Molecular insights into Hutchinson-Gilford progeria syndrome and age-associated disease

*From mechanisms to treatment strategy*

Gwladys Revêchon

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Cover picture: Microscopy picture representing progerin expression at both the protein (pink) and transcript (cyan) levels in keratinocytes from a progeroid mouse.
Molecular insights into Hutchinson-Gilford progeria syndrome and age-associated disease

From mechanisms to treatment strategy

Thesis for Doctoral Degree (Ph.D.)

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À mes parents,
Géraldine & Christian
ABSTRACT

Aging is a complex process that occurs as we grow old and is associated with a gradual decline of tissue functions. Scientists have been trying to understand the mechanisms that drive such phenomenon by studying premature aging syndromes and aging-related disorders. The Hutchinson-Gilford progeria syndrome (HGPS) is a lethal segmental genetic disease affecting children at an early age and characterized by accelerated aging-like features including alopecia, loss of subcutaneous adipose tissue and cardiovascular pathologies. This disorder primarily results from the production of a noxious protein called progerin, altering proper tissue homeostasis and functions. Strikingly, HGPS children present an accelerated vascular aging phenotype similar to that of chronic kidney disease (CKD) patients. Indeed, in addition to displaying kidney defects, CKD patients exhibit an accelerated vascular decline. Interestingly, progerin has previously been found expressed in cells and tissues from aged individuals, but its contribution to aging and aging-associated disorders remains poorly understood. Here, we investigated whether and how various levels of progerin are relevant to the development and progression of tissue pathology in HGPS, normal aging and aging-associated diseases, in order to generate a potential new treatment strategy for HGPS in particular.

In paper I, to better understand the mechanisms by which progerin accumulation disrupts tissue homeostasis, a humanized HGPS mouse model with overexpression of progerin in the skin was used. We demonstrated that progerin accumulation resulted in impaired tissue homeostasis as a consequence of an aberrant increase in symmetric cell division. Further analysis suggested a potential causal role of the Wnt/β-catenin signaling, associated with mislocalization of the nuclear envelope proteins emerin and nesprin-2.

In paper II, to investigate whether a small fraction of progerin-expressing cells in a tissue can lead to tissue pathology during aging, another humanized HGPS mouse model was employed. We showed that continuous expression of progerin in only a few preadipocytes and adipocytes is associated with fibrosis and lipoatrophy over time. This phenotype was combined with increased senescence, persistent DNA damage and cell death, which were found accompanied by macrophage infiltration and systemic inflammation. These results suggested that progerin, despite being expressed in only a low fraction of the cells of a tissue, has the potential to contribute to a common aging-associated phenotype.

In paper III, to unravel the possible involvement of progerin expression in age-associated diseases, we used arterial biopsies and blood from CKD patients. We found that progerin was expressed at low frequencies in 70% of the CKD patients arteries (in up to 7.4% of the cells), which was associated with an increase in DNA damage. When searching for the cause of progerin expression, we identified the LMNA c.1824C>T mutation in both the blood and arteries. Our data further suggested that progerin-expressing cells might arise during vascular regeneration, by proliferation of progenitor cells.

In paper IV, to examine the mechanisms associated with telomere dysfunction in HGPS, we employed various in vitro systems and a severe progeroid mouse model of skin. We demonstrated that telomere dysfunction leads to the production of non-coding RNAs, which activates the DNA damage response signaling. Treatment with telomeric sequence-specific antisense oligonucleotides not only repressed this signaling, but also significantly improved both the healthspan and lifespan of HGPS mice.

This thesis provides novel findings about the complex molecular mechanisms underlying progerin expression in HGPS, and its possible contribution to aging and CKD-related early vascular aging, all of which led to the discovery of a potential new treatment approach for HGPS.
RÉSUMÉ

Le vieillissement est un processus complexe, associé à un déclin progressif des fonctions tissulaires. Divers syndromes de vieillissement prématuré ainsi que les maladies liées à l’âge sont souvent utilisés en recherche, afin d’étudier les mécanismes à l’origine du vieillissement. Le syndrome de Hutchinson-Gilford (HGPS ou progéria), est une maladie génétique mortelle qui affecte les enfants dès leur plus jeune âge. La maladie se distingue par plusieurs caractéristiques s’apparentant au vieillissement prématuré, telles que la perte de cheveux et de tissu adipeux sous-cutané, ou encore l’apparition de pathologies cardiovasculaires. Ce syndrome résulte principalement de la production d’une protéine toxique appelée progérine, qui altère l’homéostasie et les fonctions tissulaires. Étonnamment, les enfants atteints de progéria présentent un phénotype de vieillissement vasculaire prématuré similaire à celui de patients atteints d'insuffisance rénale chronique (IRC). En effet, en plus d’altérer les fonctions rénales, l’IRC est accompagnée d’un déclin vasculaire prématuré. Par ailleurs, plusieurs études ont montré que progérine était exprimée dans les cellules et les tissus de personnes âgées, cependant, sa contribution au vieillissement et aux maladies liées à l’âge reste mal comprise.

Dans cette thèse, nous avons examiné comment différents niveaux d’expression de progérine peuvent influencer le développement de phénotypes couramment observés durant le vieillissement, mais aussi chez les patients atteints de progéria ou de maladies liées à l’âge, et ce afin de développer une nouvelle stratégie de traitement de la progéria.

Dans le premier article, afin de mieux comprendre les mécanismes par lesquels l’accumulation de progérine perturbe l’homéostasie tissulaire, nous avons utilisé un modèle murin présentant une surexpression épidermale de progérine. Ainsi, nous avons montré que l’accumulation de progérine entraînait une perturbation de l’homéostasie tissulaire en conséquence d’une augmentation anormale de division cellulaire symétrique. Une analyse plus poussée a permis de suggérer l’implication de la voie de signalisation Wnt/β-caténine, associée à une délocalisation de deux protéines de l’enveloppe nucléaire: émerine et nesprine-2.

Dans le second article, afin d’évaluer si seule une petite fraction de cellules exprimant progérine pouvait entraîner une pathologie tissulaire au cours du vieillissement, nous avons utilisé un autre modèle murin progéroïde présentant une perte de tissu adipeux sous-cutané. De ce fait, nous avons établi que l’expression continue de progérine dans seulement quelques préadipocytes et adipocytes était liée à une fibrose et à une lipoatrophie se développant au fil du temps. Ce phénotype était également associé à une sénescence accrue, à de persistants dommages à l’ADN ainsi qu’à la mort cellulaire, le tout étant accompagné par l’infiltration de macrophages au sein du tissu adipeux et d’une inflammation systémique. Ces résultats suggèrent que progérine, bien que seulement exprimée dans une faible fraction de cellules, a le potentiel de contribuer à un phénotype communément associé au vieillissement.

Dans le troisième article, pour mieux comprendre la possible implication de l’expression de progérine dans les maladies associées à l’âge, nous avons utilisé des biopsies artérielles de patients atteints d’IRC. Ainsi, nous avons constaté que progérine était exprimée à faible fréquence chez 70% des patients atteints d’IRC (fréquence allant jusqu’à 7,4% des cellules), ceci étant associé à une augmentation de dommages à l’ADN. Lors de la recherche de la cause de cette expression de progérine, nous avons pu identifier la présence de la mutation du gène LMNA, c.1824 C>T, dans les artères. Nos données suggèrent que les cellules exprimant progérine pourraient apparaître au cours de la régénérations vasculaire, via la prolifération de cellules progénitrices.
Dans le quatrième article, pour examiner les mécanismes liés au dysfonctionnement télomérique couramment observé chez les cellules de patients atteints de progéria, nous avons utilisé divers systèmes *in vitro* et un modèle murin de progéria. Nous avons ainsi démontré que le dysfonctionnement des télomères conduisait à la production d’ARN non codants, résultant en l’activation de la cascade de signalisation de la réponse aux dommages à l’ADN. L’utilisation d’oligonucléotides antisens et spécifiques aux ARN non-codants télomériques a non seulement permis de réprimer cette signalisation, mais aussi d’améliorer de manière significative la santé et la durée de vie des souris HGPS.

Ainsi, cette thèse procure de nouvelles découvertes quant aux mécanismes moléculaires sous-jacents à l’expression de progérine dans le cas de la progéria, ainsi que sa contribution au vieillissement vasculaire prématuré lié à l’IRC. Ceci a également conduit à la découverte d’une potentielle nouvelle approche thérapeutique de la progéria.
LIST OF SCIENTIFIC PAPERS

I. Accumulation of progerin affects the symmetry of cell division and is associated with impaired Wnt signaling and the mislocalization of nuclear envelope proteins
*Journal of Investigative Dermatology. 2019 May 23. pii: S0022-202X(19)31566-0.

II. Rare progerin-expressing preadipocytes and adipocytes contribute to tissue depletion over time

III. The Hutchinson-Gilford progeria syndrome mutation, LMNA c.1824C>T, is a somatic mutation in patients with chronic kidney disease

IV. Inhibition of DNA damage response at telomeres improves the detrimental phenotypes of Hutchinson-Gilford progeria Syndrome
*Accepted in Nature Communications.

* Authors contributed equally

RELATED SCIENTIFIC PUBLICATIONS

- Emerging candidate treatment strategies for Hutchinson-Gilford progeria syndrome
  Strandgren C, Revêchon G, Sola-Carvajal A, Eriksson M.

- Somatic mutagenesis in satellite cells associates with human skeletal muscle aging
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<th>Description</th>
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<tbody>
<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>ddPCR</td>
<td>Droplet digital PCR</td>
</tr>
<tr>
<td>DDR</td>
<td>DNA damage response</td>
</tr>
<tr>
<td>DSBs</td>
<td>Double-strand breaks</td>
</tr>
<tr>
<td>ECs</td>
<td>Endothelial cells</td>
</tr>
<tr>
<td>eGFP</td>
<td>Enhanced green fluorescent protein</td>
</tr>
<tr>
<td>FTIs</td>
<td>Farnesyltransferase inhibitors</td>
</tr>
<tr>
<td>HGPS</td>
<td>Hutchinson-Gilford progeria syndrome</td>
</tr>
<tr>
<td>ICMT</td>
<td>Isoprenylcysteine carboxymethyl transferase</td>
</tr>
<tr>
<td>INM</td>
<td>Inner nuclear membrane</td>
</tr>
<tr>
<td>IRES</td>
<td>Internal ribosomal entry site</td>
</tr>
<tr>
<td>LINC</td>
<td>Linker of nucleoskeleton and cytoskeleton</td>
</tr>
<tr>
<td>LRCs</td>
<td>Label-retaining cells</td>
</tr>
<tr>
<td>mTOR</td>
<td>Mechanistic target of rapamycin</td>
</tr>
<tr>
<td>NE</td>
<td>Nuclear envelope</td>
</tr>
<tr>
<td>NPCs</td>
<td>Nuclear pore complexes</td>
</tr>
<tr>
<td>OMIM</td>
<td>Online Mendelian Inheritance in Man</td>
</tr>
<tr>
<td>ONM</td>
<td>Outer nuclear membrane</td>
</tr>
<tr>
<td>SASP</td>
<td>Senescence-associated secretory phenotype</td>
</tr>
<tr>
<td>sWAT</td>
<td>Subcutaneous white adipose tissue</td>
</tr>
<tr>
<td>tASOs</td>
<td>Telomere-specific antisense oligonucleotides</td>
</tr>
<tr>
<td>teloC</td>
<td>C-rich strand of tncRNAs</td>
</tr>
<tr>
<td>teloG</td>
<td>G-rich strand of tncRNAs</td>
</tr>
<tr>
<td>TIFs</td>
<td>Telomere dysfunction induced foci</td>
</tr>
<tr>
<td>tncRNAs</td>
<td>Telomeric non-coding RNAs</td>
</tr>
<tr>
<td>VSMCs</td>
<td>Vascular smooth muscle cells</td>
</tr>
<tr>
<td>Wnt</td>
<td>Wingless/Integrated</td>
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1. INTRODUCTION

1.1 What is aging?

“Aging is an extraordinary process where you become the person you always should have
been.” - David Bowie.

Everyone is intrinsically acquainted with aging; however, everyone has their own
representation of this phenomenon. We have all seen people age and noticed how it takes
many forms and affects different individuals in distinct ways. Indeed, age-related symptoms
differ from one another, and aging is usually accompanied by a gradual decline of tissue
functions, presumably as a result of genetic and environmental factors. With aging comes a
higher risk of developing age-associated disorders, which eventually lead to death. In
essence, physiological aging is an inevitable complex multifactorial biological process. In
order to improve the quality of life and health of the elderly population, the aging research
field focuses its efforts in deciphering the various causes of such process.

In the recent years, nine cellular and molecular hallmarks thought to contribute to aging have
emerged¹. These now widely accepted characteristics can be classified into three
fundamentally interconnected categories: primary hallmarks, antagonistic hallmarks and
integrative hallmarks. Primary hallmarks are defined as causes of damage and consist of
genomic instability, telomere attrition, epigenetic alterations and loss of proteostasis.
Antagonistic hallmarks on the other hand, are defined as responses to damage, which can
have deleterious effects when exacerbated. These include cellular senescence, mitochondrial
dysfunction and deregulated nutrient sensing. Finally, integrative hallmarks are comprised of
stem cell exhaustion and altered intercellular communication, and correspond to hallmarks
altering tissue function and homeostasis¹. Yet, the precise interplay between these molecular
mechanisms remains unelucidated and most of the underlying biological bases of aging are
still not fully understood.

Several approaches can be used to investigate the features of aging, of which the study of
progeroid syndromes and age-associated diseases are prominent. Such studies not only
provide a better insight into particular disease mechanisms, but may also give the potential to
increase our knowledge about the molecular bases of physiological aging.

1.2 Progeroid syndromes

Progeroid syndromes are a class of genetic disorders characterized by several features of
premature-aging like phenotypes. These diseases mimic physiological aging and can be
classified into two categories: unimodal and segmental. Unimodal progeroid syndromes
represent accelerated-aging diseases such as xeroderma pigmentosum, where only a single
tissue presents aspects of aging. Contrastingly, segmental progeroid syndromes constitute a
family of diseases where multiple tissues are affected by defects characteristic of aging,
including Werner syndrome or Hutchinson-Gilford progeria syndrome (HGPS)².
Most of these disorders result from genetic mutations in genes involved in the DNA damage repair machinery or in the \textit{LMNA} gene, which encodes constituents of the nuclear lamina\textsuperscript{3,4}. The majority of these disorders are of particular interest in the field of aging as they can be very severe and in some cases lead to a shortened lifespan. Identification of causative genes and mechanisms are thought to be helpful in understanding the mechanisms driving aging. For that purpose, the most commonly investigated progeroid syndromes are Werner syndrome and HGPS, as they most resemble physiological aging. However, progeroid syndromes do not entirely replicate the biological changes frequently observed during aging.

\subsection*{1.3 Hutchinson-Gilford progeria syndrome}

With a reported incidence of one in 18 million newborns, the Hutchinson-Gilford progeria syndrome, commonly known as progeria (Online Mendelian Inheritance in Man (OMIM) \#176670), is a very rare genetic disease characterized by clinical features resembling that of marked premature aging. The first recorded case in the literature, a boy presenting “a very peculiar withered or old-mannish look, all his features being thin and pinched”, was described in 1886 by Jonathan Hutchinson\textsuperscript{5}. In 1895, the general practitioner mentioned briefly a second case that was then specifically depicted in 1897 by Hastings Gilford, who had followed this patient for several years until his death\textsuperscript{6}. But it was not until 2003, more than a century later, that the genetic mutation and molecular basis responsible for this disease were discovered\textsuperscript{7,8}.

\subsection*{1.3.1 Molecular basis of HGPS}

The \textit{LMNA} gene & laminopathies

HGPS is a lethal disorder, affecting children during their early years. It results from a single \textit{de novo} point mutation in the \textit{LMNA} gene. The \textit{LMNA} gene (OMIM \#150330) is located on chromosome 1q22 and consists of 12 exons. It encodes two major A-type lamin proteins: lamin A and lamin C. These two proteins are generated through alternative splicing within exon 10, along with the two minor isoforms lamin A\textsubscript{10} and lamin C\textsubscript{2}, and expressed in the eukaryotic nucleus of differentiated cells (Figure 1)\textsuperscript{9,10}. Together with the B-type, lamins B1 and B2, which are respectively encoded by the \textit{LMNB1} and \textit{LMNB2} genes during development, they represent the major components of the nuclear lamina, a fibrous protein network that underlines the surface of the inner nuclear membrane\textsuperscript{11}.

Over 450 mutations have been discovered in the \textit{LMNA} gene, causing disorders termed as primary laminopathies\textsuperscript{12}. Laminopathies are a group of heterogeneous genetic diseases caused by either \textit{de novo} or inherited mutations and resulting in defects in the nuclear lamina. This term not only describes \textit{LMNA}-related pathologies, but also encompasses diseases categorized as secondary laminopathies where mutations were found in genes encoding prelamin A-processing enzymes (such as ZMPSTE24), lamin-binding proteins (such as LAP2\textsubscript{a}, EMD, LBR, TMPO, LEMD3) or B-type lamins\textsuperscript{13-15}. To date, there are more than a dozen diseases identified as laminopathies, which can be classified in five different categories: muscular dystrophies affecting striated muscles (such as Emery-Dreifuss muscular dystrophy or dilated cardiomyopathy type 1A), lipodystrophies affecting the adipose tissue (such as
mandibuloacral dysplasia), neuropathies affecting peripheral nerves (such as Charcot-Marie-Tooth type 2 disease), segmental progeroid syndromes causing several tissues to age prematurely (such as HGPS, restrictive dermopathy or atypical-Werner syndrome) and overlapping syndromes corresponding to pathologies whose symptoms appear to be in more than one category (such as Reynolds syndrome or Pelger-Huet anomaly)\textsuperscript{16}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{From gene to proteins: the \textit{LMNA} gene. The \textit{LMNA} gene is composed of 12 exons, which are alternatively spliced at exon 10, giving rise to two similar protein products: lamin A (exon 1-12) and lamin C (exon 1-10). Lamin proteins present a central \(\alpha\)-helical rod domain, with a short globular head domain at the N-terminal extremity and a wide globular tail domain at the C-terminal extremity.}
\end{figure}

\section*{Lamin A & progerin synthesis}

Just like B-type lamins, lamin A is primarily synthetized as a precursor protein called prelamin A. In order to reach its final mature form, the precursor must undergo several post-translational modifications at its C-terminal CAAX motif (C: cysteine, A: aliphatic amino acid and X: any amino acid). In prelamin A, the CAAX sequence corresponds to cysteine, serine, isoleucine and methionine (CSIM)\textsuperscript{17}.

Prelamin A maturation occurs in four steps (Figure 2):

\begin{enumerate}
\item Farnesylation: prelamin A’s CSIM-cysteine is first farnesylated through the action of a farnesyltransferase (FTase).
\item Initial proteolysis: the terminal three amino acids, -SIM, are cleaved by a metalloprotease (ZMPSTE24 or RCE1).
\item Methylation: the farnesylated cysteine is methylated by an isoprenylcysteine carboxymethyl transferase (ICMT).
\item Terminal proteolysis: mature lamin A is produced through an endoproteolytic cleavage of the last 15 amino acids by ZMPSTE24, including the farnesylated cysteine-methyl residue\textsuperscript{17,18}.
\end{enumerate}
Despite several mutations having been reported to cause HGPS, >90% of the cases are due to a de novo heterozygous substitution in exon 11 of the LMNA gene, the c.1824C>T\textsuperscript{8,19,20}. This mutation is dominant in codon 608 and causes a substitution from GGC (glycine) to GGT (glycine), known as p.G608G. Despite being a silent mutation, it induces the activation of a cryptic splice donor site that leads to the lamin A transcript being spliced five nucleotides upstream of the mutation, creating a truncated mRNA lacking 150 nucleotides: lamin A Δ150. This, in turn, results in a truncated protein called lamin A Δ50, commonly named progerin, which contains a 50 amino-acid deletion (Val607_Gln656del) near its C-terminal region.

**Figure 2. Lamin A & progerin synthesis.** After the splicing event occurred, the maturation of prelamin A to mature lamin A takes place, involving several post-translational modifications. In HGPS, aberrant splicing leads to the deletion of 50 amino acids (AA), including the recognition site of ZMPSTE24 (RSYLLG), and the final processing step cannot be performed, thus resulting in a truncated form of prelamin A: progerin.

Like lamin A, progerin is produced from the prelamin A precursor. This truncated prelamin A undergoes the same post-translational modifications except for the terminal proteolysis step by ZMPSTE24, as the deleted 50 amino acids were containing the protease recognition site (RSYLLG motif). This results in a partially processed and toxic protein that remains farnesylated and carboxymethylated, called progerin (Figure 2). Of note, both mature lamin A and progerin proteins are generated from the mutant allele as the cryptic splice site is not continuously activated\textsuperscript{21,22}. 
1.3.2 Nuclear & cellular defects in HGPS

The nuclear envelope & the nuclear lamina

The nucleus contains the genetic material and is associated with the cytoskeleton and cytoplasm through a bilayer nuclear envelope (NE) in eukaryotic cells. The NE is composed of two lipid membranes, the inner and outer nuclear membranes (INM and ONM, respectively), in between which lies the narrow perinuclear space. Although both the INM and ONM are functionally and biochemically different, they merge at sites that are crossed by nuclear pore complexes (NPCs). NPCs allow the bidirectional translocation of macromolecules, such as mRNAs, ribosomes, proteins, lipids and signaling molecules, between the nucleus and the cytoplasm. The ONM is coated with ribosomes, as it is continuous to the rough endoplasmic reticulum, and both the ONM and INM contain various proteins that are embedded within their lipids, including lamina-associated proteins and LINC (Linker of Nucleoskeleton and Cytoskeleton) complex proteins (Figure 3).

Directly underneath the INM lie the A- and B-type lamins, type V intermediate filament proteins constituting the nuclear lamina. The lamina plays an essential role in the cell as a structural support to the NE. It defines the size and shape of the nucleus through A-type lamins, which provide nuclear stiffness and viscosity, and B-type lamins, which provide elasticity. Moreover, the nuclear lamina also takes part in gene regulation and transcription via its binding to chromatin and transcription factors, in cell differentiation, DNA replication and repair, and in NE assembly.

![Figure 3. The role of the nuclear lamina at the nuclear envelope.](image)

The nuclear lamina is located at the nuclear rim, beneath the inner nuclear membrane (INM), where it confers support and stability to the nucleus. Lamins anchor chromatin but also have numerous binding partners among the nuclear envelope proteins (e.g. LAP1/2, LBR, SUN1/2, SAMP1, MAN1, emerin) and can indirectly interact with the cytoskeleton via the LINC complex (formed by SUN1/2 and nesprins1/2), thus influencing various biological processes.
Lamins bind to numerous proteins, the vast majority being anchored to the INM. A few examples are the well-characterized lamin B receptor (LBR), lamina-associated polypeptides-1 and 2α (LAP1 and LAP2α), nesprin-1α, MAN1, emerin, SUN1 and 2 proteins and SAMP1 (Figure 3)\textsuperscript{32,35}. Through its interaction with B-type lamins, LBR was suggested to be involved in chromatin tethering, epigenetic gene silencing and cell differentiation\textsuperscript{36,38}. Similar to the LBR/B-type lamins complex, the LAP2α/A-type lamins complex was found to influence cell differentiation and proliferation, as well as chromatin organization\textsuperscript{39-42}. Lamins were also determined to play a crucial role in nesprin-1α, MAN1 and emerin localization at the NE, hence ensuring their important role in signal transduction, chromosome segregation and cell division and differentiation\textsuperscript{43-45}.

Meanwhile, emerin is also known to participate in transcriptional regulation, to bind to microtubules and stabilize actin filaments, suggesting that it can influence actin dynamics. Interestingly, emerin is also a binding partner of nesprin-1α\textsuperscript{11,46}. Nesprins are embedded at the ONM and contain a KASH transmembrane domain, which interacts in the perinuclear space with the INM SUN proteins to form the LINC complex. This complex mechanically interconnects the nucleoskeleton and the cytoskeleton as SUN proteins bind to lamins while nesprins tether directly or indirectly structural cytoplasmic fibers such as actin, intermediate filaments, centrosomes or microtubules\textsuperscript{47,53}. Of note, some isoforms such as nesprin-1α and nesprin-2β bind directly to lamins and emerin\textsuperscript{54,55}. The combination of the LINC complex and nuclear lamins forms a protein network and was suggested to be implicated in the regulation of KASH binding and localization of nesprins to the NE, as well as in cell division, chromatin rearrangement and NPCs organization\textsuperscript{56,57}. Furthermore, the A-type lamins interactor SAMP1 was established as functionally co-localizing with SUN1, and was associated with the correct distribution of emerin and organization of heterochromatin at the nuclear periphery\textsuperscript{34,58}. This giant scaffold of interlinked proteins further highlights the central role of the nuclear lamina in fundamental biological processes.

**Nuclear & cellular alterations in HGPS**

Due to the toxic effect of progerin, HGPS recapitulates many of the nuclear and cellular defects commonly observed in physiological aging, including genomic instability, telomere shortening, epigenetic changes, senescence, stem cell exhaustion and mitochondrial dysfunction (Figure 4). Indeed, mutations in lamin A alter the natural functions of the nuclear lamina, including its primary function as a structural support to the NE. This results in cells exhibiting nuclear shape abnormalities known as nuclear blebs, which are accompanied by irregular localization of NPCs. It is thought that the aberrant processing of progerin contributes to its attachment to the INM, consequently leading to nuclear architectural defects\textsuperscript{22,28}.

In addition, the cell cycle, a valuable machinery for repairing genetic damage and avoiding uncontrolled cell divisions, is affected in HGPS cells. The cell cycle represents a continuous ordered series of events leading to cell growth and proliferation composed of three states: quiescence (G0 phase, where the cell ceases to divide), interphase (G1, S and G2 phases, where the cell grows and duplicates its genetic material in preparation of cellular division) and mitotic phase (M phase, including mitosis and cytokinesis, where the cell divides into two
identical daughter cells\textsuperscript{59}. Besides manifesting an impaired chromosome partitioning during mitosis, the onset and progression of cytokinesis is delayed, and the nuclear lamina components are abnormally segregated in the cytoplasm during the G1 phase in HGPS cells\textsuperscript{26,60}.

Tightly associated with the cell cycle is cellular senescence, a steady-state of cell cycle arrest mostly resulting from oxidative stress, DNA damage, telomere attrition or metabolic changes\textsuperscript{62}. Progerin accumulation in HGPS cells and animal models generally correlates with increased senescence, and particularly with p53-dependent premature senescence\textsuperscript{63,65}. Most often, senescence coincides with the production of inflammatory components that promote the expansion of senescence to neighboring cells in a paracrine manner. This process is termed the senescence-associated secretory phenotype (SASP) and participates in the activation of immune surveillance. Accordingly, inflammation is another defect associated with progeria. Indeed, levels of pro-inflammatory cytokines were found increased in three distinct progeroid mouse models and NF-\kappaB-mediated inflammation was found to participate in the development of age-related features\textsuperscript{66,67}.

\textbf{Figure 4. Shared cellular and molecular hallmarks between aging & HGPS.} These nine cellular and molecular defects characterize the fundamental processes of HGPS pathogenesis and are commonly associated with physiological aging\textsuperscript{61}. 
HGPS cells are highly susceptible to genomic instability. They are especially characterized by impaired DNA damage-related mechanisms such as deficient DNA damage response (DDR) and double-strand breaks (DSBs) repair mechanisms, accelerated telomere shortening, epigenetic misregulation and changes in chromatin organization. The recruitment of DDR molecules like RAD51 or 53BP1 at sites of DSBs appears delayed in HGPS, on top of which both homologous recombination and non-homologous end-joining repair pathways are suggested to be dysfunctional. Meanwhile, the telomere loss observed in progeria may be explained by a decrease in TRF2 function, TRF2 being a lamin A binding partner stabilizing the formation of protective t-loops at telomeres. Gene expression, on the other hand, is regulated by chromatin organization and epigenetic changes. In HGPS, cells exhibit reduced levels of HP1α, a protein involved in heterochromatin maintenance; along with lower levels of two epigenetic marks: H3K9me3 and H3K27me3. These deficiencies impede the maintenance of genome integrity through heterochromatin loss and repression of transcription.

One of the repercussions of DNA damage accumulation is that it can participate in the deterioration of stem cell functions, thus reducing tissue homeostasis and regeneration capacity. Progeroid stem cells exhibit alterations in their proliferative capacity as an indication of stem cell exhaustion. Finally, various metabolic abnormalities are associated with HGPS. Affected pathways include glucose and fatty acid metabolism, oxidative phosphorylation and mitochondrial biogenesis, leading in some instances to mitochondrial dysfunction. A notable example is the mechanistic target of rapamycin (mTOR) signaling pathway, which regulates anabolic and catabolic processes and is especially known to monitor cell growth, proliferation and autophagy. This pathway was found downregulated in HGPS, potentially resulting in autophagy activation.

### 1.3.3 HGPS pathophysiology

Children with HGPS display no evidence of disease at birth, however within their first years of life, they rapidly begin to develop age-like features. The mean age for diagnosis is approximately 2.6 years, as they start to show classical signs of illness. Failure to thrive and growth retardation are among the first clinical symptoms, and the classical phenotypes manifest in diverse organs: skin, adipose tissue, bones and cardiovascular system (Figure 5). The skin is affected with alopecia, revealing a prominent forehead and swollen scalp veins. It is subject to epidermal hypoplasia, a reduction in elasticity and scleroderma-like changes, accompanied by a loss of subcutaneous adipose tissue, thus resulting in tight and thin skin. Characteristic facial features include a pinched nose, thin lips and a shrunken chin (Figure 5). HGPS patients experience skeletal muscle wasting and their skeleton is marked by skeletal dysplasia, where the dentition is delayed and irregular, and a diminished bone mineral density. Joint stiffness and acro-osteolysis also occur as the children age, promoting limited joint mobility and contributing to the resorption of distal phalanges. Patients suffer from atherosclerosis and cardiovascular decline, eventually leading to death at an approximate age of 14.6 years.
Figure 5. Clinical features of HGPS. HGPS patients display a strong phenotype characterized by clinical features resembling premature aging. Diagnosis can be based on the assessment of those features, which comprise growth retardation, alopecia, loss of subcutaneous fat, skeletal dysplasia and cardiovascular defects, among others. This figure was originally published in Strandgren et al., Biochem. Soc. Transac. (2017)78.

These health problems are commonly shared with normal aging, however, and for unknown reasons, other aging disorders such as neurodegeneration and dementia, chronic kidney disease or diabetes do not occur (Figure 6). The absence of phenotype in certain tissues is actually a large and unexplored area in the HGPS research field. As for the lack of neurodegeneration, it is thought that the microRNA miR9 inhibits progerin expression in neural cells from HGPS patients, thus acting as a protection against brain-associated disorders79. However, a study conducted in our lab showed that long-term overexpression of progerin in the brain of mice did not result in a phenotype, indicating that progerin does not affect neuronal cells80. Neurons are post-mitotic cells, which could explain the fact that they appear to be insensitive to progerin expression. It can also be hypothesized that progerin expression may not be of importance in some other organs, those organs potentially being protected against the toxic effects of progerin in a similar fashion. The absence of phenotype in certain tissues might also be caused by the lack of significant levels of progerin expression.
combined with the shortened lifespan of HGPS patients, as previously observed in some mouse models that fail to reproduce certain HGPS phenotypes likely as a consequence of low levels of progerin expression\textsuperscript{81,82}. Despite the presence of considerable genomic instability, affected children do not develop cancer or tumors. This is probably due to a reduced invasive capacity of tumor-initiating cells caused by changes in extracellular matrix composition and/or due to a protective mechanism to oncogenesis associated with progerin expression\textsuperscript{83,84}. HGPS being a segmental disease, organs including the liver, kidneys, brain, lungs and the gastrointestinal tract remain unaffected. In fact, it is worth noting that most affected tissues are of mesodermal and ectodermal origin.

**Figure 6.** Shared phenotype between HGPS & aging. HGPS patients display many clinical features commonly observed during normal aging, including atherosclerosis or reduced bone mineral density. However, not all aging phenotypes are recapitulated in HGPS, and patients do not present neurodegeneration or cancer. This figure was originally published in Strandgren et al., Biochem. Soc. Transac. (2017)\textsuperscript{78}. 
The adipose tissue

The mesoderm represents one of the three primary germ layers, which also include the ectoderm and the endoderm; and gives rise to mesenchymal tissues during embryogenesis. These tissues consist of connective or “soft” tissues, which can compose many organs and participate in their shape and strength. Seven major cell types are of mesenchymal origin, including adipocytes, fibroblasts, myoblasts, mesothelial cells, endothelial cells, chondroblasts and osteoblasts. The development of these cell types occurs through the differentiation of mesenchymal stem cells, which are also referred to as multipotent stromal cells.

The adipose tissue is one of the richest sources of mesenchymal stem cells. It is mainly composed of mature adipocytes, but also of various cell types forming the stromal vascular fraction, including preadipocytes, fibroblasts, endothelial cells (ECs) and hematopoietic cells. This connective tissue is found throughout the body and constitutes one of the largest organs. Different fat depots with distinct functions can be found in both humans and mice: the white adipose tissue (e.g. subcutaneous or visceral) and the brown adipose tissue. The main function of brown adipose tissue is to generate body heat. Meanwhile, white adipose tissue is known to be involved in energy storage in the form of lipid, but also in immunity, and works as an endocrine organ, secreting hormones called adipokines (e.g. leptin and resistin) and inflammatory cytokines.

One of the major physiological changes that occurs with aging is the redistribution of adipose tissue with the subcutaneous depot being reduced and the visceral depot being enlarged in humans. As previously mentioned, HGPS patients suffer from lipodystrophy, characterized by a complete loss of subcutaneous adipose tissue. Several studies have found that progerin or prelamin A accumulation impairs the differentiation into mature adipocytes in vitro, the cells being unable to commit to the terminal differentiation stage. Another interesting study further confirmed those observations and characterized the adipose tissue of progerin-expressing mice, concluding on lipodystrophic mice with increased mitochondrial biogenesis and energy expenditure. However, how progerin expression can lead to adipose tissue depletion in vivo and the mechanisms underlying such phenomenon remain little investigated.

The arteries

Arteries are part of the cardiovascular system, which consists of the heart, blood and blood vessels. Like the adipose tissue, arteries stem from the mesoderm during embryonic development. They include a blood vessel wall that can be decomposed into three specific layers: the tunica intima, the tunica media and the tunica adventitia. Each layer is made up of different cell types conferring various roles to the tissue.

The tunica intima corresponds to the innermost layer of the artery that is in direct contact with the blood, and is composed of a thin sheet of ECs embedded in the basal lamina. In that setup, endothelial cells play a structural role, giving their shape to arteries. The tunica media that is sandwiched between the tunica intima and tunica adventitia, consists of elastic tissue.
and vascular smooth muscle cells (VSMCs) able to contract and to provide shear strength. The tunica adventitia, on the other hand, is the outermost layer of the arteries and is mostly formed by collagen and fibroblasts. Arteries are known to participate in the respiration of tissues by transporting oxygenated blood from the heart.

During aging, arteries encounter structural and functional changes with the development of arteriosclerosis and the loss of distensibility\textsuperscript{95}. In HGPS, patients present an atherosclerotic phenotype characterized by a thickened intimal layer, calcification and loss of VSMCs in the media, acute fibrosis in the adventitia, indications of plaque erosion and/or rupture and inflammation\textsuperscript{96}. The vascular pathology is of particular interest in the HGPS field, as cardiovascular disease is considered as the predominant cause of death in affected children. In an \textit{in vitro} experimentation, Zhang et al. demonstrated a critical role of PARP1 in smooth muscle cell death, therefore suggesting its involvement in the loss of VSMCs associated with HGPS\textsuperscript{70}. To further comprehend the underlying cause of such vascular alterations, several animal models were developed, partially replicating the cardiovascular phenotype observed in affected children\textsuperscript{73,81,97-99}. Progerin expression was thus found associated with reduced extracellular accumulation of pyrophosphate potentially contributing to vascular calcification\textsuperscript{100}. Further investigation of the role of progerin in atherosclerosis revealed that it induces endoplasmic reticulum stress in VSMCs\textsuperscript{101}. However, cell type-specific expression of progerin implied that ECs and VSMCs are independently able to contribute to cardiovascular pathology\textsuperscript{98,99}. Altogether, this suggests that all the mechanistic links are not yet elucidated and so, additional research is needed to explore how progerin expression in arteries contributes to the tissue decline leading to premature death in HGPS patients.

**The skin**

The skin belongs to the integumentary system, which covers the body and acts as a thermoregulator and protector against environmental threats. It emerges from both ectodermal and mesodermal germ layers and develops into three main layers: the hypodermis, the dermis and the epidermis. The hypodermis is the innermost layer of the organ composed mostly of adipose tissue. It exerts metabolic functions, which comprise for instance the storage of lipids and the synthesis of vitamin D. Overlying the hypodermis is the thick hypervascular dermal layer composed of connective tissue, which harbors many sensory receptors conferring a sensation function. The most external layer is then the epidermis, which mostly consists of keratinocytes, surrounded by a few melanocytes, Langherans cells and Merkel cells. This keratinized layer is in direct contact with the external environment and thus serves its role as a protective barrier against pathogens, mechanical and chemical damage and dehydration.

In mice, the epidermal layer is usually thin compared to humans (<25\textmu m compared to >50\textmu m in thickness, respectively). It is subdivided into four main layers representing “the circle of life of keratinocytes”. The stratum basale (a.k.a. basal layer) is the lower layer in which keratinocytes are continuously renewing by a series of cell divisions. Newly formed daughter cells then migrate to the next epidermal layer, the stratum spinosum (a.k.a. spinous layer) where they lose their proliferative capacity in favor of differentiation. At this stage,
keratinocytes start to cornify and produce keratins. As cells continue their ascension towards the upper epidermal layers, they reach the stratum granulosum (a.k.a. granular layer) where they become more cornified and eventually activate programmed cell death. Finally, dead keratinocytes are stacked into several layers forming the stratum corneum (a.k.a. horny layer) and end up shedding as newly dead cells come in replacement.

The process of cell division occurring in the skin is of great importance as it governs tissue homeostasis. Stem cells have the ability to self-renew – i.e. symmetric cell division – but also to generate daughter cells committed to differentiation – i.e. asymmetric cell division. Such processes can take place via two alternative mechanisms: intrinsic or extrinsic. In intrinsic division, preferential apportioning of regulators of cell fate determinant such as ubiquitin is crucial, while in extrinsic division contact with the stem cell niche defines the potential to self-renew. In the case of skin, extrinsic division can be observed based on the orientation of the cell division towards the basement membrane: parallel division indicating symmetric cell division; perpendicular division indicating asymmetric cell division. In HGPS, skin is the first organ affected. Investigation of the skin phenotype has revealed that post-natal expression of the progeria mutation under the control of the keratin 5 promoter in mice results in early loss of epidermal stem cells. On the other hand, embryonic expression of the progeria mutation leads to severe skin abnormalities characteristic of halted development. These phenotypes of depletion of the stem cell pool and arrested skin development are suggestive of impaired homeostasis and could be evidence of defects in extrinsic cell division caused by progerin expression.

1.3.4 Treatment approaches for HGPS

In the years that followed the discovery of the gene responsible for HGPS, potential treatments rapidly emerged. The first one, a farnesyltransferase inhibitor (FTI) called Lonafarnib, was found to improve the disease phenotype both in vitro and in vivo by inhibiting the farnesylation of prelamin A. Following these promising results, a clinical trial was started and children were found to have increased body weight, improved bone structure and/or improved vascular stiffness. This treatment resulted in a significantly lower mortality rate, characterized by a mean survival increase of 1.6 years. The first treatment for progeria was thus discovered. Presently, about 30 different treatment strategies have been suggested, targeting a wide range of mechanisms, and researchers continue to find novel approaches to counteract the deleterious effects of progerin (Figure 7).

Reshaping the prelamin A processing

Although FTI treatment appeared to be a good candidate, it was later suggested that progerin may still be prenylated via an alternative pathway: geranylgeranylation. This study described that inhibiting both the addition of a farnesyl group and of a geranylgeranyl group to prelamin A’s CAAX motif resulted in a greater accumulation of the protein. In addition, they showed that reducing prenylation with a combination of aminobisphosphonates (Zoledronate) and statins (Pravastatin) ameliorated HGPS cells defects and improved both health and lifespan of a progeroid mouse model (Figure 7). Similarly, the use of mono-aminopyrimidines (Mono-
AP) resulted in improved cellular phenotypes in HGPS-derived induced pluripotent stem cells, acting as both FTIs and bisphosphonates. But prelamin A carboxymethylation is also a key process in the toxicity of progerin, and indeed, it was demonstrated that ICMT inhibition by short hairpin RNAs (shICMT) promoted the rescue of senescence and proliferation in vitro, as well as a better health- and lifespan in vivo.

**Figure 7. Targeting mechanisms of HGPS by candidate treatment strategies.** A wide spectrum of treatments for HGPS have emerged since the discovery of the mutation. Treatments aim at targeting various characteristic defects of HGPS including the prelamin A processing, downstream mechanisms affected by progerin expression (such as the progerin/lamin A interaction, mitochondrial biogenesis, inflammation, telomere dysfuncition or cellular reprogramming), but also autophagy, the splicing machinery or the mutation-causing disease.

**Targeting cellular & molecular abnormalities**

Several other approaches were established to correct the cellular and molecular abnormalities induced by progerin expression. Hence, researchers have tried to block progerin interaction with lamin A/C, as it accounts for nuclear deformations. Inhibiting such linkage by the use of a chemical compound, JH4, was shown to not only improve the nuclear morphology in vitro, but also to partly rescue HGPS-related defects and prevent early death in a systemic knock-in mouse model of HGPS. Other studies focused on targeting the downstream effectors of progerin. Thus, drugs such as the NRF2-activating drug, the NAT10-inhibitor Remodelin, the antioxidant Resveratrol targeting SIRT1 or simple supplementations of nitrites to increase nitric oxide bioavailability, or of vitamin D to counteract the vitamin D
receptor deficiency, were found beneficial to the HGPS phenotype both in vitro and in vivo\textsuperscript{119}. Furthermore, introducing human telomerase mRNA (hTERT) in HGPS cells resulted in short telomeres extension together with improved cellular capacities, while reducing NF-κB-driven inflammation with sodium salicylate hindered premature aging phenotypes in two distinct HGPS mouse models (Figure 7)\textsuperscript{66,125}. Many other drugs were found to improve the HGPS phenotype likewise. By the means of NAC (N-acetylcysteine), methylene blue, metformin, ROCK (rho-associated protein kinase) inhibitors or PPI (inorganic pyrophosphate), mitochondrial biogenesis was reinforced by restraining its dysfunction, thus regulating various cellular flaws, including reduced ROS levels, DNA damage, senescence and progerin expression\textsuperscript{100,126-130}. Finally, cellular reprogramming was investigated as a way to promote tissue regeneration. Treatments of HGPS mice with inhibitors of the reprogramming repressor DOT1L or by short-term cyclic induction of the Yamanaka factors OSKM (Oct4, Sox2, Klf4 and c-Myc) both ameliorated the premature aging defects and extended the lifespan (Figure 7)\textsuperscript{131,132}.

**Autophagy activation**

Autophagy is the natural process occurring in cells allowing for the degradation and recycling of cellular components, and for which the discovery of related mechanisms led to the award of the Nobel Prize in Physiology or Medicine to the researcher Yoshinori Ohsumi in 2016. One of the first treatment approaches of HGPS shown to target progerin turnover was using rapamycin, an inhibitor of mTOR. This strategy allowed for the autophagy-mediated clearance of progerin in vitro, resulting in reduced nuclear blebings and senescence levels (Figure 7)\textsuperscript{133}. Further in vitro studies using the rapamycin analog Temsirolimus, the antioxidant drug Sulforaphane or all-trans retinoic acids, showed similar advantageous effects\textsuperscript{134-136}. Additionally, the proteasome inhibitor MG132 was found to participate in progerin degradation partially mediated by autophagy, as well as indirectly reducing prelamin A aberrant splicing, resulting in the amelioration of the HGPS cellular phenotype (Figure 7)\textsuperscript{137}.

**Gene therapy**

Nevertheless, a compound- or drug-based therapy may not be sufficient to cure HGPS. HGPS would likely require gene therapy to some extent to eliminate the high production of progerin. Hence, antisense oligonucleotides-based therapies have previously been tested. Morpholinos directed against the lamin A splice donor site or against the aberrant exon 11 and exon 12 junction, were found efficient, considerably reducing progerin levels in HGPS cell lines or mouse model\textsuperscript{131,138}. With the use of the current genome editing technologies, two independent laboratories recently published interesting studies back-to-back. In their CRISPR/Cas9-based therapy strategies, they targeted the LMNA exon 11 upstream of the HGPS mutation of HGPS mice, thus preventing lamin A/progerin production and solely resulting in the production of lamin C. These methods markedly reduced progerin expression in vivo, which led to improved phenotypes and extended lifespan (Figure 7)\textsuperscript{139,140}. However, one could be concerned by the off-target events that may arise with such editing and further developments are required to reduce off-target effects before these approaches could be ready for patients.
HGPS clinical trials

As mentioned previously, there had been a clinical trial using FTIs in progeria patients\textsuperscript{25,112}. Based on the positive outcomes of this first monotherapy and of the studies using statins and bisphosphonates, another one rapidly followed. The second clinical trial added Pravastatin and Zoledronate to the FTI treatment. However, only the bone mineral density improved and the number of patients presenting extraskeletal calcifications and arterial plaques considerably increased\textsuperscript{141}. Interestingly, an \textit{in vitro} study from Capell and colleagues prior to the study showing beneficial effects from the combination of such drugs, inferred that progerin was not prenylated by any other processes than farnesylation\textsuperscript{106}. This suggests that the combination of these drugs may not be suitable to treat HGPS patients. Nowadays, a third clinical trial is ongoing, for which phase two was initiated back in 2017. This trial enrolled 60 HGPS children, who are currently receiving a combination of Lonafarnib and Everolimus, a rapamycin analog\textsuperscript{142}.

However, Nature is complex and intricate. Mice and humans are different species, and of all the treatment strategies developed and tested on \textit{in vitro} and \textit{in vivo} models of HGPS, not all of them might be transferrable to patients or provide the same beneficial effects. Nevertheless, a thorough assessment of treatments potential side effects or evaluation of the combination of multiple treatments in pre-clinical models is crucially needed. In addition, some treatment strategies may have contradictory effects in different models, which is why the choice of appropriate HGPS models is of critical importance. Several HGPS models were engineered (systemic mice or pigs, humanized models or tissue-specific models), which allow for the assessment of the true advantages of a given candidate treatment\textsuperscript{73,81,97,143,144}. A non-negligible point is that it is worth testing treatments in models that have acquired a phenotype, as patients present a developed disease phenotype before treatment is initiated. As the cure or a treatment with more significant benefits for HGPS patients has yet to be discovered, research is pursuing to try find new therapies that could be of potential interest to HGPS.

1.4 Age-associated diseases

Age-associated diseases correspond to a broad range of diseases that may arise as a consequence of aging, aging being a prevailing risk factor. Unlike most progeroid syndromes, age-related disorders have a high prevalence in the population and it is estimated that over 75\% of human deaths can be imputed to them\textsuperscript{145}. These include various kinds of conditions altering the healthspan such as cancers, vascular disorders (e.g. atherosclerosis), bone-related disorders (e.g. osteoporosis), metabolic disorders (e.g. type 2 diabetes), neurodegenerative diseases (e.g. dementia) and urinary system disorders (e.g. chronic kidney disease) among others (Table 1)\textsuperscript{146-149}. These pathologies are defined as chronic, meaning that they are not genetically inherited and should not be confused with accelerated-aging disorders. Interestingly, some illnesses like Alzheimer’s disease and Parkinson’s disease fall into both the chronic and accelerated categories, as they can be either inherited or sporadic. How aging impacts these diseases is generally investigated by counteracting and postponing the onset and progression of alterations commonly observed during aging in specific disease-affected tissues.
### Table 1. Overview of age-associated diseases

<table>
<thead>
<tr>
<th>BIOLOGICAL SYSTEM AFFECTED</th>
<th>AGING-ASSOCIATED DISEASE</th>
<th>SHARED HALLMARKS OF AGING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nervous system</td>
<td>Alzheimer's disease</td>
<td>Mitochondrial dysfunction, loss of proteostasis, cellular senescence, altered intercellular communication</td>
</tr>
<tr>
<td></td>
<td>Parkinson's disease</td>
<td>Cellular senescence, altered intercellular communication</td>
</tr>
<tr>
<td>Circulatory system</td>
<td>Atherosclerosis</td>
<td>Cellular senescence, telomere attrition, genomic instability, epigenetic alterations</td>
</tr>
<tr>
<td></td>
<td>Cardiovascular diseases</td>
<td>Mitochondrial dysfunction, cellular senescence, deregulated nutrient sensing, stem cell exhaustion</td>
</tr>
<tr>
<td>Respiratory system</td>
<td>Chronic obstructive pulmonary disease</td>
<td>Mitochondrial dysfunction, loss of proteostasis, cellular senescence, altered intercellular communication, telomere attrition, deregulated nutrient-sensing, stem cell exhaustion</td>
</tr>
<tr>
<td>Musculoskeletal system</td>
<td>Sarcopenia</td>
<td>Altered intercellular communication</td>
</tr>
<tr>
<td></td>
<td>Osteoporosis</td>
<td>Altered intercellular communication</td>
</tr>
<tr>
<td></td>
<td>Osteoarthritis</td>
<td>Mitochondrial dysfunction, cellular senescence, altered intercellular communication</td>
</tr>
<tr>
<td>Metabolic system</td>
<td>Type-2 diabetes</td>
<td>Cellular senescence, genomic instability, deregulated nutrient sensing</td>
</tr>
<tr>
<td>Urinary system</td>
<td>Chronic kidney disease</td>
<td>Cellular senescence, altered intercellular communication, deregulated nutrient sensing</td>
</tr>
<tr>
<td>All systems</td>
<td>Cancer</td>
<td>Cellular senescence, altered intercellular communication</td>
</tr>
</tbody>
</table>

Table derived from Wang & Bennett, 2012; Chiao & Rabinovitch, 2015; Kubben & Misteli, 2017; Franceschi et al., 2018.

### 1.5 An example of age-associated diseases: Chronic kidney disease

Chronic kidney disease (CKD) is an often-silent disease that affects 10-12% of the global population. It is a major health problem and was ranked as the 12th most prevalent cause of death, accounting for 1.1 million deaths worldwide. CKD can be defined by the presence of damaged and dysfunctional kidneys, which can threaten health. It is classified as five different stages associated with the glomerular filtration rate, which relates to the degree of severity of kidney dysfunction. Thus, CKD is often diagnosed by assessing the glomerular filtration rate, along with the serum creatinine and/or the urine albumin excretion. In most cases, CKD arises as a consequence of diabetes, hypertension and glomerulonephritis, but it can also be idiopathic or due to hereditary diseases such as polycystic kidney disease. As the disease progresses, diverse complications indicative of a premature aging process occur including anemia, osteoporosis, infections, muscle wasting, dyslipidemia, frailty, neuronal damage and cardiovascular disease (CVD).
1.5.1 CKD & CVD

CKD may be considered as a clinical example of premature vascular disease. Patients suffering from such disease have an increased risk of CVD associated with the disease progression. Cardiovascular mortality is regarded as the main cause of death in CKD patients undergoing dialysis, and is 10-20 times increased in those patients compared to the general population\textsuperscript{154}. CVD pathogenesis in CKD can be attributed to many various risk factors. Hence, hypertension is thought of as a traditional risk factor that participates in cardiac damage. Indeed, it was suggested to induce left ventricular hypertrophy, along with anemia and low glomerular filtration. Coronary ischemia, abnormal mineral metabolism, excess of proteins in the urine and inflammation are also associated with CKD-related development of CVD\textsuperscript{153}. However, the impact of CKD on the development of CVD is very complex, and all the mechanisms leading to the rapid progression of vascular disease in the context of CKD are still not entirely established.

Early vascular aging

In CKD, an apparent discrepancy between chronological and biological vascular age can be observed, thus representing an “accelerated arterial aging” phenotype. This vascular change is identified by arterial stiffening and enlargement, increased intima-media thickness and fibrotic adventitia\textsuperscript{155}\textsuperscript{157}. Such damage lead to a lower shear stress (i.e. reduced tangential force of the blood on the endothelial layer of the artery), a mechanical force normally promoting endothelial cell survival and quiescence\textsuperscript{158}. The arterial distensibility observed in CKD represents vascular calcification and is generally associated with mineral and bone disorders\textsuperscript{159}\textsuperscript{160}. Increased calcium content and calcification of the intimal and/or medial layer of the vascular wall are common features of CKD arteries, in turn leading to atherosclerosis. This phenomenon is essentially induced by circulating uremic toxins, systemic inflammatory cytokines and by impaired calcium and phosphate metabolisms in the case of renal disease\textsuperscript{161}\textsuperscript{162}. In addition, VSMCs apoptosis correlates with medial calcification. Since VSMCs exhibit phenotypic plasticity, they are hypothesized to contribute to media calcification. However, this remains debated in the cardiovascular field\textsuperscript{163}\textsuperscript{164}. This controversy emphasizes the need to further elucidate the underlying mechanisms leading to vascular damage, which could be of particular interest to understand and prevent CKD-related CVD.

1.5.2 Treatment approaches

Means of treating CKD are multiple, however, there is no cure and no specific medicines for such disease. Yet, new drugs like SGLT2 inhibitors and NRF2 agonists bring hope for better treatments of this expanding patient population\textsuperscript{165}. Current treatments aim at reducing complications and slowing down or arresting the disease progression by targeting risk factors. In the early stages of CKD, a proper lifestyle along with a healthy diet is important. Some therapies may include medications controlling the blood pressure or the cholesterol levels, whereas others may use drugs treating anemia, relieving oedema or protecting the bones\textsuperscript{166}. In end-stage renal disease, when glomerular filtration rate is <5-7 ml/min, renal replacement therapy like dialysis or kidney transplantation are needed for survival. Dialysis treatment
artificially takes on the function of healthy kidneys, thus removing extra fluid and uremic waste products from the blood. Kidney transplantation improves survival markedly, whereas the mortality rate in dialysis patients is around 20% (mostly because of CVD) and comparable to metastatic cancer disease.\textsuperscript{153} Given the high mortality rate and the important costs of renal replacement therapy, alternative treatment strategies are needed. A future treatment approach that may fit such requirements concerns the use of regenerative medicine. Indeed, restoring the kidney function with the use of stem cell therapy, for example, could be of great help to slow the disease progression\textsuperscript{167,168}. However, such treatment is not available yet, and researchers are studying the disease mechanisms in search for a suitable therapy for CKD.

\section*{1.6 Aging, HGPS & CKD: what is the link?}

It is becoming more and more evident that lamin A plays a role during physiological aging. But despite the fact that HGPS and normal aging share common mechanisms, their connection remains hazy.

\subsection*{1.6.1 Progerin expression in healthy aging}

What might explain the similitude between HGPS and normal aging is the expression of progerin. Indeed, activation of the $LMNA$ cryptic splice site resulted in low progerin transcripts levels in skin fibroblasts from healthy aged individuals. Those aged cells presented with an accumulation of nuclear abnormalities comparable to HGPS cells, including nuclear structure deformation, reduced levels of HP1 and H3K9me3, and increased DNA damage.\textsuperscript{169} Interestingly, blocking of the $LMNA$ cryptic splice site in those aged cells resulted in the reversibility of the aforementioned nuclear defects.\textsuperscript{169} This discovery was further supported by other studies that demonstrated the presence of progerin at the protein level\textsuperscript{60,170}. Progerin expression in primary human dermal fibroblasts led to mitotic defects associated with HGPS, and altogether, these findings reinforced the idea that HGPS and normal aging may have a shared cellular and molecular core.\textsuperscript{60} Indications of progerin expression and accumulation in \textit{vitro} led to the screening of a few tissues from healthy individuals of various ages. Thus, it was demonstrated that progerin is indeed produced in distinct skin regions from aged individuals. In this study, progerin mRNA levels were established as stable among the different tissue samples, whereas the protein was found to accumulate with age.\textsuperscript{170} Additional \textit{in vitro} analysis of unaffected dermal fibroblasts also confirmed the existence of progerin transcripts in very small amount and provided evidence for increased progerin mRNA in late-passage cells.\textsuperscript{171} Similarly, it was suggested that the toxic protein is also present during aging in arteries from healthy individuals, and particularly in the adventitial layer. In this case, the authors claimed positive detection of progerin-expressing cells as a punctate cytoplasmic staining.\textsuperscript{96} As a whole, these data ascertain the presence of progerin in healthy aged cells; still, the consequences of progerin production in just a few cells or overall at low levels, on normal aging remain unclear and under study.
1.6.2 HGPS to study CKD-related accelerated vascular aging

One may wonder what links HGPS to CKD. As a starter, both diseases are somewhat related to aging, HGPS being a premature aging disorder and CKD being an age-associated disease. But most strikingly, they share a common accelerated vascular aging phenotype. Briefly and as mentioned earlier, patients can present vessel plaques and vascular stiffness accompanied by inflammation in both cases. Vascular damages are characterized by thickened intima, media calcification and loss of VSMCs, and fibrotic adventitia (Figure 8).

![Figure 8. Shared vascular phenotypes and pathologies between HGPS & CKD. Characteristic features of the vascular phenotype and pathology of HGPS patients are similarly reproduced in CKD patients.](image)

Of note, lamin A and prelamin A were previously found associated with vascular calcification in nephrectomized rats and VSMCs cultured under calcifying conditions\(^ {172}\). Prior to this study, the precursor prelamin A was already described as accumulating \textit{in vitro} and \textit{in vivo} in calcifying human VSMCs\(^ {173,174}\). This accumulation was suggested to partake in persistent DNA damage, by inhibiting DDR activation, thereby promoting VSMCs osteogenic differentiation and activating senescence and the SASP, in turn contributing to vascular calcification\(^ {173,175,176}\). Besides, progerin was found expressed in arteries from healthy aged individuals, its expression increasing with aging\(^ {96}\). More recently, arterial stiffness and plaque formation were associated with progerin-expressing VSMCs in mice, and endoplasmic reticulum stress was proposed as an underlying mechanism of atherosclerosis\(^ {98,101,120}\). Altogether, these reports imply a possible role for progerin in accelerated vascular aging. The resemblance in the vasculatures of HGPS and CKD patients indicates that perhaps progerin is expressed in CKD arteries and might contribute to tissue pathology.
2. AIMS OF THE THESIS

This thesis aims at increasing the current understanding of the cellular and molecular mechanisms underlying progerin expression in the context of HGPS and more common age-associated diseases, in order to develop potential therapeutic strategies.

The specific aims were:

**Paper I**
- To understand how progerin accumulation results in depletion of the adult stem cell population and impaired tissue homeostasis.

**Paper II**
- To analyze whether expression of progerin in a low frequency of cells of the adipose tissue contributes to tissue pathology during physiological aging.

**Paper III**
- To analyze whether the progerin protein contributes to CKD-related accelerated vascular aging, and if present, to determine the cause for its expression.

**Paper IV**
- To investigate whether telomere dysfunction in HGPS results in the accumulation of telomeric non-coding RNAs.
- To evaluate the potential of telomeric sequence-specific antisense oligonucleotides as a novel treatment strategy for HGPS.
3. RESEARCH APPROACH

3.1 In vitro models of HGPS

In vitro systems are useful to answer biological questions, and for instance, they can be used to study the effect of a protein overexpression or silencing, but also to assess various treatment strategies. Several in vitro models of HGPS were used throughout the different papers.

Both papers I and IV used primary human dermal fibroblasts from HGPS patients and controls. These fibroblasts are a widely used model in progeria research given their physiologically relevant expression of progerin. In our case, HGPS patients cells were manipulated to better grasp the mechanisms underlying progerin-associated disrupted tissue homeostasis and telomere dysfunction. Paper II employed the 3T3-L1 cell line, which is derived from murine 3T3 cells. These cells are commonly used in the adipose tissue biology research field, as they are capable of differentiating into cells phenotypically resembling adipocytes. In our experimental setup, 3T3-L1 cells were transfected with various expression vectors, thus overexpressing lamin A, progerin or both in order to understand the contributions of either of these proteins to preadipocytes proliferation and differentiation. Finally, paper IV utilized human skin fibroblasts (BJ cell line) constitutively overexpressing lamin A and progerin, and doxycycline-inducible normal dermal fibroblasts that allowed for a tunable expression of progerin and lamin A in a dose-dependent manner. These particular cell models were used to further assess progerin-associated telomere dysfunction. Of note, we also manipulated an in vitro system in paper III. Human-derived primary aortic vascular smooth muscle cells were grown and supplemented with uremic serum to mimic a CKD environment and determine whether the c.1824C>T mutation could be induced by uremic surroundings.

Despite being very useful organic tools, in vitro models are not complex enough systems to entirely reflect the biology of living organisms, as they do not take into account the systemic environment. Some regulatory mechanisms occurring via paracrine and/or endocrine signaling cannot take place in Petri dishes; therefore in vivo animal models are important complements to in vitro systems.

3.2 Mouse models of HGPS

3.2.1 Conditional mouse models

In order to study HGPS and aging, our laboratory developed several mouse models based on a tetracycline-inducible system: the Tet-Off system. The Tet-Off system is a binary transgenic system that consists of a target gene downstream of a tetracycline responsive promoter element (tetop), and a transactivator (tTA) downstream of a tissue-specific promoter. This conditional model allows for the control of transgenic expression temporally by adding doxycycline, a tetracycline derivative, and spatially with the use of a tissue-specific promoter (Figure 9).
Thus, this kind of system not only gives the possibility to study transgenic expression in a given tissue, but also gives the chance to study the possibility of disease reversal by silencing the protein expression after the appearance and progression of a phenotype.

![Diagram of Tet-Off system](image)

**Figure 9. Conditional HGPS mice based on the Tet-Off system.** In the Tet-Off system, transcription of the target gene is regulated by the administration of doxycycline (Dox). In the absence of doxycycline, proteins are encoded by the transactivator (tTA), which is under the control of a tissue-specific promoter (here: NSE promoter), and will bind to the tetracycline responsive promoter element (tetop) located upstream of a target gene (here: human LMNA minigene), thus inducing its transcription. The LMNA minigene contains the coding regions of lamin A and the 1824C>T mutation. Its transcription results in the synthesis of the human lamin A, human progerin and GFP proteins.

In our mouse systems, the target gene corresponds to a human LMNA minigene, which consists of exons 1-11, intron 11 and exon 12. Exon 11 carries the most common HGPS mutation (LMNA c.1824C>T, p.G608G). This minigene allows for the production of both human lamin A and progerin, which is more physiologically relevant since the mutation is present in the heterozygous form in HGPS patients and does not create a perfect splice site (meaning that progerin is not always spliced from the mutant allele). This sequence is followed by an internal ribosomal entry site (IRES), allowing for the independent translation of enhanced green fluorescent protein (eGFP) located downstream of the IRES. At its end, the minigene includes a SV40/polyA tail facilitating mRNA stability and translation (Figure 9).

The transactivators that were used in this thesis are the keratin 5-mediated transactivator (K5-tTA) for specific transgenic expression in the skin (Paper I and IV)\(^{143,177}\), and the neuron-specific enolase-mediated transactivator (NSE-tTA) for transgenic expression mainly in the brain but also sparsely in the adipose tissue (Paper II)\(^{80,178}\). Together these mouse models allowed for the investigation of the underlying mechanisms of progerin expression and the experimentation of a new treatment strategy.
3.2.2 Knock-in and transgenic mouse models

Several systemic mouse models have been developed to study HGPS, including:

- Lmna<sup>HG/A</sup>: knock-in mice solely expressing progerin from one of the Lmna allele and lamin A/C from the other allele. These mice recapitulate symptoms of HGPS such as bone alterations, loss of subcutaneous fat and premature death, however, they display no signs of atherosclerosis<sup>179</sup>.
- BAC G608G: mice overexpressing human lamin A and progerin. Single-copy BAC mice only display a vascular phenotype resembling the one observed in HGPS patients, without alteration of the life expectancy<sup>81</sup>.
- Zmspte24<sup>-/-</sup>: mice accumulating farnesylated prelamin A and presenting many phenotypic features of HGPS including skeletal abnormalities, progressive hair loss, muscle weakness and premature death<sup>180</sup>. Of note, deletion of ZMPSTE24 likely affects other proteins as well, in addition to prelamin A.

However, the most widely used model nowadays is the Lmna<sup>G609G</sup> knock-in mouse model as these mice ubiquitously express progerin and phenocopy most clinical manifestations of HGPS. This mouse strain was generated by replacing the wild-type mouse Lmna gene by a mutated allele carrying the c.1827C>T; p.Gly609Gly mutation<sup>73</sup>. This mutation is the murine equivalent of the human HGPS c.1824C>T; p.Gly608Gly mutation. Heterozygous mice (Lmna<sup>G609G/+</sup>) display a milder phenotype than homozygous mice (Lmna<sup>G609G/G609G</sup>) due to a slower progression of the disease. Lmna<sup>G609G/G609G</sup> mice rapidly develop pronounced phenotypic features resembling those of HGPS patients, including growth retardation, abnormal bone structure, loss of adipose tissue, cardiovascular defects and premature death<sup>73</sup>. Homozygous Lmna<sup>G609G</sup> mice were used in a competitive transplantation experiment in paper III.

3.3 From mouse to human

Mouse models are complex living organisms useful to study the basis of aging, which mechanisms may be conserved among different species. However, human specimens are central actors in the current medical and scientific advancements.

In paper II, we employed subcutaneous adipose tissue biopsies originating from healthy kidney donors undergoing surgery in order to determine progerin expression levels, while in paper III, we used human blood and arterial tissue biopsies to investigate CKD-related accelerated vascular aging. Epigastric arteries were obtained from CKD stage 5 patients (i.e. with severe kidney dysfunction) undergoing living-donor renal transplantation. Given the difficulty of the procedure, control arteries were instead obtained from individuals undergoing gallbladder removal or inguinal hernia surgery. To be considered as controls, these individuals had to present no history of cardiovascular disease.
The human population is highly heterogeneous, and there are a number of confounding factors that may influence the research data. Our sample set was composed of both male and female patients from various ages with different causes for disease onset and progression, and receiving diverse treatment options.

3.4 How to study HGPS & age-associated disorders?

There are no particular methods to study HGPS and age-associated diseases. In this thesis, bright-field and confocal microscopy are the primary tools used, as they were suited for pathological examination, and spatial analysis and quantification of proteins and transcripts within specific regions of a tissue. Hence, molecular biology techniques including immunohistochemistry, in situ hybridization and immunofluorescence were broadly used first and foremost to appreciate progerin expression, but also to visualize specific aging-associated defects such as DNA damage, senescence, inflammation or telomere dysfunction. One of the major advantages of immunostainings is the possibility of multiplexing, i.e. to combine several antibodies to detect multiple proteins at once. However, co-stainings are limited by the antibodies host species and in the case of tissue immunofluorescence, by the number of microscope lasers used to excite the secondary antibodies fluorophores. That is the reason why, in some cases, FACS sorting was performed to look at gene or protein expression in specific cell types. In other instances, protein detection by an antibody was not sensitive enough, and other methods targeting DNA or mRNA were preferred. Thus, progerin expression in human tissues or SASP levels were occasionally detected by droplet digital PCR (ddPCR) or quantitative RT-PCR. In the same manner, to get a better understanding of pathways affected by progerin expression, RNA sequencing methods, expression arrays and bioinformatics analysis were applied.
4. RESULTS & DISCUSSION

4.1 Paper I. Accumulation of progerin affects the symmetry of cell division and is associated with impaired Wnt signaling and the mislocalization of nuclear envelope proteins.

Accumulation of progerin is known to ultimately result in tissue dysfunction. However, how cellular and molecular defects contribute to the disruption of tissue homeostasis remains unclear. In an attempt to elucidate such mechanisms, we hypothesized that the symmetry of stem cell division is altered, thus participating in the impaired tissue growth and renewal generally observed in progeria-like conditions (Figure 10).

![Figure 10. Graphical abstract of Paper I.](image)

Examination of postnatal day 4 HGPS mouse skin revealed defects in cell division, characterized by an increase in symmetric cell division and proliferation compared to wild-type mice. Associated with the mislocalization of the nuclear envelope proteins emerin and nesprin-2, the Wnt/β-catenin signaling pathway was found disrupted, which argued for its potential involvement in the observed aberrant cell division process in HGPS.

**Study design**

In this study, we took advantage of a conditional HGPS mouse model, defined by tissue-specific expression of the most common HGPS mutation (LMNA c.1824C>T) and leading to halted skin development. Skin samples were collected at embryonic day 18.5 (E18.5) and postnatal day 4 (PD4) from both HGPS and littermate control mice, in order to assess the symmetry of stem cell division with progressive accumulation of progerin. The epidermal stem cell population was characterized by transcriptomics, and affected pathways such as
proliferation and Wnt signaling were further validated, either in vivo or in patient and control fibroblasts. Levels of associated NE proteins were likewise assessed to try to get a global explanation of the processes leading to impaired skin homeostasis.

**Progerin accumulation favors symmetric cell division**

It has previously been demonstrated that asymmetric cell division promotes stratification and differentiation of mammalian skin, thus generating both the basal and suprabasal layers during development\textsuperscript{101}. Since our HGPS mice present epidermal hyperplasia, we first assessed the symmetry of cell division of basal epidermal stem cells. Immunofluorescence of the mitotic spindles revealed that as progerin accumulated from E18.5 to PD4 in HGPS skin, up to 66.7% of epidermal stem cells underwent symmetric division, whereas wild-type skin showed a similar distribution of asymmetric vs. symmetric cell divisions. These in vivo data are in accordance with previous in vitro results, where progerin overexpression in neural stem cells led to increased intrinsic symmetric cell division\textsuperscript{181}. Our data suggest that keratinocytes present an aberrant cell division process contributing to the impaired homeostasis observed in these HGPS mice.

**Impaired stem cell function in HGPS skin**

Since the symmetry of cell division relies on the ability of stem cells to divide, we next investigated stem cell impairment primarily in the basal layer of PD4 mouse epidermis by RNA sequencing. A total of 308 genes were found to display a significant change in gene expression between HGPS and wild-type keratinocytes. In agreement with the observed phenotype, gene ontology analysis revealed that processes such as epidermal development or regulation of cell differentiation were affected. We then applied previously described gene expression patterns to our sequencing dataset in order to determine which particular lineages of proliferative keratinocytes were affected. Indeed, there have been more than one type of stem/progenitor cells described in the skin, and several models have emerged, attempting to explain epidermal maintenance\textsuperscript{182-185}. However, these are still debated. Nevertheless, following the two stem-cell population model based on label-retaining cells (LRCs) and non-LRCs\textsuperscript{185}, we found expression patterns associated with LRCs to be especially downregulated in HGPS interfollicular epidermis. Similarly, expression patterns of hair follicle stem cells and transit-amplifying stem cells were also downregulated in HGPS hair follicles\textsuperscript{183}. These apparent gene expression repressions were further validated by expression array and in situ hybridization against keratin 15, a marker of hair follicle stem cells. Interestingly, keratin 15 was previously shown to be associated with non-homeostatic and hyperproliferative conditions\textsuperscript{186-188}. Transcriptomic analysis of genes associated with quiescence and proliferation suggested a more proliferative state in HGPS keratinocytes compared to wild-types. Similarly, at the protein level, HGPS skin presented an increased frequency of cells positive for the mitotic marker PHH3. But the balance between proliferation and differentiation is essential in skin homeostasis, and therefore, we considered expression changes in epigenetic modifiers responsible for the regulation of stemness and cell-commitment genes. Our data revealed that the epigenetic proliferation/differentiation switch was not affected by progerin accumulation in our progeroid mouse model, as supported by another study where markers of epidermal
terminal differentiation were found expressed in these same mice. However, this appears to be in contradiction with previous data from Shumaker et al., which indicated that the epigenetic mark EZH2, a known regulator of keratinocytes proliferation and differentiation, was downregulated in HGPS patient cells. Nevertheless, our data suggest that stem cells are dysfunctional and favor proliferation under progerin expression, likely resulting in disrupted skin homeostasis.

**Altered Wnt/β-catenin signaling pathway in HGPS**

To uncover the underlying pathway responsible for the shift in symmetric cell division in HGPS skin, we analyzed our RNA sequencing data using Wikipathway. This indicated the Wingless/Integrated (Wnt) signaling as being the most significantly affected pathway in progerin-expressing keratinocytes. In comparison to Notch and Hedgehog signaling pathways, which together with Wnt, are involved in the proper maintenance of stem cell equilibrium in the skin, Wnt appeared as the most significantly downregulated pathway in HGPS basal keratinocytes. Wnt signaling has been characterized as both canonical and non-canonical pathways, and more importantly, the canonical Wnt/β-catenin pathway was previously associated with the regulation of epidermal stem cell renewal. We found this pathway to be affected in both patient fibroblasts and PD4 HGPS skin, with nuclear β-catenin levels being diminished compared to controls. We attempted a rescue experiment, in which Wnt3a was supplemented to patient and control fibroblasts over a 24-hour time course. Nuclear β-catenin was found to increase over time as measured by Western blot and immunofluorescence, and the expression levels of the Wnt target gene Axin2 were likewise increased after stimulation. Despite the positive effect of such in vitro treatment, both β-catenin and Axin2 levels remained lower in HGPS compared to control cells. Of note, low Wnt signaling has been associated with symmetric cell division in developing hair follicles, thus underlining its plausible involvement in the phenotype observed in our progeroid mice.

**NE proteins mislocalization in progerin-expressing cells**

β-catenin reduction could potentially be explained by altered nuclear transportation. Indeed, the NE protein emerin was suggested to be involved in β-catenin exportation from the nucleus, and we found emerin levels to be significantly increased in our HGPS mice keratinocytes at PD4, thus suggestive of a more active exportation. But emerin is a direct interactor of lamin A, and to exclude the possibility that its accumulation at the nuclear rim results from the overexpression of both progerin and lamin A, we analyzed its levels in the skin of mice overexpressing lamin A only. The fact that emerin levels were similar to that observed in wild-type mice suggested that its accumulation was a direct consequence of progerin expression. In addition, the LINC complex protein nesprin-2 was also found to be important for the nuclear translocation of β-catenin. Interestingly, nesprin-2 levels were significantly reduced at the nuclear periphery in HGPS patients cells. This is in agreement with a previous report from Arsenovic and colleagues. This phenomenon could potentially be explained by progerin interaction with SUN1, the direct binding partner of nesprin-2, which was found to result in SUN1 aggregation. Since the diffusion of nesprins was observed upon the disruption of the SUN1-nesprin interaction, it could be inferred that the...
interaction between progerin and SUN1 reduces the binding affinity of SUN1 for nesprin-2, thus resulting in nesprin-2 mislocalization. Altogether, our results combined with previous studies suggest that the observed nuclear loss of β-catenin in progeroid keratinocytes and fibroblasts may be the outcome of overactive exportation of β-catenin by emerin or inert translocation by nesprin-2. It is worth noting that a previous report showed that overexpression of nesprin-2 benefitted HGPS mice. Furthermore, it is acknowledged that changes in the NE during cell division greatly contribute to the control of cell cycle progression, and it could therefore be hypothesized that the NE remodeling occurring with progerin expression may impact the proliferative state of cells. Emerin and nesprin-2 were found otherwise implicated in cell polarity, further supporting a role for these proteins in cell division and migration, and implying their contribution to the extensive symmetric cell division detected in HGPS keratinocytes.

4.2 Paper II. Rare progerin-expressing preadipocytes and adipocytes contribute to tissue depletion over time

Progerin expression in an entire cell population leads to tissue damage, but can a small fraction of progerin-accumulating cells contribute to tissue pathology? Progerin has been found in various occasions in cells and tissues from healthy aged individuals, but the significance of such findings is still contested given its rare occurrence in few cells of the tissues and that it was found very lowly expressed. It would be meaningful to understand whether progerin is actually involved in the tissue degradation that occurs with aging, and if its rare expression contributes to the progression of aging-associated diseases (Figure 11). In fact, this would unveil whether progerin could be used as a tool to understand mechanisms that drive physiological aging, and whether it could be an appropriate treatment target to slow the progression of age-related tissue decline.

![Graphical abstract of Paper II](image)

**Figure 11.** Graphical abstract of Paper II. Analysis of subcutaneous white adipose tissue in aging transgenic mice showed a sustained low frequency of progerin-expressing cells. This was associated with the apparition of age-associated defects including abnormal proliferation, increased DNA damage and senescence, and systemic inflammation, suggesting that even when present in a small fraction of cells, progerin is able to contribute to tissue pathology over time. Interestingly, progerin was also found at low transcript levels in human subcutaneous fat.
**Study design**

In this study, subcutaneous adipose tissues were collected from healthy human individuals (age range: 31-66 years) to evaluate the presence of progerin at the RNA level. Subsequently, we used a humanized progeroid mouse model engineered to express both lamin A and progerin under the control of the enolase promoter, which results in expression of the mutation in several tissues including the brain, skin, bone, heart and the adipose tissue\(^\text{80,92}\). These mice were referred to as rare progerin-expressing (RPE) mice. Previous gross examination of these mice revealed reduced body weights and fat depletion at 90 weeks of age\(^\text{80}\). Various organs were collected from 30-, 60- and 90-week-old RPE and control mice. Hypothalami and brainstem were used to assess progerin expression and pathological events, including apoptosis, and astrocytes and microglia activation. Inguinal subcutaneous white adipose tissues (sWAT) were collected for determination of cell type-specific progerin expression and phenotypical evidence of tissue damage such as aberrant proliferation, increased DNA damage, senescence, cell death and macrophage infiltration. Ectopic fat deposition was evaluated in the heart, liver and skeletal muscle from RPE and control mice, and sera were collected for measures of metabolic disturbances and systemic inflammation. In vitro systems were generated to further assess the detrimental characteristics of progerin expression, including proliferation and differentiation capacity of progenitor cells.

**Low levels of progerin in healthy human sWAT**

Since progerin was previously found in apparently healthy human aged cells, skin and arteries\(^\text{60,96,160-171}\), we thought to investigate whether it could also be found in human adipose tissue. Analysis of lamin A and progerin transcripts revealed that there were about 2.26 copies of the progerin transcript per 1000 copies of the lamin A transcript. This ratio of progerin to lamin A transcripts was 114.77-fold lower than that of a HGPS patient dermal fibroblasts. Such low transcript levels of progerin could not be detected at the protein level by immunostaining, probably because of the sensitivity of the antibody.

**Adipose tissue depletion with aging in RPE mice**

As our RPE mice were previously found to display a loss of subcutaneous adipose tissue\(^\text{80}\), we then investigated whether other fat depots were affected. We found that among the epididymal and interscapular brown adipose tissues, the RPE subcutaneous white adipose tissue was the most affected with aging, its proportions in 90-week-old mice being significantly smaller than that of age-matched controls and of 60-week-old RPE mice. Scanning electron microscopy revealed that 90-week-old RPE sWAT exhibited mild fibrosis and occasional loss of adipocytes in addition to smaller adipocytes compared to control sWAT as revealed by histological analysis, all of which inferred functional impairment of the tissue\(^\text{203}\). We then assessed metabolic alterations by first considering ectopic fat deposition. However, the heart, liver and muscle from 90-week-old RPE mice did not display abnormal fatty acid storage. Likewise, circulating leptin levels did not significantly differ between RPE and control mice, suggesting that sWAT metabolism was not severely disrupted despite presenting several pathological anomalies.
No involvement of the hypothalamus and brainstem in RPE mice fat depletion

Hypothalamic and brainstem neurons play a role in fat metabolism by sensing peripheral metabolic signals. They are able to control fatty acid metabolism and energy homeostasis via the autonomic nervous system and particularly the sympathetic fibers, which innervate the white adipose tissue\(^{204,205}\). Hence, given the model we were experimenting on, we studied the hypothalamus and to some extent the brainstem, in order to determine whether they were contributing to the sWAT loss observed in our RPE mice (Figure 12).

![Resting microglia](image1.png) ![Activated microglia](image2.png)

**Figure 12. No overt microglial activation.** Representative immunofluorescent staining pictures of wild-type and HGPS mice depicting microglial states (green, left: example of resting microglia, right: example of activated microglia). Analysis revealed no particular activation of microglia in the brainstem of 90-week-old HGPS mice compared to wild-types.

Progerin was not found expressed in neurons composing the hypothalamus of 60-week-old mice and pathological analysis did not reveal signs of satellitosis. Further examination by immunostainings did not show evidence of aberrant caspase 3-mediated apoptosis, nor of astrocytes activation in RPE mice compared to wild-type. Similarly, in 90-week-old brainstems, IBA1 staining showed comparable frequencies of activated microglia between RPE and control mice (Figure 12). Altogether, these findings suggested that central nervous system defects were not directly accountable for the observed loss of sWAT in our RPE mice.

**Rare progerin-expressing preadipocytes and adipocytes**

The central nervous system being apparently uninvolved in the observed sWAT loss, we then investigated potential mechanisms leading to such deficiency focusing on progerin expression directly in the sWAT. But first, we tested whether overexpression of lamin A contributed to the phenotype. Using a mouse overexpressing human lamin A only under the control of the enolase promoter, we found that the body weights, as well as the hypodermal adipose layer, were not affected in aged mice suggesting that progerin might be responsible for sWAT depletion in our RPE mice. Detection of human lamin A indicated a low frequency and distribution of the protein in the lamin A overexpressing mice skin. Hence, we proceeded to a progerin immunostaining in 30-, 60- and 90-week-old RPE mice, which
showed a sustained expression with aging, represented by low frequencies of progerin-positive cells ranging from 4.4% to 6.7%. The adipose tissue being composed of multiple cell types, we isolated the stromal vascular fraction in order to identify which of the major cell populations were expressing progerin. We found that only preadipocytes were positive for progerin and that they represented less than 1.17% of the total stromal vascular fraction. This indicated that the cell populations corresponding to the low frequency of progerin-expressing cells in RPE sWAT were preadipocytes and mature adipocytes. It should be noted that a previous study conducted on mosaic Zmpste24-deficient mice showed that accumulation of unprocessed prelamin A in about 40-60% of the cells of multiple organs did not lead to pathological alterations or premature death. This suggested that the development of prelamin A-induced premature aging most likely result from cell-extrinsic mechanisms. But we decided to test whether progerin is directly involved in the observed sWAT decline, and so we next assessed various marks of cellular aging and progeria.

**Increased cellular and molecular defects over time in RPE sWAT**

In human adipose tissue, hyperplasia is negatively correlated with cellular hypertrophy. Adipocytes being smaller in size in RPE sWAT than in controls, we started by checking proliferation. As expected, proliferation was increased in 30-week-old RPE sWAT compared to wild-type. This is consistent with other reports showing that progerin-expressing cells undergo a hyperproliferative state before plateauing and entering senescence. Similarly, proliferation was assessed in vitro, and we found that although only 5.15% of mouse preadipocytes were transfected with a vector conferring progerin expression, proliferation was significantly increased compared to cells transfected with an empty vector. Previous studies have indicated that the terminal differentiation process that gives rise to mature adipocytes was impaired upon progerin expression in various cell systems. When testing the differentiation capacity in our in vitro model, we did not observe obvious microscopic changes between the different groups. Nevertheless, analysis of differentiation regulators showed that C/EBPα and LPL were downregulated in preadipocytes expressing both lamin A and progerin. Similar trends were observed in progerin-expressing cells. However, not all of the tested markers of differentiation were affected, which suggests that terminal differentiation might only slightly be altered in our conditions.

Increased proliferation is known to contribute to cell cycle slowdown or even cell cycle arrest, which can lead to cellular senescence. We performed gene expression analysis of various markers of senescence and SASP, which resulted in a shift towards higher senescence levels in 30-week-old RPE sWAT. This was further emphasized in 90-week-old mice where p16-positive cells were found significantly increased in RPE sWAT. It was also demonstrated that persistent DNA damage, another trigger of cellular senescence, was involved in the fat decline of a premature aging mouse model. Furthermore, persistent DNA damage was also found in in vitro systems of HGPS. Analysis of DNA DSBs showed a higher frequency of γH2AX-positive cells in RPE sWAT at 60 weeks. Interestingly at both 60 and 90 weeks, the number of cells presenting elevated number γH2AX foci was significantly higher in RPE sWAT than controls.
Apoptosis can be triggered by DNA damage but also by hyperproliferation\textsuperscript{207,211,212}. We observed increased caspase-3 independent cell death in 90-weeks-old RPE sWAT compared to controls. Since adipocyte death attracts phagocytic macrophages, we performed a F4/80 staining to detect macrophage infiltration in 60-week-old sWAT. This revealed a 2-fold increase in positively stained cells in RPE sWAT. This was further confirmed by transmitted electron microscopy in 90-week-old RPE sWAT. Systemic inflammation may occur with adipose tissue depletion as the tissue produces pro-inflammatory cytokines and adipokines capable of activating immune responses\textsuperscript{40,213,214}. Systemic inflammation was found activated in RPE mice, with increased circulating levels of leukocytes and pro-inflammatory cytokines in 90-week-old mice. Taken together, all the defects present in our RPE mice sWAT (cell cycle deficiency, genomic instability, senescence, inflammation) overlap with common hallmarks of aging and HGPS\textsuperscript{1,61}, suggesting that even present in only a small fraction of cells, progerin might still be able to contribute to tissue pathology and be implicated in normal aging.

4.3 Paper III. The Hutchinson-Gilford progeria syndrome mutation, \textit{LMNA} c.1824C>T, is a somatic mutation in patients with chronic kidney disease.

It appears that only a few progerin-expressing cells in a murine tissue may be capable of contributing to tissue pathology with aging. Even though progerin was present in a small cell fraction, its levels may not be physiologically relevant in the context of human aging. Hence, we asked the question of whether progerin is expressed and involved in human tissue pathology in aging-associated diseases. The fact that HGPS and CKD patients present an almost identical vascular phenotype led us to investigate the potential association of progerin with human early vascular aging (Figure 13).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{graphical_abstract.png}
\caption{Graphical abstract of Paper III. In this study, epigastric arteries from CKD patients were scrutinized for the presence of progerin. Progerin was found expressed at the transcript and protein levels, in 70% of the patients and in up to 7% of the cells of the arteries, which was associated with activated DNA damage. Further analysis revealed that the most common HGPS mutation, \textit{LMNA} c.1824C>T, was responsible for the observed progerin expression.}
\end{figure}
Study design
Arteries were collected from CKD patients and controls, and classified into different calcification grades. Cell density was assessed along with the frequency of progerin positive cells in the tissue. Age-associated features such as proliferation, senescence and DNA damage were measured in those arteries. Arterial and blood DNA from CKD patients and controls was used to determine the somatic mutation in the *LMNA* gene that leads to progerin expression. Estimation of the effect of uremic serum was performed *in vitro* on human VSMCs, and the regenerative capacity of progerin-expressing stem cells was assessed by *in vivo* competitive transplantation of *Lmna*<sup>G609G/G609G</sup> and wild-type hematopoietic stem cells into wild-type mice recipients.

Progerin expression in CKD arteries
To determine the age-related vascular decline in our sample set, we assessed medial calcification and VSMC loss, two characteristics commonly observed in HGPS patients. Medial calcification was found correlated with increased CKD patient age, while reduced medial cell density was mostly seen in arteries displaying moderate to extensive vascular calcification. Surprisingly, screening for the presence of progerin at the protein level in 40 CKD arteries revealed that 70% of the patients displayed a small frequency of progerin-expressing cells in arterial media, forming clusters in some instances (ranging from 0.2 to 7.4%). Contrastingly, only one out of six control arteries showed a low frequency of progerin positive cells (representing 0.2%), which is in agreement with previously reported data<sup>96</sup>. As prelamin A was previously found involved in accelerated vascular aging<sup>173,174</sup>, we tested the specificity of our antibody to make sure that we were not assessing prelamin A instead of progerin. Western blot analysis confirmed the specific affinity of our antibody to progerin. In addition, prelamin A staining of the CKD arteries did not uncover the presence of prelamin A expressing cells. Further investigation of progerin expression showed that progerin transcripts could be detected by ddPCR in all CKD arteries tested but was not apparent in control arteries. Progerin transcripts were even found in CKD arteries that did not display a positive staining for the protein, suggesting that our antibody detection of progerin may not be sensitive enough to uncover all expressing cells with low levels.

To identify the mechanism responsible for such progerin expression in CKD, we analyzed the DNA of a control and 27 CKD arteries for the presence of the c.1824C>T mutation. The variant was detected in 16 of the patients’ samples, among which 13 were already found positive for progerin expression at the protein and/or RNA level. The control sample also carried the mutation and corresponded to the sample with 0.2% of progerin positive cells. In total, progerin was found at the DNA, RNA and/or protein levels in 85% of the CKD arteries (34/40) and 12.5% of the control arteries (1/8). Taken together, we have established that progerin is expressed in CKD arteries at low levels and in a small fraction of medial cells as a result of sporadic induction of the *LMNA* c.1824C>T.
Activated DNA damage and moderate senescence in CKD arteries

In an attempt to estimate the potential contribution of progerin expression to the CKD-related vascular decline, we then analyzed several cellular and molecular defects commonly associated with progerin expression. We did not find increased levels of caspase 3-mediated cell death nor any correlation between shortened telomere and increased frequency of progerin-positive cells. Analysis of senescence and SASP markers revealed a mild activation of vascular and systemic senescence. On the contrary, DNA damage was found to be increased. Analysis of the DDR markers ATR and 53BP1 showed a significant positive correlation between the increased number of progerin-positive cells and activated DDR signaling, thus suggesting that progerin expression may impact the levels of DNA damage occurring in CKD arteries, similarly consistent with previous reports demonstrating that prelamin A accumulation encourage persistent DNA damage in accelerated vascular aging\textsuperscript{173}.

Vascular regeneration as a mechanism of increased progerin expression?

After assessing progerin expression and subsequent associated defects in CKD arteries, two questions remained. How is the \textit{LMNA} c.1824C>T mutation generated? Where do these cells originate? In order to establish how the variant can be induced in the medial layer of CKD arteries, we performed an \textit{in vitro} experiment on human VSMCs, our hypothesis being that the CKD uremic environment might be capable of engendering such specific mutation. While cells presented an increased number of DNA damage foci after uremic treatment, the \textit{LMNA} c.1824C>T mutation was not detected. Strangely, the variant was observed in VSMCs before and after treatment with normal serum, suggesting that the uremic environment is not able to induce the mutation but rather negatively affect cells carrying that mutation. Our second hypothesis was that the \textit{LMNA} c.1824C>T mutation is present in VSMCs progenitors that repopulate the tissue upon vascular damage. As previous studies have demonstrated that VSMCs can proliferate under vascular damage conditions\textsuperscript{215,216}, we next addressed the proliferation status of CKD arteries. Proliferating cells were found in the medial and/or adventitial layers of nine CKD arteries out of 11, which suggested an increase in vascular regeneration, in agreement with extensive tissue damage. However, there have been debates in regard to what type of cells actually have the capacity to proliferate in arteries. Whether they are VSMC progenitors arising from the adventitial layer, dedifferentiating VSMCs or blood-circulating progenitors still remains unclear\textsuperscript{217-219}. We next assessed the presence of the \textit{LMNA} c.1824C>T mutation in blood from CKD patients and control, and found a significant increase in its fractional abundance in CKD \textit{vs.} control blood. Progerin-expressing progenitor cell functionality and ability to propagate in a tissue were then evaluated by competitive \textit{in vivo} transplantation of hematopoietic stem cells. This experiment revealed a significant increase in the initial proliferation of progeroid cells. This is in agreement with previous studies that have shown that the expression of progerin induces increased proliferation\textsuperscript{143,207}. Additionally, this established that progerin-expressing transplanted cells do not undergo senescence even after an extended time but rather are functional and able to repopulate the hematopoietic system. Taken together, our data suggest that the mutation was present in VSMCs progenitor cells from the vascular wall, which were expanded and propagated as a result of the vascular regeneration that occurred following vascular damage in CKD.
4.4 Paper IV. Sequence-specific inhibition of DNA damage response at telomeres improves the detrimental phenotypes of Hutchinson-Gilford progeria syndrome.

There are a plethora of treatment strategies for HGPS that researchers have developed over the years. However, some remain at the research lab or preclinical stage and have not been assessed in the general human population, while others may have undesirable secondary effects or would probably barely improve HGPS symptoms. As of today, there is no perfect treatment for HGPS; the current FTI strategy moderately ameliorating the health and life expectancy of patients. Until a cure is found, there is a need to discover and test novel treatment strategies. Here, we hypothesized that targeting telomere dysfunction in HGPS may rescue the phenotype and potentially extend the lifespan (Figure 14).

![Graphical abstract of Paper IV.](image)

Figure 14. Graphical abstract of Paper IV. Analysis of postnatal day 6 HGPS mice skin showed telomere dysfunction characterized by the production of telomeric non-coding RNAs resulting in DDR activation. Treating HGPS mice with telomere-specific antisense oligonucleotides (tASOs) improved the telomere dysfunction phenotype and reduced the DDR. This positively impacted cellular senescence and the proliferative capacity of keratinocytes. Most strikingly, tASOs treatment extended the maximum lifespan of HGPS mice by 44%.

**Study design**

In this study, we first made the use of various *in vitro* systems expressing progerin at different levels. Telomere dysfunction was assessed, along with the consequences of DDR inhibition at telomeres using telomere-specific antisense oligonucleotides (tASOs). We then took advantage of a severe conditional HGPS mouse model with epidermal progerin expression. To determine the impact of tASOs treatment *in vivo*, pregnant mice were injected systemically at E17.5 and newborn pups received additional tASOs intraperitoneally every three days, starting at PD2. Pup body weights, external phenotype and lifespan were...
recorded. Skin samples were collected at distinct ages and subsequently analyzed for telomere dysfunction. Effects of the tASOs treatment were measured in PD6 skin samples, including telomere dysfunction induced foci (TIFs), DDR signaling, senescence, proliferation, stemness, cell death and inflammation.

**In vitro assessment of telomere dysfunction and inhibition of telomeric DDR by tASOs**

Telomere dysfunction induces the transcription of telomeric non-coding RNAs (tncRNAs), which are essential for DDR activation and recruitment of DDR factors. Here, we first showed that these tncRNAs are accumulated in progerin-expressing cells compared to control cells. Indeed, progerin expression triggered the production of both G-rich (teloG) and C-rich (teloC) strands of tncRNAs, accompanied by an increased number of TIFs. Inhibition of telomeric DDR using tASOs complementary to either strand of tncRNAs (i.e. anti-teloC and anti-teloG) rescued the number of TIFs in progerin-expressing cells, down to the levels observed in control cells. This phenomenon was followed by an improved proliferation rate and lower senescence, without altering progerin levels. These data indicated that telomeric DDR signaling is involved in the senescence and proliferative defects commonly associated with progerin expression. Hence, targeting telomere dysfunction with tASOs may be beneficial to HGPS patients, but such assumptions needed further testing.

**tASOs-specific inhibition of telomeric DDR in HGPS mice skin**

To determine whether telomeric DDR signaling is relevant to HGPS *in vivo*, we assessed the levels of tncRNAs in both HGPS and control mice skin. Both the G-rich and C-rich strands of tncRNAs were found to be significantly increased in HGPS skin. Analysis post-tASOs treatment revealed a significant decrease in TIFs in PD6 HGPS mice treated with anti-teloG or anti-teloC, while TIFs were found elevated in HGPS *vs.* wild-type mice receiving control ASOs. In a similar fashion, DDR factors (pKAP1 and 53BP1) were overall significantly reduced, particularly in anti-teloG-treated HGPS mice. Together, these findings show that telomere dysfunction leads to telomeric DDR activation *in vivo*, which can be drastically inhibited by telomeric sequence-specific ASOs.

**Improved healthspan in HGPS mice upon tASOs treatment**

We then analyzed proliferation and senescence to determine whether these could be rescued by tASOs treatment *in vivo*. Indeed, as demonstrated in paper I, these HGPS mice display elevated numbers of proliferative keratinocytes compared to wild-types, contributing to the observed disrupted tissue homeostasis. However, the pathological proliferation that occurs in the progeroid suprabasal epidermal layer was rescued upon anti-teloG treatment. P16-associated senescence was also found increased in the basal layer of HGPS skin but was significantly reduced by anti-teloG treatment. Together, these data implied an ameliorated tissue homeostasis. We therefore checked for improvements of pathological characteristics of these HGPS mice. Globally, tASOs treatments recovered both epidermal and dermal thickness, and anti-teloG treatment was particularly efficient in reducing epidermal hyperplasia and keratinocyte atypia. In addition, tASOs-treated HGPS mice presented a more preserved stem cell pool than control ASO-treated HGPS mice. *In situ* hybridization revealed a significant reduction of keratin 15 levels in HGPS *vs.* wild-type mice, whereas these levels
were found increased by 7-fold upon anti-teloG treatment of HGPS mice. Similarly, TUNEL assay showed less evidence of cell apoptosis/necrosis in tASOs-treated HGPS mice. Hence, these results support the fact that treatment with tASOs leads to a healthier tissue in HGPS. To further support those results, we sought for signs of inflammation and found a significantly reduced number of dermal pan-leukocytes upon tASOs treatment compared to control ASO-treated HGPS mice. Likewise, in situ hybridization and qRT-PCR analyses of inflammatory cytokines showed a significant decrease in IL-1α and IL8. IL-1α being localized mostly at the epidermal granular layer and IL8 in the dermis. Taken together, all these findings argue for an overall beneficial effect of telomeric DDR inhibition.

**Lifespan extension of HGPS mice**

As the mice were being treated with the different ASOs, body weights were recorded and showed no unusual weight loss, suggesting an absence of toxicity from the administration and that the treatment was well tolerated. Macroscopic assessment of the external phenotype showed that anti-teloG-treated mice presented with a better-preserved hair growth visible from PD8 in comparison to control-treated mice. As previously described, these HGPS mice present a severe phenotype resulting in a median survival of 8 days and a maximum premature death at PD9105. HGPS mice treated with anti-teloG had a significantly improved survival, with an increase of the median lifespan by 24% and of the maximum lifespan by 44%. This was remarkable given the severity of the model. HGPS mice treated with anti-teloC, on the other hand, did not show any change in survival. Indeed, anti-teloC treatment appeared to have a milder effect than anti-teloG treatment on all of the characteristics assessed, which is most likely due to a suboptimal biodistribution of anti-teloC in the tissue.
5. CONCLUSIONS & PERSPECTIVES

Paper I

- We successfully demonstrated that progerin accumulation results in an aberrant shift towards symmetric cell division in vivo.
- Progerin expression was associated with impaired stem cell function characterized by increased proliferation, but also with disrupted canonical Wnt/β-catenin signaling characterized by reduced β-catenin levels both in patient fibroblasts and a progeroid mouse model.
- Accumulation of emerin at the nuclear rim and decreased levels of nesprin-2 were found upon progerin expression in in vitro and in vivo systems.

In summary, we propose a model in which progerin accumulation indirectly alters the proper localization of nesprin-2 and leads to emerin accumulation via their potential interaction at the NE, which eventually disrupts the Wnt/β-catenin signaling pathway. This, in turn, may lead to an abnormal cell division process characterized by cell polarity bias and proliferation increase, thus disrupting epidermal homeostasis and ultimately resulting in stem cell exhaustion.

All those mechanisms might be intricately related to each other, and our evidence fit with previous reports. However, there persist some gray areas to explain how progerin directly triggers such events. Does progerin interact with and/or sequester emerin, therefore leading to its accumulation at the nuclear rim? Does progerin interaction with SUN1 proteins disrupt the LINC complex, which could explain nesprin-2 loss at the nuclear rim? Is the nuclear transportation of β-catenin really affected by emerin accumulation coupled to nesprin-2 reduction? Could the non-canonical Wnt signaling, i.e. planar cell polarity pathway, also be involved in the observed impaired cell division? Is the gene expression downregulation observed in LRCs responsible for the increased proliferation of basal keratinocytes, or are other stem cells affected too? There remain plenty of questions to be thoroughly investigated in order to fully understand what are the downstream effects of progerin expression. It is by answering those questions and many other cellular and molecular-related questions that we will truly grasp the disease mode of action, which will allow scientists to develop better treatments for HGPS.

Paper II

- We have shown that progerin is present at very low levels in the adipose tissue of healthy individuals.
- Several of the cellular and molecular hallmarks of HGPS and aging were found replicated in the adipose tissue of our RPE mice, including disrupted cell cycle, increased DNA damage and cellular senescence, and systemic inflammation.
- We associated rare progerin-expressing cells with tissue decline over time.
In summary, we propose a hypothetical model to clarify how a low frequency of progerin-expressing cells may lead to adipose tissue decline over time. Hence, persistent exposure to a small number of progerin-expressing cells initially provokes aberrant preadipocyte proliferation, which in turn is partly responsible for abnormal cellular development and senescence. Senescence is further activated as DNA damage accumulates, and propagates to neighboring cells in a paracrine manner through activation of the SASP phenotype, all of which ends up triggering cell death and thereby encourages macrophage infiltration. The immune response is ultimately activated by the pro-inflammatory environment of the adipose tissue, resulting in systemic inflammation.

Our findings suggest that progerin expression in a small fraction of cells may participate in aging-associated pathologies. To fully understand whether a low frequency of progerin cells matters in the context of healthy aging, and if it contributes to aging-associated pathologies, it would be of interest to create a mosaic animal model. This could be achieved by using CRISPR/Cas9-based single nucleotide editing. Indeed, it was previously shown that injection of the base editor into rabbit pronuclear stage embryos to induce the HGPS mutation resulted in prematurely aged rabbits expressing progerin and displaying the typical HGPS phenotype. Thus, neonatal injection of the base editor in mice could result in low efficiency of base conversion and therefore in a low frequency of progerin expressing cells in various tissues. Using a specific virus serotype would also allow for tissue-specific editing. A tissue-specific mosaic model of progerin expression would be of great use, allowing researchers to understand which tissues are particularly affected by progerin expression, starting with the cardiovascular system where progerin has previously been found during aging.

Paper III

- We have established that progerin is expressed in a low frequency of cells present in the medial layer of CKD arteries.
- The presence of progerin expression is caused by the most common HGPS mutation $LMNA$ c.1824C>T.
- Progerin expression correlates with a DNA damage phenotype.
- Progerin expression may become more enriched by vascular regeneration, via proliferation and expansion of progenitor cells that carry the most common HGPS mutation.

It is the first time that progerin expression is identified in CKD arteries at the RNA and protein levels resulting from the induction of the $LMNA$ c.1824C>T variant. Nevertheless, the investigation of progerin’s contribution to accelerated vascular decline should be deepened. Indeed, we have demonstrated in this study that there seems to be an unusual proliferation ongoing in CKD arteries, but it is unclear if the proliferating cells are also expressing progerin, therefore, participating in clonal expansion of the progerin cell pool. The transplantation experiment seemed to show a competitive advantage of progerin-expressing hematopoietic cells, however, the relevance of such findings to vascular cells may be
contested and a more appropriate model and experimental setup could be used. In 2013, Liu and colleagues demonstrated that prelamin A promoted VSMCs calcification\textsuperscript{173}. Accumulation of prelamin A leads to similar defects to the one observed under progerin accumulation. In addition, progerin was found in arteries during physiological aging, while other studies have shown the contribution of high levels of progerin expression to VSMC calcification and atherosclerosis\textsuperscript{96,98,100}. Hence, it would be of interest to assess whether a small fraction of progerin-expressing cells (up to 7.4\%), is able to induce vascular calcification. Finally, the use of an animal model with mosaic expression of progerin in the vasculature would also be of high significance in order to establish a role for progerin in the premature vascular aging observed in CKD.

Paper IV

- We have established that telomere dysfunction in \textit{in vitro} and \textit{in vivo} models of HGPS results in DDR activation via the production of tncRNAs.
- Treatment with telomeric sequence-specific ASOs is able to repress the DDR signaling and reduce the number of telomere dysfunction induced foci \textit{in vitro} and \textit{in vivo}.
- tASOs treatments are not only able to ameliorate both the senescent and defective proliferation phenotypes, but also to improve cell death and reduce tissue inflammation to some extent.
- Anti-teloG treatment led to an astounding survival extension of HGPS mice.

Hence, targeting telomere dysfunction appears as a promising approach to combat the detrimental effect of progerin expression. ASOs have proven to be a successful tool used in the treatment of various diseases. Some were previously designed and tested in HGPS, where the effects were also found beneficial\textsuperscript{73,138}. Many ASOs are currently in advanced clinical trials, while others are already FDA approved. Since telomeric DDR is activated at dysfunctional telomeres most often during aging and cancer, it could therefore be assumed that tissue delivery would not be a problem, unlike other drugs, and that once administered, tASOs would only affect unhealthy cells.

Future perspectives

The road is long, but we might be closer to a cure for HGPS than we can imagine. With the constant development of scientific tools, research advances have grown substantially. Among the last technologies that recently emerged, base editing stands out as a potential candidate gene therapy for HGPS. Indeed, base editing is a new alternative to genome editing, allowing for the modification of a single DNA or RNA nucleotide, without creating double-stranded DNA breaks and resulting in less off-target effects\textsuperscript{222}. Adenine base editing is able to convert an A\textasciitilde{}T base pair into a G\textasciitilde{}C base pair and would thus be particularly useful in the case of HGPS, in order to correct the most common point mutation, \textit{LMNA} c.1824C\textasciitilde{}T, from a thymine to a cytosine. Therefore, base editing appears like a wonderful technology for the future of HGPS. Base substitution in only 10 to 20\% of the cells might yield
encouraging results, probably considerably slowing down the progression of HGPS and increasing the life expectancy of patients. Tailoring the delivery system to efficiently target specific organs responsible for the most lethal aspects of the disease such as the cardiovascular system, may help to attain such goals.

In the meantime, we have shown that the use of antisense oligonucleotides targeting telomere dysfunction is able to substantially improve the healthspan and lifespan of a severe progeroid mouse model. Such promising treatment should be tested in other available mouse models such as the systemic Lmna<sup>G609G</sup> mice to further assess its potential. But tASOs therapy may not only be useful for ameliorating HGPS condition, but could also potentially be applied to other age-related pathologies with telomere deficiency. The fact that CKD and HGPS patients have a common vascular phenotype characterized by early vascular aging, and that we found the LMNA c.1824C>T mutation and progerin accumulation in CKD arteries, suggests that current HGPS therapies could potentially be applied to this disease. Actually, FTIs, which are commonly used to treat HGPS patients, were previously found to significantly inhibit calcification in vascular smooth muscle cells<sup>223</sup>. Besides, we have shown that not only progerin is able to disrupt tissue homeostasis when overexpressed but also that a few progerin positive cells in a tissue could lead to detrimental pathology with aging. In addition, it is known that progerin mostly affects tissues such as skin, bones, adipose tissue and the cardiovascular system. Thus, screening for progerin expression and its associated mutation in age-associated diseases characterized by deficits in those tissues, such as osteoporosis or other cardiovascular diseases, could possibly open new paths for novel treatment strategies of more common disorders.
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7. REFERENCES


44. Liu, J. et al. (2003). MAN1 and emerin have overlapping function(s) essential for chromosome segregation and cell division in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences*, 100:4598-4603.


129. Richards, S. A. et al. (2011). The accumulation of un-repairable DNA damage in laminopathy progeria fibroblasts is caused by ROS generation and is prevented by treatment with N-acetyl cysteine. *Human molecular genetics*, **20**:3997-4004.


202. EMBL-EBI (2019). Expression atlas. Available at: https://www.ebi.ac.uk/gxa/home


