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# Clinical Trial of Protein Farnesylation Inhibitors Lonafarnib, Pravastatin and Zoledronic Acid in Children with Hutchinson-Gilford Progeria Syndrome

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**Disclosures**

Author LBG is the parent of a child who participated in the study.

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## Abstract

**Background—**Hutchinson-Gilford progeria syndrome is an extremely rare, fatal, segmental premature aging syndrome caused by a mutation in *LMNA* yielding the farnesylated aberrant protein, progerin. Without progerin-specific treatment, death occurs at an average age of 14.6 years from an accelerated atherosclerosis. A previous single-arm clinical trial demonstrated that the protein farnesyltransferase inhibitor, lonafarnib, ameliorates some aspects of cardiovascular and bone disease. This present trial sought to further improve disease by additionally inhibiting progerin prenylation.

**Methods—**Thirty-seven participants with HGPS received pravastatin, zoledronic acid and lonafarnib. This combination therapy was evaluated, in addition to descriptive comparisons with the prior lonafarnib monotherapy trial.

**Results—**No participants withdrew due to side effects. Primary outcome success was pre-defined by improved per patient rate of weight gain or carotid artery echodensity; 71.0% of participants succeeded (P<0.0001). Key cardiovascular and skeletal secondary variables were pre-defined. Secondary improvements included increased areal (P=0.001) and volumetric (P<0.001–0.006) bone mineral density, and 1.5–1.8-fold radial bone structure increases (P<0.001). Median carotid artery wall echodensity and carotid-femoral pulse wave velocity demonstrated no significant changes. Percentages of participants with carotid (5% to 50%; P=0.001) and femoral (0 to 12%; P=0.13) artery plaques and extraskeletal calcifications (34.4% to 65.6%; P=0.006) increased. Other than increased bone mineral density, no improvement rates exceeded those of the prior lonafarnib monotherapy treatment trial.

**Conclusions—**Comparisons with lonafarnib monotherapy treatment reveal additional bone mineral density benefit, but likely no added cardiovascular benefit with addition of pravastatin and zoledronic acid.

**Clinical Trial Registration—**URL: http://www.clinicaltrials.gov. Unique identifiers: NCT00879034 and NCT00916747.

### Keywords

aging; atherosclerosis; HGPS; lamin; rare disease; Progeria; laminopathy

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Hutchinson-Gilford progeria syndrome (HGPS) is an autosomal dominant, rare (population prevalence 1 in 18 million[1]), fatal, pediatric segmental premature aging disease[2]. Disease

manifestations include severe failure to thrive, scleroderma-like skin, global lipodystrophy, alopecia, joint contractures, skeletal dysplasia, global accelerated atherosclerosis with cardiovascular decline and cervical and cerebral stenoocclusive changes with debilitating strokes[2]. Without progerin-specific treatment, death at an average age of 14.6 years occurs mainly from myocardial infarction[3].

Classic HGPS is caused by a point mutation, c.1824C>T, in *LMNA*[4, 5] that activates an alternative splice site to produce a truncated lamin A protein named progerin. Lamin A, an inner nuclear membrane protein, broadly influences nuclear structure and function[6]. Post-translational farnesylation of lamin A by the zinc metalloprotease STE24 facilitates intercalation into the inner nuclear membrane where most of its functions are performed. Subsequent loss of the farnesyl anchor by the action of the zinc metalloprotease STE24 reduces the membrane binding affinity of lamin A, releasing it from the nuclear membrane[7]. Unlike lamin A, progerin's farnesyl anchor is not cleaved[5], and progerin remains more tightly associated with the nuclear envelope resulting in changes in nuclear envelope morphology and subsequent cellular damage[8].

Lonafarnib is a protein farnesyltransferase inhibitor (FTI) that reversibly binds to the farnesyltransferase CAAX binding site[9], thereby inhibiting progerin farnesylation and subsequent intercalation into the nuclear membrane. Disease phenotypes in HGPS and progeroid cell cultures[10–13], HGPS and progeroid mouse models[14–16], and human subjects[17, 18] are improved when progerin farnesylation is inhibited with an FTI.

We previously conducted a prospective single-arm clinical trial of lonafarnib for children with HGPS (NCT00425607)[17]. Lonafarnib was well-tolerated; the primary outcome measure (improved rate of weight gain) was achieved; cardiovascular distensibility, as assessed via decreased carotid-femoral pulse wave velocity ($PWV_{cf}$) and carotid artery echodensity, were improved; radial bone structural rigidity and sensorineural hearing were increased. There was preliminary evidence of decreased headache, TIA and stroke rates [18]. Other aspects of disease such as insulin resistance (IR), lipodystrophy, joint contractures, and skin were unaffected by drug treatment[17]. Lonafarnib treatment limitations may be explained by incomplete farnesyltransferase inhibition at maximum tolerated dose, potential disease-causing effects of non-farnesylated progerin, irreversibility of some aspects of disease after a critical time period, and/or some fraction of progerin undergoing alternative prenylation (geranylgeranylation)[19].

Based on the lonafarnib monotherapy outcomes, combined with preclinical data supporting inhibition of progerin prenylation upstream of its farnesylation step using combination therapy with pravastatin and zoledronic acid[10, 19], we conducted a single-arm treatment trial for children with HGPS. We hypothesized that the addition of upstream prenylation inhibitors could further improve disease phenotypes. We now report toxicity and outcomes from 37 children with HGPS treated with lonafarnib, pravastatin and zoledronic acid (triple therapy).

# METHODS

## General

Participants were 2 years of age and older with clinically and genetically confirmed c.1824 C>T, p. Gly608Gly classic HGPS, adequate organ and marrow function, reliable pre-trial body weights, and ability to travel for regular study visits. The study was approved by the Boston Children's Hospital (BCH) Committee on Clinical Investigation. Written informed consent was obtained and, when indicated, consents were translated into the parent(s)' primary language and discussions were performed with interpreters. Age-appropriate assent was also obtained. An initial feasibility study enrolled 5 participants who were naive to lonafarnib therapy; participants received triple therapy and were observed for a period of 4 weeks for significant toxicities. As no significant toxicities were observed, these participants were subsequently enrolled in a Phase 2 study without treatment interruption, along with 32 additional participants. All measures reported were determined prior to study initiation and were included as part of the trial protocol. Histories, physical examinations, and all efficacy testing was performed at BCH or Brigham and Women's Hospital, Boston MA.

## Study Drug Dosing and Administration

Trial medications were administered for a period of 40–52 months. Lonafarnib (Merck & Co., Inc.) dosing was continued or, for naive participants, initiated at 150 mg/m$^2$ twice daily. Participants experiencing drug-related grade 3 or 4 toxicity not responsive to supportive care measures were dose-reduced to 115 mg/m$^2$. Subsequently, participants were permitted to increase the dose of lonafarnib back to 150 mg/m$^2$ and monitored for tolerance. Participants were prescribed oral lonafarnib either by capsule or liquid suspension dispersed in Ora-Blend SF or Ora-Plus (Paddock Laboratories, Inc.), every 12±2 hours. Oral pravastatin (Pravachol, Bristol-Meyers Squibb) dosing was 5 mg for participants weighing under 10 kg, and 10 mg for participants weighing over 10 kg, once every 24±2 hours. Zoledronic acid (Zometa, Novartis, Inc.) was administered intravenously over 30 minutes, at baseline, months 6, 12, and 18, and at end-of-therapy. The initial infusion was 0.0125 mg/kg body weight; all other infusions were 0.05 mg/kg body weight. Serum calcium was measured immediately post-infusion and at 1–2 days post-infusion. Oral calcium (500 mg) and vitamin D (1000 IU) were supplemented daily to avoid hypocalcemia and vitamin D deficiency. Calcium supplementation was discontinued after 12 months.

## Toxicity monitoring

Participants were monitored for liver, kidney and hematological toxicity at each trial visit, and between visits when indicated symptomatically. Adverse events were monitored and recorded throughout the study during on-site visits, regularly scheduled home communications, and communications due to interim toxicities.

## Efficacy evaluations

The pre-specified primary outcome was a composite of two components relevant to disease in HGPS. The first was an increase over pre-therapy in estimated annual rate of weight gain, or a change from pre-therapy weight loss to statistically significant on-study weight gain.

This is a reliably trackable representation of the dramatic overall size deficit in HGPS. Children with HGPS have linear and individualized rates of weight gain, on average 0.44 kg/year, which remains stable over time after age 3 years[20]. Pre-trial body weights were obtained from The Progeria Research Foundation Medical and Research Database (PI L.B.G.), with parental consent (Brown University Center for Gerontology and Healthcare Research), or from participation in a lonafarnib monotherapy clinical trial (NCT00425607)[20]. A minimum of 6 weights were obtained from a calibrated medical grade scale, over a period of 6 months to 2 years. A participant was deemed improved in rate of weight gain if the participant experienced either a 10% annualized increase in rate of weight gain compared to pre-study entry, or if annualized change in weight converted from decreasing pre-study entry to increasing on-treatment. Rates of weight change were estimated by the slope of participant-specific least squares regressions versus age using data collected within the year prior to study entry and data collected during therapy.

The second component of the primary outcome was a decrease in echobrightness of the internal carotid artery (ICA) adventitia, with quantification of echodensity as a measure of vascular tissue distensibility[21]. This represents a measure of the early and pervasive cardiovascular disease in HGPS. Vascular echobrightness on ultrasound increases with tissue density. Echodensity values were quantified using ImageJ software (National Institutes of Health, Bethesda, MD) on a grayscale ranging from 0 (black) to 255 (white) according to pixel intensity, where 0 was calibrated to equal the density of intraluminal blood (preset to appear as black). All pre-specified vessel regions were captured as previously described[21]. Values were calculated using Matlab 7.9 (The Mathworks, Inc., Natick, MA). A participant was considered improved in echodensity of the deep common carotid artery adventitia if either the echodensity of the adventitia was reduced to less than or equal to 90% of the value at study entry or the patient-specific $10^{th}$ percentile of the density of the adventitia was reduced to less than or equal to 90% of the value at study entry.

Key secondary variables were pre-specified. $PWV_{cf}$, distal common carotid artery far wall intima-media thickness and plaque evaluations established using ultrasonography, 12-lead ECG, and standardized blood pressure were performed in a temperature controlled room with children in a fasting state as previously described[21], and detailed in the online supplement. Internal carotid artery flow was evaluated, but due to the complexity of analysis required to present this data, it will be included in a separate manuscript. IR was determined using homeostasis model assessment-insulin resistance = fasting (glucose)(insulin)/405 with IR defined as $\geq 2.5$.

Neuro-imaging included magnetic resonance imaging (MRI) of the brain and neck and magnetic resonance angiography (MRA) of the Circle of Willis and neck. Brain MRI consisted of sagittal and axial T1-weighted, axial T2-weighted fast spin-echo (FSE), axial fluid-attenuated inversion recovery (FLAIR), and axial diffusion weighted imaging with calculated apparent diffusion coefficient maps; brain and neck arterial imaging consisted of 3D Time of Flight MRAs; and neck imaging consisted of axial T1-weighted FLAIR and T2-weighted FSE imaging. Patients were scanned at 1.5 Tesla (General Electric Medical Systems, Milwaukee, WI) or 3 Tesla (Siemens, Erlangen, Germany) magnet strength.

Skeletal findings were evaluated as previously described[22, 23]. Dual X-ray absorptiometry (DXA) areal bone mineral density (aBMD) measures were performed using a Discovery A Scanner (Hologic, Inc.)[23]. Load bearing capacity [axial (EA), bending (EI) and torsional (GJ) rigidities] and volumetric BMD (vBMD) were calculated using peripheral quantitative computed tomography (pQCT) XCT 3000 (Stratec, Inc.) images obtained at serial cross-sections through the radius. These measures reflect the structural properties of the cancellous and cortical bones at 4%, 20%, and 50% distances from the proximal end[23].

Methods for pharmacokinetics (PK), nutritional intake, measured resting energy expenditure (MREE), and dermatologic assessments are detailed in online supplement.

### Statistics

The study was powered as follows: The pre-specified null hypothesis of interest is that the true success rate is less than or equal to 4%; the alternative is that the true success rate exceeds 4%. Specifically, the null and alternative hypotheses were: $H_0$: $\pi \leq 0.04$; $H_1$: $\pi > 0.04$, where $\pi$ is the true (unknown) overall success rate. At a one-sided 0.05 level of significance, assuming the true success rate is 0.17 (17%) or greater, 33 evaluable participants yielded 82% power to reject the null hypothesis in favor of the alternative based on the exact test of the binomial distribution.

Descriptive statistics included mean, standard deviation for symmetrically-distributed continuous variables, median and quartiles for skewed variables, and counts and percentages for categorical variables. Assessments of trends over time in continuous secondary and tertiary endpoints were assessed via parametric or non-parametric repeated measures depending on the distribution of the endpoint. These p-values do not reflect adjustment for multiple comparisons, and should be interpreted only descriptively. P-values presented are two-sided and are considered significant at the 0.05 level. All statistical analyses were carried out using SAS version 9.3.

## RESULTS

### Participants and Testing Participation

Thirty-seven participants with classic HGPS from 23 countries were enrolled in this triple drug trial. Twenty-four of the 37 participants had participated in the lonafarnib monotherapy trial (treatment non-naive) and therefore had received continuous lonafarnib treatment for at least two years prior to triple trial enrollment. Thirteen participants had no prior exposure to lonafarnib (treatment naive). Three participants were taking statins at trial entry. No participant had previous exposure to bisphosphonates.

A complete consort diagram details testing inclusion (Figure 1). Five participants did not complete the study: 2 voluntarily withdrew due to non-medical issues within 6 months of trial enrollment, and 3 died prior to study completion. Deaths were caused by trauma from a motor vehicle accident, head trauma and myocardial infarction; at ages 20, 10, and 20 years, respectively. Toxicity results are reported for all 37 enrolled participants. Pharmacokinetics (PK) are reported for the 35 participants who did not voluntarily withdraw. The primary outcome (composite of echodensity change and weight gain) is reported for 31 participants

(35 participants who did not voluntarily withdraw minus 4 participants who were *a priori* excluded from the analysis due to being under age 3 years, which is too young to establish weight gain thresholds); participants who died during the study period are imputed as primary outcome failures. Otherwise, efficacy outcomes are reported for 32 participants completing baseline and end-of-therapy measurements.

Baseline participant characteristics are presented in Table 1. Patient level treatment duration is presented in Table S1. Forty percent of participants were male. The average age at enrollment was 8.0 (+/− 4.4) years; mean age at enrollment was younger for treatment naive vs. non-naive participants (P=0.0009).

## Lonafarnib, Pravastatin, and Zoledronic acid Treatment Toxicity

Overall, therapy was well-tolerated, and no participant came off study due to treatment-related toxicity. Toxicity details were consistent with the known toxicity profiles of lonafarnib[17, 24] and zoledronic acid (http://www.pharma.us.novartis.com/product/pi/pdf/Zometa.pdf). Generally, lonafarnib-related side effects included mild diarrhea, fatigue, nausea, vomiting, anorexia, abdominal pain, and elevated liver function tests (Table S2). There were no pravastatin-related side effects identified. Zoledronic acid-related side effects included post-infusion flu-like symptoms and hypocalcemia, at rates significantly lower than those previously published for non-HGPS pediatric studies (Table S3)[25]. Within 48 hours of successive zoledronic acid infusions (baseline, months 6,12,18 and end-of-study), participants developed one or more flu-like symptoms at rates of 11.1%, 25.7%, 14.3%, 2.9% and 6.7%, respectively. Participants developed hypocalcemia at rates of 5.6%, 5.7%, 8.6%, 0% and 3.3%, respectively. Overall, 23/37 (62%) participants experienced post-infusion side effects.

## Primary Outcomes

Overall, 22/31 (71.0%) participants (9 treatment naive and 13 non-naive) succeeded under the prospectively established primary outcome measure of success (P<0.001 vs. a pre-specified performance goal of 4% success rate), which required success for either weight gain or echodensity (Table 2). Individually, weight gain success was achieved in 15 of 31 (48.4%) participants (4 treatment naive and 11 non-naive), while echodensity success was achieved in 11 of 35 (31.4%) participants (8 treatment naive and 3 non-naive). However, only 6 of 35 (12.9%) participants (all four participants too young to be included in the weight gain analysis experienced echodensity failure) succeeded for both outcome measures, which implies that these two outcome measures may not be clinically related. Patient level data on primary outcome measures is presented in Table S1.

## Secondary Outcomes

**Weight and Nutritional Findings—**There was no significant difference between average daily energy intake, fat or carbohydrate intake, or MREE for participants who succeeded versus those who failed the weight outcome (Table S4). Protein intake was increased for the success group when assessed in the 10% rate of weight gain group (P=0.04), and nearly significant for the 50% rate of weight gain group (P=0.07). This is

partly supported by an increase in lean body mass by DXA for the 50% success group (P=0.02), but not by the 10% success group (P=0.27; Table S5).

**Cardiovascular and Neurovascular Findings—**Carotid artery wall echodensity and carotid-femoral pulse wave velocity ($PWV_{cf}$) represent measures of arterial structure and function[21]. Mean carotid artery wall echodensity of the intima-media, near or deep adventitia, as well as $PWV_{cf}$ demonstrated no significant changes overall nor within naive and non-naive subgroups (Figure 2), presumably representing no overall change in vascular stiffness.

Prevalence of carotid artery plaque significantly increased during the triple trial, with 5% (n=2) of participants at baseline vs 50% (n=14) at end of study; (P<0.001). Plaque was identified in the superficial femoral arteries (SFA) as well (0% baseline and 13% (n=4) at end-of-study, though not statistically significant (P=0.13) (Table 3). These are the first atherosclerotic SFA plaques identified in HGPS.

IMT was within the normal range (means ±SDs = 0.42–0.44±0.03–0.07) with no significant changes between baseline and end-of-therapy at trial entry and end-of-therapy overall (n=64 vessels; P=0.47), and in naive (n=24 vessels; P=0.25) and non-naive (n=40 vessels; P=0.24) subgroups.

Left ventricular hypertrophy (LVH) is the predominant ECG abnormality in HGPS[17, 21]. One of 32 (3%) patients had LVH at study entry. This participant remained positive, plus 7 additional participants developed LVH by end-of-therapy (8/32 (25%); p = 0.016). Of the 3 patients who died during the triple trail (not considered in the denominator of 32 above), 1 had no LVH and 2 had LVH at baseline and at 12-month study visits.

Triple therapy yielded decreasing trends (P=0.065) in diastolic blood pressure (BP) in relation to both chronologic and height-age normal comparison values (Table 3)[21]. Systolic BP was decreased for height-age, but remained the same for chronologic age.

IR rates increased during triple therapy, primarily in the non-naive population (Table 3). Mean serum leptin levels were extremely low at study entry (females 0.95±1.04; males 0.95±0.81) and did not change significantly at end-of-therapy (females 0.66±0.0.51, n=11, P>0.25; males 0.59±0.27 ng/ml; n=11, P=0.21).

Two participants developed new brain infarcts on MRI during the study period. Of the 5 participants (14%) who had infarcts on their baseline brain MRIs, 1 had an infarct while on therapy and 4 did not develop additional infarcts during the study period. Three of 37 (8.1%) participants experienced new TIAs; one of these also experienced a new infarct. Headache frequency decreased from 1.2/week to 0.81/week.

**Skeletal Findings—**There were significant improvements in absolute and height-adjusted areal bone mineral density (aBMD) (P<0.001), and radial vBMD at all sites (P <0.001–0.006; Figure 3, Table S6). There was marked improvement in all structural rigidity parameters, at all sites. Axial, bending, and torsional rigidities improved by 1.6-fold, 1.5-fold and 1.8-fold, respectively (P<0.001–0.03; Figure 3, Table S7).

Extraskeletal calcifications were detected by X-ray, primarily located in the digital tufts, but also at various locations throughout the body[22]. Prevalence rates increased from 34.4% (n=11/32) at baseline to 65.6% (n=21/32) of participants at end-of-study (P=0.006). Calcifications were also observed as calcific skin eruptions in 3 participants demonstrated these at baseline, and 7 participants exhibited new eruptions while on triple therapy. Mean serum total protein, calcium, vitamin D, phosphorous and calcium-phosphate product were within normal ranges pre-therapy and at end-of-therapy (Table S8).

A minority of participants experienced new hip dislocations (3/37 participants; 8%), shoulder dislocations (3/37 participants; 8%), appendicular fractures (6/37; 16%), and skull fractures (3/37; 8%).

**Lonafarnib Pharmacokinetics—**PK characteristics were similar to those previously published in HGPS and non-HGPS pediatric participants (Figure S1 and online supplement)[17, 24]. Mean time to maximum drug concentration was 3.5 h (n=34). Average maximum concentration was 2.67±1.2 μg/ml. Six participants' baseline and peak pK values indicated that they were not at steady state, likely due to medication noncompliance. Results did not change significantly when these PK values were omitted from the analysis.

**Additional Pertinent Negatives—**Several additional measures were abnormal pre-therapy, and did not change significantly with treatment. These included joint contractures, typical HGPS-related X-ray findings, and cranial hair counts[17].

**Age Association versus Treatment Effect—**To help assess whether results for the key outcomes were due to the therapeutic regimen versus natural history of disease, we analyzed cross-sectional associations of LVH, IR, PWV, extraskeletal calcification, and echodensity, with participant age at baseline, in approximately 38 participants, prior to initiation of lonafarnib or triple therapy treatment. LVH and IR prevalence were positively associated with age ($p \leq 0.017$). After adjusting for age, the increase in LVH and IR incidence from baseline to end of triple trial discussed above was no longer significant ($p \geq 0.159$). There was a trending but non-statistical linear relationship between PWVcf and age (P=0.13), and between extraskeletal calcification and age (P=0.08). After adjusting for age, the increase in PWVcf and calcification with triple therapy was not significant (P=0.216 and 0.11, respectively.) While these results support that worsening over the trial period could be in part due to the natural history of disease in HGPS with increased age, it does not limit the possibility that triple treatment may still effect these variables. For example, though the age-adjusted increase in extraskeletal calcification across the trial was not significant, the odds ratio for calcification incidence at end of trial versus baseline was still 2.0 adjusting for age (it was 3.6 unadjusted). Thus the adjusted odds ratio after adjusting for age remained greater than 1, albeit not significantly, indicating still a potential relationship with calcification and triple therapy. Echodensity was not associated with age (P=0.73) in this cohort.

## Triple Therapy Comparisons with Previous Lonafarnib Monotherapy Trial Results

Success of the primary outcome measure for the previously published lonafarnib monotherapy trial required ≥50% increase in annual rate of weight gain from pre-to post-

therapy. Because many of our triple trial participants had already been treated with lonafarnib for 2 years and had an opportunity to increase rates of weight gain on monotherapy, the current trial design required only a 10% increase in annual rate of weight gain in order to be considered a success for the weight component of the primary endpoint. When applying the more stringent ≥50% increase to the current triple therapy trial results, triple therapy improved rate of weight gain (29.0% of participants) with similar results for treatment naive (33.3%) and non-naive (27.2%) (Table 2). Successful increase in rate of weight gain on monotherapy did not necessarily portend success in rate of weight gain on triple therapy. Of nine participants who succeeded in gaining ≥50% in rate of weight gain on monotherapy and entered triple therapy, two succeeded in gaining ≥50% on triple therapy. Of the 13 participants who failed to gain ≥50% in rate of weight gain on monotherapy and entered triple therapy, four went on to succeed in gaining ≥50% on triple therapy.

Overall, baseline participant characteristics (Table 1) were similar to those of the lonafarnib monotherapy trial previously reported[2, 17], though the naive triple therapy subgroup was younger on average. BMD was the only outcome that improved in the current trial and showed no significant improvement during the lonafarnib monotherapy trial (Figure 3, Table S6). Skeletal rigidities improved, but rates were below that of the lonafarnib monotherapy trial; 1.5–1.8-fold improvement compared to the nearly 3-fold improvement noted with monotherapy treatment (Figure 3, Table S7).

In addition, a variety of treatment outcomes were not significantly different from those experienced after 2 years of lonafarnib monotherapy in the prior treatment trial. These include average daily energy intake, macronutrients, or MREE for participants who succeeded versus those who failed the weight outcome (Table S4); normal carotid artery IMT at baseline and end-of-study (Figure 2); rates of on-therapy joint dislocations, fractures (see online supplement), transient ischemic attacks (TIAs) and stroke[18].

Importantly, rates of several outcomes indicating disease progression that could not be accounted for by increasing participant age were significantly accelerated in the current trial, where a similar acceleration was not detected in the prior lonafarnib monotherapy trial. In contrast to the current study, the prior lonafarnib monotherapy trial showed no participants developed new carotid artery plaques, and no SFA plaques were detected at baseline or end-of-study; there was no significant increase in rates of extraskeletal calcifications (prevalence rates went from 29% (n=8/25) to 44% (n=11/25; P=0.45) of participants).

## DISCUSSION

We report results from a single-arm combination therapy clinical treatment trial for children with HGPS. This trial followed a single-arm farnesyltransferase inhibitor (lonafarnib) monotherapy trial for HGPS, and added two prenylation inhibitors, zoledronic acid and pravastatin, to lonafarnib treatment. Lonafarnib toxicity profile and pK were unaffected by addition of pravastatin and zoledronic acid, and all drugs were well tolerated, overall.

For the composite primary outcome measure, each participant's pre-trial rate of weight gain or carotid artery echodensity was used as his or her own control. In children with HGPS, rate

of weight gain is linear over time after age 3 years. We hypothesized this variable to be a surrogate measure of overall disease status. Rate of weight gain represents the severe growth impairments in HGPS, while carotid artery wall echodensity is expected to increase in the aging population[26], and therefore approximates an element contributing to the cardiovascular decline that causes mortality in HGPS. Since we previously showed that carotid artery echodensity is a hallmark of vascular disease in HGPS after age 3 years[21], this cardiovascular endpoint was included as a second primary outcome measure. Using these two predefined measures, the composite primary study outcome was achieved. However, since there was little cross-over between success for participants between weight and carotid artery echodensity, it is unlikely that rate of weight gain is a surrogate for cardiovascular health.

Though the primary outcome measure for this trial was successful, overall, the results are mixed, presenting the authors with several significant concerns. The previously conducted lonafarnib monotherapy trial yielded cohesive evidence for cardiovascular benefit, with $PWV_{cf}$ and echodensity improvements, and evidence of stable plaque status. In contrast, benefit assessed by overall change in $PWV_{cf}$, echodensity, BP, and insulin resistance was not achieved by adding zoledronate and pravastatin to lonafarnib treatment. Though outcomes reflecting skeletal structure were improved, they did not change above the improvement change rates seen with lonafarnib alone, even in the treatment naive subgroup.

Some systems were positively affected by treatment. Stroke rate was extremely low, and participants experienced increases in overall bone size along with significant improvements in structural properties of the bone. Bone changes for the treatment-non-naive group improved by a smaller margin than the treatment-naive group, likely due to the improvements already achieved with prior monotherapy that created an improved status at baseline when compared to the treatment-naive group.

Our group previously published that the BMD of children with HGPS is in the normal range when measured directly by pQCT, but that aBMD is in a slightly low range (aBMD Z-score $<-1.0$) when estimated by DXA because bone thickness and cross-sectional geometry are ignored by the latter method. Lonafarnib monotherapy did not change these outcomes[23]. In this study, the apparent increase in both aBMD and vBMD of patients in the triple therapy cohort reflects the inhibition of osteoclastic bone resorption by zoledronic acid which curtailed endosteal remodeling of the intramedullary canal of the diaphysis (20% and 50% forearm sites) and augmented trabecular bone mass at the spine and radial metaphysis (4% site). The radial cortical wall thickness increased since the cross-sectional area of the intramedullary canal remained constant, but periosteal surface continued to expand with growth.

Some findings imply that there may be calcium build-up in various tissues, including vasculature. This tendency is supported by *in vitro* studies showing that progerin promotes abnormal VSMC matrix production [27] and impaired mitochondrial function resulting in reduced ATP production and impaired synthesis of PPi resulting in decreased extracellular pyrophosphate [28]. Progerin also causes osteogenic differentiation of human mesenchymal stem cells[29]. Clinically, outcomes reveal an increase in carotid and femoral arterial plaques

with ultrasound (potentially contributing to the appearance of LVH by ECG), and an increase in extraskeletal calcifications by X-ray. Since we found normal levels of calcium, phosphate, and calcium-phosphate product in serum, increased serum levels of calcium and phosphate cannot account for this finding[30]. Both vascular plaques and extraskeletal calcifications occur in HGPS without experimental treatments, but rates observed on-study were increased with triple therapy. The increased rate of appearance of atherosclerotic plaques compared with the monotherapy study implies that their development may have been exacerbated by triple therapy. Whether inhibition of osteoclastic activity by zoledronic acid contributed to these events should be further investigated.

There were a variety of challenges and study limitations. We conducted a single-arm study that included both participants naive to lonafarnib therapy, as well as those previously treated with lonafarnib. HGPS has a prevalence of 1 in 18 million living individuals, and this study was able to enroll much of the identified population worldwide. Much of that population had been enrolled in the previous monotherapy trial and therefore, no model for control arm pairing was possible. Secondary outcome analysis compares historical data from the lonafarnib monotherapy trial, a 2-year, 25 participant study, to the current 3–4 year, 35 participant trial; both are imbalanced comparisons. However, whereas extended treatment with triple therapy could have provided additional time for treatment benefit over and above lonafarnib monotherapy, this did not occur even with comparisons encompassing only the treatment-naive cohort. In addition, it is possible that the effects of lonafarnib, pravastatin, and zoledronic acid interact in ways that preclude the effects of any one of these drugs administered as a monotherapy. This synergy could occur via effects of on the lamin A and progerin maturation pathway, or through diverging influences on cellular signaling that interact downstream.

We previously demonstrated that prenylation inhibition extends estimated lifespan in children with HGPS[3]. The published study compared both historic and concurrent untreated control participants with those participating in the prior lonafarnib monotherapy treatment trial grouped with current triple therapy trial participants, in part because the majority of participants took part in both trials. Lonafarnib inhibits farnesylation, the statin pravastatin inhibits HMG-CoA reductase, and the bisphosphonate zoledronate inhibits farnesyl-pyrophosphate synthase. Each enzyme functions along the protein prenylation pathway. Given that lonafarnib monotherapy provided evidence for cardiovascular benefit in HGPS, and triple therapy does not provide evidence for additional benefit, it is likely that lonafarnib, and not zoledronate and pravastatin, was mainly responsible for estimated lifespan extension. Overall, given the ability to demonstrate a small improvement in survival using lonafarnib, a drug that likely affects progerin prenylation at modest levels at its maximum tolerated dose, it will be important to investigate additional candidate drugs or strategies aimed at depleting progerin from the nucleus. A variety of promising preclinical studies have begun to address this crucial issue [31]

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Glossary

| | |
|---|---|
| **PK** | pharmacokinetics |
| **MREE** | measured resting energy expenditure |
| **$PWV_{cf}$** | carotid-femoral pulse wave velocity |
| **aBMD** | areal bone mineral density |
| **pQCT** | peripheral quantitative computed tomography |
| **vBMD** | volumetric bone mineral density |
| **IR** | insulin resistance |

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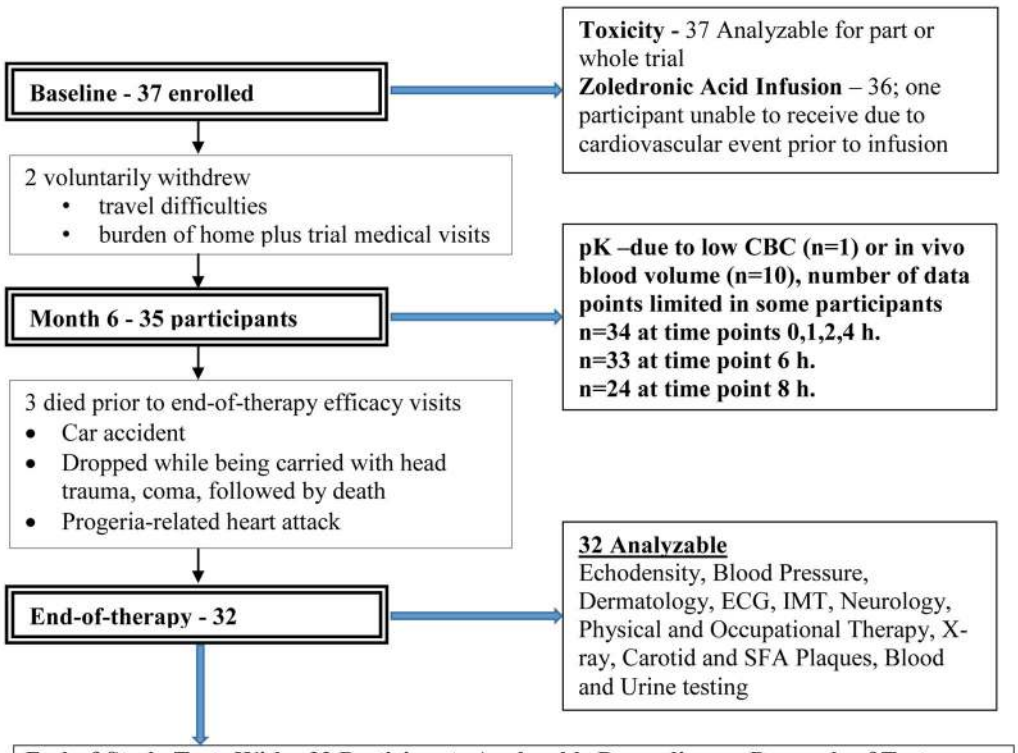
## Clinical Perspective

**What Is New?**

- In a prior clinical trial, the protein farnesyltransferase inhibitor, lonafarnib, ameliorated some aspects of cardiovascular and bone disease in children with HGPS.
- This present trial sought to further improve health, by adding zoledronic acid and pravastatin to lonafarnib treatment.
- The composite primary study outcome, increased rate of weight gain and decreased carotid artery echodensity, was achieved.
- Overall, participants experienced increased bone density, size, and structural properties; however, unlike the prior lonafarnib monotherapy, mean carotid-femoral pulse wave velocity and mean carotid artery adventitial echodensity were not improved.
- In addition, rates of carotid and femoral arterial plaques and extraskeletal calcifications increased.

**What are the clinical Implications?**

- Comparisons with prior lonafarnib monotherapy treatment reveal additional bone mineral density benefit, but likely no added cardiovascular benefit with addition of pravastatin and zoledronic acid.
- Since increased bone fracture is not a disease feature, the addition of the combination of statin and bisphosphonate to lonafarnib therapy is not recommended for clinical treatment of HGPS.
- Though not an inherent feature of HGPS, it is reasonable to consider statins for the treatment of lipid abnormalities when clinically indicated.

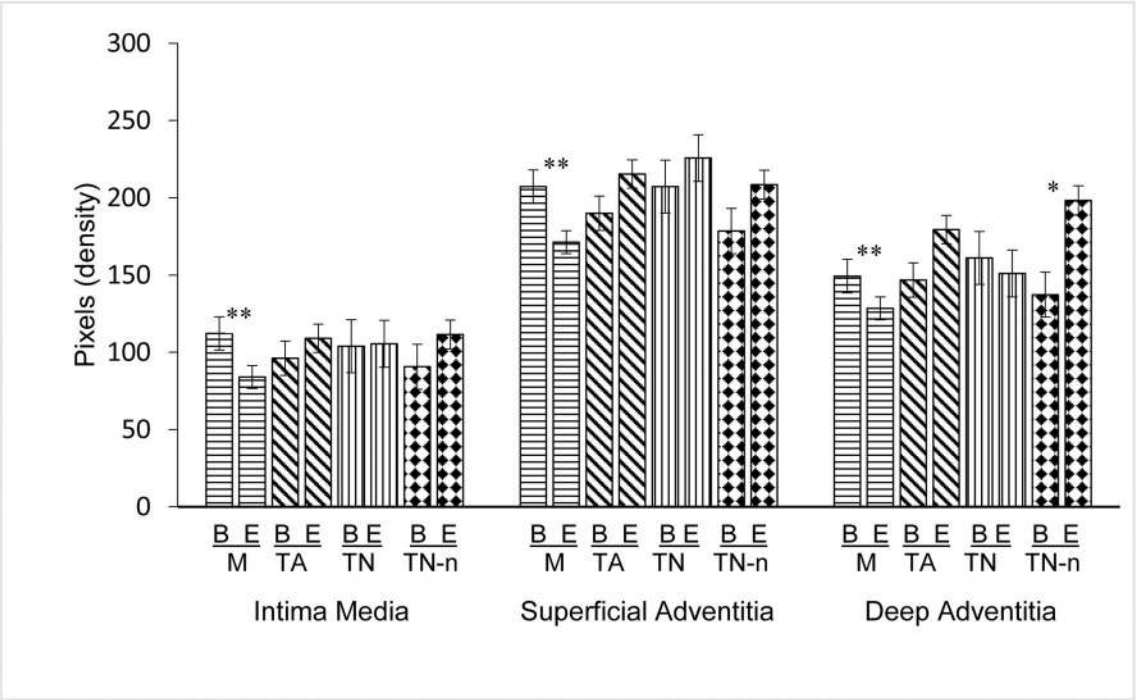



**End-of-Study Tests With <32 Participants Analyzable Depending on Demands of Test**

| Test Name | # Performed/ Analyzable | Reasons for Omissions |
|---|---|---|
| Zoledronic Acid Infusion | 30 | 1 withheld due to unstable respiratory status, 1 withheld due to investigator recommendation |
| Rate of Weight Gain | 28 | 4 participants too young for valid HGPS weight curve |
| cf Pulse Wave Velocity | 23 | 9 had malfunction with ECG-to-ultrasound communication |
| Nutrition | 25 | 7 participant families did not complete food record |
| MREE | 29 | 3 participants too young to perform |
| MRI | 26 | 4 participants too young to perform; 2 had discomfort |
| pQCT | 26 | 6 participants too young to perform |
| DXA | 31 | 1 participant too young to perform |

**Figure 1.**
Consort Diagram of Trial Inclusion and Testing

A.



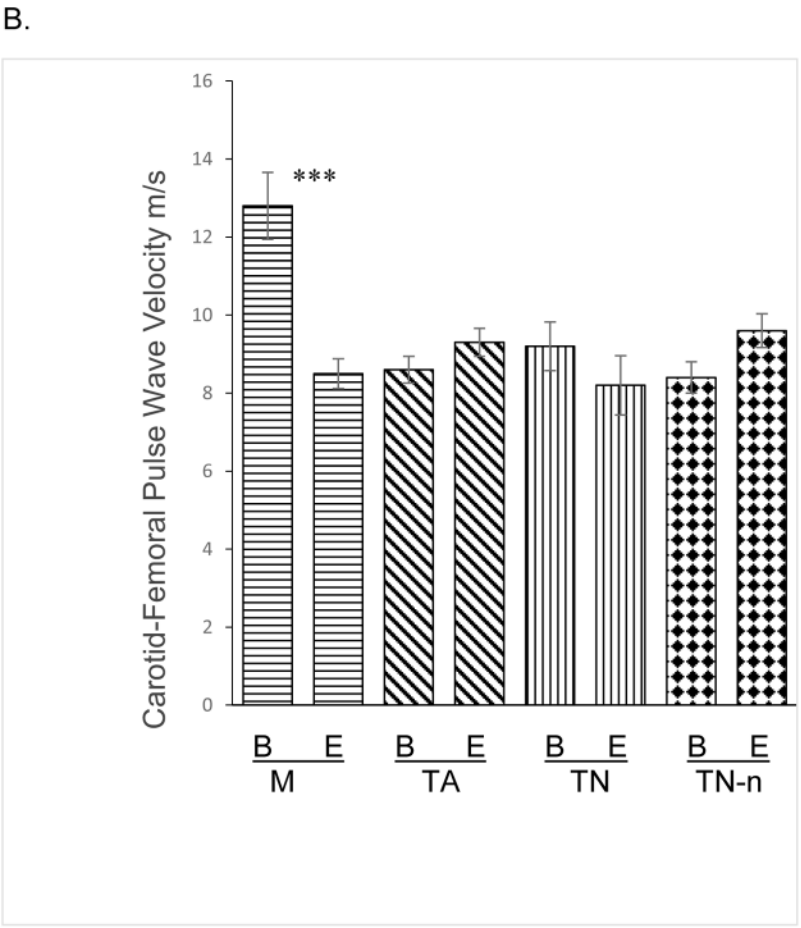
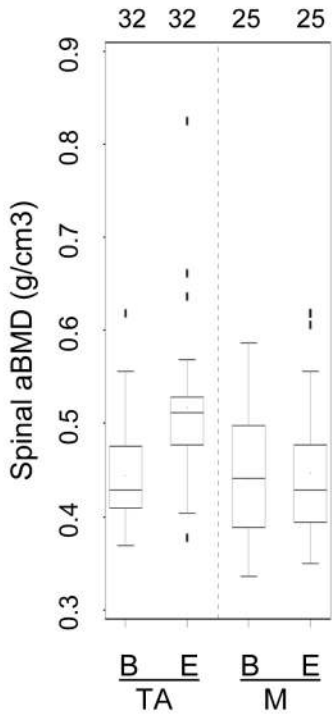



**Figure 2.**

Cardiovascular Outcomes Comparing Triple Therapy Whole Cohort, Naive and Non-naive Subgroups with Lonafarnib Monotherapy. All bars show mean (±SE); P-values between adjacent bars: ***=<0.0001,**= <0.001, *=<0.05.; B-baseline, E = end of study, M=lonafarnib monotherapy, T= triple therapy, N-naive participants, N-n=non-naive participants A. Carotid artery echodensity significantly decreased with monotherapy (n=24), but not with triple therapy (n=30) regardless of naive or non-naive entry status. B. PWV: The monotherapy cohort entered the trial with significantly higher PWV, and significantly improved with monotherapy (n=19) (P=0.0025), but not with triple therapy (n=23) (P>0.05) regardless of naive or non-naive entry status.

A.



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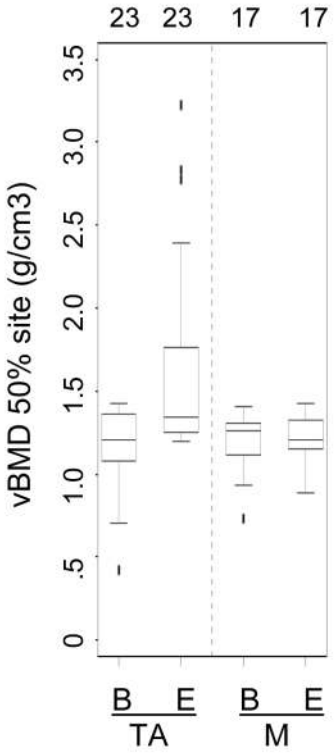

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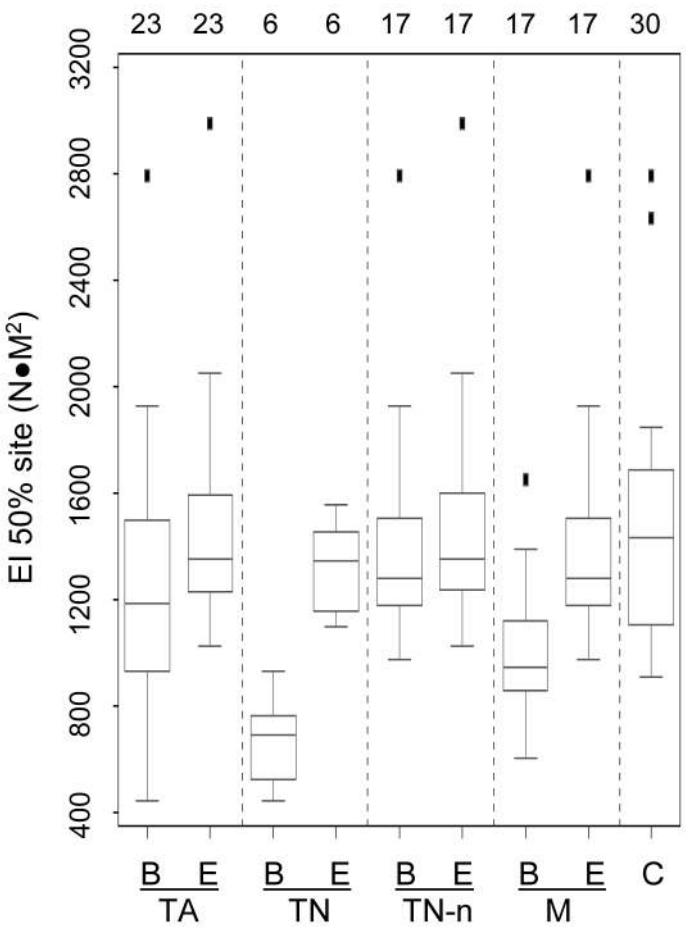


**Figure 3.**

Box plots of a) height-adjusted spine aBMD b) vBMD at the 50%$^{ile}$ radial site c) bending (EI) rigidity measured at the 50%$^{ile}$ radial site in the various patient groups. Interquartile ranges (IQR; 75$^{th}$ and 25$^{th}$ percentiles) are top and bottom box edges, respectively. Horizontal lines within boxes represent medians. Lower and upper whiskers show the extreme points that fall within Q1-1.5 x IQR and Q3+ 1.5 x IQR. *, **, and NS represent P values <0.05, <0.001, and >0.05, respectively. B-baseline, E = end of study, M=lonafarnib monotherapy, T= triple therapy, N-naive participants, N-n=non-naive participants, C=non-HGPS controls. n for each participant group listed above each box at top of graph.

## Table 1

Baseline Participant Characteristics (Mean±S.D.)

| | Triple Therapy All (n=35)* | Triple Therapy Naive (n=13) | Triple Therapy Non-Naïve (n=22)* | Lonafarnib Monotherapy Trial (n=25) |
|---|---|---|---|---|
| Males (n) % (n) | 14 (40.0) | 5 (38.5) | 9 (40.9) | 11 (44.0) |
| Age at Enrollment (Y) | 8.0±4.4 | 5.0±4.4 | 9.8±3.3 | 7.5±3.2 |
| Treatment Duration (Y) | 3.3±0.7 | 3.4±0.6 | 3.3±0.7 | 2.2±0.1 |
| Weight at Enrollment (kg) | 11.0±2.7 | 9.7±1.6 | 11.8±3.0 | 10.5±2.7 |
| Weight Z-score | −3.96±0.7 | −3.6±0.8 | −4.2±0.5 | −4.2±0.6 |
| Height-age (Y) | 3.6±1.8 | 2.6±1.4 | 4.2±1.8 | 3.4±1.6 |
| Standing Height (cm) | 96.1±13.6 | 87.6±10.9 | 101.1±12.7 | 94.9±11.9 |
| Standing Height Z-score | −4.8±1.7 | −3.7±1.9 | −5.5±1.1 | −5.0±1.1 |
| Segmental Height (cm) | 98.6±13.8 | 90.0±10.5 | 103.7±13.0 | 95.9±11.8 |
| Segmental Height Z-score | −4.3±1.8 | −3.1±2.2 | −5.1±1.1 | −4.8±1.1 |
| BMI | 11.9±1.4 | 12.8±1.7 | 11.4±1.0 | 11.5±1.1 |

Two participants who withdrew from the study prior to 6 months on study are omitted.

## Table 2

Success of Primary Outcome Measure, and Contributing Components *

| SUCCESS CRITERIA | All Participants | Naive Participants | Non-Naive Participants |
|---|---|---|---|
| *Primary Outcome* Weight Gain **OR** Echodensity | 71.0% (n=22/31) ** | | |
| Echodensity * | 31.4% (n=11/35) | 61.5% (n=8/13) | 13.6% (n=3/22) |
| **Subgroup That Increased Rate of Weight Gain ≥10%** | | | |
| Weight Gain | 48.4% (n=15/31) | 44.4% (n=4/9) | 50.0% (n=11/22) |
| Weight Gain **OR** Echodensity | 71.0% (n=22/31) ** | 100% (n=9/9) | 59.1 (n=13/22) |
| Weight Gain **AND** Echodenisty | 17.1% (n=6/35) | 38.5% (n=5/13) | 4.6% (n=1/22) |
| **Subgroup That Increased Rate of Weight Gain ≥50%** | | | |
| Weight Gain | 29.0% (n=9/31) | 33.3% (n=3/9) | 27.2% (n=6/22) |
| Weight Gain **OR** Echodensity | 51.6% (n=16/31) | 89.0% (n=8/9) | 36.4 (n=8/22) |
| Weight Gain **AND** Echodenisty | 15.4% (n=4/35) | 37.5% (n=3/13) | 5.6% (n=1/22) |

* Includes 3 participants who died (counted as failures); excludes 2 participants who voluntarily withdrew prior to 6 months on study.; weight analyses exclude 4 participants due to age < 3 years (as pre-specified in the statistical plan); weight analyses include success for participants who achieved switch from negative to positive slope (as pre-specified in the statistical plan); echodensity analyses include 2 participants who did not have end-of-therapy echodensity measurements, counted as echodensity failures.

** Predefined primary outcome measure result; significantly greater than the hypothesized value of 4% (P<0.001)

**Table 3**

Plaque Burden, Blood Pressure*, and ECG Pathology (Number (%) positive)

| | Triple Therapy All (n=32) | | | Triple Therapy Naive (n=12) | | | Triple Therapy Non-naive (n=20) | | | Lonafarnib Monotherapy (n=25**) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **B** | **EOS** | **P value** | **B** | **EOS** | **P-Value** | **B** | **EOS** | **P value** | **B** | **EOS** | **P value** |
| Carotid Artery Plaque | 2 (5) | 16 (50) | <0.001 | 0 (0) | 4 (33) | 0.13 | 2 (10) | 9 (45) | 0.016 | 3 (12) | 3 (12) | 1.00 |
| Superficial Femoral Artery Plaque | (0)*** | 4 (13) | 0.13 | 0 (0) | 1 (8) | 1.00 | 0 (0)*** | 3 (15) | 0.25 | 0 (0) | 0 (0) | 1.00 |
| Left Ventricular Hypertrophy | 1 (3) | 8 (25) | 0.016 | 0 (0) | 2 (17) | 0.5 | 1 (5) | 6 (30) | 0.063 | 1 (4) | 2 (8) | 0.38 |
| SBP Elevated for Chronologic Age | 6 (19) | 1 (3) | 0.125 | 4 (33) | 1 (8) | .375 | 2 (10) | 0 (0) | 0.500 | 7 (28) | 3 (12) | 0.289 |
| DBP Elevated for Chronologic Age | 9 (28) | 2 (6) | 0.065 | 6 (50) | 0 (0) | 0.031 | 3 (15) | 2 (10) | 1.000 | 8 (32) | 5 (20) | 0.581 |
| SBP Elevated for Height-age | 8 (25) | 8 (25) | 1.000 | 3 (25) | 3 (25) | 1.000 | 5 (25) | 5 (25) | 1.000 | 12 (48) | 8 (32) | 0.289 |
| DBP Elevated for Height-age | 11 (34) | 4 (13) | 0.065 | 4 (33) | 2 (17) | 0.625 | 7 (35) | 2 (10) | 0.125 | 16 (64) | 9 (36) | 0.119 |
| Insulin Resistance | 8/31 (25.8) | 16/31 (51.6) | 0.02 | 2/11 (18) | 3/11 (27) | 1.00 | 6/20 (30) | 13/20 (65) | 0.02 | 8/24(33) | 9/24 (37.5) | 1.00 |

* Elevated BP is defined as at or above the 95%ile

** one participant did not receive ECG at end-of-therapy; therefore n=24

*** 5 participants were too young to tolerate baseline assessment. End-of-therapy assessment showed no plaque; therefore baseline was assumed negative.