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

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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 Hutchinson-Gilford progeria syndrome 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 because of side effects. Primary outcome success was predefined 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 predefined. Secondary improvements included increased areal ($P=0.001$) and volumetric ($P<0.001$–0.006) bone mineral density and 1.5- to 1.8-fold increases in radial bone structure ($P<0.001$). 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 the addition of pravastatin and zoledronic acid.

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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 Hutchinson-Gilford progeria syndrome.
- 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 the addition of pravastatin and zoledronic acid.
- Because increased bone fracture is not a disease feature, the addition of the combination of statin and bisphosphonate to lonafarnib therapy is not recommended for the clinical treatment of Hutchinson-Gilford progeria syndrome.
- Although not an inherent feature of Hutchinson-Gilford progeria syndrome, it is reasonable to consider statins for the treatment of lipid abnormalities when clinically indicated.

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 steno-occlusive 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, the farnesyl anchor of progerin 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 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 a farnesyltransferase inhibitor.

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; and radial bone structural rigidity and sensorineural hearing were increased. There was preliminary evidence of decreased headache, transient ischemic attack, 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 the 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 with clinically and genetically confirmed c.1824 C>T, p.Gly608Gly classic HGPS, adequate organ and marrow function, reliable pretrial body weights, and ability to travel for regular study visits. The study was approved by the Boston Children's Hospital Committee on Clinical Investigation. Written informed consent was obtained,

and when indicated, consents were translated into the parents' primary language and discussions were performed with interpreters. Age-appropriate assent was also obtained. An initial feasibility study enrolled 5 participants who were naïve to lonafarnib therapy; participants received triple therapy and were observed for a period of 4 weeks for significant toxicities. Because 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 before study initiation and were included as part of the trial protocol. Histories, physical examinations, and all efficacy testing were performed at Boston Children's Hospital or Brigham and Women's Hospital, Boston, MA.

Study Drug Dosing and Administration

Trial medications were administered for a period of 40 to 52 months. Lonafarnib (Merck & Co, Inc) dosing was continued or, for naïve 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 by either 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 <10 kg and 10 mg for participants weighing >10 kg once every 24±2 hours. Zoledronic acid (Zometa, Novartis, Inc) was administered intravenously over 30 minutes; at baseline; at months 6, 12, and 18; and at the 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 after infusion and at 1 to 2 days after 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 hematologic 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 as a result of interim toxicities.

Efficacy Evaluations

The prespecified primary outcome was a composite of 2 components relevant to disease in HGPS. The first was an increase in estimated annual rate of weight gain or a change from pretherapy 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 of 0.44 kg/y, which remains stable over time after 3 years of age.[20] Pretrial body weights were obtained from The Progeria Research Foundation Medical and Research Database (principal investigator, L.B.G.) with parental consent (Brown University Center for Gerontology and Healthcare Research, Providence, RI) 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 a 10% annualized increase in rate of weight gain compared with before study entry or if the annualized change in weight converted from decreasing before study entry to increasing while on treatment. Rates of weight change were estimated by the slope of participant-specific least-squares regressions versus age from data collected within the year before study entry and data collected during therapy.

The second component of the primary outcome was a decrease in echobrightness of the internal carotid artery 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 with ImageJ software (National Institutes of Health) on a gray scale 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 prespecified vessel regions were captured as previously described.[21] Values were calculated with Matlab 7.9 (Mathworks, Inc). A participant was considered improved in echodensity of the deep common carotid artery adventitia if either the echodensity of the adventitia was reduced to ≤90% of the value at study entry or the patient-specific 10th percentile of the density of the adventitia was reduced to ≤90% of the value at study entry.

Key secondary variables were prespecified. PWV_{cf}, distal common carotid artery far wall intima-media thickness, and plaque evaluations established with 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-only Data Supplement. Internal carotid artery flow was evaluated, but because of the complexity of analysis required to present this information, it will be included in a separate manuscript. IR was determined from the homeostasis model assessment-IR: fasting (glucose)(insulin)/405, with IR defined as ≥2.5.

Neuro-imaging included magnetic resonance imaging of the brain and neck and magnetic resonance angiography of the circle of Willis and neck. Brain magnetic resonance imaging consisted of sagittal and axial T1-weighted, axial T2-weighted fast spin-echo, axial fluid-attenuated inversion recovery, and axial diffusion-weighted imaging with calculated apparent diffusion coefficient maps. Brain and neck arterial imaging consisted of 3-dimensional time-of-flight magnetic resonance angiographies. Neck imaging consisted of axial T1-weighted fluid-attenuated inversion recovery and T2-weighted fast spin-echo imaging. Patients were scanned at 1.5-T (General Electric Medical Systems) or 3-T (Siemens) magnet strength.

Skeletal findings were evaluated as previously described.[22,23] Dual x-ray absorptiometry areal bone mineral density (aBMD) measures were performed with a Discovery A Scanner (Hologic, Inc).[23] Load-bearing capacity (axial, bending, and torsional rigidities) and volumetric BMD were calculated with peripheral quantitative computed tomography 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, nutritional intake, measured resting energy expenditure, and dermatological assessments are detailed in online-only Data Supplement.

Statistics

The study was powered as follows: The prespecified null hypothesis of interest is that the true success rate is ≤4%; the alternative hypothesis is that the true success rate exceeds 4%. Specifically, the null and alternative hypotheses were as follows: H_0, $\pi \leq 0.04$; and H_1, $\pi > 0.04$, where π is the true (unknown) overall success rate. At a 1-sided 0.05 level of significance, assuming that the true success rate is ≥0.17 (≥17%), 33 evaluable participants yielded 82% power to reject the null hypothesis in favor of the alternative on the basis of the exact test of the binomial distribution.

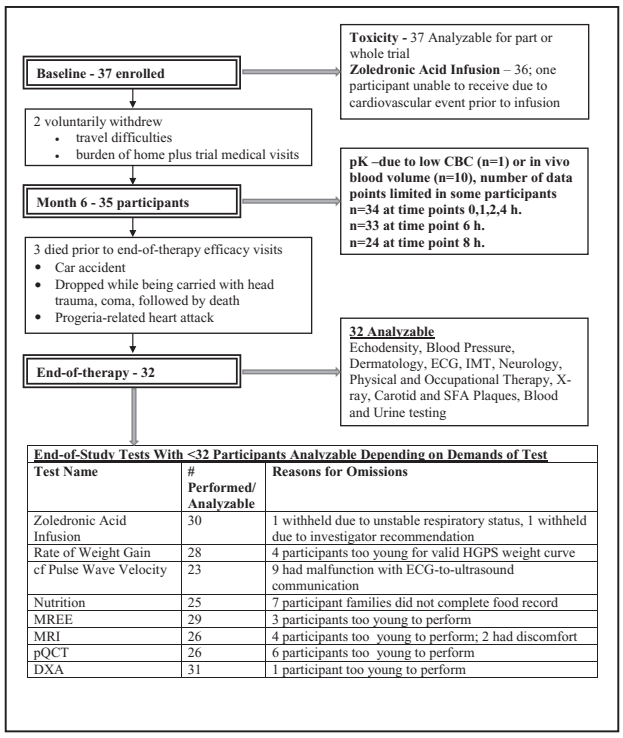
Descriptive statistics included mean and standard deviation for symmetrically distributed continuous variables, median and quartiles for skewed variables, and counts and percentages for categorical variables. Trends over time in continuous secondary and tertiary end points were assessed via parametric or nonparametric repeated measures, depending on the distribution of the end point. These *P* values do not reflect adjustment for multiple comparisons and should be interpreted only descriptively. *P* values presented are 2-sided, except for the primary outcome analysis, and are considered significant at the 0.05 level. All statistical analyses were carried out with SAS version 9.3 (SAS Institute).

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 nonnaïve) and therefore had received continuous lonafarnib treatment for at least 2 years before enrollment in the triple trial. Thirteen participants had no prior exposure to lonafarnib (treatment naïve). Three participants were taking statins at trial entry. No participant had previous exposure to bisphosphonates.

A complete CONSORT (Consolidated Standards of Reporting Trials) diagram details testing inclusion (Figure 1). Five participants did not complete the study: 2 voluntarily withdrew because of nonmedical issues within 6 months of trial enrollment, and 3 died before study completion. Deaths were caused by trauma from a motor vehicle accident, head trauma, and myocardial infarction at 20, 10, and 20 years of age, respectively. Toxicity results are reported for all 37 enrolled participants. Pharmacokinetics 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



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 (Consolidated Standards of Reporting Trials) Diagram of Trial Inclusion and Testing. CBC indicates complete blood count; DXA, dual x-ray absorptiometry; HGPS, Hutchinson-Gilford progeria syndrome; IMT, intima-media thickness; MREE, measured resting energy expenditure; MRI, magnetic resonance imaging; pK, pharmacokinetics; pQCT, peripheral quantitative computed tomography; and SFA, superficial femoral artery.

who were a priori excluded from the analysis because of being <3 years of age, 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 I in the online-only Data Supplement. 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 naïve than for nonnaïve participants (*P*=0.0009).

Lonafarnib, Pravastatin, and Zoledronic Acid Treatment Toxicity

Overall, therapy was well tolerated, and no participant came off study because of 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

Table 1. Baseline Participant Characteristics

	Triple Therapy All (n=35)*	Triple Therapy Naïve (n=13)	Triple Therapy Nonnaïve (n=22)*	Lonafarnib Monotherapy Trial (n=25)
Male sex, 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
Body mass index, kg/m^2	11.9±1.4	12.8±1.7	11.4±1.0	11.5±1.1

Values are mean±SD.
*Two participants who withdrew from the study before 6 months on study are omitted.

mild diarrhea, fatigue, nausea, vomiting, anorexia, abdominal pain, and elevated liver function tests (Table II in the online-only Data Supplement). No pravastatin-related side effects were identified. Zoledronic acid–related side effects included postinfusion flu-like symptoms and hypocalcemia, at rates significantly lower than those previously published for non-HGPS pediatric studies (Table III in the online-only Data Supplement).[25] Within 48 hours of successive zoledronic acid infusions (baseline; months 6, 12, and 18; and end of study), participants developed ≥1 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 of 37 participants (62%) experienced postinfusion side effects.

Primary Outcomes

Overall, 22 of 31 participants (71.0%; 9 treatment naïve and 13 nonnaïve) succeeded under the prospectively established primary outcome measure of success ($P<0.001$ versus a prespecified 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 participants (48.4%; 4 treatment naïve and 11 nonnaïve), whereas echodensity success was achieved in 11 of 35 participants (31.4%; 8 treatment naïve and 3 nonnaïve). However, only 6 of 35 participants (12.9%) succeeded for both outcome measures, which implies that these 2 outcome measures may not be clinically related. All 4 participants too young to be included in the weight gain analysis experienced echodensity failure. Patient-level data on primary outcome measures are presented in Table I in the online-only Data Supplement.

Secondary Outcomes

Weight and Nutritional Findings

There was no significant difference between average daily energy intake, fat or carbohydrate intake, or measured resting energy expenditure between participants who succeeded and those who failed the weight outcome (Table IV in the online-only Data Supplement). Protein intake was increased for the success group when assessed in the 10% rate of weight gain group ($P=0.04$) and was nearly significant for the 50% rate of weight gain group ($P=0.07$). This is supported in part by an increase in lean body mass by dual x-ray absorptiometry for the 50% success group ($P=0.02$) but not for the 10% success group ($P=0.27$; Table V in the online-only Data Supplement).

Cardiovascular and Neurovascular Findings

Carotid artery wall echodensity and PWV_{cf} represent measures of arterial structure and function.[21] Mean carotid artery wall echodensity of the intima-media, near or deep adventitia, and PWV_{cf} demonstrated no significant changes overall or within the naïve and nonnaïve subgroups (Figure 2), presumably representing no overall change in vascular stiffness.

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

Intima-media thickness was within the normal range (0.42–0.44±0.03–0.07 [mean±SD]) with no significant changes between baseline and the end of therapy

Table 2. Success of Primary Outcome Measure and Contributing Components*

Success Criteria, Primary Outcome	All Participants, % (n/N)	Naïve Participants, % (n/N)	Nonnaïve Participants, % (n/N)
Weight gain or echodensity	71.0 (22/31)†		
Echodensity*	31.4 (11/35)	61.5 (8/13)	13.6 (3/22)
Subgroup with increased rate of weight gain ≥10%			
Weight gain	48.4 (15/31)	44.4 (4/9)	50.0 (11/22)
Weight gain or echodensity	71.0 (22/31)†	100 (9/9)	59.1 (13/22)
Weight gain and echodensity	17.1 (6/35)	38.5 (5/13)	4.6 (1/22)
Subgroup with increased rate of weight gain ≥50%			
Weight gain	29.0 (9/31)	33.3 (3/9)	27.2 (6/22)
Weight gain or echodensity	51.6 (16/31)	89.0 (8/9)	36.4 (8/22)
Weight gain and echodensity	15.4 (4/35)	37.5 (3/13)	5.6 (1/22)

*Includes 3 participants who died (counted as failures); excludes 2 participants who voluntarily withdrew before 6 months in the study. Weight analyses exclude 4 participants with age <3 years (as prespecified in the statistical plan); weight analyses include success for participants who achieved the switch from negative to positive slope (as prespecified 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).

overall (n=64 vessels; P=0.47) and in the naïve (n=24 vessels; P=0.25) and nonnaïve (n=40 vessels; P=0.24) subgroups.

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

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

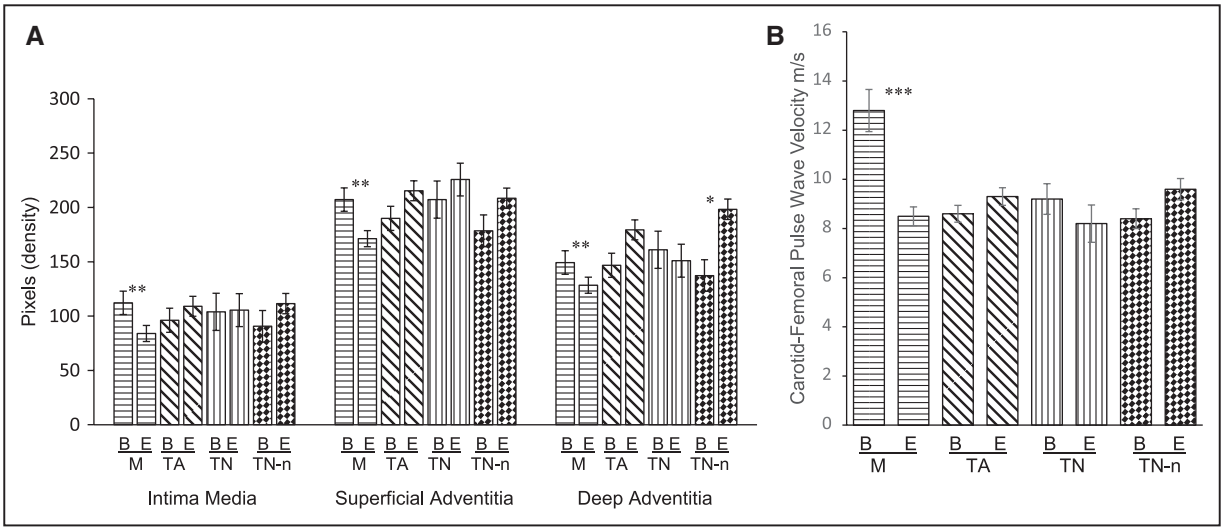


Figure 2. Cardiovascular outcomes comparing triple-therapy whole cohort and naïve and nonnaïve subgroups with lonafarnib monotherapy.

A, Carotid artery echodensity significantly decreased with monotherapy (n=24) but not with triple therapy (n=30) regardless of naïve or nonnaïve entry status. **B**, Carotid-femoral pulse wave velocity (PWV_{cf}). The monotherapy cohort entered the trial with significantly higher PWV_{cf} and significantly improved with monotherapy (n=19; P=0.0025) but not with triple therapy (n=23; P>0.05) regardless of naïve or nonnaïve entry status. All bars show mean (±SE). A indicates all participants; B, baseline; E, end of study; M, lonafarnib monotherapy; N, naïve participants; N-n, nonnaïve participants; and T, triple therapy. P values between adjacent bars: ***P≤0.0001, **P≤0.001, and *P≤0.05.

Table 3. Plaque Burden, Blood Pressure,* and Electrocardiographic Pathology

	Triple Therapy All (n=32)			Triple Therapy Naïve (n=12)			Triple Therapy Nonnaïve (n=20)			Lonafarnib Monotherapy (n=25)†		
	B, n (%)	EOS, n (%)	*P* Value	B, n (%)	EOS, n (%)	*P* Value	B, n (%)	EOS, n (%)	*P* Value	B, n (%)	EOS, n (%)	*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 (0)‡	4 (13)	0.13	0 (0)	1 (8)	1.00	0 (0)‡	3 (15)	0.25	0 (0)	0 (0)	1.00
LVH	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 chronological age	6 (19)	1 (3)	0.125	4 (33)	1 (8)	0.375	2 (10)	0 (0)	0.500	7 (28)	3 (12)	0.289
DBP elevated for chronological 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
IR	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

Values are n (%) positive. B indicates baseline; DBP, diastolic blood pressure; EOS, end of study; IR, insulin resistance; LVH, left ventricular hypertrophy; and SBP, systolic blood pressure.

*Elevated BP is defined as ≥95th percentile.

†One participant did not receive an ECG at the end of therapy; therefore, n=24.

‡Five participants were too young to tolerate baseline assessment. End-of-therapy assessment showed no plaque; therefore, baseline was assumed to be negative.

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

Two participants developed new brain infarcts on magnetic resonance imaging during the study period. Of the 5 participants (14%) who had infarcts on their baseline brain magnetic resonance images, 1 had an infarct while on therapy, and 4 did not develop additional infarcts during the study period. Three of 37 participants (8.1%) experienced new transient ischemic attacks; 1 of these participants also experienced a new infarct. Headache frequency decreased from 1.2 to 0.81 per week.

Skeletal Findings

There were significant improvements in absolute and height-adjusted aBMD (*P*<0.001) and radial volumetric BMD at all sites (*P*<0.001–0.006; Figure 3 and Table VI in the online-only Data Supplement). There was marked improvement in all structural rigidity parameters at all sites. Axial, bending, and torsional rigidities improved by 1.6-, 1.5-, and 1.8-fold, respectively (*P*<0.001–0.03; Figure 3 and Table VII in the online-only Data Supplement).

Extraskeletal calcifications, detected by x-ray, were located primarily in the digital tufts but also at various locations throughout the body.[22] Prevalence rates increased from 34.4% (n=11 of 32) at baseline to 65.6% (n=21 of 32) of participants at the end of the study (*P*=0.006). Calcifications were also observed. Three participants demonstrated calcific skin eruptions 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 before and at the end of therapy (Table VIII in the online-only Data Supplement).

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

Lonafarnib Pharmacokinetics

Pharmacokinetic characteristics were similar to those previously published in HGPS and non-HGPS pediatric participants (Figure I and data in the online-only supplement).[17,24] Mean time to maximum drug concentration was 3.5 hours (n=34). Average maximum concentration was 2.67±1.2 μg/mL. Six participants' baseline and peak pharmacokinetic values indicated that they were not at steady state, likely because of medication noncompliance. Results did not change significantly when

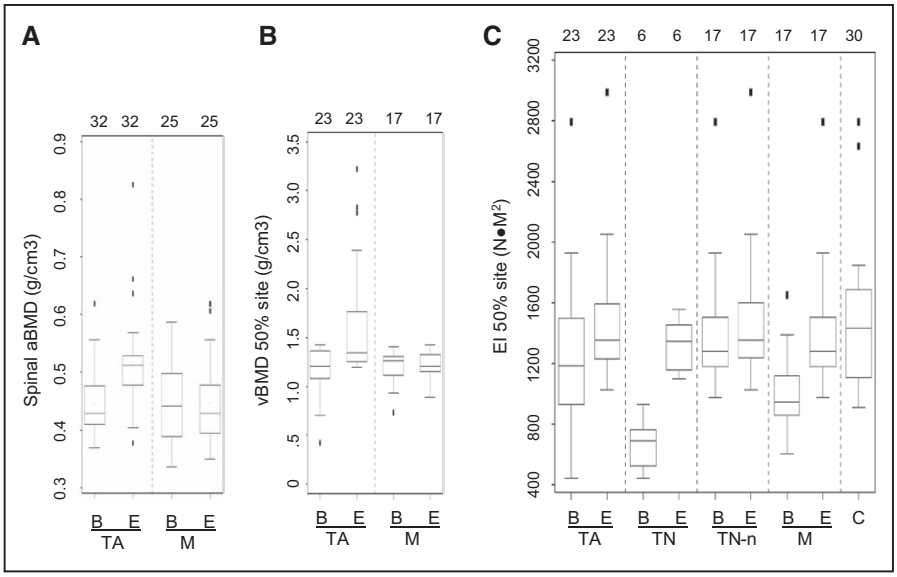


Figure 3. Box plots of (A) height-adjusted spine areal bone mineral density (aBMD), (B) volumetric bone mineral density (vBMD) at the 50th percentile radial site, and (C) bending (EI) rigidity measured at the 50th percentile radial site in the various patient groups.

Interquartile ranges (IQRs; 75th and 25th 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× IQR and Q3+1.5×IQR. n for each participant group is listed above each box at the top of the graph. B indicates baseline; C, non–Hutchinson-Gilford progeria syndrome controls; E, end of study; M, lonafarnib monotherapy; N, naïve participants; N-n, nonnaïve participants; and T, triple therapy. *P<0.05 and **P<0.001 (P>0.05 is not significant).

these pharmacokinetic values were omitted from the analysis.

Additional Pertinent Negatives

Several additional measures were abnormal before 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 attributable to the therapeutic regimen versus the natural history of disease, we analyzed cross-sectional associations of LVH, IR, PWV_{cf}, extraskeletal calcification, and echodensity with participant age at baseline in ≈38 participants before the initiation of lonafarnib or triple-therapy treatment. LVH and IR prevalence were positively associated with age (P≤0.017). After adjustment for age, the increase in LVH and IR incidence from baseline to the end of the triple trial discussed above was no longer significant (P≥0.159). There were trending but nonstatistical linear relationships between PWV_{cf} and age (P=0.13) and between extraskeletal calcification and age (P=0.08). After adjustment for age, the increase in PWV_{cf} and calcification with triple therapy was not significant (P=0.216 and P=0.11, respectively). Although these results support that worsening over the trial period could be due in part to the natural history of disease in HGPS with increased age, it does not limit the possibility that triple treatment may still affect these variables. For example, although the age-adjusted increase in extraskeletal calcification across the trial was not significant, the odds ratio for calcification incidence at the end of the trial compared with baseline was still 2.0 with adjustment for age (3.6 unadjusted). Thus, the odds ratio after adjustment for age remained >1, albeit not significantly, still indicating 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 before to after 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 to be considered a success for the weight component of the primary end point. When the more stringent ≥50% increase was applied to the current triple-therapy trial results, triple therapy improved the rate of weight gain (29.0% of participants) with similar results for treatment-naïve (33.3%) and nonnaïve (27.2%) participants (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 9 participants who succeeded in gaining ≥50% in rate of weight gain on monotherapy and entered triple therapy, 2 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, 4 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] although the naïve triple-therapy subgroup was younger on average. BMD was the only outcome that improved in the present trial and showed no significant improvement during the lonafarnib monotherapy trial (Figure 3 and Table VI in the online-only Data Supplement). Skeletal rigidities improved, but rates were below that of the lonafarnib monotherapy trial (1.5- to 1.8-fold improvement compared with the nearly 3-fold improvement noted with monotherapy treatment; Figure 3 and Table VII in the online-only Data Supplement).

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, and measured resting energy expenditure for participants who succeeded versus those who failed the weight outcome (Table IV in the online-only Data Supplement); normal carotid artery intima-media thickness at baseline and the end of the study (Figure 2); and rates of on-therapy joint dislocations, fractures (see the online-only Data Supplement), transient ischemic attacks, 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 present trial, whereas a similar acceleration was not detected in the prior lonafarnib monotherapy trial. In contrast to the present study, the prior lonafarnib monotherapy trial showed that no participants developed new carotid artery plaques, and no superficial femoral artery plaques were detected at baseline or the end of the study; additionally, there was no significant increase in rates of extraskeletal calcifications (prevalence rates went from 29% [n=8 of 25] to 44% [n=11 of 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 2 prenylation inhibitors, zoledronic acid and pravastatin, to lonafarnib treatment. The lonafarnib toxicity profile and pharmacokinetics were unaffected by the addition of pravastatin and zoledronic acid, and all drugs were well tolerated overall.

For the composite primary outcome measure, each participant's pretrial 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 3 years of age. We hypothesized this variable to be a surrogate measure of overall disease status. Rate of weight gain represents the severe growth impairments in HGPS, whereas 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. Because we previously showed that carotid artery echodensity is a hallmark of vascular disease in HGPS after 3 years of age,[21] this cardiovascular end point was included as a second primary outcome measure. With the use of these 2 predefined measures, the composite primary study outcome was achieved. However, because there was little crossover between success for participants between weight and carotid artery echodensity, it is unlikely that rate of weight gain is a surrogate for cardiovascular health.

Although the primary outcome measure for this trial was successful, overall, the results are mixed, presenting 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, blood pressure, and IR was not achieved by adding zoledronate and pravastatin to lonafarnib treatment. Although outcomes reflecting skeletal structure were improved, they did not change above the improvement change rates seen with lonafarnib alone, even in the treatment-naïve subgroup.

Some systems were positively affected by treatment. The 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-nonnaïve group improved by a smaller margin than for the treatment-naïve group, likely as a result of the improvements already achieved with prior monotherapy that created an improved status at baseline compared with the treatment-naïve group.

Our group previously published that the BMD of children with HGPS is in the normal range when measured directly by peripheral quantitative computed tomography but that aBMD is in a slightly low range (aBMD Z score <−1.0) when estimated by dual x-ray absorptiometry 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 volumetric BMD 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 because the cross-sectional area of the intramedullary canal remained constant, but the periosteal surface continued to expand with growth.

Some findings imply that there may be calcium buildup in various tissues, including vasculature. This tendency is supported by in vitro studies showing that progerin promotes abnormal vascular smooth muscle cell matrix production[27] and impaired mitochondrial function, resulting in reduced ATP production and impaired synthesis of pyrophosphate, 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. Because 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 naïve to lonafarnib therapy and 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; 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, with the current 3- to 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-naïve 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 on the lamin A and progerin maturation pathways or through diverging influences on cellular signaling that interact downstream.

We previously demonstrated that prenylation inhibition extends the estimated life span of 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 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 that triple therapy does not provide evidence for additional benefit, it is likely that lonafarnib, not zoledronate and pravastatin, was mainly responsible for estimated life-span extension. Overall, given the ability to demonstrate a small improvement in survival with 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]

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DISCLOSURES

Dr L.B. Gordon is the parent of a child who participated in the study. The other authors report no conflicts.

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FOOTNOTES

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

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SUPPLEMENTAL MATERIAL

Methods

Lonafarnib Pharmacokinetic Analysis: Plasma concentrations of lonafarnib were determined at 150 mg/m2 at 0, 1, 2, 4, 6, and 8 h post-dose by HPLC/ion chromatography (IC) tandem mass spectrometry (6). Lonafarnib pharmacokinetics (PK) were determined using noncompartmental analyses. The lower limit of quantitation for lonafarnib was 5 ng/mL with a linear standard curve over a concentration range of 5–2,500 ng/ml. Individual plasma lonafarnib concentrations were used for PK analysis using model-independent methods (7). The area under the plasma-concentration time curve from time 0 to 12 h after dose [AUC(0–12)] was calculated using the linear trapezoidal method, where concentration at 0 h also was used as an estimate of plasma concentration at 12 h for each concentration–time profile (steady state achieved at 6 and 18 mo.).

Cardiovascular: Twelve-lead ECG, and BP measures were performed. Manual cuff pressures were assessed according to standardized protocol. Either a pediatric 12- to 19-cm or infant size 8- to 13-cm BP cuff was selected for each child, based on the size that would allow the bladder to cover 80% of the upper arm. Height-age was determined by calculating the median age in the general population of a child with the height of each patient with HGPS, using Centers for Disease Control and Prevention sex-specific pediatric growth curves (http://www.cdc.gov/growthcharts/).

Carotid-femoral pulse wave velocity (PWV_{cf}) was determined by measuring the propagation time of the pressure pulse from the carotid to femoral arteries[1].

Propagation time (Δtcf) was calculated by measuring the time lag between the R-wave of the simultaneous ECG and the arrival of the arterial pulse at both the carotid (Δtc) and femoral (Δtf) arteries. The distance between the carotid and femoral arteries (lcf) was measured and recorded. PWV_{cf} was calculated using the formula PWV_{cf} = lcf/Δtcf.

Diagnostic carotid artery ultrasonography was performed in an ICAVL accredited laboratory using established protocols[2]. A Philips iU22 ultrasound machine (Philips, Eindhoven, The Netherlands) equipped with a 17-5 MHz broadband linear-array transducer was utilized.

Carotid plaque was defined as protrusion from the intimal surface into the lumen with a local intimal medial thickness greater than 1.5 mm. Plaques were described by echobrightness, surface characteristics and whether they were circumferential or not. Carotid stenoses were graded using velocity ratios, and pulsed wave Doppler was performed with appropriate angle correction. Mean distal ICA velocity was calculated using a formula that adds 1/3 of the peak systolic velocity plus 2/3 of the end diastolic velocity, as previously described[3]. Gray map 5 was used on all studies and after adjusting overall gain so that intra-luminal blood appeared black. Digital gain compensation was kept perpendicular.

Distal common carotid artery far wall intima-media thickness (IMT) was measured from the intima-lumen border to the media-adventitia border over a 2-centimeter segment according to standard protocol[1] using edge-detection software (Medical Imaging Applications, Coralville, IA)[4].

Measured Resting Energy Expenditure:

Resting energy expenditure (REE) was measured using the Vmax 29n metabolic cart after a minimum four hour fasting period. Patients were awake and resting comfortably in bed. With the subject in the supine position a transparent canopy was placed over their head. The Vmax 29n measured the inspiratory concentration of O^2 (F_iO^2) and the difference between F_iO^2 and expiratory concentrations of O^2 (F_eO_2) with a paramagnetic differential oxygen sensor. REE was calculated using the Weir equation and was expressed in Kcals/day. Percent predicted REE was calculated using the Schofield normative data[5].

Nutrition: Seven day food records were collected at baseline and 2 year visits. Food records were recorded by parents in the parents' native languages and translated with an interpreter in the presence of dietary staff. Nutrient analysis of macronutrients was completed using the nutrient analysis program Nutrition Data System for Research, versions 2008-2012 (University of Minnesota, Minneapolis, MN). Individual macronutrient intake was then compared to the recommended dietary intake per age [6].

Dermatologic Evaluations: Full skin examinations were performed and photography was obtained. Hair was examined including severity (mild, moderate, severe) and pattern of alopecia. Hair counts were obtained from a 2 x 2cm2 area on the vertex scalp. Severity of skin atrophy was graded (mild, moderate, or severe). Calcinosis cutis was noted on the affected body areas (upper or lower trunk, arms or legs). Nail dystrophy was noted on specific fingernails and toenails. Sclerodermatous skin changes were noted on the affected body areas (upper or lower trunk, arms or legs).

Results

Rates of new hip dislocations (3/37 subjects; 8%), shoulder dislocations (3/37 subjects; 8%), appendicular fractures (6/37; 16%), and skull fractures (3/37; 8%) did not significantly differ between lonafarnib monotherapy study (1/26; 4%, 2/26; 8%, 4/26; 15%, 2/26; 8% respectively) and triple therapy study.

At the 10% rate of weight gain cutoff, participants with weight gain success experienced a gain in fat (P=0.03) and bone mineral (P=0.03), but not lean body mass (P=0.27), above that of participants without weight gain success (Table S4). At the 50% cutoff rate of weight gain cutoff, participants with weight gain success experienced a gain in muscle (P=0.02), but not fat (P=0.53) or bone mineral (P=0.82), above that of participants without weight gain success. The 50% cutoff results agree with those of the former lonafarnib trial, where the threshold was also a 50% increase in rate of weight gain. These results imply that whole body composition by DXA may not be sensitive enough to detect between group differences at lower increased rates of weight gain.

Table S1: Individual Participant Data							
Pt #	Age (yr)		Trial Duration (mo)	Weight (kg)		Deep Adventitial Echodensity (pixels)	
	B	EOS		B	EOS	B	EOS
1	8.6	12.3	44.4	10.94	12.7	134	102
2	9.2	12.6	40.8	13.2	14.2	32.5	103
3	11.7	15.1	40.8	13.3	12.35	88	125
4	11.2	14.5	39.6	14.5	17.4	85	73
5	12.8	16.3	42.0	20.5	21.3	85	156
6	11.0	14.4	40.8	8.5	8.7	123	151
7	7.0	10.4	40.8	10.38	11.9	110	190
8	11.9	15.3	40.8	14.7	17.4	147	198
9	6.2	9.6	40.8	10.1	12.4	90	183
10	5.6	8.9	39.6	8.58	10.3	18	118
11	13.0	16.7	44.4	11.94	14.1	190	120
12	9.6	13.1	42.0	9.84	11.6	14	193
13	13.9	17.3	40.8	16.5	18.9	117	189.5
14	9.3	13.0	44.4	10.44	12.4	111	119
15	11.3	14.7	40.8	12.63	12.7	121	145.5
16	7.1	10.9	45.6	8.92	10.6	1	146
17	5.7	9.1	40.8	10.2	12.7	169	166
18	5.1	8.8	44.4	8.08	10	45	152
19	11.1	14.5	40.8	10.33	12.3	127	179
20	9.2	13.5	51.6	11.88	14.9	88.5	152
21	2.5	6.3	45.6	8.3	11.2	177	164.5
22	2.5	6.4	46.8	9.6	11.7	47	121
23	2.2	5.9	44.4	9.3	11	139	100
24	3.2	6.7	42.0	7.85	9.6	203	127
25	4.3	7.9	43.2	10.43	11.4	75	56
26	3.0	6.6	43.2	9.41	11	159	72
27	4.2	7.7	42.0	8.09	9.5	117	164
28	3.5	6.9	40.8	9.9	11.8	24	176
29	2.1	5.6	42.0	9.07	12.4	148	94
30	3.2	6.6	40.8	9.77	12.1	25	0
31	6.0	9.3	39.6	9.27	11.3	130	108
32	10.8	14.0	38.4	10.33	12.7	177	106
33	17.5	18.9	16.8	14.02	NA	147	NA
34	5.9	7.6	20.4	8.43	NA	97	NA
35	18.4	19.9	18.0	12.93	NA	72	NA
36	5.5	6.0	6.0	7.03	NA	0	NA
37	10.6	11.1	6.0	11.15	NA	159	NA
NA = not assessed due to early participant exit from study							

Table S2: Toxicities Possibly Related to Treatment, Excluding Zoledronic Acid Post-infusion Toxicities n (%)						
Toxicity	**Grade**	**No. subjects exhibiting toxicity during entire trial period* (n = 37)**	**Number of subjects exhibiting toxicity during time period specified (months on treatment)**			
			0- 6 (n = 37)	**6-12 (n = 37)**	**12 -18 (n = 35)**	**18-end (n = 35)**
Gastrointestinal						
Diarrhea	1	17 (45.9)	14 (37.8)	9 (24.3)	11 (31.4)	8 (22.9)
	2	1 (2.7)	0	1 (2.7)	0	0
	3	1 (2.7)	0	0	0	1 (2.9)
Dyspepsia	1	10 (27.0)	7 (18.9)	2 (5.4)	1 (2.9)	3(8.6)
Vomiting	1	12 (32.4)	9 (24.3)	3 (8.1)	2 (5.7)	2 (5.7)
	2	1 (2.7)	0	0	1 (2.9)	0
Constitutional						
Anorexia	1	11(29.7)	8 (21.6)	3 (8.1)	1 (2.9)	3 (8.6)
	2	2 (5.4)	2 (5.4)	0	0	0 (5.7)
Fatigue	1	14 (37.8)	3 (8.1)	7 (18.9)	3 (8.6)	3 (8.6)
Headache	1	0	1 (2.7)	0	0	0
	2	1 (2.7)	0	0	0	1 (2.9)
Nausea	1	8 (21.6))	5 (13.5)	3 (8.1)	2 (5.7)	0
Weight Loss	1	5 (13.5)	5 (13.5)	1 (2.7)	0	0
Organ Function						
Elevated AST	1	5 (13.5)	4 (10.8)	4 (10.8)	6 (17.1)	5 (14.3)
	2	3 (8.1)	2 (5.4)	0	1 (2.9)	0
Elevated ALT	1	4 (10.8)	4 (10.8)	6 (16.2)	7 (20.0))	7 (20.0)
	2	3 (8.1)	1 (2.7)	3 (8.1)	2 (5.7)	2 (5.7)
	3	4 (10.8)	2 (5.4)	0	1(2.9)	1 (2.9)
Elevated Alkaline Phosphatase	1	1 (2.7)	0	1 (2.7)	1 (2.9)	0
Elevated CPK	1	2 (5.4)	0	1 (2.7)	3 (8.6)	2 (5.7)
Low ANC	1	1 (2.7)	1 (2.7)	0	0	1 (2.9)
	2	1 (2.7)	0	1 (2.7)	1 (2.9)	0
Low ALC	1	2 (5.4)	1 (2.7)	2 (5.4)	1 (2.9)	1 (2.9)
Low Hemoglobin	1	2 (5.4)	2 (5.4)	0	0	0
	2	2 (5.4)	2 (5.4)	1 (2.7)	1 (2.9)	0
Low Platelets	1	1 (2.7)	1 (2.7)	1 (2.7)	0	0
Low WBC	1	7 (18.9)	2 (5.4)	2 (5.4)	5 (14.3)	3 (8.6)
Metabolic						
Elevated Magnesium	1	2 (5.4)	0	0	0	1 (2.9)
Elevated Potassium	1	2 (5.4)	1(2.7)	0	0	1 (2.9)
Low CO_2	1	4 (10.8)	3 (8.1)	2 (5.4)	1 (2.9)	3 (8.6)
Low Sodium	1	2 (5.4)	1(2.7)	1 (2.7)	0	0
Other						
Colitis	2	1 (2.7)	0	0	0	1 (2.9)
Dry Mouth	1	1 (2.7)	0	0	1 (2.9)	1 (2.9)
*Per subject count is once for that subject's highest toxicity grade.						

Table S3: Toxicities Post-zoledronic Acid Infusion (first 48 hours), Possibly Related to Zoledronic Acid							
Toxicity	**Grade**	**No. subjects exhibiting toxicity during entire trial period* (n = 37)**	**Baseline (n = 36)**	**6 mo. (n = 35)**	**12 mo. (n = 35)**	**18 mo. (n = 34)**	**End-of-therapy (n = 30)**
Abdominal Pain	1	3 (8.1)	0	2 (5.7)	1 (2.9)	0	0
Chills	1	1(2.7)	1(2.8)	0	1 (2.9)	0	0
Dehydration	1	1(2.7)	0	1 (2.9)	0	0	0
Diarrhea	1	1 (2.7)	0	1 (2.9)	0	0	0
Fatigue	1	2 (5.4)	1 (2.8)	1 (2.9)	1 (2.9)	0	0
	2	1 (2.7)	1 (2.8)	0	0	0	0
Fever	1	11 (29.7)	4 (11.1)	7 (20.0)	3 (8.6)	1 (2.9)	1 (3.3)
	2	1 (2.7)	0	1 (2.9)	0	0	0
Flu-like Syndrome	1	1 (2.7)	0	1 (2.9)	0	0	0
Headache	1	3 (8.1)	0	2 (5.7)	1 (2.9)	0	0
Hypocalcemia	1	1 (2.7)	1 (2.8)	1 (2.9)	1 (2.9)	0	0
	2	3 (8.1)	1 (2.8)	1 (2.9)	1 (2.9)	0	1 (3.3)
	3	1 (2.7)	0	0	1 (2.9)	0	0
Muscle/joint/body Pain	1	7 (20.0)	3 (8.3)	2 (5.7)	0	0	0
	2	4 (10.8)	4 (11.1)	2 (5.7)	1 (2.9)	0	1 (3.3)
Nausea	1	1 (2.7)	0	1 (2.9)	0	0	0
Neuropathy	1	1 (2.7)	0	0	0	0	1 (3.3)
Vomiting	1	4 (10.8)	0	2 (5.7)	1 (2.9)	0	1 (3.3)
	2	1(2.7)	0	1 (2.9)	0	0	0
*Per subject count is once for that subject's highest toxicity grade.							

Table S4. Nutritional Factors Contributing to Rate of Weight Gain Success vs. Failure: Median Change In Daily Intake (lower, upper quartiles)							
	Energy (kcal/d)	**Fat (g/d)**	**CHO (g/d)**	**Protein (g/d)**	**N**	**MREE (kcal/d)**	**N**
Triple Therapy							
10% Achieved	53.3 (-140, 215)	0.2 (-4, 5)	6.1 (-13, 34)	2.4 (-5.05, 13.01)	13	43 (-25,145)	15
10% Not achieved	-265.4 (-328, -14)	-6.6 (-16, 4)	-24.8 (-46, -5)	-6.1 (-15.86, -4.86)	8	13 (-57,135)	13
P-value	0.11	0.59	0.41	0.04		0.70	
Triple Therapy							
50% Achieved	53.3 (-198, 215)	0.8 (-10, 9)	6.1 (-13, 34)	2.4 (-10.75, 15.62)	7	43 (-25,133)	9
50% Not achieved	-126.0 (-271, 209)	-2.8 (-9, 3)	-18.4 (-42, 17)	-4.9 (-12.86, 10.58)	14	38 (-57,145)	19
P-value	0.43	0.62	0.30	0.07		0.96	
Lonafarnib Monotherapy							
50% Achieved	1.5 (- 275, 309)	-8.3 (-20, 17)	2.5 (-7, 41)	-2.0 (-11.00, 16.20)	8	85 (56,111)	9
50% Not achieved	-12.0 (-258, 252)	-3.3 (-11, 12)	5.0 (-26, 37)	4.4 (-2.40, 15.40)	15	74 (-55,204)	16
P-Value	0.76	0.94	0.51	0.78		0.97	

Table S5. Whole Body DXA Evaluation of Body Composition Factors Contributing to Rate of Weight Gain Success vs. Failure: Median Change (lower, upper quartiles)

	Fat mass (g)	Lean tissue mass (g)	BMC (g)
Triple Therapy			
10% Achieved (n=15)	674.1 (595.6, 846.6)	1082.9 (889.4, 1623.2)	124.0 (94.1, 147.5)
10% Not achieved (n=13)	557.90 (323.7, 699.2)	860.70 (457.7, 1400.6)	74.5 (64.8, 98.6)
P-value achieved vs. not achieved	0.03	0.27	0.03
Triple Therapy			
50% Achieved (n=9)	661.0 (595.6, 720.2)	1527.8 (1047.7, 1655.3)	97.6 (85.1, 128.2)
50% Not achieved (n=19)	629.5 (400.8, 732.1)	889.4 (-38.7, 1400.6)	98.6 (71.2, 128.1)
P-value achieved vs. not achieved	0.53	0.02	0.82
Lonafarnib Monotherapy			
50% Achieved (n=9)	-124.2 (-208.7, 488.8)	720.3 (615.8, 1041.6)	27.1 (21.0, 39.4)
50% Not achieved (n=16)	99.2 (-4.5, 277.6)	408.0 (177.5, 835.4)	15.48 (-13.7, 38.8)
P-Value	0.90	0.01	0.52

Table S6. Effect on Bone Density and Extraskeletal Calcifications*								
	Triple Therapy Trial				**Lonafarnib Monotherapy Trial**			
	Baseline	**End-of -study**	**N**	**P value**	**Baseline**	**End-of -study**	**N**	**P value**
aBMD by DXA								
Whole Body (g/cm^2)	0.49	0.54	31	<0.001	0.49	0.49	25	0.12
Height-adjusted Z Score Whole Body**	-2.75 (-3.20,-2.10)	-2.11 (-2.80,-1.20)	19	<.001	-2.44 (-3.20,-2.00)	-2.96 (-3.50,-2.40)	13	0.04
Spine (g/cm^2)	0.44	0.52	32	<0.001	0.44	0.45	25	0.342
Height-adjusted Z Score Spine*	-1.68 (-2.50,-0.80)	-0.74 (-1.30,-0.10)	19	0.001	-1.72 (-3.00,-1.00)	-1.54 (-2.50,-0.70)	13	0.35
vBMD (g/cm^3) (median; quartiles) by pQCT								
4% site	0.73 (0.67,0.83)	0.89 (0.86,1.55))	24	<0.001	0.62 (0.56, 0.71)	0.74 (0.69,0.84)	18	0.05
20% site	1.20 (1.08,1.32)	1.30 (1.24,1.76)	24	0.006	1.20 (1.01,1.28)	1.23 (1.11,1.32)	18	0.30
50% site	1.20 1.08,1.36)	1.34 (1.25,1.76)	23	<0.001	1.26 (1.12, 1.30)	1.21 (1.15,1.33)	17	0.96
Extraskeletal Calcifications, Subject # (%) Positive, by X-ray	11 (34.4)	21 (65.6)	32	0.0063	8 (29)	11 (44)	25	0.4531

*Significance was maintained when groups were broken into naïve and non-naïve triple therapy groups (data not shown)

**Normative values for DXA BMD Z-scores are generally available for ages over 3 years, with more information available for the spine and whole body compared to the hip. Therefore, no Z-scores were generated for subjects with height-age under age 3 years[7].

Table S7. pQCT structural evaluations; median (lower quartile, upper quartile)													
Site	**Triple Trial All (N=23-24)**			**Triple Trial Naïve (N=6)**			**Triple Trial non-naïve (N=17-18)**			**Lonafarnib monotherapy (N=17-18)**			**Non-HGPS Normal Controls (N=30)**
	Baseline	**End-of -study**	**P value**	**Baseline**	**End-of -study**	**P value**	**Baseline**	**End-of -study**	**P value**	**Baseline**	**End-of -study**	**P value**	**N/A**
EI ($N\bullet M^2$)													
4%	695 (353,994)	855 (696,1076)	<0.001	249 (129,291)	765 (721,866)	0.03	823 (621,1071)	891 (671,1159)	<0.001	408 (305,642)	823 (621,1071)	<0.001	730 (375,1127)
20%	891 (567,1187)	1131 (722,1289)	<0.001	276 (237,495)	1070 (862,1122)	0.03	1088 (646,1205)	1175 (698,1301)	<0.001	738 (500, 840)	1088 (646,1205)	<0.001	1102 (727,1368)
50%	785 (529,1100)	952 (827,1192)	<0.001	287 (124,361)	942 (755,1054)	0.03	879 (774,1105)	952 (838,1196)	<0.001	541 (458, 715)	879 (774,1105)	<0.001	1032 (702,1290)
EA (MN)													
4%	2.32 (1.44, 2.83)	2.66 (2.34, 3.14)	<0.001	0.98 (0.72,1.34)	2.5 (2.41,2.62)	0.03	2.56 (2.05,3.18)	2.84 (2.27,3.53)	<0.001	1.29 (1.06,1.70)	2.56 (2.05, 3.18)	0.0002	2.16 (1.12,3.64)
20%	2.18 (1.60, 2.76)	2.70 (2.24,3.17)	<0.001	0.96 (0.71,1.70)	2.46 (2.14,2.69)	0.03	2.47 (2.11,2.81)	2.84 (2.43,3.23)	<0.001	1.36 (1.02, 1.63)	2.47 (2.11, 2.81)	<0.0001	2.45 (1.77,2.97)
50%	2.14 (1.51, 2.98)	2.78 (2.23, 3.26)	<0.001	0.81 (0.40,1.05)	2.77 (2.39,2.83)	0.03	2.64 (2.05,3.03)	2.88 (2.23,3.31)	<0.001	1.37 (1.18, 1.77)	2.64 (2.05, 3.03)	<0.0001	2.51 (1.93,3.13)
GJ ($N\bullet M^4$)													
4%	754 (416,1095)	868 (711,1087)	0.0003	264 (148,331)	781 (738,879)	0.03	886 (714,1149)	904 (686,1176)	0.03	454 (323,743)	886 (714,1149)	<0.001	742 (388,1139)
20%	925 (593,1233)	1148 (739,1312)	<0.001	285 (245,514)	1085 (875,1134)	0.03	1122 (718,1241)	1190 (717,1312)	<0.001	759 (514,862)	1122 (718,1241)	<0.001	1114 (738,1380)
50%	798 (536,1121)	961 (850,1203)	<0.001	292 (129,368)	952 (771,1062)	0.03	904 (786,1138)	961 (855,1215)	<0.001	551 (466,726)	904 (786,1138)	<0.001	1044 (713,1301)

Table S8. Bone-related serum values (Mean±S.D.; N=32)			
	Baseline	**EOS**	**P value**
Protein (mg/dl)	7.3±0.4	7.2±0.5	0.215
Calcium(mg/dl)	10.1±0.5	9.7±0.4	<.0001
Phosphorous(mg/dl)	4.8±0.6	4.4±0.6	0.002
25-hydroxy vitamin D (mg/dl)	40.3±15.8	30.6±16.3	<.0001
Calcium-phosphorous product (mg^2/dl^2)	48.0±6.9	42.2±6.9	0.0001
Normal values: calcium = 8.4-10.5 mg/dl; phosphorous under age 13 y. = 3.0-5.7 mg/dl; phosphorous over age 13 y. = 2.7-4.9 mg/dl; 25-hydroxy vitamin D=30-80 mg/dl; Calcium-phosphorous product ≤60			

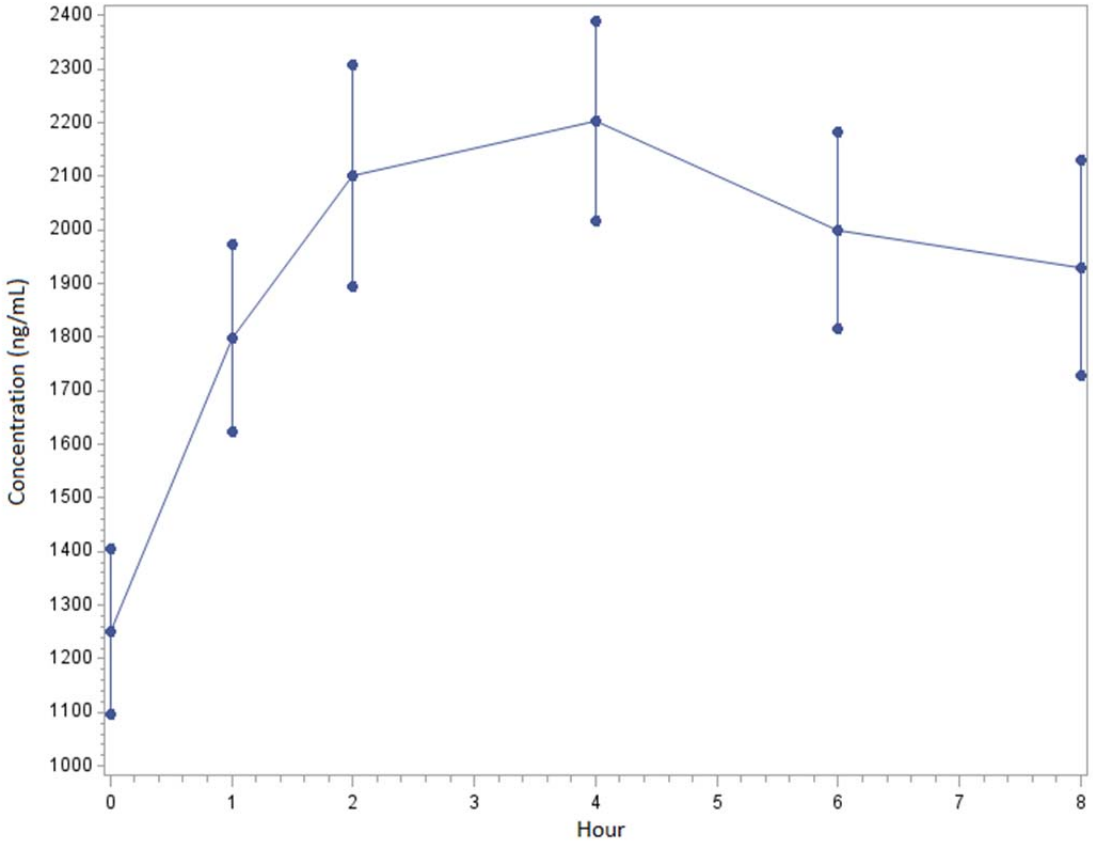

Figure S1 Lonafarnib pharmacokinetics. Mean plasma lonafarnib concentration (y axis) over an 8-h sampling period (x axis). Area under the curve 15.7± 1.2 μg·h−1·mL−1

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