The Progeria Research Foundation
11th International Scientific Workshop
Poster Abstracts

#1

Abstract Poster

Title: Patient Survey: COVID-19 Infection and Vaccination in Children and Young Adults with Progeria

Author(s): Kelsey Tuminelli, MS[1]; Justine Maestranzi, BA[1]; Wendy E. Norris, MS[2]; Leslie B. Gordon, MD, PhD[1,2,3]

Institution: [1]The Progeria Research Foundation (PRF), [2]Hasbro Children's Hospital, [3]Warren Alpert Medical School of Brown University

Introduction:
COVID-19 began to spread in late 2019 and remains highly prevalent, still leading to morbidity and mortality throughout the world. Those living with comorbidities, including Progeria and its associated heart disease, are thought to be more susceptible to COVID-19 symptoms and mortality. This study aims to survey the Progeria community to evaluate its members' COVID-19 infection and recovery rates, levels of concern, vaccination rates, and related themes.

Methods:
Using an IRB approved questionnaire, adults with Progeria and parents of children with Progeria were surveyed between April 5 and August 9, 2022. The survey results were evaluated using descriptive statistics, and were compared to world data to draw conclusions on how a global rare disease community may respond to a pandemic differently in comparison to the general population, and why.

Results:
A total of 83 questionnaires were completed by 13 adults with Progeria and 70 parents of children with Progeria. Those with Progeria ranged in age from 19 months to 44 years, including 50 males and 33 females. Thirty-one percent of children and adults with Progeria had contracted COVID-19 by the time of questionnaire completion, compared to an estimated 44% of the world's population having contracted COVID-19 by mid-November, 2021, 5 months earlier. The peak in COVID-19 case numbers for the Progeria community occurred in January 2022, which is mirrored by world data. The known COVID-19 mortality rate in the Progeria community is 2.1%, compared to the current 1.1% global mortality rate. At the beginning of the pandemic, 70% of questionnaire respondents identified as "very concerned" about COVID-19 exposure and infection, with their top cited reason being the belief that members of the Progeria community are in extra danger due to their Progeria and related health conditions. Seventy percent of respondents identified with a decreased level of concern at the time of survey completion. Thirty-five percent of children and adults with Progeria were fully vaccinated, compared to 58.6% of the world's population. Of the 60% unvaccinated (n = 50/83), 48% did not have access to vaccinations due to age requirements or place of residence (n = 24/50), and 52% (n = 26/50) remained unvaccinated for other reasons, which we explored in more detail.

Conclusions:
COVID-19 infection rates were lower than expected based on world population data. The Progeria community may have better protected itself against COVID-19 risk factors due to the majority's belief that having Progeria put them at a greater risk of COVID infection and negative prognosis. The COVID-19 induced mortality rate was almost twice as high in the Progeria community as in the general population, reinforcing the need for cautionary behaviors and vaccinations for this subpopulation. This may be due to the Progeria community's vaccination rate being about half that of the world population, in addition to this population's higher risk factors. Stronger and more widely distributed messaging around the benefits of vaccination could help this population to overcome fears and doubts around vaccination. Data collected in this survey will help PRF to respond effectively to Progeria community members seeking guidance around COVID-19 prevention, care, and vaccination in the future.

#2

Abstract Poster

Title: PRF by the Numbers: The Progeria Research Foundation Programs

Author(s): Justine Maestranzi, BA[1]; Kelsey Tuminelli, MS[1]; Wendy E. Norris, MS[2]; Christina Sollecito, BS[1]; Leslie B. Gordon, MD, PhD[1,2,3]

Institution: ***[1]The Progeria Research Foundation, [2]Hasbro Children's Hospital, [3]Warren Alpert Medical School of Brown University***

The mission of The Progeria Research Foundation (PRF) is to discover treatments and the cure for Hutchinson-Gilford Progeria Syndrome and its aging related disorders, including heart disease.

PRF By the Numbers is an online data sharing tool originating from PRF's programs and services. We take raw data collected through our programs and services, remove any personal information to protect the participant, and present it to you in a format that is engaging and informative. PRF By the Numbers is updated quarterly throughout the year to communicate longitudinal progress for the programs that support PRF's mission.

This poster highlights the longitudinal progress made through the programs and services offered by PRF, including The PRF International Registry, The PRF Diagnostics Program, The PRF Call and Tissue Bank, The PRF Medical and Research Database, PRF Research Grants Program, The Clinical Trial Funding and Co-Coordination, and The PRF Handbook Creation and Distribution.

#3a

Abstract Poster

Title: Plasma Progerin in Animal Models of Hutchinson-Gilford Progeria Syndrome

Author(s): Wendy Norris, MS[1]; Francis Collins, MD, PhD[2]; Mike Erdos, PhD[2]; Vicente Andres, PhD[3,4]; Tom Misteli, PhD[5]; Mudaiah Puttaraju, PhD[5]; Roland Foisner, PhD[6]; Kan Cao, PhD[7]; Jay Humphrey, PhD[8]; W. Ted Brown, MD, PhD[9]; Frank Rigo, PhD[10]; Frank Bennet, PhD[10]; Leslie Gordon, MD, PhD[1,11,12]

Institution: [1]Hasbro Children's Hospital, Providence, RI; [2]National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; [3]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; [4]CIBER de Enfermedades Cardiovasculares (CIBERCV), Spain; [5]National Cancer Institute, National Institutes of Health, Bethesda, MD; [6]Medical University of Vienna, Vienna, Austria; [7]University of Maryland, College Park, MD; [8]Yale University and Yale School of Medicine, New Haven, CT; [9]University of Sydney, Sydney, Australia; [10]Ionis Pharmaceuticals, Carlsbad, CA; [11]Alpert Medical School of Brown University, Providence, RI; [12]The Progeria Research Foundation, Peabody, MA

Introduction: Hutchinson-Gilford progeria syndrome (HGPS) is an ultra-rare, fatal, premature aging disease caused by the toxic protein, progerin. Recently, a sensitive, specific progerin immunoassay has been developed that detect progerin in human plasma samples. A variety of animal models of HGPS have been generated, including mouse and pig. Each model has displayed commonalities with the human disease, most importantly the cardiovascular disease that results in early death for children with HGPS. A strategy for longitudinal intra-animal progerin testing had remained lacking. This study sought to evaluate plasma progerin in various animal models of HGPS, with and without progerin-targeted treatment.

Methods: Previously stored frozen animal plasma or serum samples were contributed from Progeria scientists worldwide in a collaborative effort to understand the genotype/phenotype relationship between plasma progerin using a highly sensitive and specific progerin bioassay. Plasma progerin was measured using a single molecule counting (SMC™) immunoassay (EMD Millipore). All mouse models evaluated had a C57Bl/6 background. Only experiments that included baseline pre-therapy samples were evaluated for longitudinal effects of treatment on progerin levels.

Results: There was no significant difference in mean progerin between plasma and serum ($p<0.05$). Plasma progerin was low in C57Bl/6 control mice (0.4±0.4 ng/ml, N=11; range 0.2-1.3 ng/ml). C57Bl/6 mice that were fed a low protein or high protein diet had no detectable levels of progerin (n=4 each). High levels were detected in the G608G homozygotes (1,334±305 ng/ml, N=310; range 464-2,499 ng/ml), and heterozygotes (569 ±184 ng/ml, N=92; range 341-1,402 ng/ml); G609G homozygotes (335 ±197ng/ml, N=28; range 64-748 ng/ml), and heterozygotes (85±18 ng/ml, N=21; range 46-120 ng/ml); $Lmna^{HGPSrev/HGPSrev}$ mice (224±158 ng/ml, N=7; range 95-519 ng/ml), and TA+/VF+ endothelial-specific mice (147±73 ng/ml, N=4; range 42-206 ng/ml). Heterozygous G608G minipig plasma measured 44±21 ng/ml, (N=8; range 25-93 ng/ml), similar to human HGPS patient levels (unpublished data). Serum progerin levels in the vascular smooth muscle-specific ApoE-/-, LCS+/+, SM22Cre+/- mouse model was 30 ±10 ng/ml (N=4; range 22-44 ng/ml). A natural history study of G608G homozygotes and heterozygotes demonstrated a positive association between the age at collection and progerin levels ($p<0.0001$). Progerin-targeted treatment showed a significant decrease of progerin in some, but not all, instances.

Conclusions: A sensitive, specific progerin assay has been developed that can detect progerin levels in plasma and serum from a variety of animal models. This assay can be used as to test intra-animal progerin longitudinally in various animal models, which has not previously been possible. Due to the wide variances in baseline levels within HGPS animal models, baseline and longitudinal intra-animal samples should be obtained for progerin-targeted treatments.

#3b

Abstract Poster

Title: The Progeria Research Foundation Cell and Tissue Bank and Diagnostic Programs

Author(s): Wendy Norris, MS[1]; Kelsey Tuminelli, MS[2]; Leslie Gordon, MD, PhD[1,2,3]

Institution: [1]Hasbro Children's Hospital, Providence, RI; [2]The Progeria Research Foundation, Peabody, MA; [3]Alpert Medical School of Brown University, Providence, RI

The Progeria Research Foundation (PRF) Cell and Tissue Bank was established in 2002, with Institutional Review Board Approval from Hasbro Children's Hospital, Providence, RI. Its main goal is to stimulate research on Progeria, aging and cardiovascular disease by providing researchers worldwide with genetic and biological material from patients with Hutchinson-Gilford Progeria Syndrome (HGPS or Progeria) and their family members. Since its inception, the PRF Cell and Tissue Bank has provided cell lines and other biological material to over 223 basic science laboratories in 28 countries (133 in the United States and 90 internationally), yielding contributions to 112 peer-reviewed publications.

The PRF Cell and Tissue Bank currently provides 214 cell lines from affected children and their family members, including both immortalized and non-immortalized dermal fibroblast lines, lymphoblast lines and induced pluripotent stem cell lines. Biological materials such as urine, plasma, serum, DNA and autopsy tissue are also available for special projects and collaborations. The Bank also offers the farnesyltransferase inhibitor Zokinvy (lonafarnib) for pre-clinical studies, provided by Eiger BioPharmaceuticals.

The PRF Cell and Tissue Bank ensures sufficient availability of cells and other biological materials for research leading to a better understanding of the biologic basis for disease in Progeria, its relationships to generalized aging, discoveries leading to new treatments for children with Progeria, and most importantly, the discovery of a cure for Progeria.

The PRF Diagnostic Testing Program is a research-based Progeria testing program. It was established in 2004 to provide genetic sequence testing for Progeria-causing mutations in association with a CLIA-approved diagnostics testing facility. Eligibility for testing is determined by registration with the PRF International Registry and one or more of the following: proband phenotypic presentation, family history (prenatal testing), or a relative with a confirmed progeroid mutation. LMNA sequencing has been performed for 142 individuals with suspected progeria, leading to the diagnosis of 84 classic HGPS, 13 non-classic HGPS, 10 non-progerin producing laminopathies and 35 negative cases, some of which have been further analyzed using whole exome sequence testing in collaboration with other investigators. Testing has been performed for patients in 44 countries, many of which do not offer this type of genetic testing. The program has provided a pathway for diagnostic confirmation, helped physicians and families avoid misdiagnoses, and supported patient inclusion in Progeria clinical trials that uniformly require confirmed mutational analysis.

#4

Abstract Poster

Title: Transcriptional analysis of progeria patient derived fibroblasts suggests deficits in the mesenchymal lineage

Author(s): *Nishita Gogia,* Rebeca San Martin [1], Priyojit Das[2], Jacob T. Sanders[1,3], Ashtyn Hill[1], Rachel Patton McCord[1]*

Institution: 1 Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, 309 Ken and Blaire

Mossman Bldg. 1311 Cumberland Ave, Knoxville, TN, 37996, USA

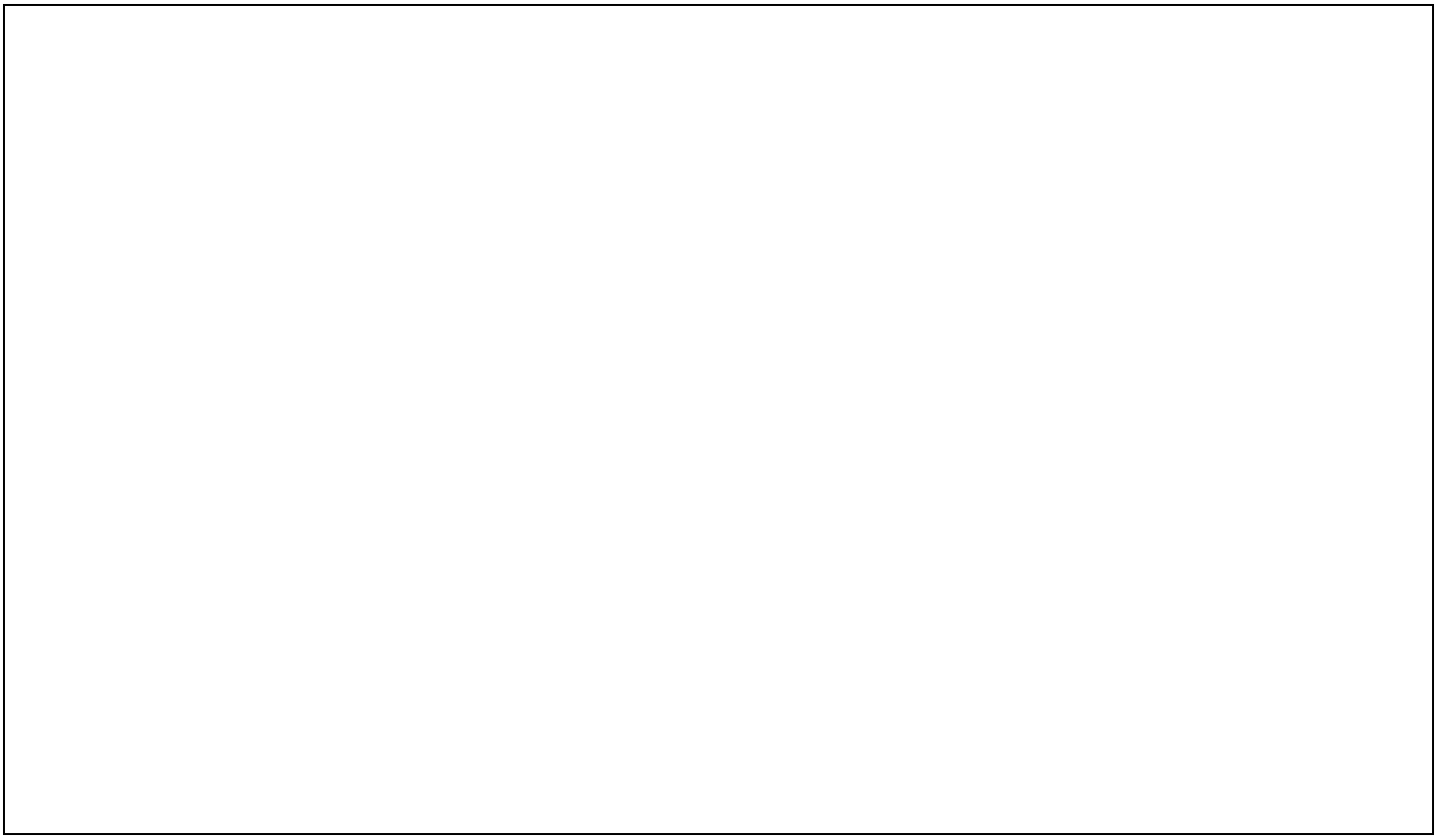
2 UT-ORNL Graduate School of Genome Science and Technology, University of Tennessee, 309 Ken and Blaire

Mossman Bldg. 1311 Cumberland Ave, Knoxville, TN, 37996, USA

3 Department of Pathology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas TX 75390, USA

*Corresponding author: rmccord@utk.edu ; (865)-974-3149

The presence of a mutant Lamin A protein in the cells of Hutchinson-Gilford Progeria Syndrome patients leads to altered genome architecture, nuclear morphology and epigenetic states which in turn leads to defects in cells and tissues of the mesenchymal lineage. Here, we report the transcriptional status of many different patient-derived HGPS fibroblasts, which in chronic pathological conditions are sentinels of the global MSC health and status. These fibroblasts carry abnormal transcriptional signatures, centering around five main functional hubs: DNA maintenance and epigenetics, bone development and homeostasis, blood vessel maturation and development, fat deposition and lipid management, and processes related to muscle growth. Transcriptional analysis of the patients within an age group of four to seven years old shows alterations in the cohort of genes related to the endochondral ossification and chondrogenic commitment. We propose that this alteration in early commitment, along with the defects described in the five functional hubs, carry developmental consequences, based on the depletion of tissue-resident and vasculature-associated MSC pools. These gene expression findings motivate us to quantify potential MSC depletion in Progeria patient fibroblasts and to evaluate their ability to differentiate into adipogenic, osteogenic and chondrogenic lineages.



#5

Abstract Poster

Title: Endothelial-to-mesenchymal transition triggered by dysfunctional vascular smooth muscle cells contributes to accelerated atherosclerosis in progeria

Author(s):

Magda R. Hamczyk[1,2,3,*], Rosa M. Nevado[1,2,*], Pilar Gonzalo[1,2], María J. Andrés-Manzano[1,2], Paula Nogales[1], Jacob F. Bentzon[1,4], Carlos López-Otín[3], Vicente Andrés[1,2,**]

*equal contribution ** corresponding author

Institution:

[1] Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain.
[2] Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Spain.
[3] Departamento de Bioquímica y Biología Molecular, Instituto Universitario de Oncología (IUOPA), Universidad de Oviedo, Spain.
[4] Department of Clinical Medicine, Aarhus University, Denmark.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare disease caused by progerin, a mutant form of lamin A. Patients show premature aging and die during childhood mainly from atherosclerosis complications. We recently found that expressing progerin in vascular smooth muscle cells (VSMCs) is sufficient to accelerate atherosclerosis and death in *Apoe*-deficient mice, at least in part through an intrinsic mechanism involving activation of endoplasmic reticulum stress in VSMCs. However, it remained to be elucidated whether VSMC-specific progerin expression also contributes to atherogenesis by provoking alterations in neighboring endothelial cells (ECs).

In this study, we analyzed EC phenotype in two atheroprone mouse models of HGPS, with systemic or VSMC-specific progerin expression. *En face* aorta immunofluorescence studies revealed altered EC shape together with augmented LDL permeability and leukocyte recruitment in both HGPS mouse models. Aortic root immunofluorescence experiments also revealed a prominent accumulation in both models of cells expressing the *bona fide* EC markers CD31, vWF, and ERG within atherosclerotic plaques. Moreover, a subset of these ECs was proliferating and expressed mesenchymal markers, such as N-cadherin and collagen III, indicating that luminal ECs in atheroma plaques of HGPS animals undergo endothelial-to-mesenchymal transition (endMT). Accordingly, in both HGPS models RT-qPCR analysis showed upregulation of *Snai1*, and to a lesser extent *Zeb2*, two transcription factors typically involved in endMT.

We next analyzed TGFβ signaling, the most common trigger of endMT. Atheroma plaques of both the ubiquitous and the VSMC-specific progeria models showed upregulation of TGFβ1 and its downstream effector pSMAD3, without changes in TGFβ2. Consistent with this, VSMC-specific progeria mice treated with the pSMAD3 inhibitor SIS3 showed reductions in leukocyte recruitment, adventitial thickening, VSMC loss, and atherosclerosis burden in the thoracic aorta. Moreover, SIS3 treatment decreased collagen III and collagen IV area and the number of CD31-positive cells in atherosclerotic plaques, suggesting that TGFβ signaling inhibition partially represses progerin-induced endMT. In summary, progerin-induced VSMC alterations promote EC dysfunction and endMT via TGFβ1/pSMAD3, identifying this signaling pathway as a candidate target for progeria treatment.

Funding: Work in the V.A. laboratory is supported by the Spanish Ministerio de Ciencia e Innovación (MCIN)/Agencia Estatal de Investigación (AEI)/10.13039/501100011033 (grant PID2019-108489RB-I00) with co-funding from the European Regional Development Fund ("A way to build Europe"), the Progeria Research Foundation (PRF) (award PRF 2019–77), and Asociación Progeria Alexandra Peraut. The CNIC is supported by the MCIN, the Instituto de Salud Carlos III, and the Pro-CNIC Foundation and is a Severo Ochoa Center of Excellence (grant CEX2020-001041-S funded by MCIN/AEI/10.13039/501100011033). R.M.N is supported by the Ministerio de Educación, Cultura y Deporte (pre-doctoral contract FPU16/05027). M.R.H. is supported by the MCIN (post-doctoral contract IJC2019-040798-I).

#6

Abstract Poster

Title: Progerin expression in endothelial cells does not provoke premature aging or atherosclerosis in atheroprone mice

Author(s):

Magda R. Hamczyk[1,2,3], Pilar Gonzalo[1,2], Rosa M. Nevado[1,2], Ricardo Villa-Bellosta[4], María J. Andrés-Manzano[1,2], Carlos López-Otín[3], Vicente Andrés[1,2,*]

* corresponding author

Institution:

[1] Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain.
[2] Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Spain.
[3] Departamento de Bioquímica y Biología Molecular, Instituto Universitario de Oncología (IUOPA), Universidad de Oviedo, Spain.
[4] Departamento de Bioquímica y Biología Molecular, Universidad de Santiago de Compostela (USC), Spain.

Atherosclerotic disease is one of the most severe clinical problems in Hutchinson-Gilford progeria syndrome (HGPS), and is the proximal cause of death in most patients. We previously showed that *Lmna*$^{G609G/G609G}$ mice with systemic progerin expression develop aggravated atherosclerotic disease when crossed to an atherogenic background (either *Apoe*$^{-/-}$ or *Ldlr*$^{-/-}$). We have also investigated the role of vascular smooth muscle cells (VSMCs) and myeloid cells in progerin-driven atherogenesis; however, the role of endothelial cells (ECs) in atherosclerosis remains unexplored in HGPS.

Here, we investigated the contribution of ECs to HGPS-related atherosclerosis by generating an atheroprone mouse model with EC-specific progerin expression induced by tamoxifen treatment. On average, 94.5% of ECs (CD31^{+}) expressed progerin in the aortas of 16-week-old *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*Cdh5-Cre*^{ERT2} mice, whereas non-ECs (CD31^{-}) in the adventitia, media, and atheroma plaque did not show substantial expression of the mutant protein, confirming specificity of the model. No progerin-positive cells were found in *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$ control mice. Heterozygous *Apoe*$^{-/-}$*Lmna*$^{LCS/+}$*Cdh5-Cre*^{ERT2} mice and homozygous *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*Cdh5-Cre*^{ERT2} mice both had body-weight and survival curves similar to their controls (*Apoe*$^{-/-}$*Lmna*$^{LCS/+}$ and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$, respectively). After eating a high-fat diet for 8 weeks, 16-week-old *Apoe*$^{-/-}$*Lmna*$^{LCS/+}$*Cdh5-Cre*^{ERT2} and *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*Cdh5-Cre*^{ERT2} mice showed no evidence of accelerated atherosclerosis as assessed by Oil Red O staining in the thoracic aorta, the most affected aortic region in progeria mouse models that express progerin ubiquitously. Likewise, EC-specific mouse models with heterozygous or homozygous progerin expression showed no alterations in any of the hematological parameters analyzed. Furthermore, the aortas of 16-week-old fat-fed *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*Cdh5-Cre*^{ERT2} mice showed no alterations to adventitial thickening, medial collagen content, medial lipid accumulation, or medial VSMC loss. Finally, leukocyte recruitment to the aortic wall was not increased in 16-week-old *Apoe*$^{-/-}$*Lmna*$^{LCS/LCS}$*Cdh5-Cre*^{ERT2} mice fed normal chow.

We thus found no aging or vascular phenotype in atheroprone mice with EC-specific progerin expression. Taken together with our previous finding that VSMC-specific progerin expression leads to accelerated atherosclerosis and death in atheroprone mice, these results indicate that the vascular alterations in HGPS originate from progerin expression in VSMCs, and not ECs.

Funding: Work in the V.A. laboratory is supported by the Spanish Ministerio de Ciencia e Innovación (MCIN)/Agencia Estatal de Investigación (AEI)/10.13039/501100011033 (grant PID2019-108489RB-I00) with co-funding from the European Regional Development Fund ("A way to build Europe"), the Progeria Research Foundation (PRF) (award PRF 2019–77), and Asociación Progeria Alexandra Peraut. The CNIC is supported by the MCIN, the Instituto de Salud Carlos III, and the Pro-CNIC Foundation and is a Severo Ochoa Center of Excellence (grant CEX2020-001041-S funded by MCIN/AEI/10.13039/501100011033). Work in the C.L.O. laboratory is supported by the European Research Council (ERC-Advanced Grant, grant number 742067). R.M.N is supported by the Ministerio de Educación, Cultura y Deporte (pre-doctoral contract FPU16/05027). M.R.H. is supported by the MCIN (post-doctoral contract IJC2019-040798-I).

#7

Abstract Poster

Title: Impact of Progerin Expression on Adipogenesis in Hutchinson-Gilford Progeria Skin-Derived Precursor Cells

Author(s): Ramona Hartinger, Farah Najdi, Peter Krüger, Felix Fenzl, Karima Djabali

Institution: Epigenetics of Aging, Department of Dermatology and Allergy, TUM School of Medicine, Technical University of Munich (TUM), Garching, Germany

Hutchinson-Gilford Progeria syndrome (HGPS) is a rare genetic disease causing premature aging symptoms similar to physiological aging, like vascular diseases, subcutaneous fat loss and lipodystrophy, loss of bone mineral density or alopecia. Mostly, HGPS is generated by a single de novo mutation in the gene encoding lamin A (LMNA) (c.1824 C>T; p.G608G). Thereby, a cryptic splice site in the exon 11 is generated, resulting in a truncated lamin A protein, named progerin. The accumulation of progerin is toxic to the cells and causes changes in cellular dynamics, nuclear dysfunction, premature senescence and apoptosis. In this study, we examined the impact of cellular senescence on adipogenesis using HGPS skin-derived precursor cells (SKPs). We analyzed the differentiation potential of SKPs isolated from pre-established human primary fibroblast cultures using recently reported low-pH stress method. To determine the influence of cellular senescence, we used fibroblast cultures with a senescence index of 5% and 30%. The differentiation efficacy of HGPS SKPs was lower compared with control SKPs. Hence, in both control and HGPS cultures, SKPs derived from late fibroblast passages (characterized by a high senescence index) exhibited reduced capability to differentiate into adipocytes. This finding indicates that increased number of senescent cells create an inflammatory milieu that negatively impact adipogenesis. To reduce inflammation, we used baricitinib, an FDA approved and selective JAK 1/2 inhibitor. Our in vitro studies show that baricitinib treatment could promote HGPS adipogenesis by delaying senescence and inflammation.

#8

Abstract Poster

Title: Cardiac abnormalities in a mouse model of HGPS rescued by inhibiting Lamin A-progerin interaction

Author(s): So-mi Kang, Bae-Hoon Kim, Bum-Joon Park

Institution: Pusan National University

Hutchinson-Gilford progeria syndrome (HGPS) is a very rare genetic disease with a premature aging syndrome including cardiovascular disease. In *in vitro* and *in vivo* HGPS models, the therapeutic drug progerinin ameliorates premature aging characteristics of HGPS, suggesting a strong drug candidate for HGPS. However, the effect of progerinin on the cardiac function has not been determined yet in $Lmna^{G609G}$ mice, HGPS model mice. This study is aimed to validate the effect of HGPS drug progerinin on improving the cardiac function and histological alterations of $Lmna^{G609G}$ mice. To do this, $Lmna^{G609G}$ mice are fed with either control diet or progerinin diet, and the cardiac function in these mice was determined at the various time points of the diet feeding period by echocardiography. Blood and tissue samples of mice were acquired for pathological analysis at the end of echocardiography. The evaluation of *in vivo* cardiac parameters by echocardiography revealed that progerinin has significant improvement in the key cardiac function indicators stroke volume, ejection fraction and fractional shortening. Daily progerinin treatment shows protective effects on maintaining elastin fibers organization and reduction of collagen deposition on aortic wall in HGPS mice. Analysis of blood serum by using proteome profiler reveals that the levels of cytokines and chemokines of HGPS mice are partially recovered as similar to the levels of wild type mice. These results demonstrate the effective improvement of cardiac function and pathologies of HGPS mice by the long-term daily treatment of progerinin and will pave the way to develop the therapeutic strategy for treatment of patients with HGPS.

#9

Abstract Poster

Title:

Author(s): Crystal Kennedy, George Truskey

Institution: Duke University

The primary pathology of the rare Hutchinson-Gilford Progeria Syndrome (HGPS) is atherosclerosis – the blockage and stiffening of arteries - leading to stroke or heart attack. The direct effect of HGPS on vasculature has been characterized and includes arterial calcification, smooth muscle cell loss from the arterial medial layer, and thickening of the outer adventitial layer. However its effect on the endothelial lining has only more recently been investigated. The endothelium is mechanosensitive layer, directly in contact with and affected by the shear stress exerted by blood flow. Healthy endothelium responds to laminar flow by aligning in the flow direction and upregulating genes such as eNOS which increase vascular tone. Endothelial dysfunction is an early event in general atherosclerosis and is characterized by impaired eNOS expression. As such we set out to examine whether there is a difference in molecular response to laminar shear stress between healthy and HGPS endothelium. To do this, healthy and HGPS induced pluripotent stem cells were differentiated to endothelial cells (viECs), cultured to confluency on glass sides and exposed to steady laminar flow in a parallel plate flow channel at a shear stress of 12 dynes/cm^2 for 24 hours. These cells and their static controls were imaged and quantified in ImageJ for alignment to flow. RNA was isolated from these cells and pair-ended sequencing was performed to determine differences in the genetic response to flow between healthy and HGPS viECs. Resulting RNA sequences were processed and analyzed for differential expression between the flow and static conditions for each cell type, using DESeq2 and the Gene Set Enrichment Analysis (GSEA) tools in R. Image analysis shows that alignment in response to 24-hour flow is impaired in HGPS viECs in comparison with healthy viECs. Differential expression analysis also shows a diminished genetic response to flow in HGPS viECs when compared with healthy viECs, with a smaller number of significantly expressed genes and a lower magnitude of gene expression change after flow in HGPS. Using GSEA to categorize significantly expressed genes, it was found that there are differences in the gene sets altered after flow between healthy and HGPS viECs. An example of this is the 'KRAS Signaling Downregulation' gene set, whose genes are uniquely over-represented in HGPS viECs after flow. ZTBT16 and TGFβ2 are 2 genes in this set that show vast upregulation after flow in HGPS, but not in healthy viECs. These genes have been implicated in eNOS reduction and fibrosis induction respectively, which are both indicators of endothelial dysfunction in response to flow. Thus far, RNAseq results show differences in the gene expression profiles of healthy and HGPS viECs when exposed to flow. This supports our hypothesis that HGPS endothelium has altered molecular responses to flow as compared with healthy endothelium, thus potentially contributing to atherogenesis in HGPS patients.

#10

Abstract Poster

Title: ***Abnormal Cortical Bone Structure and Strength in Hutchinson-Gilford Progeria Syndrome***

Author(s): Raymond J. Kreienkamp, Alicia Pendleton, Daniel J. Schiferl, Leslie B. Gordon, Catherine M. Gordon

Institution: Boston Children's Hospital

Bone strength is influenced by multiple mechanical and hormonal factors, including skeletal muscle, which produces the largest load on bone and is integral for bone health. Patients with Hutchinson-Gilford Progeria Syndrome (HGPS) exhibit reduced bone mineral density (BMD), although muscular strength is preserved, with muscle volume proportional to body mass. To analyze the muscle-bone unit in HGPS, peripheral quantitative computed tomography (pQCT) was utilized to visualize bone and muscle composition of the forearm of participants with HGPS and healthy control participants (matched for age, ancestry, and pubertal stage). Patients with HGPS had notable changes in cortical architecture of the radius and ulna at the 66% site. A blinded-analysis of bone demonstrated that HGPS patients developed "star" and "hook" shaped cortical bone, that was different in structure from healthy controls. 18% of patients with HGPS had changes in both radius and ulna cortical structure, whereas no controls had structural changes in both bones. The stress-strain index (SSI), a surrogate marker for bone strength, was then computed at these sites, which was reduced in HGPS patients. SSI can be modulated by differences in torsional forces applied to bone, suggesting that contractures or other potential compensatory movements, may drive this change in HGPS. As a result, although patients with HGPS do not appear to have an increased fracture risk, they have abnormalities in cortical bone architecture. Treatments aimed at improving disease phenotype should also monitor whether they impact restoration of normal cortical architecture.

#11

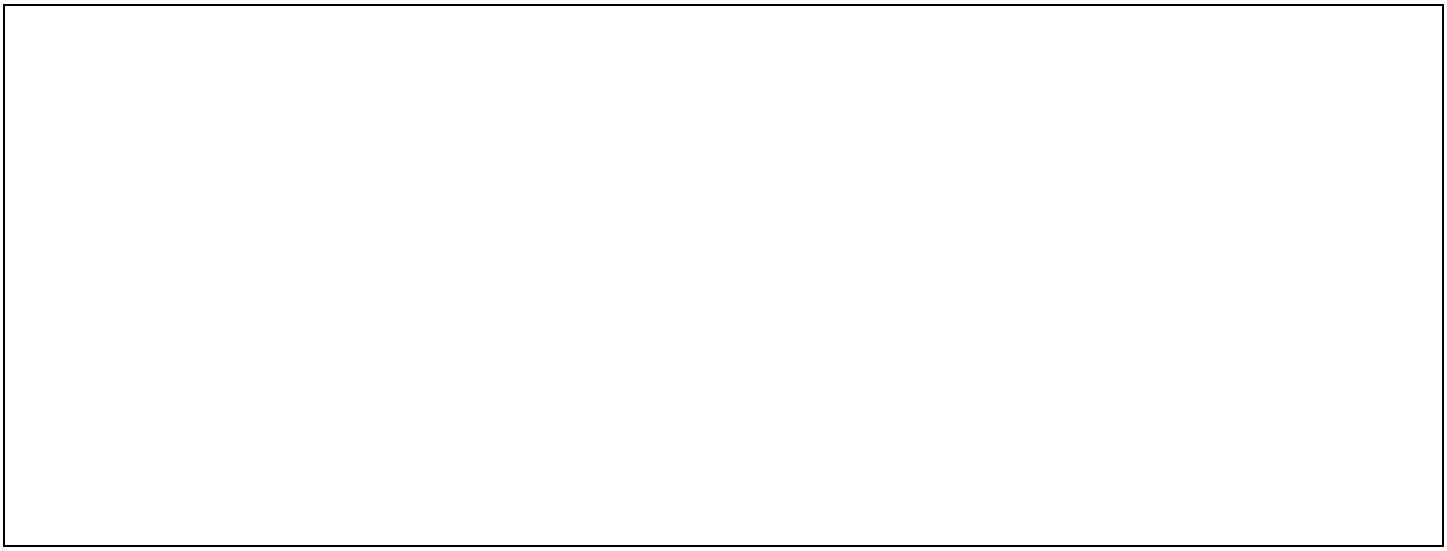
Abstract Poster

Title: Baricitinib reduces Inflammation and cellular toxicity of Lonafarnib and improves cellular homeostasis in progeria

Author(s): Peter Krüger, Rouven Arnold, Liu Chang, Denize Cagla Togan, Eva Lederer, Felix Fenzl, Ramona Hartinger, Moritz Schroll, Karima Djabali

Institution: Epigenetics of Aging, Department of Dermatology and Allergy, TUM School of Medicine, Technical University of Munich (TUM), Garching, Germany

The farnesyltransferase inhibitor (FTI) lonafarnib is FDA approved for treatment of HGPS patients. Lonafarnib ameliorates HGPS disease, however, it is not a complete cure. The inhibition of the farnesyltransferase leads to the emergence of cellular side effects including genomic instability, binucleated and donut-shaped nuclei. Hence, FTI also causes an increased frequency of cytoplasmic DNA occurrence leading to the activation of the cGAS-STING-STAT1 pathway. Recently, we showed that the JAK-STAT signaling pathway is overactivated in HGPS cells. Moreover, we confirmed that inhibition of JAK1/2-STAT1/3 signaling using an FDA approved compound baricitinib ameliorated the state of chronic inflammation and delayed senescence in HGPS cells. Importantly, lonafarnib/baricitinib combination treatment improved HGPS cellular defects over and above lonafarnib alone. To provide the preclinical data to assess the efficacy of this new combination therapy and determine whether it can be considered as a future treatment for children with HGPS, we are currently testing this medication in a mouse model of HGPS.



#12

Abstract Poster

Title: Functional significance of the progeria somatic mutation in aging and cardiovascular disorders

Author(s): P Machtel, G Revêchon, D Whisenant, M Eriksson

Institution: Department of Biosciences and Nutrition, Center for Innovative Medicine, Karolinska Institute, Huddinge, Sweden

Background:
Although progeria is a systemic disease, the most serious consequences affect the cardiovascular system. Thus, the main causes of death of HGPS patients in their early teens are cardiovascular complications. Previous data has identified progerin in non-HGPS samples and we have found the *LMNA* c.1824C>T as a somatic mutation in arteries of chronic kidney disease patients (CKD). HGPS and CKD patients have a similar vascular phenotype, which is characterized by atherosclerosis, arterial stiffness and calcification. However, functional significance of the somatic mutation in CKD remains uncertain.

Methods:
To address this, we first investigated the process of regeneration and apoptosis in the arteries of a progeria mouse model (*Lmna*$^{G609G/G609G}$)(Osorio et al. 2011). Samples from aortic arch were stained for markers of proliferation and apoptosis. Then we designed a mosaic mouse model with only a certain fraction of progeroid cells (*Lmna*$^{LCS/LCS}$) in the vascular wall (*Myh11*-CreERT2). To monitor potential propagation and clonality of this mutation, we use lineage tracing.

Results:
We have generated a mosaic mouse model with a fraction of cells that express the mouse equivalent to the common HGPS mutation and progerin. The vascular phenotype is being analyzed. Time-course evaluation of the systemic mouse model (*Lmna*$^{G609G/G609G}$) showed intensified vascular smooth muscle cell loss at 10 weeks of age accompanied by adventitia thickening, as previously described by several labs. This allows us to map the specific time points that will be applied and compared to our newly developed mosaic model.

Conclusions:
We managed to generate a mosaic mouse model of progeria. It allows us to test the functional contribution of the HGPS somatic mutation to vascular pathologies.

References:
Osorio FG, et al., Splicing-directed therapy in a new mouse model of human accelerated aging. Sci Transl Med. 2011 Oct 26;3(106):106ra107

#13

Abstract Poster

Title: Lack of evidence for a direct causal role of endothelium-specific progerin expression in HGPS-associated cardiovascular phenotype and premature death

Author(s): Ignacio Benedicto[1,2], Rosa María Carmona[2], Ana Barettino[2,3], Carla Espinós-Estévez[2], María J Andrés-Manzano[2,3], Cristina González-Gómez[2,3], Pilar Gonzalo[2,3], Beatriz Dorado[2,3], Vicente Andrés[2,3].

Institution: [1]Universidad Complutense de Madrid, Madrid, Spain; [2]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; [3]CIBER en Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder caused by a mutation in the *LMNA* gene that results in the synthesis of an aberrant protein called progerin, which provokes accelerated aging and dramatically reduces lifespan. The most clinically relevant feature of HGPS is the development of severe cardiovascular alterations, including massive loss of vascular smooth muscle cells (VSMCs), vessel stiffening, vascular calcification and fibrosis, and generalized atherosclerosis, as well as electrical, structural, and functional anomalies in the heart. As a result, most HGPS patients die of myocardial infarction, heart failure, or stroke in their mid-teens. Solid evidence from mouse models indicates that progerin expression in VSMCs plays a key role in vascular alterations and reduced lifespan in HGPS. However, information is scarce about whether progerin expression in endothelial cells (ECs) has a direct causal role in HGPS. To tackle this issue, we generated mouse models with EC-specific progerin expression and suppression, the latter together with lamin A restoration. Mice with EC-specific progerin expression did not develop heart fibrosis compared with progerin-free controls and showed normal cardiac electrical and functional properties, body weight, and lifespan. We also found that mice with progerin suppression and lamin A restoration only in ECs exhibited the HGPS-associated cardiac electrical and functional alterations characteristic of ubiquitous progerin expression, and did not show improved body weight and survival. Moreover, excessive atherosclerosis burden was undistinguishable between mice with ubiquitous progerin expression and those with EC-restricted progerin suppression and lamin A restoration. In marked contrast, suppressing progerin and restoring lamin A in VSMCs was sufficient to reduce atherosclerosis burden to the low level seen in control mice lacking progerin. Our mouse studies thus strongly suggest that progerin expression in ECs is not a direct cause of the HGPS-associated cardiovascular phenotype and premature death, and that progerin suppression only in ECs does not ameliorate cardiovascular pathology and fails to prolong survival, thus reinforcing the notion that VSMC dysfunction and loss are major drivers of HGPS-related vascular pathology and associated premature death. Future strategies to treat HGPS through gene editing or RNA technologies should consider focusing on eliminating progerin expression in VSMCs rather than in ECs.

Funding: Work in the V.A. laboratory is supported by the Spanish Ministerio de Ciencia e Innovación (MCIN)/Agencia Estatal de Investigación (AEI)/10.13039/501100011033 (grant PID2019-108489RB-I00) with co-funding from the European Regional Development Fund (“A way to build Europe”), the Progeria Research Foundation (PRF) (award PRF 2019–77), and Asociación Progeria Alexandra Peraut. The CNIC is supported by the MCIN, the Instituto de Salud Carlos III,

and the Pro-CNIC Foundation and is a Severo Ochoa Center of Excellence (grant CEX2020-001041-S funded by MCIN/AEI/10.13039/501100011033). A.B. is supported by the Ministerio de Ministerio de Ciencia, Innovación y Universidades (predoctoral contract BES-2017-079705), C.E.-E. by Fundación "la Caixa" (grant LCF/BQ/DR19/1170012), and I.B. by the Comunidad de Madrid (grants 2017-T1/BMD-5247 and 2021-5A/BMD-20944).

#14

Abstract Poster

Title: Defining disease-relevant transcriptional profiles at the single-cell resolution for treatment of premature vascular aging

Author(s): Lara G. Merino[1*], Gwladys Revêchon[1*], Daniel Whisenant[1], Santhilal Subhash[1], Giuseppe Mocci[2], Christer Betsholtz[2], Maria Eriksson[1]

Institution: **1.** Department of Biosciences and Nutrition, Karolinska Institutet **2.** Department of Medicine, Karolinska Institutet

[*]**Shared first authorship**

Background: The average life expectancy of HGPS patients is 14.6 years, with the main cause of death being myocardial infarction or stroke. Detailed analysis of the vascular pathology of HGPS patients has shown progressive vascular degeneration concurrent with disease progression. Diseased arteries display vascular smooth cell (VSMC) loss, adventitial fibrosis, accumulation of proteoglycans and elastic tissue fragmentation. We aim to describe how the cellular composition of the vasculature changes as HGPS develops at single-cell resolution to identify vascular cell subsets particularly susceptible to premature aging. We also intend to explore the role of long non-coding RNAs (lncRNAS) in HGPS pathophysiology.

Experimental approach. We used *Lmna*$^{G609G/G609G}$ mice, which are engineered to express the murine equivalent of the human *LMNA* c.1824C>T mutation. We performed single-cell RNA-sequencing (scRNA-seq) on cells derived from the aortic arch of *Lmna*$^{G609G/G609G}$ and WT mice. Mice of different ages (6, 10 and 12 weeks) were included to study progressive changes in the vasculature parallel to HGPS development. Additionally, *Lmna*$^{G609G/G609G}$*/Acta2-GFP* mice were used to enrich for aortic arch VSMCs and perform scRNA-seq specifically in these cells, due to their extensive loss in HGPS.

Results: We have observed that *Lmna*$^{G609G/G609G}$ mice progressively accumulate a subset of stressed VSMC. Furthermore, fibroblasts from these mice acquire chondrogenic and inflammatory features.

Conclusion. We have shown that the cellular composition of the vascular wall changes with HGPS progression in the *Lmna*$^{G609G/G609G}$ mice. These alterations could underlie the vascular degeneration observed in HGPS. Further analysis of this data will allow the detection of genes driving this phenotype, as well as the role of long non-coding RNAs in HGPS pathophysiology. Overall, this strategy can lead to the identification of new potential therapeutic targets for HGPS treatment.

#15

Abstract Poster

Title: Progerin suppression and lamin A restoration in adipose tissue reduces lipodystrophy, ameliorates vascular alterations, and extends lifespan in progeroid mice

Author(s): Carla Espinós-Estévez[1], Cristina González-Gómez[1,2], María González-Amor[1,2], Pilar Gonzalo[1,2], María J. Andrés-Manzano[1,2], Víctor Fanjul[1], Álvaro Macías[1], David Mendoza[1], Beatriz Dorado[1,2], Ignacio Benedicto[1,4], Vicente Andrés[1,2]

Institution: [1]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; [2]CIBER en Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain.

Hutchinson-Gilford progeria syndrome (HGPS, or progeria) is an extremely rare genetic disease caused by the expression of a mutant variant of prelamin A called progerin. The disease is characterized by cardiometabolic disorders and premature aging and death. There is no cure for HGPS, and the natural history of the disease remains ill-defined. Lipodystrophy is severe in HGPS animal models and patients; however, its possible role in triggering progerin-dependent cardiometabolic alterations and premature death remains largely unexplored.

To tackle this question, we crossed *Lmna*$^{HGPSrev/HGPSrev}$ mice ubiquitously expressing progerin *(HGPSrev)*[1] with *FABP4Cre*$^{tg+/wt}$ mice expressing Cre recombinase in preadipocytes and their descendents[2]; the resulting *HGPSrev-FABP4Cre* mice express progerin ubiquitously except in fat depots, where progerin is suppressed and lamin A expression restored. qPCR and immunofluorescence analysis confirmed adipose–tissue-specific progerin suppression and lamin A restoration in *HGPSrev-FABP4Cre* mice, which showed normal body weight up to ≈70 weeks of age; partial improvement in the content of total, perivascular, and subcutaneous fat; and a 37% increase in median lifespan (74.6 weeks versus 54.4 weeks in *HGPSrev* mice, $p<0.0001$).

Histological studies in *HGPSrev* mice (with ubiquitous progerin expression) revealed a number of alterations in perigonadal and inguinal white adipose tissue that were absent or partially prevented in *HGPSrev-FABP4Cre* mice: collagen deposition; higher numbers of adipocytes/mm^2; and reduced adipocyte area, perimeter, and Feret diameter. Interestingly, lifespan extension in *HGPSrev-FABP4Cre* mice was not accompanied by amelioration of the electrocardiographic alterations observed in *HGPSrev* mice, but correlated with a partial improvement in aortic alterations despite abundant progerin expression in aorta.

Our results suggest that lipodystrophy contributes significantly to HGPS through an imbalance in the secretion of adipokines by damaged adipocytes, and that adipose–tissue-specific progerin suppression is sufficient to lessen lipodystrophy, leading to reduced vascular damage and extended lifespan. We are currently performing transcriptomic and metabolomic studies to shed light on the mechanisms underlying the cross-talk between adipose tissue and the cardiovascular system in HGPS and to identify potential therapeutic targets.

REFERENCES

1. Sánchez-López, A. *et al.* Cardiovascular progerin suppression and lamin A restoration rescue Hutchinson-Gilford progeria syndrome. *Circulation* **144**, 1777–1794 (2021).
2. He, W. *et al.* Adipose-specific peroxisome proliferator-activated receptor γ knockout causes insulin resistance in fat and liver but not in muscle. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 15712–15717 (2003).

Funding: Work in the V.A. laboratory is supported by the Spanish Ministerio de Ciencia e Innovación (MCIN)/Agencia Estatal de Investigación (AEI)/10.13039/501100011033 (grant PID2019-108489RB-I00) with co-funding from the European Regional Development Fund ("A way to build Europe"), Fundació Marató TV3 (grant 38/C/2020), the Progeria Research Foundation (PRF) (award PRF 2019–77), and Asociación Progeria Alexandra Peraut. The CNIC is supported by the MCIN, the Instituto de Salud Carlos III, and the Pro-CNIC Foundation and is a Severo Ochoa Center of Excellence (grant CEX2020-001041-S funded by MCIN/AEI/10.13039/501100011033). C.E.-E. is supported by Fundación "la Caixa" (grant LCF/BQ/DR19/1170012), and I.B. by the Comunidad de Madrid (grants 2017-T1/BMD-5247 and 2021-5A/BMD-20944).

#16

Abstract Poster

Title: Abolishing the prelamin A ZMPSTE24 cleavage site leads to progeroid phenotypes with near-normal longevity in mice

Author(s): Yuexia Wang[a,b], Khurts Shilagardi[c], Trunee Hsu[d,e], Kamsi O. Odinammadu[c], Takamitsu Maruyama[f,1], Wei Wu[a,b], Chyuan-Sheng Lin[b,g], Christopher B. Damoci[g], Eric D. Spear[c], Ji-Yeon Shin[a,b], Wei Hsu[e,1,2], **Susan Michaelis*[c,3]**, and Howard J. Worman*[a,b,3] (*Co-Corresponding authors)

Institution: [a]Dept. of Medicine, Vagelos College of Physicians and Surgeons, Columbia Univ., New York, NY; [b]Dept. of Pathology and Cell Biology, Vagelos College of Physicians and Surgeons, Columbia Univ., New York, NY; [c]Dept. of Cell Biology, Johns Hopkins Univ. School of Medicine, Baltimore, MD; [d]Case Western Reserve Univ., Cleveland, OH; [e]Dept. of Biomedical Genetics, Univ. of Rochester Medical Center, Rochester, NY; [f]Dept of Dentistry, Univ. of Rochester Medical Center, Rochester, NY

Prelamin A is a farnesylated precursor of lamin A, a nuclear lamina protein. Accumulation of the farnesylated prelamin A variant progerin, with an internal deletion including its processing site, causes HGPS. Loss of function mutations in *ZMPSTE24*, which encodes the prelamin A processing enzyme, lead to accumulation of full-length farnesylated prelamin A and cause related progeroid disorders. **Some data suggest that prelamin A also accumulates with physiological aging**. *Zmpste24*$^{-/-}$ mice die young, at ~20 weeks**. Because ZMPSTE24 has functions in addition to prelamin A processing, we generated a mouse model to examine effects solely due to the presence of permanently farnesylated prelamin A**. These mice have an L648R amino acid substitution in prelamin A that blocks ZMPSTE24-catalyzed processing to lamin A. The *Lmna*$^{L648R/L648R}$ mice express only prelamin and no mature protein. Notably, nearly all survive to 65-70 weeks, with approximately 40% of male and 75% of female *Lmna*$^{L648R/L648R}$ mice having near-normal lifespans of 90 weeks (almost 2 years). Starting at ~10 weeks of age, Lmna$^{L648R/L648R}$ mice of both sexes have lower body masses than controls. By ~20-30 weeks of age, they exhibit detectable cranial, mandibular and dental defects similar to those observed in *Zmpste24*$^{-/-}$ mice, and have decreased vertebral bone density compared to age- and sex-matched controls. Cultured embryonic fibroblasts from *Lmna*$^{L648R/L648R}$ mice have aberrant nuclear morphology that is reversible by treatment with a protein farnesyltransferase inhibitor. **These novel mice provide a model to study the effects of farnesylated prelamin A during physiological aging.**

#17

Abstract Poster

Title: The farnesyltransferase inhibitor lonafarnib improves nuclear morphology in ZMPSTE24-deficient patient cells with the progeroid disorder MAD-B.

Author(s): Kamsi Odinammadu, Khurts Shilagardi, and Susan Michaelis.

Institution: Department of Cell Biology, The Johns Hopkins School of Medicine, Baltimore, MD 21205

Several related progeroid disorders are due to defective post-translational processing of prelamin A, the precursor of the nuclear scaffold protein lamin A, encoded by *LMNA*. Prelamin A is first farnesylated at its C-terminus and subsequently this farnesylated C-terminal segment is cleaved off by the zinc metalloprotease ZMPSTE24. The premature aging disorder Hutchinson Gilford progeria syndrome (HGPS) and a related progeroid disease, mandibuloacral dysplasia (MAD-B), are due to mutations in *LMNA* and *ZMPSTE24*, respectively, that result in failure to process and accumulate permanently farnesylated prelamin A. The farnesyl transferase inhibitor (FTI) lonafarnib is known to correct the aberrant nuclear morphology of HGPS patient cells and improves the health-span and life-span in children with HGPS. Importantly, and in contrast to a previous report, we show here that FTI treatment also improves the aberrant nuclear phenotypes in MAD-B patient cells with a mutation in *ZMPSTE24*. We also present evidence that, as expected, patient cells with *LMNA* mutations that alter residues at a distance from the ZMPSTE24 cleavage site are proficient in prelamin A processing, and their nuclear morphology defects do not improve with FTI treatment. Additionally, we examine for the first time, prelamin A processing in fibroblasts from two patients with the laminopathy mutation *LMNA-R644C*. Despite the proximity of residue R644 to the prelamin A cleavage site between Y646 and L647, neither patient cell line shows a prelamin A processing defect, and both have normal nuclear morphology. This work clarifies the processing status in a variety of laminopathy patient cells and supports the hypothesis that MAD-B patients that are prelamin A processing-deficient due to a *ZMPSTE24* mutation may benefit from FTI treatment.

#18

Abstract Poster

Title: Somatic mutations in progeria models

Author(s): Miguel Araujo-Voces, Samuel Pis and Victor Quesada

Institution: Departamento de Bioquímica y Biología Molecular, Universidad de Oviedo (Spain)

Among the nine hallmarks of aging, genomic instability stands out as a causal event with irreversible consequences for cells. Stochastic somatic mutations are also very difficult to detect as most of them affect only one cell. Somatic mutations affecting pluripotent cells, and therefore their descendant cells, may be more physiologically relevant. If cells accumulate genomic damage in progeric individuals, this might set a theoretical limit to life expectancy that would not be treated with conventional therapies. To study this trait, we have compared the rate of somatic mutation in organoids derived from single progenitor cells from bone marrow, colon crypts and lungs in *Zmpste24*$^{-/-}$ mouse models of progeria compared to those of their healthy littermates. Our results suggest that the mutational rates of both progeric and wild-type mice in the same tissues are very similar throughout the life expectancy of progeric mice. Likewise, the mutational patterns in both groups are similar, and consistent with the expected accumulation of genomic damage through aging. These results suggests that genomic instability is not a bottleneck in the life expectancy of progeric mice.

#19

Abstract Poster

Title:Improving the quality of life in progeria: a first trial in the murine Lmna $^{G609G/G609G}$ model

Author(s):Schena Elisa, Stefano Squarzoni, Elisabetta Mattioli,Costanza Bonfini, Cristina Capanni, Catia Barboni, Federico Parenti, Anna Zaghini, Giovanna Lattanzi.

Institution:

Hutchinson–Gilford progeria syndrome (HGPS) causes premature aging in children, with adipose tissue, skin and bone deterioration, and cardiovascular impairment. The quality of HGPS patients' life is compromised under many aspects requiring continuous and skillful effort to be managed and overcome. The final goal of this project is identifying a therapeutic strategy able to improve the quality of life in progeria. Recently, we demonstrated that inhibition of interleukin-6 activity by Tocilizumab, a neutralizing antibody raised against interleukin-6 receptors, counteracts progeroid features in both HGPS fibroblasts and *Lmna*$^{G609G/G609G}$ progeroid mice and extends the life span of *Lmna*$^{G609G/G609G}$ progeroid mice. We had also noticed that locomotor activity was preserved, while skin and hair deterioration and kyphosis were delayed in Tocilizumab-treated *Lmna*$^{G609G/G609G}$mice. Based on these results, we decided to test the possibility that adding Tocilizumab to currently used clinical protocols for progeria, based on Lonafarnib or Everolimus, could at least improve the quality of life of HGPS patients. Thus, we treated *Lmna*$^{G609G/+}$ and *Lmna*$^{G609G/G609G}$ progeroid mice with Tocilizumab in combination with Lonafarnib or Everolimus. Surprisingly, we observed that both combined treatments improve the quality of life in progeroid mice as assessed by frailty index. This work suggests to explore the combination of Tocilizumab and Lonafarnib or Tocilizumab and Everolimus in clinical trials for HGPS.

#20

Abstract Poster

Title: Drug Treatment and Adenine Base Editing Improve HGPS Phenotype in Vascular Cells

Author(s): Nadia Abutaleb[1], Kevin Shores[1], Daniel Gao[2,3], David Liu[2,3], George A. Truskey[1]

Institution: [1]Department of Biomedical Engineering, Duke University, [2]Broad Institute, [3]Harvard University

The objective of this project was to evaluate the therapeutic effect of either the combination of Lonafarnib and Everolimus or adenine base editing on vascular cells from Hutchinson-Gilford Progeria Syndrome (HGPS) patients. Endothelial (viECs) and smooth muscle (viSMC) cells were differentiated from iPSCs derived from HGPs patients. We treated these cells with Lonafarnib, a farnesyl transferase inhibitor (FTI) that reduces progerin accumulation in the nuclear membrane, Everolimus, a rapamycin analog that increases autophagy-mediated progerin clearance, or a combination of the two. Everolimus at 0.1 μM reduced the number of nuclear abnormalities in both viECs (25% to 10%, $p<0.01$) and viSMCs (35% to 20%, $p<0.01$). The combination of 1 μM Lonafarnib and 0.05 μM Everolimus reduced reactive oxygen species (ROS) levels in viECs (44% decrease, $p<0.0001$) and viSMCs (50% decrease, $p<0.05$), increased proliferation in viECs (460% increase, $p<0.0001$) and viSMCs (200% increase, $p<0.01$), and reduced the amount of DNA damage in viECs (57% decrease, $p<0.01$) and viSMCs (46% decrease, $p<0.01$). This combination treatment also increased nitric oxide (NO) production by 425% ($p<0.01$) in viECs. Treatment with 1 μM Lonafarnib alone significantly increased flow-mediated expression for all genes except NOS3 ($p\leq0.01$). The combination Lonafarnib and Everolimus treatment did not further improve flow-mediated gene expression over Lonafarnib alone.

To assess the effects of adenine base editing, HGPS iPSCs were transduced with a lentivirus containing an adenine base editor (ABE) that corrects the c.1824 C>T mutation in the *LMNA* gene. We differentiated these corrected iPSCs into viECs and viSMCs and found 99% correction efficiency for both cell types. ABE treatment reduced the number of nuclear abnormalities in viECs (35% to 15%, $p<0.0001$) and viSMCs (25% to 12%, $p<0.0001$), increased cellular proliferation in viECs (80% increase, $p<0.05$) and viSMCs (46% increase, $p<0.05$), reduced ROS levels in viECs (20% decrease, $p<0.05$) and viSMCs (26% decrease, $p<0.0001$), and reduced the amount of the DNA damage in viECs (46% decrease, $p<0.01$) and viSMCs (74% decrease, $p<0.0001$). NO production was also increased in the viECs (238%, $p<0.001$), as well as flow-mediated expression for all genes except NOS3 and GCLC ($p\leq0.01$). Overall, both traditional drug treatment and genetic engineering therapies significantly improve the HGPS phenotype in vascular cells.

#21

Abstract Poster

Title: Disrupting the LINC complex as a therapeutic route to treating HGPS

Author(s):, *Jinqiu Zhang[1], Rafidah Mutalif[1], Yin Loon Lee[2], Brian Burke[1], and Colin L Stewart[1]*

Institution: *[1]ASTAR Skin Research Laboratories, A*STAR, Singapore,[2]Neuvocor, Singapore*

Most Lamin A mutations primarily affect muscle tissues resulting in diseases that include Dilated Cardiomyopathy (DCM), Muscular Dystrophies (Emery-Dreifuss, Limb-Girdle), and Progeria. Many nuclei in these diseased tissues show elevated levels of the nuclear envelope LINC complex protein SUN1. In mice that rapidly die from *Lmna*-induced DCM, deleting *Sun1* increases their survival from one month to more than one year. Here, we show that in a G609G HGPS mutant mouse line, loss of *Sun1* extends longevity by 60%, with improved postnatal growth, reduced vascular smooth muscle cell loss and fibrosis in the aorta. Together these results identify the SUN1-LINC complexes as a potential therapeutic route to treating DCM and HGPS.

We developed a dominant negative Sun1 minigene (DN-SUN1) that, on introduction into *Lmna*-DCM hearts, disrupts the LINC complex in cardiomyocytes, significantly ameliorating many of the *Lmna*-induced cardiac pathologies, with lifespan extension to more than one year with near normal cardiac function. We are extending our AAV-mediated gene therapy approach to determine whether it could suppress disease progression in HGPS by expressing the DN-SUN1 under the control of various promoters.

In parallel with these *in vivo* studies, we produced cardiomyocytes from iPS stem cells derived from HGPS patients. These HGPS cardiomyocytes show differences in their rates of contraction and Ca^{2+} gating compared to cardiomyocytes derived from a revertant (normal) HGPS line. Disrupting the SUN1-LINC complex in the HGPS cardiomyocytes significantly restores contractility and Ca^{2+} gating to near normal levels, suggesting that targeting the LINC complex in human HGPS cardiomyocytes may have therapeutic benefits.

#22

Abstract Poster

Title: Single cell transcriptomics identifies endothelial YAP/TAZ signaling as a key mediator of progeria-associated vascular alterations

Author(s): Ana Barettino, Cristina González-Gómez, Maria J. Andrés-Manzano, Carlos R. Guerrero, Rosa M. Carmona, Yaazan Blanco, Marina Muñoz, Ricardo Garcia, Ignacio Benedicto*, Vicente Andrés*

*, equal contribution

Institution: Centro Nacional de Investigaciones Cardiovasculares (CNIC)

Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare incurable disease characterized by the expression of progerin, an aberrant protein produced as the result of a *de novo* point mutation in the *LMNA* gene. HGPS patients present premature aging and typically die from complications of atherosclerosis. Understanding vascular disease onset and progression and identifying new therapeutic targets are both critically dependent on the identification of molecular and functional alterations in the heterogeneous cell subsets present in the arterial wall. Here, we used single-cell transcriptomics to provide the first characterization of the cellular landscape of the aorta in HGPS mice. Compared with wild-type aortas, the aortas of HGPS mice showed a marked increase in immune cell content, together with significant transcriptional alterations in resident macrophages, fibroblasts, vascular smooth muscle cells, and endothelial cells (ECs). Bioinformatics analysis showed gene expression changes in progeroid aortic ECs consistent with extracellular matrix (ECM) alterations, increased leukocyte extravasation, and the activation of the YAP/TAZ mechanosensing pathway, which was validated by qPCR, western blot, and immunofluorescence assays. *En face* atomic force microscopy experiments on decellularized aortas demonstrated stiffer sub-endothelial ECM in progerin-expressing mice, and echocardiographic assessment of the aortas of live HGPS mice revealed disturbed blood flow, both of which are potential inducers of the YAP/TAZ pathway in ECs. Drug-based inhibition of the YAP/TAZ pathway *in vivo* reduced leukocyte infiltration into the *tunica intima* in HGPS aortas and decreased atherosclerosis burden in atheroprone *Apoe*$^{-/-}$ HGPS mice. Our findings identify YAP/TAZ signaling as a potential therapeutic target for HGPS-associated atherosclerosis and open a new avenue for drug development.

Funding: Work in the V.A. laboratory is supported by the Spanish Ministerio de Ciencia e Innovación (MCIN)/Agencia Estatal de Investigación (AEI)/10.13039/501100011033 (grant PID2019-108489RB-I00) with co-funding from the European Regional Development Fund (“A way to build Europe”), the Progeria Research Foundation (PRF) (award PRF 2019–77), and Asociación Progeria Alexandra Peraut. The CNIC is supported by the MCIN, the Instituto de Salud Carlos III, and the Pro-CNIC Foundation and is a Severo Ochoa Center of Excellence (grant CEX2020-001041-S funded by MCIN/AEI/10.13039/501100011033). A.B. is supported by the Ministerio de Ministerio de Ciencia, Innovación y Universidades (predoctoral contract BES-2017-079705), and I.B. by the Comunidad Autónoma de Madrid (grants 2017-T1/BMD-5247 and 2021-5A/BMD-20944).

#23

Abstract Poster

Due by October 12, 2022

Title: Transient expression of an adenine base editor corrects the Hutchinson-Gilford progeria syndrome mutation and improves the skin phenotype in mice

Author(s): Daniel Whisenant[1]*, Kayeong Lim[2]*, Gwladys Revêchon[1], Haidong Yao[1], Martin O. Bergo[1], Piotr Machtel[1], Jin-Soo Kim[2], Maria Eriksson[1]

***Authors contributed equally**

Institution:

[1]Department of Biosciences and Nutrition, Center for Innovative Medicine, Karolinska Institutet, Huddinge, Sweden.

[2]Center for Genome Engineering, Institute for Basic Science (IBS), Dajeon, South Korea.

Please provide all information in the block below. Email completed form to: Workshop@progeriaresearch.org.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare premature ageing disorder caused by a point mutation in the LMNA gene (LMNA c.1824 C > T), resulting in the production of a detrimental protein called progerin. Adenine base editors recently emerged with a promising potential for HGPS gene therapy. However, adeno-associated viral vector systems currently used in gene editing raise concerns, and the long-term effects of heterogeneous mutation correction in highly proliferative tissues like the skin are unknown. Here we use a non-integrative transient lentiviral vector system, expressing an adenine base editor to correct the HGPS mutation in the skin of HGPS mice. Transient adenine base editor expression corrected the mutation in 20.8-24.1% of the skin cells. Four weeks post-delivery, the HGPS skin phenotype was improved, and clusters of progerin-negative keratinocytes were detected, indicating that the mutation was corrected in both progenitor and differentiated skin cells. These results demonstrate that transient non-integrative viral vector mediated adenine base editor expression is a plausible approach for future gene-editing therapies.

#24

Abstract Poster

Title: DNA editing partially rescues bone dysplasia in a mouse model of Hutchinson-Gilford Progeria Syndrome.

Author(s): Wayne A. Cabral[1], Caleb M. Grenko[1], Urraca L. Tavarez[1], Diana Yeritsyan[2], Indeevar Beeram[2], Kaveh Momenzadeh[2], Narisu Narisu[1], Luke W. Koblan[3,4,5], David R. Liu[3,4,5], Ara Nazarian[2], Michael R. Erdos[1] and Francis S. Collins[1]

Institution: *1.* Molecular Genetics Section, Center for Precision Health Research, NHGRI, NIH, Bethesda, MD, USA
2. Carl J. Shapiro Department of Orthopedic Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA.
3. Broad Institute of Harvard and MIT
4. Department of Chemistry and Chemical Biology, Harvard University
5. Howard Hughes Medical Institute, Harvard University, Cambridge, MA, USA.

Hutchinson-Gilford Progeria Syndrome (HGPS) is a premature aging disorder that affects tissues of mesenchymal origin. Most individuals with HGPS harbor a *de novo* c.1824C>T (p.G608G) mutation in the gene encoding lamin A (*LMNA*), which activates a cryptic splice donor site resulting in production of a toxic protein termed “progerin”. Clinical manifestations include growth deficiency, lipodystrophy, sclerotic dermis, cardiovascular defects and bone dysplasia. We have previously described the generation of a transgenic murine model that carries a human BAC harboring the common mutation, G608G, which in the double-copy state recapitulates the phenotypic features of HGPS in skin, adipose, vascular and skeletal tissues, and is therefore well-suited for testing therapeutic interventions that target either the mutation or mutant gene product in the context of the human sequence. Here we report the efficacy of Adenine Base Editor (ABE)-mediated correction *in vitro* and *in vivo*. Utilizing lentiviral-mediated delivery of ABE to murine-derived osteoblast cultures in the absence of any selective reagents we found that, *in vitro*, with as little as 30-40% gene correction, synthesis of progerin protein was reduced by 60-70%, while normal lamin A increased by 25-70%. Osteoblast mineralization capability was increased by 10-37%. For *in vivo* experiments, following AAV9-mediated delivery of ABE retro-orbitally at 3 days (P3) or 14 days of age (P14) gene correction levels of 14 and 22%, respectively, were obtained in long bones of treated mice by 6 months, and was sufficient for partial improvement of the bone phenotype. Micro-CT imaging of femora from treated mice revealed a 10% increase in trabecular BMD, compared to untreated littermates, with restoration of trabecular bone volume and cortical thickness to wild-type values. Although geometrically predicted resistance to deformation (Moment of Inertia) was not significantly improved, mechanical testing of femora demonstrated restoration of elastic properties, including yield load and stiffness, as well as plastic properties, such as increased fracture load, to wild-type values and suggest improvement of bone material properties in treated versus untreated mice. Gene expression profiling of ABE-treated tibiae revealed normalization of elevated osteoclast markers that was associated with reduction of Rankl/Opg ratios, and reduction of elevated expression of osteocyte markers. This work demonstrates the possibility of delivering a locus-specific base editor to bone, and suggests that this system might be specifically tailored in the future for application to other monogenic disorders that cause bone dysplasias.

#25

Title: **Optimized delivery of Lipid-Nanoparticle Encapsulating Telomerase mRNA to Reverse Vascular Senescence in Progeria Mice**

Author(s): ***Anahita Mojiri, Elisa Morales, Chiara Mancino, Luay Boulahouache. Kusum Gorantla, Christian Boada, Francesca Taraballi, John P. Cooke***

Institution: Houston Methodist Research Institute

Background
Hutchinson-Gilford Progeria Syndrome (HGPS) is an accelerated aging syndrome associated with premature vascular disease and death due to heart attack and stroke. A spontaneous mutation in lamin A (progerin) alters nuclear morphology and gene expression, resulting in HGPS. Current available treatments only modestly increase the lifespan of affected children, and the need for new therapeutic approaches is evident. We have previously shown that human telomerase (hTERT) mRNA can rescue the phenotypes and in vitro functions of senescent progeria endothelial cells and that the lentiviral delivery of mouse TERT (mTERT) rejuvenates the vasculature of progeria mice in different organs in vivo. To translate this therapy for clinical use, a delivery system capable of maintaining mRNA integrity and preventing its immunogenicity is needed. Here, we hypothesize that the development of a lipid nanoparticle (LNP)-based drug delivery system for mouse-TERT mRNA would allow us to successfully protect and deliver the cargo to an HGPS mouse model and recapitulate the rejuvenation seen previously with the lentiviral delivery.

Materials/Methods
Our LNP formulation showed consistency with current literature for LNPs' size, polydispersity index, surface charge, and encapsulation efficiency. Luciferase mRNA was used to investigate the LNP's mRNA expression profile. In addition, we have used DiD fluorescence marker in the LNPs to investigate the biodistribution of the LNPs in the mice organs. A dose-dependent toxicity study was conducted to reveal the safest dose for treatment.

Results
The LNP's biodistribution and mRNA expression profile 24 hours post-injection is predominantly hepatic. A dose-dependent toxicity study revealed post-injection that 2mg/kg is the safest dosage to be used for prevention of acute toxicity. An ongoing life extension study aims to test efficacy of long-term administration of mRNA-LNP therapy.

Conclusions
Preliminary data suggest that vascular rejuvenation using telomerase mRNA is a promising and highly translational approach for progeria and other age-related diseases.

#26

Abstract Poster

Title: Role of low basal Nitric Oxide in the adverse symptoms of Hutchinson-Gilford Progeria Syndrome: How a biofilm of commensal Ammonia Oxidizing Bacteria might help

Author(s): David R Whitlock dwhitlock@aobiome.com

Institution: AOBiome Therapeutics

Progeria Research Foundation 11th International Scientific Workshop, November 2-4, 2022, Cambridge, MA

Please provide all information in the block below. Email completed form to: Workshop@progeriaresearch.org.

HGPS recapitulates many symptoms characteristic of old age; many symptoms characteristic of low nitric oxide, including hypertension, oxidative stress, atherosclerosis, tortuous blood vessels, thin skin, insulin resistance, osteoporosis, and cardiovascular disease.

My poster from 2016 [1] shows many characteristic vascular traits of HGPS derive through vascular remodeling under conditions of low nitric oxide, including hypertension, tortuous vessels, capillary rarefaction. Nitric oxide is an exceedingly important signaling molecule that is generated at a site, diffuses a distance and then activates a sensor (heme, sGC, superoxide, any radical, Zn-thiols, RS-, or many others (figure 1).

All of these NO signaling pathways work together and are 'in sync', where they are cooperatively regulated. The background level of NO is not a 'constant', rather it is a global control parameter that physiology moves up and down so as to adjust all NO signaling pathways, simultaneously, with no threshold. NO is already an 'active' signaling molecule, actively controlling the pathways it is involved in. There is 'no threshold' for changes in that NO level to affect the outcome of those NO pathways. This is what makes using nitric oxide as a therapeutic so difficult, NO diffuses everywhere, is active at picomolar levels in myriad tissue compartments. (see figure 2)

Nitric oxide pathways derived from resident Ammonia Oxidizing Bacteria (AOB) (figure 3,4) are from deep evolutionary time, from before the Precambrian, and across the entire evolutionary tree, including humans.

Figure 5 shows multiple episodes of a spontaneous physiological effect mediated through nitric oxide (nocturnal tumescence), coincident with instrumental measure of spontaneous nitric oxide production by neurogenic ammonia release to a long term (years) persistent biofilm of AOB, indicating that NO release by AOB is under full and purposeful physiological feedback control.

In other words, physiology can and does increase NO levels in the vascular beds of the penis, sufficient to cause tumescence, by releasing ammonia to the scalp where a resident AOB biofilm generate NO and nitrite. If HGPS is triggered/exacerbated by low NO, it is extremely likely that physiology can compensate by non-thermal sweating to generate NO once the normally resident biofilm of AOB on the skin is restored; reducing the severity of a particular case, or delaying its progression. This could be particularly important and effective in very early childhood, before low NO has caused capillary rarefaction and cardiovascular damage.