Children with Hutchinson-Gilford progeria syndrome (HGPS) age rapidly due to a rare de novo mutation which causes accumulation of a shortened form of prelamin A—called progerin—at the nuclear envelope.\(^1,2\) Progerin is toxic and causes misshapen nuclei, cell senescence, a host of aging-related disease phenotypes, and death in the teenage years from myocardial infarction or stroke.\(^3\) Because progerin is methylated by the enzyme ICMT at a carboxyl-terminal cysteine residue, earlier studies hypothesized that targeting ICMT might be an effective anti-HGPS therapy. These studies showed that targeting ICMT with genetic strategies improves phenotypes and extends survival in mouse models of progeria and that early-stage ICMT inhibitors can overcome senescence and improve phenotypes of cells from HGPS patients. However, further studies were not possible due to the lack of ICMT inhibitors with ample bioavailability and pharmacological properties. In this issue of *ACS Central Science*, Marcos-Ramiro and co-workers take a big step forward by synthesizing and validating a potent ICMT inhibitor that can be used in vivo, which improved key phenotypes of mice with progeria and extended survival.\(^4\) Although several hurdles remain, these results set the stage for future clinical trials in children with HGPS.

Understanding HGPS etiology and the significance of targeting ICMT requires at least a basic understanding of prelamin A’s complicated maturation process. In normal cells, prelamin A and other so-called CAAX proteins (e.g., RAS) undergo three posttranslational processing steps at a carboxyl-terminal CAAX motif: 1. farnesylation of the cysteine residue (the “C” in CAAX) by farnesyltransferase (FTase); 2. endoproteolytic removal of the −AAX; and 3. methylation of the newly exposed farnesylcysteine residue by isoprenylcysteine carboxyl methyltransferase (ICMT) (Figure 1). These steps render CAAX proteins hydrophobic, and in the case of prelamin A they target the protein to the inner surface of the nuclear envelope. Prelamin A is unique in that it is the only CAAX protein that undergoes a fourth processing step where the 15 carboxyl-terminal amino acids—including the farnesyl-methyl-cysteine residue—are cleaved off by ZMPSTE24; mature lamin A is thus formed from the amino-terminal portion (Figure 1). The HGPS mutation activates a cryptic splice site which causes a 50-amino acid internal deletion of prelamin A, which removes the ZMPSTE24 recognition site; hence, the truncated prelamin A protein, progerin, with its farnesyl-methyl-cysteine residue, accumulates at the nuclear envelope.

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Progerin triggers nuclear shape abnormalities and cell senescence, hair loss, stunted growth, bone abnormalities,
sclerotic skin, vascular defects, and death from myocardial infarction or stroke at a median age of 13 years. The identification of the HGPS mutation set off a flurry of studies to determine if inhibiting progerin farnesylation might be a therapeutic strategy. FTase inhibitors (FTIs)—previously developed for anti-RAS cancer therapy and failed due to an inherent resistance mechanism—were found to reduce nuclear shape abnormalities and delay symptoms and improve survival in mouse models of progeria. Last year, the FTI lonafarnib was approved as the first targeted therapy for children with HGPS—a milestone for the field. Although lonafarnib improves phenotypes and extends survival in children with HGPS, the effect is modest. One potential problem is that FTIs were developed for anticancer therapy and have potent antiproliferative effects. A potential explanation is that there are many dozen farnesylated proteins in cells, and several of them are involved in proliferation and growth mechanisms; in addition, farnesylation is important for their function. Thus, although lonafarnib reduces the toxicity of progerin and delays symptoms, it is unlikely to stimulate growth and development of children with HGPS. We would argue that an effective HGPS therapy should overcome senescence of cells and allow proliferation to proceed.

Targeting ICMT in HGPS therapy was first proposed in 2013 following studies which had shown that knockout of Icmt causes little or no phenotypic effects in mouse bone marrow, liver, and lung—suggesting that targeting ICMT with a small-molecule drug may cause low levels of toxicity. Moreover, targeting ICMT was found to interfere with prelamin A and progerin processing and mislocalize the proteins away from the nuclear rim. Progerin is toxic and causes all HGPS disease phenotypes. The farnesyltransferase inhibitor lonafarnib prevents progerin farnesylation and is the only approved targeted therapy for HGPS. In the current study, Marcos-Ramiro and co-workers develop a new inhibitor of progerin methylation (ICMT inhibitor Compound 21; UCM13207) and show that it improves survival in mice with HGPS.

Figure 1. Posttranslational processing of prelamin A and progerin. (A) In wild-type normal cells, the carboxyl-terminal CAAX-motif (i.e., CSIM) of prelamin A is modified in four posttranslational processing steps: 1. The cysteine residue (i.e., the “C” in CSIM) is lipidated by the farnesyltransferase (FTase). 2. The last three amino acids (i.e., the SIM) are proteolytically removed by RAS converting enzyme (RCE1) or zinc metalloprotease ste24 homologue (ZMPSTE24). 3. The newly exposed farnesylcysteine residue is methylated by ioprenylcysteine carboxymethyltransferase (ICMT). 4. The 15 carboxyl-terminal amino acids, including the farnesyl-methyl-cysteine residue, are lost due to upstream recognition (shown in red) and cleavage by ZMPSTE24. Mature lamin A is formed from the amino-terminal portion of the protein. (B) In HGPS cells, the G609G mutation activates a cryptic splice site which causes an internal deletion of 50 amino acids near the carboxyl terminus of prelamin A, which results in the production of a truncated protein called progerin. Progerin undergoes steps 1, 2, and 3 of the prelamin A processing steps. However, the internal deletion of 50 amino acids removes the ZMPSTE24 cleavage site, which results in accumulation of farnesylated and methylated progerin at the nuclear rim. Progerin is toxic and causes all HGPS disease phenotypes. The farnesyltransferase inhibitor lonafarnib prevents progerin farnesylation and is the only approved targeted therapy for HGPS. In the current study, Marcos-Ramiro and co-workers develop a new inhibitor of progerin methylation (ICMT inhibitor Compound 21; UCM13207) and show that it improves survival in mice with HGPS.
Enter Marcos-Ramiro and co-workers who have now produced an ICMT inhibitor (UCM-13207, Cpd21) that is bioavailable and has promising pharmacokinetic properties. Their drug improves both cellular and in vivo phenotypes of HGPS, including parts of the vascular phenotype, and extends survival of mice with progeria. The study represents an important step in the preclinical validation of this therapeutic strategy and raises hopes that clinical trials might be possible in the not-too-distant future.

There are both overlapping and distinct effects of Cpd21 in the current study compared with the two previous studies which used gene targeting and C75. Both the current and previous studies show that targeting ICMT mislocalizes progerin, alleviates senescence, and stimulates proliferation of cells from mice and children with HGPS. Both also show that targeting ICMT does not influence the characteristic nuclear blebbing phenotype of progerin-expressing cells. Moreover, the magnitude of the effects of these three approaches is comparable. Whereas the earlier studies find that blocking progerin methylation reduces its turnover and causes the protein to accumulate in the nucleoplasm, Cpd21 was found to increase progerin turnover and reduce its levels in cells and tissues. The latter result—reducing the levels of a toxic protein—is obviously more attractive from a therapeutic perspective, and it raises the questions of whether Cpd21 causes off-target effects that trigger progerin degradation or whether it influences LMNA transcription, splicing at the G609G site, or mRNA turnover. The current study did not distinguish between these possibilities.

What are the hurdles for taking this drug to clinical trials—other than conventional chemistry, toxicity, and funding issues associated with drug development? A potential problem is that since lonafarnib was shown to improve clinical phenotypes and survival of children with HGPS, it was proposed that all new drug candidates would have to be used in combination with this drug. The reason this is a problem is that progerin must be farnesylated for methylation to occur (see Figure 1); thus, targeting ICMT would be pointless in the presence of lonafarnib—at least from the perspective of our current biochemical and mechanistic understanding of progerin processing. For this requirement to be removed, Cpd21 would need to demonstrate outstanding performance in continuing preclinical validation experiments, and the results of the current study should be independently verified in other labs. Considering the severity of HGPS, it seems urgent to get going on those experiments.

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Notes
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