$^{G609G/G609G}$ nuclear-targeted tdTomato mice, before appreciable aortic medial VSMC loss, and were more frequent in aortic regions more prone to VSMC loss in progeroid animals.

Progerin unquestionably causes VSMC death, since arterial VSMC numbers increase when progerin expression is reduced, either by correcting the HGPS-causing mutation or by modulating aberrant *Lmna* mRNA splicing [57,74–76]. Moreover, TUNEL assays have evidenced VSMC apoptosis in *Lmna*$^{G609G/G609G}$ aortas [68,77], which also showed increased protein expression of cytochrome C and apoptosis-inducing factor, components of the caspase-dependent and caspase-independent apoptotic pathways, respectively [77]. Nonetheless, future studies are warranted to assess whether other forms of cell death, such as pyroptosis or ferroptosis, may be involved in progerin-induced VSMC loss. Increased VSMC death in *Lmna*$^{G609G/G609G}$ aortas was linked to augmented poly(ADP-Ribosyl)ation, a posttranslational modification that regulates DNA repair and causes a drop in nicotinamide adenine dinucleotide (NAD+) levels, promoting DNA damage accumulation and cell death program activation. The aortic phenotype of

Lmna$^{G609G/G609G}$ mice was ameliorated upon inhibition of poly [ADP-ribose] polymerase 1, the enzyme responsible for poly(ADP-Ribosyl)ation and a major consumer of NAD+ [77]. Early mechanisms underlying VSMC death in HGPS arteries were investigated in a bulk RNAseq analysis of the aortic medial layer of 8-week-old athero-susceptible *Apoe*$^{-/-}$ *Lmna*$^{G609G/G609G}$ mice, before the appearance of manifest vascular disease [70]. This study revealed activation of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) in progerin-expressing medial VSMCs. Alleviation of this stress response by treatment with tauroursodeoxycholic acid diminished VSMC loss and atherosclerosis in the aorta. ER stress and the associated UPR are therefore probable early mechanisms contributing to VSMC death and accelerated atherosclerosis in HGPS [70].

Two recent scRNA-seq studies of *Lmna*$^{G609G/G609G}$ aortas provided a more comprehensive characterization of progerin-induced VSMC alterations. Analysis of samples from 14-week-old mice confirmed that progerin triggers a switch from a highly contractile VSMC phenotype to a dedifferentiated and dysfunctional phenotype featuring ECM remodeling, ER stress response activation, and ultimately apoptosis [34]. Moreover, sc-RNAseq analysis of *Lmna*$^{G609G/G609G}$ aorta at postnatal days 100, 140, and 168, corresponding respectively to early, intermediate and end-stage disease, detected elevated VSMC expression of genes related to chondrogenesis and osteogenesis, processes that manifested at late disease stages, after apoptosis induction [68].

To investigate the role of VSMCs in the origin of HGPS vascular disease, investigators have generated several mouse models with cell-type–specific progerin expression or suppression using Cre-lox systems (Box 3) (Table 1 and Figure 1, Key Figure). In one model, progerin expression was directed to VSMCs by crossing *Lmna*$^{LCS/LCS}$ mice with *SM22α-Cre* mice, in which Cre expression is controlled by the *Tagln* (a.k.a. *SM22α*) promoter. *In vivo* and *ex vivo* functional analyses of *Lmna*$^{LCS/LCS}$*SM22α-Cre* mice revealed increased arterial stiffness and depressed aortic contraction in 13–15-week-old mice versus age-matched *Lmna*$^{LCS/LCS}$ controls [59,72]. By 38 weeks, the aortic media of *Lmna*$^{LCS/LCS}$*SM22α-Cre* mice was devoid of VSMCs, which were replaced by collagen.

Nevertheless, median survival was the same as that of *Lmna*$^{LCS/LCS}$ controls [35], and aortic cross-sections showed no signs of atherosclerosis.

Despite the importance of atherosclerosis in HGPS, accelerated atherosclerotic disease (determined as increased aortic lipid accumulation and/or exacerbated development of aortic root plaques) has been observed in only 3 **atheroprone mouse models** with ubiquitous progerin expression: *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice [35], *Ldlr*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice [61], and *HGPSrev* mice injected with a hypercholesterolemia-inducing adeno-associated virus encoding a mutant form of mouse **PCSK9** (rAAV8-mPCSK9^{D377Y}) [78]. The impact on atherosclerosis of VSMC-specific progerin expression was investigated by crossing *Lmna*$^{LCS/LCS}$*SM22α-Cre* mice with an *Apoe*-deficient mouse model featuring severe hypercholesterolemia [35]. At 16 weeks, *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22α-Cre* mice showed VSMC loss in the aortic media that was accelerated when animals were fed a HFD for the last 8 weeks. The cause of VSMC death in this model was linked, at least in part, to ER stress and the UPR [70]. Diminished VSMC content in these mutant mice was accompanied by increased medial accumulation of collagen and lipids [35]. Experiments with fluorescently-labeled LDL detected higher LDL retention in the aortic media of *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22α-Cre* mice than in *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ controls, despite similar serum cholesterol in both groups. VSMC alterations in *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22α-Cre* mice were accompanied by a series of pro-atherogenic changes in other aortic layers: the tunica adventitia was thickened, and ECs in the tunica intima showed evidence of increased permeability to LDL particles, enhanced immune cell recruitment, and EndMT [35,79]. At 16 weeks, *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22α-Cre* animals also showed accelerated atherosclerosis, especially in the thoracic aorta, a region typically only mildly affected by atherosclerosis in *Apoe*$^{-/-}$ mice [35]. Aortic atheromas in HFD-fed *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22α-Cre* mice had a low content of αSMA-positive VSMCs and thin and disorganized fibrous caps. These are characteristics of an unstable plaque phenotype, and accordingly these mice showed evidence of plaque disruption and thrombus formation. Although *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22α-Cre* mice had no overt whole-body phenotype and were indistinguishable from *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ control littermates in the first months of life, they stopped gaining weight from around 20 weeks and died

prematurely (median survival: 8 months). Analysis of cardiovascular tissue of *Apoe*$^{-/-}$ *Lmna*$^{LCS/LCS}$*SM22α-Cre* mice at 51 weeks—close to their maximum survival—revealed severe aortic and coronary atherosclerosis, plaque calcification, and frequent chondroid metaplasia in lesions. Moreover, the hearts of these animals had perivascular and interstitial fibrosis, which, together with an unstable plaque phenotype featuring low amounts of collagen and αSMA-positive VSMCs, suggests vulnerability to microinfarctions. Overall, the cardiovascular alterations observed at ages close to maximum survival indicate that *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*SM22α-Cre* mice typically die of atherosclerosis-related complications. Accordingly, treatment of these mice with tauroursodeoxycholic acid, a chemical chaperone that alleviates ER stress and the UPR, reduced VSMC loss, inhibited atherosclerosis, and extended lifespan by 35% [70].

The effects of suppressing progerin expression in VSMCs have been investigated in *HGPSrev-SM22α-Cre* mice [62] (Box 3). Aortas from 1-year-old *HGPSrev-SM22α-Cre* mice, unlike aged-matched *HGPSrev* mice with ubiquitous progerin expression, were structurally indistinguishable from those of age-matched wild-type controls, showing no VSMC loss and normal collagen accumulation and leukocyte recruitment [62,78]. *HGPSrev-SM22α-Cre* animals lived 63% longer than *HGPSrev* mice, and their median survival was similar to that of wild-type controls [62]. Atherosclerosis development in *HGPSrev-SM22α-Cre* mice was studied after administration of rAAV8-mPCSK9^{D377Y} and HFD feeding to induce hypercholesterolemia and atherosclerosis [78]. These HFD-fed atheroprone *HGPSrev-SM22α-Cre* mice showed no aortic VSMC loss or medial fibrosis, and plaque development was similar to that in wild-type animals.

These studies indicate that progerin-expressing VSMCs play a causal role in the development of HGPS-associated vascular disease, including atherosclerosis. However, progerin expression or repression in these studies was driven by the *SM22α* (*Tagln*) promoter, which can induce Cre-mediated recombination in cardiomyocytes, fibroblasts, myeloid cells, and smooth muscle cells of intestinal muscularis externa [35,62,70,80]. The more recently developed *Myh11-CreER*^{T2} and *Itga8-CreER*^{T2} models provide more

specific VSMC targeting [81,82], and it would therefore be interesting to validate and refine the published findings in these newer inducible Cre mouse lines.

ECs in mouse models of HGPS

Ubiquitous progerin expression in *Lmna*$^{G609G/G609G}$ mice is associated with impaired vasorelaxation in response to acetylcholine, strongly suggesting endothelial dysfunction [32,72,83]. It is therefore of interest to study EC molecular alterations in HGPS mouse models in order to elucidate their potential contribution to the HGPS cardiovascular phenotype and to identify endothelial therapeutic targets. Recent studies have demonstrated elevated expression of pro-inflammatory factors such as VCAM-1 and P-selectin in lung and aortic ECs of progeroid *Lmna*$^{G609G/G609G}$ and *BACG608G* mice, indicating a general state of endothelial activation in HGPS [34,46,83]. This was accompanied by increased leukocyte accumulation in the aortic intimal layer of *Lmna*$^{G609G/G609G}$ mice [34], a feature also observed in *HGPSrev* mice [78] and in atheroprone *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice, where it was accompanied by increased endothelial permeability to LDL [79]. These findings strongly suggest a key role of ECs in progerin-induced vascular inflammation and atherosclerosis onset. scRNA-seq analysis revealed activation of the mechanosensing **YAP/TAZ pathway** in the aortic ECs of *Lmna*$^{G609G/G609G}$ mice [34]. The authors of this study found that the aortas of progeroid mice have enhanced subendothelial ECM stiffness and disturbed blood flow, well-known YAP/TAZ pathway inducers. Moreover, verteporfin-induced YAP/TAZ inhibition reduced endothelial activation, intimal leukocyte accumulation, and atherosclerosis burden in *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice. These positive effects occurred without changes to circulating leukocyte numbers, aortic collagen content, or aortic blood flow pattern, suggesting that the verteporfin-mediated decrease in EC activation and atherosclerosis burden was due to direct inhibition of endothelial YAP/TAZ signaling [34]. Another study found that VCAM-1 expression in the aortic ECs of *BACG608G* mice was decreased by treatment with a lentivirus encoding mouse telomerase, which also reduced endothelial DNA damage and extended lifespan by ≈20% [46]. Moreover, atheroprone *Apoe*$^{-/-}$*Lmna*$^{G609G/G609G}$ mice showed an abnormally high atheroma content of cells

expressing endothelial markers, with clear signs of extensive EndMT [79]. This was accompanied by elevated plaque levels of the EndMT trigger TGF-β1, as well as enhanced phosphorylation of the TGF-β1 target SMAD3. Treatment with SIS3 (specific inhibitor of SMAD3) ameliorated the progerin-induced vascular phenotype, including reductions in intimal leukocyte accumulation, adventitia:media ratio, VSMC loss, intraplaque ECM deposition, and atherosclerosis burden, although the effect on atherosclerosis burden did not reach statistical significance. Targeting endothelial activation and EndMT are therefore promising strategies for the development of HGPS treatments and merit further investigation.

Several groups have used conditional mouse models to study the contribution of endothelial progerin expression to HGPS-associated features, generating some strikingly different outcomes (Table 1 and Figure 1). In contrast to *Lmna*$^{G609G/G609G}$ mice with ubiquitous progerin expression, *Lmna*$^{LCS/LCS}$*Tie2Cre* mice with progerin expression in ECs under the control of the *Tek* (a.k.a. *Tie2*) promoter showed no increased arterial stiffness or defective vasoreactivity [59,72]. In agreement with these findings, *Prog-Tg* mice, which express human progerin and lamin A in ECs under the control of the EC-specific *Cdh5* promoter, did not show impaired acetylcholine-mediated vasorelaxation [84]. Nevertheless, pathological features of *Prog-Tg* mice included endothelial transcriptional alterations (upregulation of genes related to fibrosis, inflammation, and senescence), leukocyte accumulation in liver and lung, and abnormal bone microstructure with reduced vascular density [85,86]. Additional HGPS-associated alterations in *Prog-Tg* mice included reduced body weight, heart fibrosis and hypertrophy, diastolic dysfunction, adventitial thickening, and premature death [84]. The effects of EC progerin expression have also been investigated by crossing *Tie2*-Cre mice with *Lmnaf/f* mice to generate *Lmnaf/f;TC* mice [83]. Alterations in these mice included body weight reduction, premature death, bone alterations, reduced capillary density and defective neovascularization in muscle, decreased acetylcholine-mediated aortic vasorelaxation (a feature absent from *Prog-Tg* or *Lmna*$^{LCS/LCS}$*Tie2Cre* mice [72,84]), and cardiac anomalies, including hypertrophy and reduced ejection fraction, the latter of which was not observed in *Prog-Tg* mice, *Lmna*$^{LCS/LCS}$*Cdh5-Cre*^{ERT2} mice, or in mice with ubiquitous

progerin expression [84,87,88]. Lung ECs from *Lmnaf/f;TC* mice had reduced expression of the nicotinamide adenine dinucleotide–dependent deacylase Sirt7, and treatment with adeno-associated viruses expressing Sirt7 under the control of the EC-specific *Icam2* promoter improved muscle neovascularization and prolonged lifespan [83]. More recent studies in atheroresistant mouse models (*Lmna*$^{LCS/LCS}$*Tie2Cre* and *Lmna*$^{LCS/LCS}$*Cdh5-Cre*^{ERT2}) and atheroprone *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*Cdh5-Cre*^{ERT2} mice showed that animals with EC-specific progerin expression lack multiple features of ubiquitous progerin expression, namely body weight reduction, premature death, VSMC loss, adventitial thickening, medial accumulation of ECM proteins, excessive atherosclerosis, altered aortic blood flow, aortic endothelial EndMT and YAP/TAZ activation, heart fibrosis, and cardiac dysfunction and electrical alterations [34,79,88]. Moreover, none of these models showed intimal leukocyte accumulation, suggesting a lack of general vascular inflammation. This conflicts with the data from *Prog-Tg* mice [86], but is consistent with another mouse model of *Tie2* promoter-driven progerin expression (*Tie2-Cre;Lmna*$^{LCS/LCS}$*;Rosa26*$^{tdTomato/tdTomato}$), in which the lungs showed no altered expression of inflammation-related genes in the absence of pro-inflammatory stimuli [50]. Moreover, in *HGPSrevCdh5-Cre*^{ERT2} mice, specific progerin suppression and lamin A restoration in ECs did not protect animals from intimal leukocyte accumulation, and these mice exhibited VSMC loss, vascular fibrosis, aggravated atherosclerosis, body weight reduction, and premature death to the same extent as *HGPSrev* mice with ubiquitous progerin expression [78].

The discrepancies between these studies likely derive from the differing natures of the models and specific limitations regarding progerin expression, including progerin amount, endothelial specificity, and the developmental stage at which progerin expression starts. Thus, 1) non-endogenous promoters can generate supraphysiological progerin levels, as in *Prog-Tg* mice [84]; 2) *Tie2* promoter-driven recombination is not fully EC-specific and can induce progerin expression in certain leukocyte populations [89]; 3) models derived from *Lmna*$^{LCS/LCS}$ mice lack lamin A expression, and therefore the nuclear lamina has a different protein composition from that of wild-type mice; and 4) the use of the tamoxifen-inducible *Cdh5-Cre*^{ERT2} cassette to achieve EC-specific progerin expression or

suppression in 1.5-month-old mice does not address possible effects of progerin during development and the first weeks after birth [78,88]. Additional mouse models and experimental strategies are therefore needed to determine if EC progerin expression is a causal factor in the development of the HGPS cardiovascular phenotype. However, multiple lines of evidence demonstrate that *SM22α* promoter-driven progerin expression or suppression profoundly modulates endothelium-related pathology [35,78,79], clearly pinpointing VSMCs as promising targets for gene editing strategies aimed at preventing the HGPS vascular phenotype (Box 4). Regardless of whether HGPS-associated endothelial alterations derive from progerin expression in ECs or in other cell types, these alterations can be targeted by pharmacological treatments that ameliorate HGPS symptoms, such as verteporfin [34] or SIS3 [79]. Cell therapies with EC-like stem cells have also shown potential in progeroid mice to improve bone structure and tissue neovascularization capacity, ameliorate body weight loss, and extend lifespan [90].

Concluding remarks

HGPS is an early-onset genetic disease that resembles many characteristics of physiological aging, with CVD underlying premature death in most patients. Investigators have therefore focused on vascular cell types, especially VSMCs and ECs, as targets for possible HGPS therapies. However, the extreme rarity of HGPS entails challenges for studying disease mechanisms and evaluating treatments. To overcome this limitation, many *in vivo* and *in vitro* disease models have been developed. Studies using animal models with ubiquitous progerin expression have revealed alterations in VSMCs and ECs; however, these models do not address the causal role of these cell types in the appearance of the complex, multiorgan aging phenotype. *In vitro* studies with progerin-expressing VSMCs and ECs have exposed numerous defects in both cell types, while sometimes producing conflicting results given the inability of cell culture systems to reproduce the structural and functional complexity of the arterial wall. To dissect the cellular and molecular causes of accelerated vascular disease in HGPS, investigators have generated several conditional mouse models with progerin expression or suppression. Models with VSMC-specific progerin expression have consistently identified

VSMC alterations as the main trigger of HGPS vascular disease, including atherosclerosis and atherosclerosis-associated premature death, and these alterations are ameliorated or abolished in *in vivo* models of progerin suppression in VSMCs. In contrast, mouse models of EC-specific progerin expression have produced varied results, with some studies reporting no or few pathological alterations and others reporting severe phenotypes and a lifespan reduction similar to progeria models with ubiquitous progerin expression (see Outstanding Questions). However, it is significant that the HGPS vascular or aging phenotypes were not rescued by suppression of progerin in ECs *in vivo*. Together, these findings suggest that future gene editing therapies aimed at correcting the HGPS-causing mutation might be more effective if targeted to VSMCs rather than ECs. But although progerin-expressing VSMCs appear to be the primary driver of premature vascular disease in HGPS, the vascular phenotype can also be mitigated by targeting EC alterations, which are partly secondary to VSMC defects, as demonstrated by treatment with SIS3 and verteporfin. Further studies are warranted to validate and refine recent findings on the contribution of different cell types to HGPS symptoms and to design cell-type–targeted therapies for HGPS that might be more feasible than whole-body treatments.

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Clinician's Corner and Text Boxes (in the order they appear in the text)

Clinician's Corner

- HGPS is caused by the expression of the mutant protein progerin in most body cells. Multiple organs are affected, with a critical disease hallmark being generalized atherosclerosis typically in the absence of conventional risk factors. Patients die at an average age of 14.6 years, with the main causes being myocardial infarction, heart failure, and stroke.
- The sole therapy approved for HGPS is lonafarnib, a farnesyltransferase inhibitor that reduces progerin toxicity by preventing posttranslational farnesylation. Clinical benefits of lonafarnib include improved vascular compliance, enhanced bone mineralization, better hearing, reduced headaches, and a lifespan extension of 2.5−5 years.
- Combined therapy with lonafarnib and everolimus—an mTOR inhibitor that promotes progerin clearance via autophagy—is currently being tested in a phase I/II clinical trial (NCT02579044). Additional clinical trials are planned to assess the therapeutic potential of progerinin, an inhibitor of the interaction between progerin and lamin A, and SRP-2001, a molecule that blocks progerin expression (https://www.progeriaresearch.org/clinical-trials/). For older HGPS patients with calcific aortic stenosis, modified transcatheter aortic valve replacement and modified apico-aortic valve replacement are high-risk surgical interventions to be considered.
- A cure for HGPS will be achieved by suppressing progerin expression. In progeroid mouse models, gene editing approaches to correct the HGPS-causing mutation, as well as the use of ASOs to prevent progerin expression, have been shown to ameliorate the HGPS phenotype and significantly increase lifespan, even without complete elimination of progerin from all tissues. However, technical, ethical, and safety challenges must be addressed before these approaches can be applied clinically. In the meantime, developing novel therapies to increase the quality of life and survival of HGPS patients is crucial. Focusing on treatments that target the

cell types most susceptible to progerin-induced damage, particularly within the cardiovascular system, may offer effective and less complex alternatives to systemic therapies.

- Research involving HGPS mouse models has identified VSMCs as critical targets of progerin toxicity. Mice expressing progerin predominantly in VSMCs developed HGPS phenotypes comparable to those with ubiquitous progerin expression, whereas progeroid mice with suppressed progerin expression in VSMCs showed significant vascular improvements and increased lifespan. Conversely, endothelial-specific progerin elimination provided no therapeutic benefit. These findings distinguish VSMCs as primary contributors to vascular disease in HGPS, suggesting that VSMC-targeted progerin suppression could yield substantial benefits for patients.

Box 1. The heterogeneous cellular landscape of the vessel wall

The blood vessel wall is composed of several cell layers, each contributing to its structural and functional integrity. The outermost layer is the adventitia, which supports overall vessel structure and mainly contains fibroblasts, immune cells, and ECM. The media lies internal to the adventitia and primarily consists of VSMCs, which contract and dilate in response to stimuli, thus changing blood vessel volume and local blood pressure. Upon atherosclerosis-associated vascular injury, ‘differentiated’ VSMCs undergo a phenotypic transformation into proliferating, ECM-secreting, and migrating cells (‘synthetic phenotype’) that generate a subendothelial fibrous cap that protects against plaque rupture [91]. The intima, the innermost layer, is lined by an EC monolayer in direct contact with blood. ECs regulate vascular tone, hemostasis, barrier function, and inflammation, and are key players in atherosclerosis onset and progression [92]. Vascular cell types communicate extensively through physical interactions, paracrine signaling, and the exchange of extracellular vesicles. These interactions are vital for vascular homeostasis, and their disturbance can drive vascular disease. For example, abnormal EC–VSMC crosstalk can trigger inflammation, low nitric oxide bioavailability, and excessive VSMC proliferation, key factors in the development of atherosclerosis and other vascular diseases.

Box 2. *In vitro* experimental systems: useful but with caveats

In vitro systems constitute powerful platforms for studying the HGPS vascular phenotype and screening for new therapeutics. Progerin overexpression in primary VSMCs and ECs induces multiple molecular, cellular, and functional alterations that may play a role in the development of HGPS-associated alterations, and could therefore constitute therapeutic targets. However, overexpression systems are hampered by the risk of supraphysiological progerin expression, which could produce non-physiological effects. To tackle this issue, investigators have adopted strategies based on iPSCs generated from skin fibroblasts obtained from HGPS patients. VSMCs and ECs differentiated from these iPSCs express physiological amounts of progerin under the control of the endogenous *LMNA* promoter. The use of human VSMCs and ECs rather than animal cells is also likely to ease the translation of any therapeutic strategy identified with these systems to the clinic. Because vascular homeostasis is highly dependent on molecular VSMC–EC crosstalk, experimental approaches should include both cell types and study how progerin expression in VSMCs impacts neighboring ECs and vice versa [37,46,47]. Given the critical role in vascular homeostasis of endothelial mechanotransduction upon blood flow sensing, it is also important to use devices that allow exposure of ECs to physiological shear stress, such as **tissue engineered blood vessels (TEBVs)** [48,93]. However, these *in vitro* approaches have important limitations: individual studies tend to rely on a restricted set of stem cell clones; stem cells may not fully differentiate into authentic VSMCs or ECs or may not replicate the diverse characteristics of VSMCs and ECs from different tissues; and *in vitro* systems lack systemic factors, circulating hematopoietic cells, and tissue-resident immune cells that might modulate the action of VSMCs and ECs in the development of HGPS-associated vascular alterations *in vivo.* Findings from *in vitro* approaches should therefore be supported by data from *in vivo* HGPS models that provide the full biophysical, functional, cellular, and molecular complexity of the cardiovascular system.

Box 3. Progerin expression or suppression in specific cell types

Cre-lox mice are useful tools for studying the causal role of cell-type–specific progerin expression in the development of the HGPS phenotype. *Lmna*$^{LCS/LCS}$ (lamin C-STOP) mice harbor an HGPS-causing mutation in *Lmna* exon 11 (c.1827C>T; p.G609G, equivalent to human *LMNA* c.1824C>T; p.G608G). However, the presence of a *neomycin resistance* gene in intron 10 prevents progerin expression and leads to expression of the lamin C isoform only [58,94]. In crosses of *Lmna*$^{LCS/LCS}$ mice with a Cre-expressing mouse line, the loxP-flanked *neomycin resistance* cassette undergoes Cre-dependent excision and the mice produce progerin (together with lamin C and residual lamin A) in the cell type of interest. Targeted suppression of progerin is possible with *HGPSrev* mice. These mice ubiquitously express progerin and lack lamin A; crossing *HGPSrev* mice with an appropriate Cre mouse line suppresses progerin and restores lamin A expression in the cell type of interest [62].

Box 4. Gene editing: the future cure for HGPS?

Extensive efforts have been dedicated to developing therapeutic strategies in mouse models that target the genetic cause of HGPS. Antisense oligonucleotides (ASOs) designed to inhibit the HGPS-causing aberrant mRNA splicing have been successfully used to reduce progerin expression. Treatment of progeroid mice with ASOs reduced senescence markers, reduced lipodystrophy, ameliorated the HGPS-associated vascular phenotype, and increased lifespan [58,74,76,95]. Gene editing strategies have also shown great potential to reduce progerin expression and improve the HGPS phenotype *in vivo*. This was accomplished by postnatal administration of adeno-associated viruses carrying either CRISPR/Cas9 reagents to create indels in the *Lmna* gene [75,96] or adenine base editors to correct the HGPS-causing mutation [57,97]. These approaches increased body weight, improved the muscle and skin phenotypes, ameliorated several cardiovascular alterations, and induced very significant lifespan extensions. Application of gene editing technologies in the clinical settings will require steps to minimize viral vector dose and thus avoid potential off-target effects and detrimental immune responses to viral infection. This optimization would be furthered by the identification of the cell types in which progerin elimination yields most benefit, since targeted delivery of the gene

editing machinery to particular tissues would probably be more efficient and require lower doses than systemic approaches. Recent studies using progeroid *HGPSrev* mice, genetically engineered to enable cell-type–specific progerin elimination and lamin A restoration, showed that suppressing progerin in VSMCs, but not in ECs, prevented HGPS-associated VSMC loss, vascular fibrosis, exacerbated atherosclerosis, and premature death [62,78]. VSMCs therefore stand out as strong candidates for the development of cell-type–targeted gene editing therapies.

Glossary

Atheroprone mouse models: unlike humans, mice do not develop atherosclerosis spontaneously, probably because of their relatively low levels of LDL and high levels of high-density lipoprotein (HDL) in blood. Promoting atherosclerosis in mice thus requires manipulation of the expression of key proteins involved in lipid homeostasis. Apolipoprotein E-deficient mice (*Apoe*$^{-/-}$ mice), LDL receptor deficient mice (*Ldlr*$^{-/-}$ mice), and mice treated with rAAV8-mPCSK9^{D377Y} (see below) are examples of well-established atheroprone models that feature hypercholesterolemia and develop atherosclerosis, which can be accelerated by feeding with a HFD.

ECM: extracellular matrix. Complex network of macromolecules including collagens, proteoglycans, elastin, and many other proteins that form the non-cellular scaffold of all tissues. The ECM provides physical support to cellular constituents, as well as key biochemical and biomechanical cues required for tissue morphogenesis, differentiation, and homeostasis. ECM alterations play a key role in disease.

EndMT: endothelial-to-mesenchymal transition. Sequential process whereby ECs undergo phenotypic changes toward a mesenchymal state that gives rise to a variety of cell types, including fibroblasts, osteoblasts, and VSMCs.

HGPS: Hutchinson-Gilford progeria syndrome, or progeria. Ultrarare genetic and pediatric disease in which children show symptoms typical of the third age, including

severe atherosclerosis and associated CVD. HGPS patients die at an average age of 14.6 years, mainly due to myocardial infarction, heart failure, or stroke.

iPSCs: induced pluripotent stem cells. Cells derived from *in vitro* somatic cell reprogramming to revert to pluripotency, thereby regaining the capacity to differentiate into multiple cell types. The generation of iPSCs derived from skin fibroblasts of HGPS patients allows *in vitro* studies in human cell types with key roles in HGPS, such as VSMCs and ECs, which would otherwise be almost impossible to obtain due to restricted access to human HGPS vessel biopsies or fresh autopsies.

PCSK9: proprotein convertase subtilisin/kexin type 9. An important regulator of cholesterol metabolism through lysosomal-dependent degradation of the LDL receptor. A single injection of adeno-associated virus carrying a gain-of-function PCSK9 mutant (rAAV8-mPCSK9^{D377Y}) provokes hypercholesterolemia and atherosclerosis development in mice fed a HFD.

TEBVs: tissue engineered blood vessels. TEBVs are arteriole-scale perfusable 3D systems in which iPSC-derived VSMCs are incorporated within a dense collagen gel, forming tubular structures whose lumen is then coated with iPSC-derived ECs and exposed to different physiological blood flow patterns.

YAP/TAZ pathway: YAP and its homolog TAZ are transcriptional regulators whose function is modulated in part by a set of physical cues. In the absence of activating stimuli, phosphorylated YAP/TAZ remain sequestered in the cytoplasm, where they eventually undergo ubiquitin-mediated proteasomal degradation. After mechanical stimulation (e.g., increased ECM stiffness or oscillatory flow), YAP/TAZ become dephosphorylated and translocate to the nucleus, where they regulate gene expression by interacting with TEAD transcription factors.

Table 1. HGPS-associated alterations in body weight, lifespan, and cardiovascular features in conditional mouse models with progerin expression or suppression in VSMCs or ECs.

Genetic manipulation		Progerin expression in VSMCs		Progerin expression in ECs					Progerin suppression in VSMCs		Progerin suppression in ECs	
Promoter controlling progerin expression or suppression		*Tagln*[1]		*Cdh5*[2]			*Tek*[3]		*Tagln*[1]		*Cdh5*[2]	
Atheroprone background		N	Y	N		Y	N		N	Y	N	Y
Mouse model		*Lmna*$^{LCS/LCS}$*SM22αCre*	*Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ *SM22αCre*	*Lmna*$^{LCS/LCS}$*Cdh5Cre*^{ERT2}	*Prog-Tg*	*Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ *Cdh5Cre*^{ERT2}	*Lmna*$^{LCS/LCS}$*Tie2Cre*	*Lmnaf/f;TC*	*HGPSrevSM22αCre*	*HGPSrevSM22αCre* + AAV-PCSK9^{D377Y}	*HGPSrevCdh5Cre*^{ERT2}	*HGPSrevCdh5Cre*^{ERT2} + AAV-PCSK9^{D377Y}
Body weight reduction		NR	Y	N	Y	N	NR	Y	Y	NR	Y	NR
Lifespan shortening		N	Y	N	Y	N	NR	Y	N	NR	Y	NR
Vascular phenotype	VSMC loss	Y^{qo}	Y	N	N	N	NR	Y^{qo}	N	N	Y	NR
	Adventitial thickening/fibrosis	Y^{qo}	Y	N	Y	N	NR	Y^{qo}	NR	NR	NR	NR
	Medial collagen accumulation	Y^{qo}	Y^{qo}	N	NR	N	N	Y^{qo}	N	N	Y	NR
	Vascular stiffening (wire myography)	Y	NR	NR	NR	NR	N	NR	NR	NR	NR	NR
	Subendothelial ECM stiffening (AFM)	NR	NR	NR	NR	NR	Y	NR	NR	NR	NR	NR
	Altered vessel contraction (Phenyl.)	Y	NR	NR	NR	NR	N	NR	NR	NR	NR	NR
	Altered vessel relaxation (Ach.)	N	NR	NR	N	NR	N	Y	NR	NR	NR	NR
	Altered aortic hemodynamics	NR	NR	NR	NR	NR	N	NR	NR	NR	NR	NR
	Exacerbated atherosclerosis	NR	Y	NR	NR	N	NR	Y^{qo,7}	NR	N	NR	Y
	Medial calcification	NR	NR	NR	N	NR	NR	NR	NR	NR	NR	NR
	Plaque calcification	NR	Y	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Intimal leukocyte accumulation	NR	Y	N	NR	N	N	NR	N	NR	Y	NR
	ER stress in the aortic media	NR	Y	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Increased endothelial permeability	NR	Y	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Aortic endothelial YAP/TAZ activation	NR	NR	NR	NR	NR	N	NR	NR	NR	NR	NR
	Aortic EndMT	NR	Y	NR	NR	N	NR	NR	NR	NR	NR	NR
	Impaired endothelial mechanosensing	NR	NR	NR	Y	NR	NR	NR	NR	NR	NR	NR
Cardiac phenotype	Interstitial fibrosis	NR	Y	N	Y	NR	NR	Y^{qo}	NR	NR	NR	NR
	Perivascular fibrosis	NR	Y	N	Y	NR	NR	Y^{qo}	NR	NR	NR	NR
	Hypertrophy	NR	NR	N[5]	Y[6]	NR	NR	Y[8]	NR	NR	NR	NR
	Electrical conduction anomalies	NR	Y[4]	N	NR	NR	NR	NR	NR	NR	NR	NR
	Reduced ejection fraction	NR	NR	N	N	NR	NR	Y	NR	NR	NR	NR
	Diastolic anomalies	NR	NR	N	Y	NR	NR	NR	NR	NR	NR	NR
	Heart rate anomalies	NR	NR	N	N	NR	NR	Y	NR	NR	NR	NR
References		[35,59,72]	[35,70,79]	[88]	[84]	[79,88]	[34,59,72]	[83]	[62,78]	[78]	[78]	[78]

Ach., acetylcholine; AFM, atomic force microscopy; ECM, extracellular matrix; EndMT, endothelial-to-mesenchymal transition; ER, endoplasmic reticulum; Phenyl., phenylephrine; N, no; NR, not reported; Y, yes. ^{qo}Qualitative observation (no quantification). [1]Also known as *SM22α*. [2]Also known as VE-cadherin. [3]Also known as *Tie2*. [4]Significant anomalies in 26-week-old but not 16-week-old mice. [5]Analyzed by echography. [6]Analyzed by echography and heart weight/body weight and heart weight/tibia length ratios. [7]Based on histological observations of aortic structure, without clear signs of atherosclerotic plaques. Note that no atherosclerosis was found in

Lmna$^{G609G/G609G}$ mice with ubiquitous progerin expression after 8 weeks of HFD feeding, as examined by aortic oil red O staining [35].
[8]Analyzed by heart weight/body weight ratios.

FIGURE LEGENDS

Figure 1. Effects of progerin expression or suppression in vascular smooth muscle or endothelial cells. The roles of vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) in the origin and development of cardiovascular disease and other phenotypic signs of HGPS have been investigated in mouse models genetically engineered to induce or suppress progerin expression in a cell-type–specific manner. Progerin expression in VSMCs recapitulates most of the vascular pathological features present in HGPS patients and mouse models with ubiquitous progerin expression, including VSMC loss, arterial fibrosis, vascular stiffening, and exacerbated atherosclerosis. Progerin suppression in VSMCs, but not in ECs, prevents HGPS-associated vascular pathology and premature death. The effects of EC-specific progerin expression vary significantly depending on the mouse models used. While some models are phenotypically indistinguishable from progerin-free mice, others exhibit typical HGPS signs, including cardiovascular pathology and reduced lifespan. The figure shows representations of mouse arterial cross-sections, where progerin-expressing cells are depicted as cells with white nuclei. This figure was created using BioRender (https://biorender.com/) licensed to V.A.

Highlights

- The cardiovascular system is especially affected by progerin, a mutant lamin A variant that causes HGPS.
- VSMCs and ECs are key arterial cells that are profoundly altered in HGPS animal models. VSMC alterations include reduced contractility, altered ECM production, ER stress, osteochondrogenic features, and massive death. ECs show increases in the expression of pro-inflammatory factors, leukocyte recruitment, and LDL permeability, as well as altered mechanosensing and EndMT.
- Tissue-specific progerin expression or suppression in mice has been instructive in delineating the causal role of VSMCs and ECs in HGPS-related CVD.
- VSMC alterations seem to be the initial trigger for most HGPS vascular manifestations. Thus, VSMCs are regarded as both ‘victims’ at the frontline of progerin-induced damage and ‘villains’ that propagate the pathology to neighboring cells through harmful crosstalk.

Outstanding Questions

- How can we better model HGPS vascular disease *in vitro* (e.g. by using TEBVs, organoids, or organ-on-a-chip models including VSMCs, ECs, and other cell types such as fibroblasts and immune cells)?
- Would the use of the newly-generated Cre mouse lines (*Myh11-CreER^{T2}* and *Itga8-CreER^{T2}*) to express or suppress progerin in VSMCs produce the same outcomes obtained with the *SM22α-Cre* mouse line?
- Why do different mouse models of EC-specific progerin expression have seemingly contradictory phenotypes?
- Does progerin expression in ECs play a causal role in the appearance of any of the cardiovascular symptoms of HGPS?
- Which EC phenotypic changes in HGPS are secondary to alterations in neighboring VSMCs and which stem from progerin expression in ECs?
- What is the precise sequence of molecular and cellular events during the course of HGPS-linked CVD?
- Is crosstalk between VSMCs, ECs, and immune cells important for HGPS disease progression?
- Is HGPS-related CVD influenced by changes in the provision of systemic factors by non-vascular tissues?
- Can we identify molecular biomarkers of CVD initiation and progression in HGPS?
- Would a polypill combining drugs with beneficial effects in preclinical trials improve the quality of life and lifespan of HGPS patients beyond the benefits of lonafarnib treatment alone?
- Which technical approach will work best for the delivery of VMSC-targeted therapies in HGPS patients?

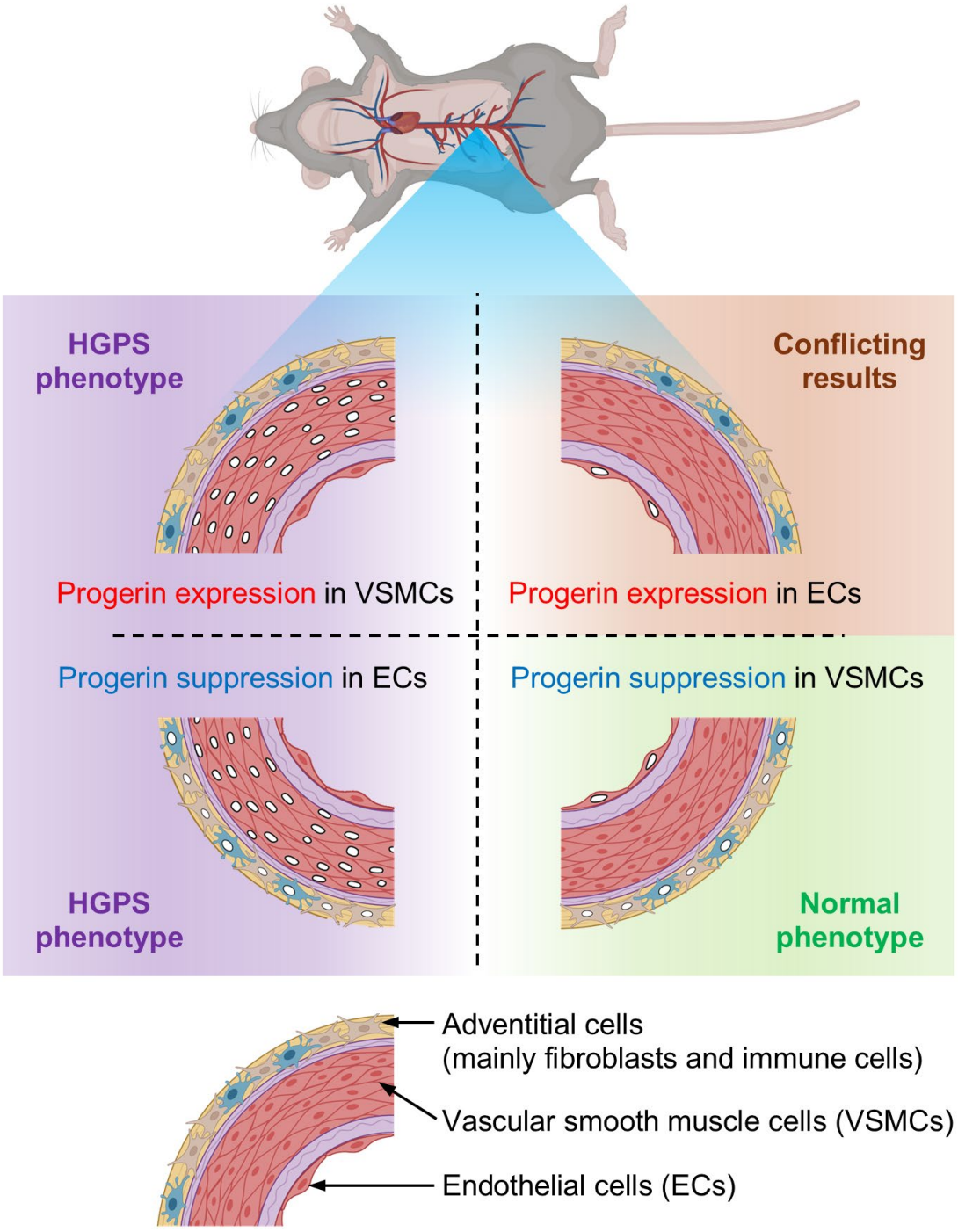
HGPS phenotype
Conflicting results
Progerin expression in VSMCs
Progerin expression in ECs
Progerin suppression in ECs
Progerin suppression in VSMCs
HGPS phenotype
Normal phenotype
Adventitial cells
(mainly fibroblasts and immune cells)
Vascular smooth muscle cells (VSMCs)
Endothelial cells (ECs)

FIGURE 1