

Meeting Report

Highlights of the 2007 Progeria Research Foundation Scientific Workshop: Progress in Translational Science

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IN the spring of 2007, a 2-year clinical drug trial began at Children's Hospital Boston (1) with the goal to advance the quality of life for children with Hutchinson-Gilford progeria syndrome (henceforth *progeria*), a rare (frequency 1 in 4 million), multisystem, and inevitably fatal disease that claims young lives due to myocardial infarctions and strokes between ages 7 and 20 years (2). The journey to the first clinical trial for progeria has been facilitated by a series of collaborative scientific workshops organized by The Progeria Research Foundation (PRF) and supported by agencies interested in aging, cardiovascular disease, rare disease research, and genetics (http://www.progeriaresearch.org/2007_prf_workshop_on_progeria.html). These meetings have provided a concentrated forum to facilitate the collective thinking of clinicians and scientists about progeria, forge collaborations in this little-known field, and accelerate the discovery of new ways to push the field forward toward treatments and cure. The first PRF Scientific Workshop in 2001 helped to identify nuclear blebbing as an important phenotypic marker of progeria cells (noted by Anthony Weiss; University of Sydney, Australia), and to recognize a translocation on chromosome 1 in the cells cultured from a progeria patient (W. Ted Brown, New York State Institute for Basic Research in Developmental Disabilities), pointing geneticists in the direction of the gene mutation for progeria (3). In 2003, the second workshop was held just 3 months after publication of the gene defect in progeria, which is typically a sporadic autosomal dominant disease caused by a C → T mutation at nucleotide 1824 of the *LMNA* gene encoding lamin A (4,5). Importantly, the mutation results in a persistence of an aberrant form of a farnesylated-prelamin A molecule (4) now called progerin. The attendees now included experts in the field of lamin biology and laminopathies. The 2004 Progeria Workshop, held within the National Human Genome Research Institute (NHGRI), was held specifically to discuss the role of stem cells and the potential for stem cell transplantation in progeria, an area of continuing interest for the field. At the general Progeria Workshop in 2005, two mouse models of progeria were unveiled and, although they contained identical mutations, they yielded very different phenotypes, mimicking various portions of the human disease (6,7).

Early *in vitro* data on a potential role of farnesyltransferase inhibitors (FTIs) in treating progeria were presented by four laboratories, demonstrating that FTI could reverse nuclear blebbing in cells expressing progerin (8–11). Armed with data demonstrating that treatment of newly developed cellular assays with FTIs improves or reverses some of the effects of progerin accumulation, a clinical trial treating children with progeria with an FTI was initiated in May 2007 (1) (www.clinicaltrials.gov).

The present article, detailing the 2007 PRF Scientific Workshop (November 12–14, Boston), emphasizes the complexities of progerin and lamin A biology, and the need to continue to look for the most effective protocols for correcting the effects of the lamin A defect in progeria. All speakers presented preliminary data that had not been peer-reviewed at the time of presentation. In the months since the workshop, some data have been published (referenced within this article), and we look forward to additional peer-reviewed publications on the work presented at this meeting over the coming year.

The essence of translational research was represented in the presentations, which brought forth the most important issues in progeria today—the effects of progerin and lamins and their binding partners on the functioning of cells, systems, mouse models, and humans; the connection between progeria, aging, and cardiovascular disease in the general population; careful analysis of FTI effects on progeria at all levels (summarized in Table 1); and strategies for future disease treatments and cure. In his introduction at the workshop, *Francis Collins* (Director, NHGRI) noted that a 4-year period between gene discovery and a clinical drug trial is “. . . an unprecedented feat for a rare genetic disease.” For progeria patients and their families, who have struggled to live within a void of information, the clinical trial represents “. . . a source of light coming up after a long, long night” and hope has replaced “. . . a sense of fear and helplessness on each passing birthday” (Subbarao, father of Priya, a 15-year-old with progeria).

GETTING TO KNOW THE CHILD WITH PROGERIA

For a disease as rare as progeria, scientists often have no exposure to the very population they wish to serve. During

Table 1. Workshop-Presented Studies of Effectiveness of FTIs on Progeria Cells*

Presenter	Cell/Tissue Type	Cellular Defect	FTI Effect
Collins	Progeria BAC transgenic HGPS ^{+/+} mouse arteries	Tissue pathology: progressive loss of VSMC and appearance of PG	Improved carotid arteries: more VSMC, less PG
Comai	Progeria fibroblasts and transfected progerin-expressing normal fibroblasts	Over-expressing progerin increased rate of cell death	No improvement
Lammerding	Progeria fibroblasts	Increased nuclear rigidity and early cell death at later passages after mechanical stretch	Reversal
Lammerding	Progeria fibroblasts	Wound-healing deficit	Reversal
Lammerding	Progeria fibroblasts	Proliferation deficit after mechanical stretch	No improvement
Paschal	Progeria fibroblasts and transfected progerin-expressing HeLa cells	NPC abnormality-TPR mislocalization	Reversal
Paschal	L647R cells, in which cleavage by Zmpste24 is lacking	Inverted RAN gradient	Reversal
Zou	Progeria fibroblasts	DSBs	No improvement
Sinensky	Transfected progerin-expressing HeLa cells	Farnesylated progerin was present	Geranylgeranylated progerin appeared
Lopez-Otin	Zmpste24 ^{-/-} fibroblasts	Farnesylated prelamin A was present	Farnesylated prelamin A levels decreased; geranylgeranylated prelamin A appeared

Notes: *Data are listed in order of appearance in article text.

FTI = farnesyltransferase inhibitor; BAC = bacterial artificial chromosome; HGPS = Hutchinson-Guilford Progeria Syndrome; VSMC = vascular smooth muscle cells; PG = progeria; DSBs = double-strand breaks; NPC = nuclear pore complexes; TPR = translocated promoter region.

the first evening of the workshop, participants had a unique chance to hear from children with progeria and their families during a panel discussion. *Dr. Scott Berns* (PRF and Warren Alpert Medical School of Brown University), the father of a child with progeria, moderated the family session. Through interpreters, Sammy (12 year old from Italy) and Priya (15 year old from India), displayed an uncanny ability to describe their lives with eloquence, poise, and wisdom—their frail and aged physical appearance belying their lively spirits. Priya shared that she enjoys traveling, and Sammy liked to skateboard, swim, and is well assimilated into school life. Sammy's parents, Laura and Amerigo, spoke about how their son "...gives them the strength to keep on and to not give up, and always look at the good side of the day." Sammy has given them an "...opportunity to enjoy life in a different way ...to enjoy every day of your life with your child ...". Keith and Molly Moore (U.S.A.), parents of Zachary, who recently passed away, described the selfless traits of their son as well as other children with progeria, relating how children with progeria "...care so much for others, and Zach understood that the research might not help him directly and personally, but it certainly would help others" (12). In concluding, young Sammy wanted to especially "...thank all the doctors and all the researchers that are putting such an effort into trying to help me out and all the other kids with the disease."

DESIGNING THE FIRST CLINICAL DRUG TRIAL

The ultimate goals of disease-related research are to find treatments and a cure—in this case to improve the health and increase the life spans of children with progeria. Designing a clinical trial that has the greatest chance of assessing the efficacy of an intervention on the disease in a statistically significant manner is a key element in this process. For a disease such as progeria, in which fewer than 50 patients are identified worldwide at any one time, this is

especially challenging. To that end, a clinical trial using FTI to treat progeria is currently being conducted at Children's Hospital, Boston. Several of the trial team members presented baseline clinical studies of the major systems affected in progeria, trial design, and measures of efficacy that will likely serve as models for future treatment trials in the field.

Mark Kieran (Dana Farber Cancer Institute), principal investigator of the clinical trial team, reviewed the rationale for the clinical trial design, drug selection, and parameters for assessing drug efficacy. The selection of the FTI lonafarnib was made on the basis of two lines of evidence: the addition of FTI to progeria fibroblasts reverses nuclear blebbing (8–11), and its low toxicity profile in adults and children with cancer (13). However, because lonafarnib has not previously been administered to children with progeria, it will be important to carefully track the toxicity profile, pharmacokinetics, and efficacy of lonafarnib in a variety of organ systems to determine whether there is any benefit to using this FTI in progeria clinically. To quantitate the effect of drug treatment in a measurable way, Dr. Kieran stressed the importance of first documenting the natural history of the disease, including an understanding of the breadth and variability in disease phenotype from patient to patient. Because the number of children with progeria is often too small to see population-based changes, for some clinical measures each child must serve as his or her own control for assessing drug effect, comparing pretreatment measures to posttreatment measures. Because disease in progeria is progressive, any measures improving with treatment would be considered drug-related changes. The primary clinical parameter for measuring treatment efficacy is rate of weight gain, which is linear for each child after approximately age 2 years in progeria (14), and therefore provides an opportunity to assess whether FTI affects rate of weight gain over a 2-year treatment period. Secondary parameters of efficacy

include measures of abnormality in other well-known features of the disease, such as alopecia, short stature, subcutaneous fat, bone integrity, limb abnormalities, auditory and dental abnormalities, and cardiovascular disease. While these features are important to the disease process, the ability to statistically assess treatment effect on these systems is less reliable than rate of weight gain. Although cardiovascular disease is clinically the most important determinant of health and longevity in progeria, it is currently not a feasible primary outcome measure for a clinical trial, but is followed as a secondary measure. Some patients in the study have already had myocardial infarctions or strokes, and the damage incurred cannot predictably be reversed. In addition, the ages for participants in the trial range from 3 to 16 years. Because the most accurate assessment of cardiovascular improvement would be an increase in mean age of death, the clinical trial with a cardiovascular primary parameter would require a 10- to 15-year commitment. Hence, rate of weight gain is used as a surrogate marker of general health in progeria.

In addition to rate of weight gain, other more focused features such as bone health could change in a measurable way within the 2-year treatment period. *Catherine Gordon* (Children's Hospital, Boston) presented the preliminary data on the bone phenotype for 28 children, measured using two-dimensional dual-energy x-ray absorptiometry. Because the body habitus of children with progeria is drastically different from their age-matched peers, calculation adjustments must be made for height-age. Remarkably, the clinical histories indicate that children with progeria do not have a higher risk of fracturing their weight-bearing bones, although they are very playful and active. She suspects that fracture rates in progeria children are not increased because the cortex of weight-bearing long bones is of normal density by x-ray (14), in contrast to other low weight-disorders such as anorexia nervosa and cystic fibrosis, where fracture rates are increased and cortical bone is thinned on x-ray. In support of this hypothesis, preliminary data demonstrated that bone density z-scores move from an uncorrected osteoporotic range of -2 to -4, where we would expect some spontaneous bone breaks similar to osteogenesis imperfecta, to a height-age corrected osteopenic range of -1 to -2. Further studies of bone quality are in progress, using peripheral quantitative computed tomography to track the three-dimensional cortical and trabecular characteristics with and without FTI treatment.

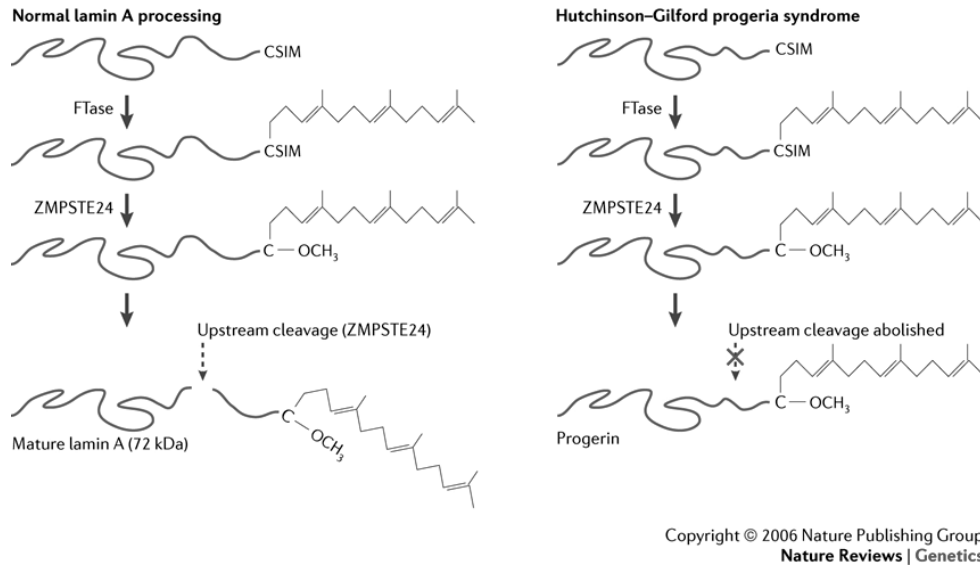
THE CARDIOVASCULAR PHENOTYPE IN PROGERIA AND PROSPECTS FOR VASCULAR TREATMENT WITH FTI

Cardiovascular events in progeria include hypertension, myocardial infarction, congestive heart failure, and stroke. *Elizabeth Nabel* (National Heart, Lung and Blood Institute) and *Marie Gerhard-Herman* (Brigham and Women's Hospital) discussed recent findings on the vascular phenotype and function in children with progeria. It was the consensus of both presenting clinicians that the disease progression of progeria blood vessels does not fit the typical atherosclerotic description that includes: a) preservation of the vascular smooth muscle cells (VSMC), b) intimal

thickening, c) atherosclerotic plaque (rich lipid core) with ruptured cap and superimposed thrombosis, and d) some proliferating smooth muscle cells. Instead, Drs. Nabel and Gerhard-Herman propose that progeria vascular phenotype resembles an arteriosclerosis, which is characterized by hardening of the vessels and vascular stiffness, typically seen in the normal aging populations. Vessel cross-sections at autopsy of one 20-year-old patient revealed no indication of vasculature inflammation (15). In addition, children with progeria do not have abnormal serum cholesterol levels, which is a factor in chronic lipid-driven inflammation (16). Clinical studies at both the National Institutes of Health (NIH) clinical center (17) and Children's Hospital, Boston, revealed that the intima-media thickness of the progeria carotid artery is normal. Furthermore, in agreement with Stehbens and colleagues (15), Dr. Nabel presented autopsy cross-sections from a child with genetically confirmed progeria, and the histology showed that the vascular media no longer contained smooth muscle cells and the elastic structure was destroyed and replaced with extracellular matrix or fibrosis. She hypothesized that the primary loss of smooth muscle cells initiates vascular remodeling by secondary replacement with matrix in large and small vasculature.

In a study of 28 children with progeria at Brigham and Women's Hospital (Boston), Dr. Gerhard-Herman measured global vascular stiffening and, through imaging techniques, observed vessel tortuosity, profound adventitial thickening, and a depleted media layer. For children with an occluded internal carotid artery, there was an overall loss of the trilaminar architecture. Functional studies demonstrated high femoral pulse wave velocities, and decreased vasodilatory response to nitrous oxide stimulation induced by ankle flexion. Overall, the vessel stiffness, tortuosity, and adventitial brightness (the molecular composition not yet identified), as observed in imaging studies, support a model of arteriosclerosis similar to what is observed in aging. Remarkably, the blood velocities are in the normal range and flow is still laminar, implying that there is either a tremendous reserve capacity in the vasculature, or there are compensatory mechanisms helping to preserve function. Complementary data gathered at the NIH studying a cohort of 15 patients revealed initially normal vascular compliance that decreases with increasing age, and a number of children displayed left atrial enlargement (17). Blood pressure and heart rate were in the normal range in younger children, while the older children had increasing blood pressures and variable heart rates. Electrocardiograms were normal in most, although a few had long Q-T intervals suggesting fibrosis of the conduction system. In summary, the progeria vasculature is characterized by global stiffness, tortuosity, and a loss of smooth muscle cells in the media with subsequent extracellular replacement. Smooth muscle cell dropout is unique to progeria, while global stiffness and tortuosity are observed in the normal aging population.

The vascular remodeling characteristics in children with progeria are similar to vascular events observed in a transgenic progeria mouse model that expresses human progerin, created in Francis Collins' laboratory (7). Within the descending aorta (cross-section histology), smooth muscle



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Figure 1. Posttranslational processing of prelamin A to mature lamin A (left) and preprogerin to progerin (right) (57). C = cysteine; S = serine; I = isoleucine; M = methionine; FTase = farnesyl transferase. [Reproduced by permission of the publisher.]

cells begin to “drop-out” by 5 months, and by 20 months there is a paucity to a complete absence of smooth muscle cells. The remaining endothelial cells are picnotic, similar to progeria cells grown *in vitro* when progerin is over-produced. There is substantial adventitial thickening, and the anatomical changes correlate with functional changes; the blood pressure response to a vasodilator is blunted and includes a protracted recovery time to achieve baseline values. The NIH progeria mouse model mimics the human vascular disease, and is therefore a viable model for assessing treatment effects on progeria. At the 2007 workshop, Dr. Collins reported on studies from his laboratory where transgenic progeria mice began daily treatment with FTI at two different phases of development: at time of weaning (0–9 months) and later between 6–9 months. Whereas the carotid arteries of untreated mutant mice demonstrated a progressive loss of VSMC and excessive appearance of proteoglycans with age, matched progeria mice treated with intermediate to high doses of FTI had significantly more VSMC, and little evidence of proteoglycan accumulation. The results with the mice treated between 6 and 9 months give us the first evidence that FTI may have a beneficial effect in reversing vasculature change brought about by progeria.

EFFECTS OF PROGERIN AND LAMIN IMBALANCE ON CELL STRUCTURE AND FUNCTION

To continue developing treatment prospects in progeria, and to understand its role in normal aging, it is essential to study not only the effects of FTI, but also the biology of lamins, progerin, and related molecules at the cellular level. In normal cells, lamins form a concentrated layer along the inner rim of the nucleus, called the nuclear lamina, and are also found dispersed within the nucleoplasm (18). Lamins play a critical role in a number of essential cellular functions, such as the transcription of RNA polymerase II,

DNA replication, the organization of the chromosome territories, and epigenetic modulations within the nucleus. Lamin A is processed via a series of posttranslational steps (Figure 1): prelamin A is farnesylated, carboxymethylated, and cleaved twice to produce mature lamin A. Elements for this pathway include initial farnesylation by a farnesyltransferase, removal of the three C-terminal amino acids, and methyl esterification of the now C-terminal farnesyl cysteine. This is followed by integration of prelamin A into the nuclear membrane, and cleavage of 15 amino acids and the attached farnesyl group from its C-terminal end by Zmpste24 to produce mature lamin A. Mature lamin A is then released from the nuclear membrane (19). Typically in progeria, a single silent C to T transition mutation at nucleotide 1824 (Gly608Gly) activates a cryptic splice site, resulting in the deletion of 150 messenger RNA bases in the 3' portion of exon 11 (4). The aberrant protein, progerin, lacks the cleavage site for removal of its C-terminal farnesylated peptide by Zmpste24 endoprotease, and therefore remains bound to the inner nuclear membrane. Hence, progerin resembles an aberrant form of farnesylated-prelamin A. However, whereas prelamin A is a transient, intermediate protein, which is present at very low levels in the cell, progerin is a nontransient protein whose concentration builds with successive cell divisions.

Several workshop presentations addressed progerin's influences on lamin A and its binding partners, its role in architectural changes in the nuclear membrane, and subsequent effects on cell function that include: increase in apoptosis and disruption of binding with lamin-binding proteins, increased nuclear rigidity, sluggish wound healing *in vitro*, and altered cell proliferation and chromosomal distribution. Lucio Comai's (University of Southern California) studies stressed the importance of determining the cellular effects of progerin and excess prelamin A on cell growth (20). Dr. Comai used a lentivirus system to create

two cell lines, progerin expressors and prelamin A over-expressors, from normal human fibroblasts. Over-expression of either prelamin A or progerin significantly increased the amount of cell death compared to wild-type controls in a passage-independent manner. When progerin-expressing cells lost progerin expression, apoptosis decreased to wild-type levels. Apoptosis also decreased to wild-type levels when prelamin A overexpressors were transfected with additional *Zmpste24* DNA, which decreased prelamin A protein levels and increased mature lamin A levels. As expected, over-expressing *Zmpste24* did not rescue progerin-expressing cells, because progerin lacks the *Zmpste24* recognition site. Adding FTI to progerin-expressing cell cultures did not improve rates of cell death, presumably because the excess nonfarnesylated prelamin A created with FTI exposure counteracted any improvements in growth that decreasing progerin may have caused. Studies by *Stephen Young* (UCLA) provided additional evidence for the toxic effects of farnesylated prelamin A. Dr. Young showed that certain clinically used HIV protease inhibitors that cause lipodystrophy reminiscent of that seen in progeria also create a *Zmpste24* blockade and a build-up of farnesylated prelamin A in normal fibroblasts (21). Thus, it is crucial to study not only the effects of progerin, but that of other proteins that are affected by the presence of progerin or by treatments that create an imbalance of lamin A-related proteins.

Jan Lammerding (Harvard Medical School) studied the effects of accumulated progerin on nuclear rigidity, wound healing in vitro, and cell cycling, and asked whether FTIs reverse abnormalities. When strain is applied to wild-type fibroblast nuclei, they remain stiff (22). Fibroblasts cultured from a lamin A/C deficient mouse model were twice as fragile as wild-type cells, and after 24 hours of mechanical strain, cell death significantly increased. In sharp contrast, while progeria fibroblasts showed normal rigidity at early passages (when progerin levels and nuclear blebbing are at a minimum), late-passage nuclei show dramatically *increased* rigidity over wild-type fibroblasts. Although the lamin A/C deficient fibroblasts and progeria fibroblasts displayed vastly different nuclear rigidities, nuclear strain elicited early cell death in both cell types over controls. In addition, whereas wild-type cells enter S phase and G2 phase in response to mechanical stretch, progeria cells did not proliferate in response to stretch. Serum-starved progeria fibroblasts had a slow and incomplete response to an in vitro wound healing assay as well. The ability of FTIs to affect these cellular abnormalities was mixed. Nuclear rigidity and wound-healing deficits were reversed in FTI-treated progeria cultures. However, FTI treatment did not promote cell proliferation after mechanical strain.

Within the nucleus, lamin A monomers first dimerize, then dimers associate in a head-to-tail fashion and finally associate laterally (23). The 50-amino acid deletion in progerin probably does not affect its ability to dimerize, as normal lamin A does, because the necessary components for this function are not deleted. Hence progerin can form dimers with itself or normal lamin A, thereby influencing lamin A's interactions with nuclear pores, chromatin, and lamin binding partners. For example, the formation of lamin

A and lamin B are separate yet interdependent; the failure of one lamin-type to properly form can interfere with the formation of another lamin type (24). Progerin build-up with increasing cell passage leads to progressively increased percentages of blebbed nuclei (25). *Robert Goldman* (Northwestern University) referred to these blebs as "sophisticated structures" worthy of close examination to determine their influence on cell function, and began by hypothesizing that progerin's affect on other lamin types is central to the blebbing defect. In the region of the blebs, there is an absence of heterochromatin but euchromatin can be identified surrounded by lamin A, a configuration not observed in other regions where the nuclear membrane appears to be formed normally (26). Dr. Goldman and his group suggest that the blebs contain transcriptionally active DNA and that this is providing clues regarding the role of mutant lamin A in progeria.

Complementary chromosome positioning studies were explored by *Joanna Bridger* (Brunel University, U.K.), who supports the hypothesis that A-type lamins (and/or emerin) are involved in translocating and anchoring chromosomes to the nuclear membrane or nuclear matrix in nonrandom distributions according to cell cycle status, and that lamin mutations perturb this balance. Normally, upon cell cycle exit, gene-poor chromosomes move from the nuclear periphery to more interior positions (27,28). The distribution profile of chromosome position within the nucleus during cell cycling, quiescence, and senescence all differ. Dr. Bridger had earlier reported that proliferating fibroblasts from laminopathy patients, including progeria, exhibited chromosome distributions characteristic of nonproliferating (senescent or quiescent) normal fibroblasts, where the largest chromosomes are located at the periphery of the nucleus and the smallest at the interior. At the workshop, Dr. Bridger reported new experiments that distinguished the chromosome distribution in senescent from quiescent cells based on the position of chromosome 10, which she finds located at the periphery in quiescent cells and at the interior in senescent cells. In proliferating progeria fibroblasts, however, chromosome 10 was located at the periphery, a position found only in the nonproliferating, quiescent state in normal cells. Several other proliferating laminopathy cultures also displayed the quiescent positioning. This chromosome positioning was not changed when progeria or laminopathy cells became senescent. The consequences of aberrant chromosome 10 location on transcription in progeria will be the subject of Dr. Bridger's future studies.

Bryce Paschal (University of Virginia) reported on progerin-induced changes in the nuclear pore complexes (NPCs), channels through which all transport between the nucleus and the cytoplasm takes place (29). He has found major changes both in NPC structure and in the transport machinery and signals. The structural change is in the position of the *nucleoplasmic basket*, a structure located at the pore on the nucleoplasmic side. It consists of two proteins: TPR (translocated promoter region), the key constituent, and Nup 153, a facilitator of its assembly. The entire basket structure is disassembled and reassembled at every mitosis. When Dr. Paschal looked for the localization of TPR in progeria fibroblasts by immunostaining, he

obtained a striking result: Instead of being in the basket, TPR is out in the cytoplasm. The same result is obtained when human epithelial cells are transfected with progerin. Administration of an FTI to the culture corrected the TPR localization back into the nuclear envelope of progeria cells.

Progerin also had a major effect on localization of a transport regulator. While small molecules can diffuse passively through the NPC channel, macromolecules must enter as a complex with a member of the superfamilies of importin or exportin proteins, depending on the direction of transport (29). Once one of these complexes has traversed the pore, it is dissociated with the involvement of a Ras-related GTPase called RAN. In normal cells, there is a gradient of RAN concentration across the pore, with more RAN in the nucleus than in the cytoplasm.

Dr. Paschal found that the RAN protein gradient was disrupted in epithelial cells when lamin A was depleted with siRNA. He therefore examined the effect of several laminopathy mutations on RAN localization. Whereas cells from dilated cardiomyopathy and Emery-Dreifuss muscular dystrophy mutations that express a mutant form of lamin A had normal RAN gradients, the RAN protein gradient is dramatically disrupted in both progeria cells and in cells with the mutant lamin A L647R, in which cleavage by *Zmpste24* is lacking. While in normal cells most of the RAN is in the nucleus, progeria cultures have an inverted gradient—there is more RAN in the cytoplasm than in the nucleus. In the L647R cultures, this effect was rescued by adding an FTI to the culture. It will be important to assess whether FTI rescues the RAN progerin gradient in progeria cells.

PROGERIA AND AGING

Hutchinson-Gilford progeria syndrome is named for its resemblance to aging: “pro” = prematurely and “geras” = aged. It is a *segmental progeroid syndrome* (30), since some, but not all, of the patients’ characteristics resemble accelerated aging. Over the last several years, a growing body of evidence for cellular and molecular overlaps between progeria and normal aging has been uncovered. Early investigations compared several properties, for example, telomere length (31) and distribution of mRNA species (32). The discovery that progeria is caused by a mutation in lamin A, which had not previously been implicated in mechanisms of aging, brought in an entirely new question: Are defects in lamin A implicated in normal aging? The first positive evidence was reported by Scaffidi and Misteli in 2006 (33), who showed that cell nuclei from normal individuals have acquired defects similar to progeria patients, including changes in histone modifications and increased DNA damage. Younger cells show considerably less of these defects. They further demonstrated that the age-related effects are the result of the production of low levels of preprogerin mRNA produced by activation of the same cryptic splice site that functions at much higher levels in progeria, and is reversed by inhibition of transcription at this splice site. Cao and colleagues (34), studying the same phenomenon, showed that, among interphase cells in fibroblast cultures, only a small fraction of the cells contained progerin.

The percentage of progerin-positive cells increased with passage number, suggesting a link to normal aging.

A large amount of the data presented at this workshop underscored the value of studying progeria as a cellular and organismal model for some key aspects of the aging process. The evidence that progerin is present in normal aging was considerably strengthened at the workshop by *Karima Djabali* (Columbia University) (35). Dr. Djabali measured progerin mRNA and protein (using a monoclonal antibody she created that recognizes progerin but not lamin A) in cells from skin biopsies taken from progeria patients and from 150 normal participants with ages ranging from newborn to 97 years. Dr. Djabali found low levels of progerin mRNA in all tissue samples, but found progerin protein only in skin samples taken from elderly participants, within dermal fibroblasts, and in a few terminally differentiated keratinocytes. Furthermore, using this same antibody, Dr. Djabali detected a dramatic increase of progerin-containing cells in normal dermal fibroblast cultures as passage number increased. The discovery of progerin in the skin biopsies provided the first evidence for the in vivo accumulation of progerin during normal aging. The presence of progerin in normal individuals implies that individuals without progeria tolerate a low level of progerin as part of normal aging. With respect to treating progeria, it is encouraging and suggests that effective therapy may be achieved with a balance between prelamin, lamin A, and progerin rather than a complete elimination of the progerin molecule.

Francis Collins’ latest research is asking a central question in the search for influences of *LMNA* or progerin production on longevity in the general population. Prompted by the Djabali evidence of increased amounts of progerin with age, Dr. Collins is looking for variations in DNA sequence around the *LMNA* gene in fibroblasts of long-lived people that may give them a selective advantage over those individuals who die at younger ages. In an experiment that examined cells from more than 1000 people aged greater than 94 years, he found that four single nucleotide polymorphisms (SNPs) differed from the general population ($p = 2 \times 10^{-5}$). Four of four centenarians analyzed also contained these same SNPs. The probability of this finding being random is 7×10^{-8} . Further study will ask whether increases or decreases in progerin production are linked to these SNPs.

Normal cellular senescence is marked by increasing rates of DNA damage and a decline in the ability to repair this damage (36). Progeria cells have been shown to accumulate double-stranded DNA (dsDNA) breaks and impaired DNA repair (37–39). *Yue Zou* (East Tennessee State University) presented results on the mechanism of this failure (40). Normally, in response to formation of dsDNA breaks, histone H2AX is rapidly phosphorylated and then recruits various DNA repair proteins, together forming foci for DNA repair. In progeria fibroblasts, the phosphorylated protein, called gamma-H2AX, was unable to recruit the DNA repair enzymes Rad51 and Rad50. Instead, the damage recognition protein XPA (xeroderma pigmentosum group A) was recruited. XPA normally participates only in excision repair systems and has no known role in dsDNA repair. Similar results were obtained from restrictive dermatopathy cultures.

Dr. Zou confirmed the identity of XPA by immunoprecipitation, and XPA siRNA knock-down experiments reversed these findings in progeria cultures. Thus, XPA likely plays a key role in defective dsDNA repair in progeria. Importantly, Dr. Zou and colleagues found that FTI treatment could not reduce the accumulated DNA breaks in progeria cells, suggesting that FTI may not effectively treat this aspect of disease (41).

FUTURE TREATMENT POSSIBILITIES THROUGH A BETTER UNDERSTANDING OF THE BIOLOGY OF DISEASE IN PROGERIA: STEM CELLS, ALTERNATIVE PRENYLATION, COMBINATION CHEMOTHERAPY, AND A NOVEL HIGH-THROUGHPUT SCREENING SYSTEM

Stem Cells

For several presenting researchers, stem cells took center stage for two reasons. First, there is the question of whether or not progeria is a disease characterized by stem-cell depletion, whereby there is an inadequate supply of stem cells for replenishing prematurely dying mesenchyme-derived cell populations (VSCM, adipocytes, fibroblasts, and osteocytes). Second, the lack of lamin A expression in immature cells presents stem-cell therapy as a viable strategy for treatment, or even reversal, of disease in progeria. The therapeutic potential of mesenchymal stem cells is being tested in numerous clinical and preclinical trials (42).

In his introductory presentation, *Huber Warner* (University of Minnesota) advanced his hypothesis that depletion of stem cells is a major factor in causing the tissue changes observed in progeria (43). The depletion may arise from the increased turnover of differentiated cells, which are lost at an accelerated rate (44). This hypothesis has been further analyzed in the literature. *Prolla* (45) suggested that the regenerative capacity of tissues with high turnover might be reduced due to the exhaustion of progenitor cells. Mesenchymal stem cells are believed to be involved in expression of symptoms of progeria, since most mesenchymal stem cells express lamins A/C (46), while some committed hematopoietic cell lines do not (47). *Gotzmann* and *Foisner* (48) point out that deregulated or impaired tissue regulation and deregulated cell-type plasticity might account for nearly all pathological conditions detected in laminopathies. *Halaschek-Wiener* and *Brooks-Wilson* (49) argue that depletion of mesenchymal stem cells can help account for the specific segmental nature of progeria.

The current published literature supports the view that lamin A, and hence progerin, is produced by differentiated cells, and undifferentiated cells do not transcribe or translate either molecule. However, *Carlos Lopez-Otin* (Universidad de Oviedo, Spain) described experiments in which hair follicle-derived stem cells are severely affected by defects in the lamin processing pathway. His *Zmpste24*^{-/-} progeria mouse model (50) produces no mature lamin A and suffers from a build-up of farnesylated prelamin A. The homozygous null mouse displays many features of human laminopathies, including severe growth retardation and premature death, dilated cardiomyopathy (DCM), muscular

dystrophy (EDMD), lipodystrophy (progeria), and loss of fur and whiskers. Dr. Lopez-Otin's preliminary studies show that, unlike their wild-type counterparts, many hair follicle-derived stem cells from these mice were quiescent, showed profound nuclear blebbing, and the accumulation of heterochromatin in two large masses in the centers of many cell nuclei. In addition, clonogenic assays showed that these stem cells have a profound proliferation defect, abnormal accumulation of p16, and an absence of beta-catenin, suggesting a loss of *wnt* signaling. Importantly, when an *LMNA* allele was crossed into *Zmpste24*^{-/-} mice, levels of farnesylated prelamin A decreased, lamin A was synthesized, and all measured cellular defects were normalized (39). Hence, in this system, stem-cell function was profoundly affected by nuclear defects and the ratio of farnesylated prelamin A to mature lamin A.

In light of this interest in the possibility that stem-cell depletion causes many of progeria's phenotypes, *Irina Conboy* (University of California at Berkeley) was invited to present her work on mechanisms causing the decline of tissue regenerative potential in normal aging skeletal muscle in response to injury and on regeneration using embryonic stem cells. She demonstrated both that a youthful biochemical milieu can restore cell vitality, and that old cells have regenerative capacity that can be tapped.

Skeletal muscle has its own source of stem cells, called satellite cells. Satellite cells are located at the basement membrane of terminally differentiated muscle fibers and can be identified and purified. Dr. Conboy has carried out both in vivo and in vitro experiments in which stem cells from young animals were exposed to niche elements from old animals and vice versa (51). She has discovered that the decline of regenerative capacity with age results from changes in the stem cell niche, not in the stem cells themselves. A key element is age-related impairment of the up-regulation of the Notch ligand Delta after muscle injury (52). Exposure of satellite cells from old mice to young serum enhanced the expression of Notch ligand Delta as well as proliferation and regenerative capacity of aged satellite cells. Forced activation of Notch by adding a Notch-specific antibody restored regenerative potential to old muscle.

Dr. Conboy has extended her studies to examine the effects of aged systemic milieu on the function of human embryonic stem cells (hESCs) (53). Aged mouse serum dramatically inhibited the self-renewal and proliferative potential of hESCs and satellite cells, as measured by expression of markers of stem-cell functionality, rate of cell proliferation, and the capacity for myogenic differentiation. When hESCs are cultured in serum-free medium instead of immediate transfer to medium containing aged serum, the cells are no longer susceptible to the effects of aged serum. This implies that hESCs have produced one or more anti-aging factors.

Together, these experiments suggest that the regenerative outcome of stem-cell replacement will be determined by a balance between negative effects of aged tissues on transplanted cells and positive effects of embryonic cells on the endogenous regenerative capacity. This result indicates that a complex interplay between negative regulation by the

aged niche and positive regulation by hESCs will likely determine the success of hESC-based cell replacement therapies. Dr. Conboy's observations in striated muscle provide a well-tested pathway for studying the key questions of the role of stem cells and aging in progeria, and are easily adaptable to the progeria mouse model.

Tom Misteli and Paola Scaffidi (National Cancer Institute) presented the first evidence supporting the hypothesis that stem-cell depletion is a factor in organismal symptoms of progeria (53). The first step of their approach was to determine what gene expression programs are affected by progerin. The experimental system consisted of an immortalized skin fibroblast cell line that expresses inducible green fluorescent protein (GFP) progerin. In this system, significant progerin accumulation was first observed 5 days after induction and reached a plateau after 10 days at levels similar to those found in cells from progeria patients. This system allowed analysis of time-dependent changes in transcriptional profiles in response to progerin expression and compared to expression of GFP-tagged wild-type lamin A as a control.

Among genes known to influence differentiation, progerin induction led to up-regulation of several genes in the Notch signaling pathway, most prominently Hes 1, Hes 5, and Hey 1. This up-regulation was also found in progeria cells. Consistent with a direct role of nuclear progerin in their regulation, these genes are all in the downstream portion of the Notch pathway, and no increase was found in cleaved Notch expression or in the upstream cleavage reaction. This result dovetailed with Dr. Conboy's observations that Notch is important for maintenance of stem cells (including mesenchymal stem cells) and differentiation pathways, and points to the Notch pathway as a key element in progeria.

Drs. Misteli and Scaffidi further explored the effects of progerin on differentiation by inducing stem cells to go down various differentiation pathways (54). Preliminary results showed that the rate of osteogenesis was increased, adipogenesis was blocked, and chondrogenesis was not affected. They hypothesize that progeria leads to the activation of part of the Notch pathway that causes differentiation, and that accelerated aging in progeria patients is largely the result of stem-cell dysfunction.

ALTERNATIVE PRENYLATION AND THE NEED FOR COMBINATION CHEMOTHERAPY IN PROGERIA

Posttranslational processing to create lamin A involves several forms of prelamin A, both farnesylated and unfarnesylated (Figure 1). FTI treatment presumably leads to unusually increased cellular levels of nonfarnesylated forms of prelamin A and progerin, and could theoretically promote alternative prenylation (geranylgeranylation). The influences of nonfarnesylated prelamin A and progerin on cell function and disease status have yet to be intensely explored. Michael Sinensky (East Tennessee State University) has begun to examine alternative prenylation (geranylgeranylation) of prelamin A and progerin under the influence of FTI. He finds that, in cells treated with an FTI, several important cellular events occur: First, geranylgeranyl

is incorporated into prelamin to yield a geranylgeranylated intermediate. He also finds that Zmpste24 does cleave both farnesylated and geranylgeranylated prelamin A, and therefore mature lamin A is produced even in the complete absence of farnesylation. However, he finds that the farnesylated prelamin A is a 20-fold better substrate than geranylgeranylated prelamin A. Therefore, processing of geranylgeranylated prelamin A is slow, resulting in abnormal accumulation of nonfarnesylated prelamin A within cells. Progerin also became geranylgeranylated in cells treated with FTI, creating a new prenylated protein. Prelamin A accumulation can affect cell cycling (55). There is a paucity of data available comparing the behavior of geranylgeranylated versus farnesylated prelamin A or progerin, and this area of research may be essential to designing future treatments for progeria since alternative prenylation can alter the behavior and function of cells (56).

In a set of experiments with tremendous breadth, Carlos Lopez-Otin examined the potential role of lamin intermediates in disease, found support for alternative prenylation of prelamin A, and effectively treated a progeria mouse model using combination chemotherapy that interrupts progerin production at multiple points (57). The effects of lamin intermediates were first addressed. Dr. Lopez-Otin created a "triple mutant" mouse model that has the complete absence of both the Zmpste endonuclease and farnesyltransferase postnatally and is therefore the genetic equivalent of complete farnesyltransferase inhibition. Hence, only nonfarnesylated prelamin A is created in this mouse after birth. Strikingly, the phenotype of these mice mimicked the Zmpste24^{-/-}-only mice, without phenotype rescue. Dr. Lopez-Otin hypothesized that the phenotype was caused either by the accumulation of nonfarnesylated prelamin A, or by an alternatively prenylated prelamin A product. To resolve this issue, he turned to cell culture, and determined which species of prelamins were present in Zmpste24^{-/-} cells with and without FTI treatment. As expected, farnesylated prelamin A was present in Zmpste24^{-/-} cell extracts. However, when FTI was added to Zmpste24^{-/-} cell cultures, the amount of farnesylated prelamin A decreased, and geranylgeranylated prelamin A appeared. By extrapolation from FTI treated Zmpste24^{-/-} cultured cells to triple mutant mice, he hypothesized that the absence of farnesylation in Zmpste24^{-/-} mice does not improve the phenotype in vivo because geranylgeranylated prelamin A is created, and this product may be at least partly responsible for disease phenotype in the triple mutant mice.

Dr. Lopez-Otin went on to treat Zmpste24^{-/-} mice with two commercially available drugs, statins and bisphosphonates. These drugs block two different steps in the mevalonate pathway, upstream from farnesyltransferase and geranylgeranyltransferase (Figure 2). Treatment of Zmpste24^{-/-} mice with statins and bisphosphonates increased their 20-week life span by 80%. There was also impressive rescue of bone phenotypes, and cellular blebbing was substantially reduced. Further research is needed to understand the mechanism responsible for extended life span in treated mice, since bisphosphonates home specifically to bone. However, Dr. Lopez-Otin presents us with the first preliminary evidence that combination chemotherapy

using FTI along with drugs that affect both progerin production and alternative prenylation could be efficacious.

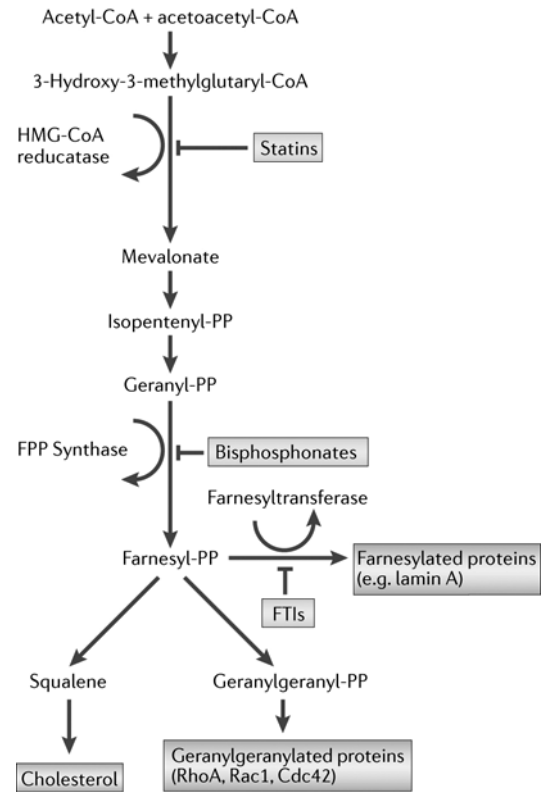
A POWERFUL NEW TESTING STRATEGY FOR DRUG DISCOVERY IN PROGERIA

Drug discovery is an important direction to pursue in progeria, especially in light of the possibility that drugs that help these children may also be beneficial to the normal aging population and a large fraction of men and women afflicted with cardiovascular disease. High-throughput screening (HTS) is an essential first step in drug development. The key to HTS is to develop an assay that easily detects some visible change in the target cells, due to exposure to a chemical compound that can be quickly and reliably read by a sensor. Nuclear blebbing has been used to measure improvement in progeria cells in response to various drugs such as FTIs. However, for testing chemical libraries on a large scale, such morphological assays are unsuitable. Tom Misteli and Cordelle Tanega (National Cancer Institute) have developed what could be a breakthrough testing strategy. The Misteli laboratory has previously showed that blocking the cryptic splice site that is activated by the progeria mutation with a complementary oligonucleotide restores the phenotype of progeria fibroblasts to normalcy (58). Because satisfactory methods for system-wide introduction of oligonucleotides to humans do not yet exist, Misteli has embarked on a search for small molecules that specifically inhibit the alternate splicing created in exon 11. The work is being carried out at a fully automated high-throughput system for drug screening at the NIH Chemical Genome Center.

Dr. Misteli devised a system based on dual fluorescence reporters that indicates whether the cryptic splice site in *LMNA* is used or not. After development, the assay was characterized using the available oligonucleotide and optimized for HTS. In pilot experiments, a small library of 1279 compounds was tested at seven concentrations, thereby producing a titration curve on the first screen. A large screen of ~150,000 compounds followed, and led to the identification of ~100 compounds based on their response profiles as well as structural and pharmacological considerations. Experiments are now under way to test these compounds in progeria fibroblasts, looking at cell phenotypes, splicing activity, and specificity.

DISCUSSION

The presentations, posters, and discussion at the 2007 Progeria Workshop showed extraordinary progress at all levels—from basic science, mouse and human studies that better define the biological effects of progerin on disease pathogenesis and on aging and cardiovascular disease in the general population, to the first clinical drug trial for progeria. The discovery that progerin is found in normal human fibroblasts in an age-dependent manner establishes a new link between progeria and aging. This is the best example yet of how studies on progeria may be valuable to understanding human aging. In addition, preliminary studies on samples from people over the age of 94 years found distinguishing single-nucleotide polymorphisms in the



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Figure 2. The prenylation pathway—opportunities for therapy (59). Potential for therapy (i.e., statins, bisphosphonates, and FTIs [farnesyltransferase inhibitors]) at three points along the pathway. FPP = farnesyl pyrophosphate; HMG-CoA = hydroxymethylglutaryl co-enzyme A; PP = pyrophosphate. [Reproduced by permission of the publisher.]

vicinity of the *LMNA* gene, a step toward identifying *LMNA* genotypes involved in longevity. Links between progerin, progeria, and the cardiovascular disease in aging are still in their early stages, as most experiments have been conducted on fibroblast cultures and should be extended to vascular cell types in future studies. However, pathological and clinical studies in humans and mice point to a nonlipid-driven vascular stiffening shared by both progeria patients and elderly individuals.

As the current drug trial for progeria is testing an FTI, there was much focus on the biological mechanisms that involve farnesylation during posttranslational steps that lead to lamin A. In a progeria model mouse, the first evidence was presented that FTI may reverse the damaging effects of progeria *in vivo*, even when administered after vascular damage has occurred. The importance of this result becomes obvious when one recalls that progeria is not diagnosed until significant damage to organ systems has taken place. In the numerous basic science studies on cellular effects in progeria, investigators often checked whether FTI treatment affected the cellular damage under study. As shown in Table 1, some but not all were reversed by FTI treatment.

Whether this will translate into some, but not complete, clinical improvement with FTI remains to be determined.

Pivotal factors for disease phenotype that emerged during this workshop included the balance between multiple prelamin A forms, mature lamin A, and progerin, the presence of alternative prenylation, and epigenetic changes modifying chromatin structure. It is highly likely that, as with most diseases, improving quality and longevity of life for children with progeria will require intense and multipronged research. The emerging data hold promise that progeria may be amenable to single or combination chemotherapy, stem-cell therapy, and genetic therapies. In addition, this field of study has already brought an entirely new gene (*LMNA*) and its protein products (lamin A and progerin) into the aging and cardiovascular fields. Future research should concentrate on understanding the influences of balancing prelamin A, lamin A, and progerin not only in progeria, but on health and longevity in all of us.

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