THE MAX AND VICTORIA DREYFUS FOUNDATION

THE PROGERIA RESEARCH FOUNDATION INTERNATIONAL WORKSHOP 2005

SUMMARY REPORT

I. Introduction

The most recent workshop of the Progeria Research Foundation (PRF) was held in Boston, Massachusetts this past November 3-5. The scientific program was organized by ***Drs. Leslie Gordon, Frank Rothman and Christine Harling-Berg*** (Brown U.) and attracted approximately 100 participants- clinicians, scientists and students dedicated to understanding and curing Progeria. They arrived from nine countries, making this workshop truly international and the largest Progeria workshop to date. For the first time, a poster session was necessary, since the explosion of Progeria research over the past year has produced more data than our oral platform could accommodate. In another first, the platform included a very special round table conversation between families of children with Progeria and workshop participants. ***Dr. Scott Berns*** (Brown U.), Chairman of the Board for PRF, physician, and a parent of a child with Progeria, moderated this insightful exchange, which gave scientists a rare opportunity to realize the potential impact of their research on the lives of children with Progeria and their families. Support for the conference was provided by the Office of Rare Diseases (NIH), the National Heart, Lung, and Blood Institute (NIH), The Ellison Medical Foundation, Celgene Corporation, The Max and Victoria Dreyfus Foundation, and The Progeria Research Foundation.

II. Highlights

A. *Phenotypic Reversal of the HGPS Phenotype in Cultured Cells*

Four presentations showed evidence for the reversal of the HGPS phenotype in cultured HGPS cells by farnesyl transferase inhibitors (FTIs). ***Francis Collins*** (Director, NHGRI, NIH), ***Loren Fong*** (UCLA), ***Thomas Glover*** (U. of Michigan) and ***Susan Michaelis*** (Johns Hopkins U.) examined disease phenotype by analyzing the fraction of cells with dramatically misshapen nuclei, characterized by blebs and evaginations, and the distribution of the mutated form of lamin A, named "progerin." Treating cell cultures with FTI decreased the percentages of abnormally shaped nuclei in a dose-dependent manner. FTI-treated cells simultaneously showed both lamin A and progerin relocation from the nuclear membrane to the nucleoplasm. The finding indicates that the improved nuclear shape was induced by the removal of progerin from the inner nuclear membrane, where it has its primary influence on nuclear structure. Exploring both the mechanism of disease and potential treatment of Progeria, ***Paula Scaffidi*** (NCI, NIH) presented data showing the phenotypic reversal of HGPS fibroblasts and lymphocytes by inhibiting transcription at the mutationally altered splice site. ***Junko Oshima*** (U. Washington) used RNA interference to produce reversal of Progeria phenotype in cell cultures. The Scaffidi and Oshima studies prove that genetic therapies are important tools for exploring Progeria biology and potential disease treatment.

B. *Improvement of the Progeria Phenotype in a Mouse Model*

In an important transition from *in vitro* to *in vivo* studies on the potential for treating Progeria with FTI, ***Stephen Young*** (UCLA) treated a mouse model of Progeria which lacks the enzyme ZMPSTE24 and thus cannot farnesylate either lamin A or progerin, with FTIs. When mice were treated with FTI orally starting at 5 months of age, they did not develop the major elements of the Progeria phenotype in this mouse model. The FTI-treated mice demonstrated increased body size, lack of osteoporosis and bone fractures, increased grip strength and increased percentage survival at 20 weeks compared to Progeria mice which did not receive FTI.

C. *Natural History of Progeria*.

Initial data were presented on a clinical natural history study of 10 children with HGPS, designed to create objective measures of disease status that could be followed progressively and used in clinical trials. Data from the numerous clinical tests carried out over a six day period in hospital demonstrated significant, quantifiable abnormalities in growth and body composition, bone density, joints, endocrine and vascular function (***Leslie Gordon***, Brown U. and ***Richard Cannon,*** NHLBI, NIH).

D. *FTI-Treatment for Cancer in Children*

Mark Kieran (Harvard U., Boston), a leading clinical trialist for the field of FTI, compared and contrasted tolerability and efficacy for cancer of two FTI's currently used in clinical trials involving children. Together with the phenotypic reversal studies and clinical studies described above Sections A., B., and C., these results have set the stage for a clinical trial using FTI to treat Progeria.

E. *A Broader View on Laminopathies*

Clues to HGPS pathophysiology may also be gleaned from the more than 10 other diseases resulting from mutation in the LMNA gene, which affect striated muscles, peripheral nerves, or adipose tissue. In HGPS several of the same phenotypes are observed suggesting a real continuum within the different types of laminopathies. ***Gisell Bonne,*** (INSERM, Paris) presented her view of this continuum of lamin-associated diseases, described her disease database constructed to collate and compare these rare diseases. ***W. Ted Brown*** (New York State IBR in Developmental Disabilities, Staten Island) commented on the mutations and phenotypes of several patients with various laminopathies, and ***Raoul Hennekam*** (U. Amsterdam, London U.) also presented his view on the clinical characteristics of progeria that need further study. In other, more focused presentations, ***Nicholas Levy*** (U. Marseilles, France) compared Restrictive Dermopathy, newly identified as a laminopathy by Dr. Levy, to progeria both genetically and phenotypically and ***Abhimanyu Garg*** (Southwestern Medical Center, Dallas) discussed his studies of insulin resistance and diabetes in lipodystrophies.

F. *Biological and Biochemical Studies of Disease Phenotypes in HGPS and Other Laminopathies*

1. ***Katherine Wilson*** (Johns Hopkins U.) proposed that actins are key to HGPS nuclear abnormalities, by failing to develop the usual lamin-associated network of stable filaments and therefore lead to mechanical instability.

2. ***Robert. Goldman*** (Northwestern U.) presented the loss of peripheral heterochromatin as a morphological hallmark of HGPS progeria nuclei . One mechanism for this loss is significant alterations in the state of histone methylation. This finding links histone methylation to the hundreds of changes in gene regulation observed in HGPS fibroblasts.

3. ***Richard Lee*** (Harvard U., Boston) found reduction in nuclear stiffness and increases in nuclear instability in fibroblasts by using sophisticated equipment to stretch individual cells from several laminopathies, suggesting important roles for nuclear mechanics and mechanotransduction .

4. ***Karima Djabali*** (Columbia U.) has developed a much needed antibody specific for progerin, which does not bind to normal lamin A or C. In earlier cytochemical work, antibodies against normal lamin A and C were used to localize progerin as well as the normal lamins. The polyclonal antibody specific to progerin has been used to demonstrate tissue specific patterns of accumulation of progerin. Results to date include a cellular age-dependence of the accumulation, and, given that HGPS children die of heart attacks and strokes following cardiovascular disease, the striking observation that in skin biopsies, progerin accumulates primarily in the nuclei of vascular cells.

5. ***Elizabeth Nabel*** (Director, NHLBI, NIH) presented an analysis of the cardiovascular system of a new HGPS mouse model developed by the laboratory of Francis Collins. This model carries a human bacterial artificial chromosome that harbors the HGPS mutation, but does not display the external phenotypes seen in HGPS. However, these mice develop a progressive loss of VSMCs in the media of the carotid artery and descending aorta, eventually resulting in their complete depletion. This and other structural changes lead to deficits in vascular function. The structural changes are strikingly similar to those reported in autopsies of children with HGPS and will open the field up for its first comprehensive studies of vascular disease in Progeria. Turning to the human disease process, ***Catherine Shanahan*** (U. Cambridge, England) discussed the mechanisms of VSMC dysfunction and the potential role for nesprins in Progeria, given their importance in VSMC stress response, cytoskeletal integrity, lamin and actin function.

G. *Linking Aging and Initiation of Cellular Senescence in Mouse Models*

Although HGPS children have some, but not all, characteristics of normally aged people, no link at the molecular or cellular level had been previously established. ***Carlos Lopez-Otin*** (U. de Oviedo, Spain) used the ZMPSTE24 $^{-/-}$ progeria mouse model to discover marked up-regulation of a number of genes whose expression results from activation of the tumor suppressor gene p53. P53 activation is in turn, linked to organismal aging and to the initiation of a cellular senescence program, thus establishing a molecular link between a premature aging syndrome and normal aging. In mice with mutations in Lmna and in p16/p19, ***Colin Stewart*** (NCI) demonstrated premature cellular death and defects in differentiation of myoblasts that help us understand the link between mesenchymal tissues and progeria. ***Maria Eriksson*** (Karolinska Inst., Sweden) has developed a tissue-specific HGPS mouse model to investigate epidermis and VSMC in disease process.

III. Summary and Conclusions by George Martin (U. Washington)

The highlights listed above demonstrate the extraordinary progress in HGPS research in the two and a half years that passed between identification and publication of the responsible gene and the workshop. The fast track toward a preclinical trial is perhaps unprecedented. Basic research continues apace leading to discoveries which may impact not only treatment of HGPS and other laminopathies, but also provide useful clues to mechanisms of aging and heart disease. The 2005 PRF Workshop continued to provide a meeting ground for both clinical and basic science investigators that was conducive to informal as well as formal exchanges, and served as a breeding ground for collaborations.