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A super-agonist of growth hormone–releasing hormone causes rapid improvement of nutritional status in patients with chronic kidney disease

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Chronic kidney disease is frequently associated with protein-energy wasting related to chronic inflammation and a resistance to anabolic hormones such as insulin and growth hormone (GH). In this study, we determined whether a new GH-releasing hormone super-agonist (AKL-0707) improved the anabolism and nutritional status of nondialyzed patients with stage 4–5 chronic kidney disease randomized to twice daily injections of the super-agonist or placebo. After 28 days, this treatment significantly increased 24-h GH secretion by almost 400%, without altering the frequency or rhythmicity of secretory bursts or fractional pulsatile GH release, and doubled the serum insulin-like growth factor-1 level. There was a significant change in the Subjective Global Assessment from 'mildly to moderately malnourished' to 'well-nourished' in 6 of 9 patients receiving AKL-0707 but in none of 10 placebo-treated patients.
By dual-energy X-ray absorptiometry, both the mean fat-free mass and the body mineral content increased, but fat mass decreased, all significantly. In the AKL-0707-treated group, both serum urea and normalized protein equivalent of nitrogen appearance significantly decreased with no change in dietary protein intake, indicating a protein anabolic effect of treatment. Thus, our study shows that stimulation of endogenous GH secretion by AKL-0707 overcomes uremic catabolism of patients with advanced chronic kidney disease.

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Chronic kidney disease (CKD) is frequently associated with protein-energy wasting related to chronic inflammation and resistance to the actions of anabolic hormones, such as insulin and growth hormone (GH).[1] The resulting complex of malnutrition, inflammation, and accelerated atherosclerosis (known as the MIA syndrome) is a major cause of excessive morbidity and mortality observed in these patients.[2]

Molecular mechanisms of uremic GH resistance include impaired postreceptor signaling,[3] reduced bioavailability of the downstream mediator insulin-like growth factor-1 (IGF-1) due to increased plasma protein binding,[4,5] and possibly impaired IGF-1 target tissue signaling.[6] Resistance to endogenous GH can be partially overcome by administration of recombinant human GH (rhGH) at pharmacological doses.[7–12] rhGH is an approved treatment of growth failure in children with CKD and causes anabolic effects[7–11] and an improved quality of life[11,12] in adults undergoing dialysis,[7–11] but clinical experience in adults with pre-dialytic CKD is lacking.

Spontaneous pulsatile GH secretion is mainly regulated by the interplay of two hypothalamic hormones, namely GH-releasing hormone (GHRH) and somatostatin. Administration of GHRH by subcutaneous injection or continuous infusion enhances endogenous GH secretion while preserving the physiological pulsatile GH secretion pattern.[13,14] Therefore, stimulation of endogenous episodic GH release by

GHRH administration may provide a more physiological activation of the somatotropic hormone axis than the direct subcutaneous injection of rhGH, which results in the exposure of target tissues to a single large daily wave of GH. Although human GHRH has been demonstrated to stimulate statural growth in children, its clinical use was largely abandoned because of its apparent slightly inferior growth-promoting efficacy relative to rhGH.

AKL-0707 ([D-Ala^2, D-Tyr^{10}, D-Ala^{15}, Lys^{22}]hGHRH (1–29)NH_2) is a poly-substituted analog and super-agonist of human GHRH. Relative to the native GHRH peptide, AKL-0707 binds to the human GHRH receptor *in vitro* with at least 900 times higher affinity and exhibits at least 25-fold increased resistance to proteolysis in the human plasma.[15] These properties make the compound a promising therapeutic alternative to rhGH. We carried out a placebo-controlled randomized clinical trial to determine the efficacy of AKL-0707 in stimulating endogenous GH secretion and reversing uremic catabolism in adult patients with CKD-associated protein-energy wasting.

RESULTS

Patients

A total of 28 subjects were randomized: 13 to AKL-0707 and 15 to placebo. Participant flow is presented in Figure 1. Baseline characteristics were similar in both groups at the time of screening (Table 1). A total of 26 subjects completed the study as planned; 1 subject per group discontinued the study for reasons other than adverse events (AEs). The first subject was enrolled on 23 August 2005 and the last subject completed the study on 23 March 2007.

GH/IGF-1

The mean area under the plasma GH concentration vs time curve was similar in the two groups at baseline, and increased ~9.5-fold during the initial 4 h after drug injection ($P<0.0001$) and 5-fold in the 24-h concentration profile ($P<0.005$) after AKL-0707 as compared with placebo (Table 2, Figure 2). The difference in GH plasma concentrations was brought about by commensurate increases in the mass of GH released per hormone pulse and basal GH secretion, without changes in GH pulse frequency, fraction of pulsatile secretion, or regularity of pulses.

Table 1 | Baseline characteristics of the study subjects by treatment randomization

	AKL-0707 (*n*=13)	Placebo (*n*=15)
Male (*n*)	6	9
Diabetic (*n*)	4	3
Hypertension (*n*)	12	14
Age, years	61.8 ± 9.5	63.4 ± 10.5
Body weight, kg	62.8 ± 10.0	64.6 ± 8.1
Body mass index, kg/m^2	23.7 ± 3.2	22.8 ± 2.8
Fat-free mass (DXA), kg	43.8 ± 10.9	46.4 ± 8.3
Fat mass (DXA), kg	17.1 ± 9.5	16.3 ± 6.1
Total body bone mineral content, kg	2.2 ± 0.7	2.4 ± 0.5
Protein intake, g/kg per day	1.0 ± 0.3	1.0 ± 0.4
Energy intake, kcal/kg per day	26.9 ± 7.2	27.1 ± 7.9
nPNA, g/kg per day	1.2 ± 0.6	1.0 ± 0.5
Glomerular filtration rate, ml/min per 1.73 m^2	20.4 ± 5.8	17.9 ± 8.0
Serum creatinine, mg/dl	3.1 ± 0.7	3.8 ± 1.3
Serum urea, g/dl	130 ± 40	124 ± 40
Serum albumin, g/l	39.6 ± 6.7	40.4 ± 5.3
Serum bicarbonate, mmol/l	23.0 ± 5.4	22.0 ± 4.0
Serum IGF-1, ng/ml	345 ± 157	375 ± 164
Serum IGFBP-1, ng/ml	75.0 ± 39.1	75.8 ± 25.3
Serum IGFBP-3, mg/ml	4.0 ± 0.8	4.5 ± 1.1
IGF-1/IGFBP-3 molar ratio	0.30 ± 0.12	0.30 ± 0.08

Abbreviations: DXA, dual-energy X-ray absorptiometry; IGF-1, insulin-like growth factor 1; IGFBP-1, insulin-like growth factor binding protein-1; IGFBP-3, insulin-like growth factor binding protein-3; nPNA, normalized protein equivalent of nitrogen appearance.
Data are mean ± s.d., unless given otherwise.

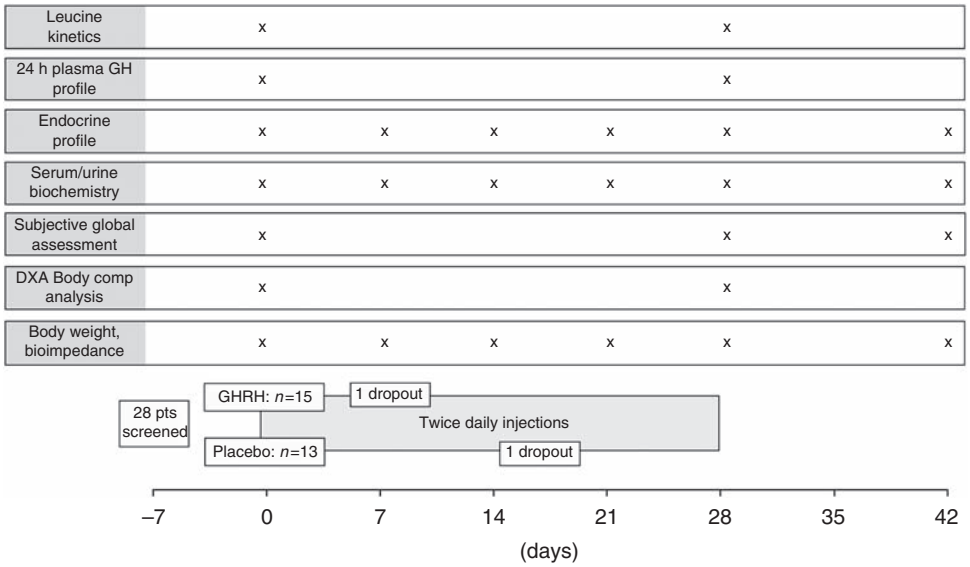

Figure 1 | Trial design and patient flow. DXA, dual-energy X-ray absorptiometry; GHRH, growth-hormone-releasing hormone; pt, patient.

Table 2 | Effects of AKL-0707 on 24-h growth hormone concentration/secretion profiles

	AKL-0707 N=12	Placebo N=14	Difference or ratio of means*	95% CI	P-value
Area under the curve (μIU/ml min) at 4 h					
Baseline	413 (366)	403 (385)	Ratio		
Day 28	5103 (5053)	525 (707)	9.49	(4.4; 20.3)	0.0001[a]
Area under the curve (μIU/ml min) at 24 h					
Baseline	3771 (2140)	3389 (1666)	Difference		
Day 28	18,580 (13 363)	4146 (3528)	11,585	(4571; 18,598)	0.0032[b]
Mean pulse frequency (per 24 h)					
Baseline	8.09 (2.81)	10.54 (3.72)	Difference		
Day 28	9.07 (3.49)	8.76 (3.19)	0.8	(−2.2; 3.8)	0.577[b]
Inter-pulse regularity					
Baseline	2.35 (0.63)	2.87 (2.29)	Difference		
Day 28	1.95 (0.30)	2.01 (0.66)	−0.0	(−0.5; 0.4)	0.877[b]
Mass per pulse (mU/l)					
Baseline	11.3 (7.0)	8.9 (9.4)	Difference		
Day 28	43.9 (36.2)	10.8 (14.8)	26.5	(7.2; 45.8)	0.009[b]
Basal secretion rate (mU/l per 24 h)					
Baseline	26.9 (19.8)	19.7 (18.3)	Ratio		
Day 28	118 (102)	34.3 (25.1)	2.53	(1.34; 4.79)	0.007[a]
Pulsatile secretion rate (mU/l per 24 h)					
Baseline	98.2 (49.3)	70.1 (45.4)	Ratio		
Day 28	457 (526)	98.5 (102)	2.81	(1.15; 6.89)	0.026[a]
Total secretion rate (mU/l per 24 h)					
Baseline	125 (64)	90 (45)	Ratio		
Day 28	574 (594)	133 (115)	2.72	(1.23; 5.99)	0.016[a]
% Pulsatile secretion rate					
Baseline	79.0 (9.1)	75.2 (22.1)	Difference		
Day 28	71.3 (16.2)	70.2 (14.5)	−0.4	(−11.6; 10.8)	0.945[b]

Abbreviations: ANOVA, analysis of variance; ANCOVA, analysis of covariance; CI, confidence interval.
Treatment comparison with ANCOVA, assuming log-normal distribution. *Selection of method of choice was based on distribution of residuals.
Data are given as mean (s.d.).
[a]Treatment comparison with nonparametric ANCOVA.
[b]ANOVA for repeated measures, assuming normal distribution.

Plasma IGF-1 concentrations doubled within 3 weeks in patients receiving AKL-0707 ($P<0.01$–0.001 vs controls from day 7 to day 28) (Figure 3). Conversely, plasma IGF-binding protein (IGFBP)-1 levels decreased by ~30% ($P<0.01$ vs controls at day 28). Plasma IGFBP-3 was stimulated slightly in the AKL-0707 group ($P=0.13$). The molar IGF-1/IGFBP-3 ratio increased from 0.28 ± 0.12 to 0.49 ± 0.09 in the AKL-0707 group ($P<0.0001$), but remained unchanged in the placebo group (0.30 ± 0.08 to 0.34 ± 0.11). Two weeks after discontinuation of AKL-0707, IGF-1, and IGFBPs, plasma concentration levels had returned to baseline (and placebo control) levels.

Leucine kinetics

Results of leucine kinetics studies are provided in Table 3. Leucine oxidation and flux, as well as calculated rates of protein synthesis and breakdown were similar in the two treatment groups at baseline and after 4 weeks of treatment, irrespective of whether normalization was performed by body weight or by fat-free mass (FFM).

Body mass and composition

In patients receiving AKL-0707, their body weight increased within the 28-day treatment period by a mean of 1.2 kg, compared with 0.1 kg with placebo-treated patients (mean difference 0.95%, 95% confidence interval (95% CI): 0.2–1.8, $P<0.05$) (Figure 4). Correspondingly, the mean body mass index increased by 0.42 kg/m^2 in the AKL-0707 group compared with 0.03 kg/m^2 after placebo (ratio of means 1.0, 95% CI: 1.00–1.03, $P<0.05$).

Body composition analysis performed by dual-energy X-ray absorptiometry (DXA) and bioimpedance (BIA) showed that the increase in body mass was the net effect of reciprocal changes in FFM and fat mass (FM) (Figure 4). On day 28, mean FFM had increased in subjects receiving AKL-0707 according to both DXA (1.83 kg) and BIA (3.3 kg), and had decreased in the placebo group (1.39 kg by DXA; 0.8 kg by BIA). Conversely, FM decreased in the AKL-0707 group (−0.51 kg by DXA; −1.8 kg by BIA) and increased in the placebo group (1.21 kg by DXA; 1.3 kg by BIA). Changes in FFM and FM differed significantly between the treatment

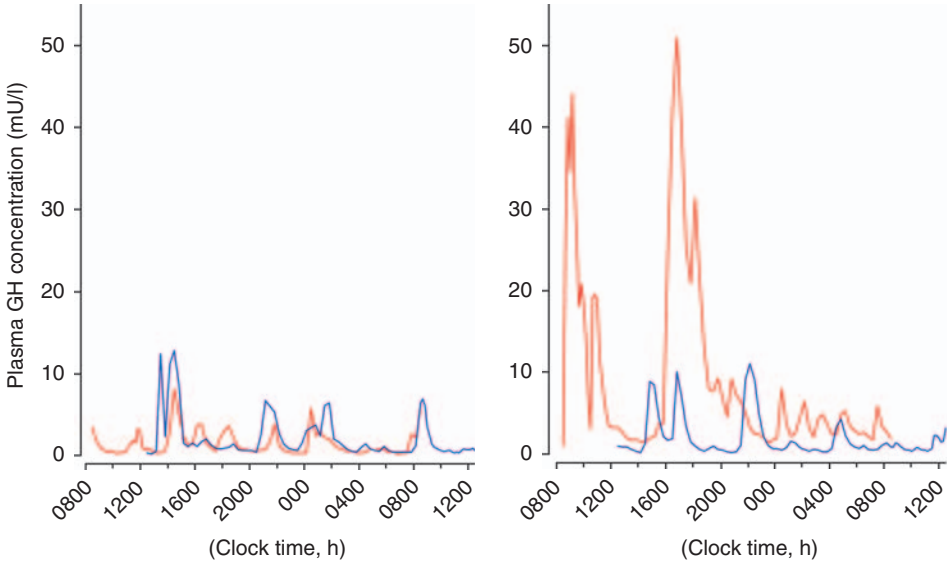

Figure 2 | Representative 24-h growth hormone concentration profiles at baseline (day 0/1, blue) and after 4 weeks of treatment (day 28/29; red). Left panel shows a patient from the placebo group; right panel shows a patient from the AKL-0707 group. The 24-h profiling was started at 0800 hours on day 0 and at 1230 hours on day 28. Injections were performed at 0800 and 1600 hours on day 28/29 profiles. GH, growth hormone.

groups, irrespective of the methodology used ($P<0.05$–0.01, Figure 4).

Bone mineral content by DXA increased by 38 ± 41 g with AKL-0707 as compared with no change with placebo (adjusted mean difference 40 g, 95% CI: 10–70 g; $P<0.05$).

Subjective Global Assessment

Among patients rated as 'mildly to moderately malnourished' at baseline, six out of nine subjects in the AKL-0707 group, but none out of seven in the placebo group, appeared 'well-nourished' after 28 days of therapy ($P<0.01$ for overall change) (Figure 5). The Subjective Global Assessment (SGA) ratings remained unchanged in each patient at the follow-up visit 2 weeks after discontinuation of therapy.

Biochemical parameters of nutrition

Serum urea levels decreased from 130 ± 43 to 96 ± 26 mg/dl in the AKL-0707 group ($P<0.01$) as compared with unchanged levels in placebo-treated patients (124 ± 40 to 137 ± 46 mg/dl), resulting in an adjusted mean difference of -44 (95% CI: -62 to -25 mg/dl) ($P<0.0001$). Correspondingly, the normalized protein equivalent of nitrogen appearance decreased from 1.16 ± 0.56 to 0.79 ± 0.29 g/kg per day in the AKL-0707 group ($P<0.01$) and remained constant in the placebo group (1.00 ± 0.53 to 1.01 ± 0.40 g/kg per day). Serum urea levels returned to baseline 2 weeks after termination of treatment with AKL-0707. Serum albumin levels did not change significantly in either group and did not differ at day 28 (37.7 ± 6.3 vs 39.0 ± 8.3 g/l). In addition, no significant effects were observed for serum pre-albumin, transferrin, leptin, and adiponectin. Protein intake remained unchanged, similar to AKL and placebo administration (day 28: 1.0 ± 0.4 vs 1.0 ± 0.3 g/kg per day, NS), and so did energy intake (25 ± 8.7 vs 27 ± 9 gkcal/kg per day, NS).

Biochemical parameters of metabolic control

Significant differences between the treatment groups were also noted with respect to changes in fasting insulin and glucose levels in nondiabetic subjects. Fasting insulin levels increased from 9.7 ± 3.4 to 12.4 ± 4.3 μU/ml in the AKL-0707 group ($P<0.05$), resulting in a 2.1 (95% CI: 1.5–3.0; $P<0.0001$) ratio of means relative to placebo. Fasting glucose increased from 4.9 ± 0.3 to 5.3 ± 0.7 mmol/l (ratio of means 1.1 (95% CI: 1.0–1.2; $P<0.005$)). There was no significant effect of AKL-0707 on insulin resistance as assessed by homeostatic model assessment index either in the nondiabetic (2.9 ± 1.0 vs 1.6 ± 1.0) or in the total intention-to-treat population (9.7 ± 19.5 vs 3.9 ± 7). In addition, no effect on HbA1c levels was detected.

Mean cholesterol values decreased from 5.1 ± 1.0 to 4.7 ± 0.6 mmol/l in subjects receiving AKL-0707 as compared with no change in the placebo group ($P<0.05$ for treatment effect).

Safety

The two groups were generally matched with respect to AEs (Table 4). A total of 70 treatment-emergent AEs were reported in 12 subjects randomized to AKL-0707, and 58 AEs in 14 subjects randomized to placebo. The most frequently reported AEs were injection site bruising, followed by red blood cell count decrease and injection site pain, blood glucose increase, and hemoglobin decrease. Four serious AEs were reported in three subjects, but they were considered not study drug related. Three out of four serious AEs occurred in the placebo group.

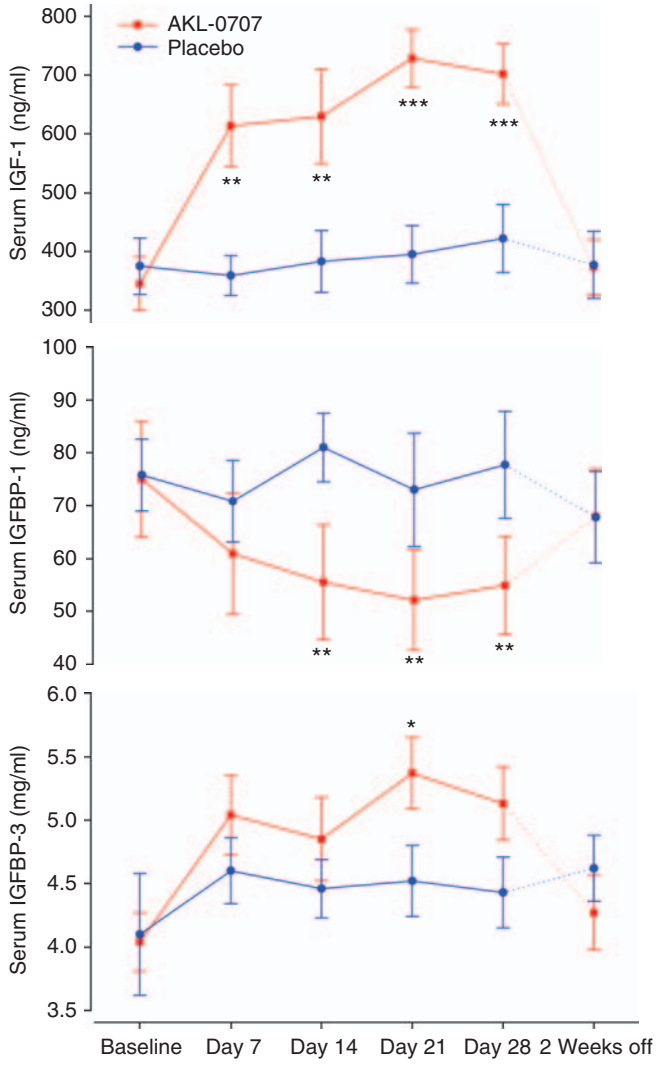

Figure 3 | Plasma concentrations of insulin-like growth factor 1 (IGF-1) and IGF-binding protein-1 and protein-3 before, during, and after treatment in 12 patients completing 4 weeks of treatment with AKL-0707 (red, squares) and in 14 patients completing 4 weeks of placebo administration (blue, circles). Nonparametric ANCOVA was used to assess differences between treatment groups in plasma IGF-1; ANOVA was used for repeated measurements to assess IGFBP-1 and IGFBP-3 differences (*$P<0.05$, **$P<0.001$, ***$P<0.0001$). ANOVA, analysis of variance; ANCOVA, analysis of covariance; IGFBP, insulin-like growth factor binding protein.

DISCUSSION

In this placebo-controlled study, we demonstrated the rapid and profound effects of a novel GHRH super-agonist on the endocrine and nutritional status and body composition of malnourished patients with advanced pre-dialysis CKD.

AKL-0707 treatment increased circulating GH concentrations by almost fivefold. This response was brought about by an amplification of the physiological pulsatile GH secretion pattern, with proportionate increases in the mass of GH secreted per pulse and basal secretion rate but no change in the frequency or orderliness of GH pulses. Qualitatively similar results have been achieved with native GHRH in the elderly.[13]

The restoration of GH secretion to a juvenile level was associated with a doubling of serum IGF-1, a marked suppression of IGFBP-1, and a slight increase in IGFBP-3 concentrations in serum. This endocrine response to GH serves to increase IGF-1 bioavailability at the tissue level. Low free IGF-1 and high IGFBP-1 levels are characteristic of GH insufficiency in CKD and have been linked to poor statural growth in uremic children.[5,16] Treatment with rhGH at pharmacological doses increases circulating IGF-1 and IGFBP-3 and decreases IGFBP-1 in pediatric CKD and adult hemodialysis patients.[10,17] The endocrine effects of AKL-0707 were readily reversible; serum IGF-1 and binding protein concentrations reached baseline levels within 2 weeks of discontinuation of the drug.

GHRH super-agonist treatment was remarkably efficacious in improving the state of nutrition and general well-being of patients. SGA is a widely used, reliable, and efficient method of standardized nutritional assessment at bedside in nephrology, surgery, and oncology.[18] Within 4 weeks of blinded AKL-0707 administration, six out of nine patients who were rated mildly to moderately malnourished by SGA at baseline became well-nourished, as compared with no change in controls receiving placebo.

This considerable clinical benefit corresponded to marked changes in body composition. An average weight gain of 1 kg was observed, which was the net effect of an increase in FFM and a reduction in FM. FFM gains were noted in 11 of 12 patients completing the AKL-0707 treatment period, averaged 1.8 kg according to DXA, and contrasted with a continued loss of FFM in the placebo group. This protein anabolic effect of 4-week GHRH analog treatment is comparable with the dose-related 1.9–3.1 kg gain of FFM observed by DXA after 6 months of rhGH therapy in a recent placebo-controlled trial of adult patients on maintenance hemodialysis.[11] The significant decreases in serum urea concentrations by 26% and in urinary nitrogen appearance by 32% at unchanged protein intake provide biochemical confirmation of the protein anabolism induced by AKL-0707.

At the same time, FM decreased significantly with AKL-0707 as compared with an increase in controls. The reduction in adipose tissue mass most likely reflects augmented GH release, as GH pulses directly promote lipolysis.[19] Lipolytic effects have been commonly observed in patients receiving rhGH for various indications including healthy elderly subjects,[20] with conflicting results in hemodialysis patients.[11,21] rhGH is an effective treatment for HIV-associated visceral fat accumulation ('lipodystrophy'). Recent data have suggested that GHRH analogs, possibly by augmenting endogenous pulsatile GH secretion patterns, might be particularly effective in reducing abdominal fat accumulation.[22,23] In this context, it is noteworthy that AKL-0707 also decreased total plasma cholesterol levels as compared with placebo-treated controls.

Table 3 | Results of leucine kinetic studies

	AKL-0707 N=13	Placebo N=15	Adjusted mean difference or 50% median point estimate	95% CI	P-value
Leucine oxidation (μmol/kg h)					
Baseline mean (s.d.)	7.7 (4.5)	9.8 (6.9)			
Day 28 mean (s.d.)	10.2 (4.9)	9.4 (11.2)			
Change from baseline to day 28 mean (s.d.)	2.4 (5.7)	0.8 (10.7)	1.1	(−6.0; 8.2)	0.75
Leucine flux (μmol/kg h)					
Baseline mean (s.d.)	101.5 (21.5)	94.0 (24.7)			
Day 28 mean (s.d.)	103.1 (31.3)	92.4 (21.1)			
Change from baseline to day 28 mean (s.d.)	1.2 (30.1)	−3.2 (25.5)	7.5	(−12.0; 27.0)	0.43
Protein synthesis (μmol/kg h)					
Baseline mean (s.d.)	93.7 (20.4)	84.2 (26.2)			
Day 28 mean (s.d.)	92.9 (29.7)	83.0 (22.3)			
Change from baseline to day 28 mean (s.d.)	−1.2 (30.8)	−4.0 (24.9)	6.9	(−10.9; 24.7)	0.43
Protein breakdown (μmol/kg h)					
Baseline mean (s.d.)	97.5 (21.5)	90.0 (24.7)			
Day 28 mean (s.d.)	99.0 (31.2)	88.4 (21.1)			
Change from baseline to day 28 mean (s.d.)	1.2 (30.1)	−3.2 (25.5)	7.5	(−11.9; 27.0)	0.43

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval.
Statistical comparisons were made by ANCOVA assuming normal distribution

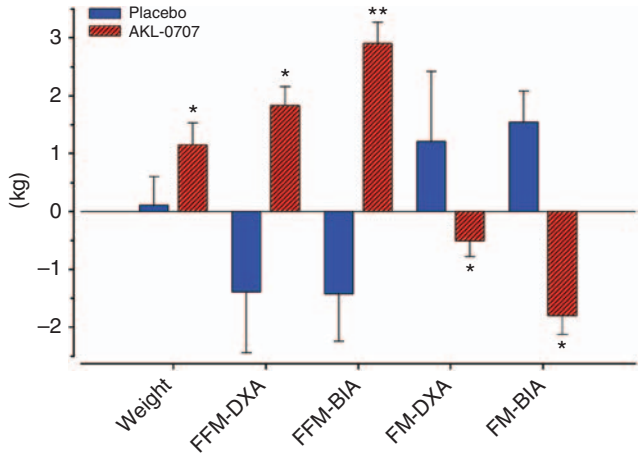

Figure 4 | Changes in mean body weight, fat-free mass, and fat mass measured by dual-energy X-ray absorptiometry or bioimpedance during 4 weeks of either AKL-0707 ($n = 12$) or placebo administration ($n = 14$). Dashed red bars denote AKL-0707 and blue bars represent placebo. Asterisks (*) denote significant differences between treatment groups (*$P < 0.05$, **$P < 0.01$). BIA, bioimpedance; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; FM, fat mass.

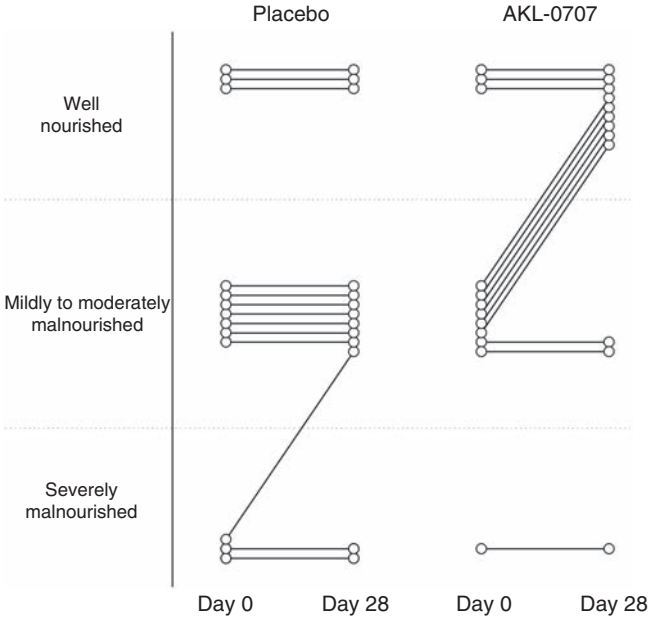

Figure 5 | Subjective Global Assessment of nutritional status before and after 4 weeks of treatment with AKL-0707 or placebo. The change in nutritional status differed significantly between treatment groups ($P < 0.01$).

Another notable finding by DXA was the small but significant increase in total body mineral content in AKL-0707-treated subjects as compared with those treated with placebo. Although long-term (>6 months) rhGH substitution tends to improve bone mineralization in adults with GH deficiency,[24] no effect or even a transient reduction in lumbar spine mineralization was noted on dialysis in patients receiving rhGH.[12] GH functions on bone both by stimulating local IGF-1 production and by direct GH receptor activation on osteoblasts, osteoclasts, and osteocytes.[25] The latter mechanism may be susceptible to the temporal pattern of GH exposure and be more effectively activated by GHRH analog-induced augmented endogenous pulsatile GH secretion as compared with the more tonic GH profile obtained by subcutaneous rhGH injections.

In view of the clinically visible improvement in nutritional status, markedly increased FFM, and substantial reduction in serum urea and normalized protein equivalent of nitrogen

Table 4 | Adverse and serious adverse events reported during the study period

Event	AKL-0707 (*N*=13)	Placebo (*N*=15)
Number of patients (% of total patients; number of adverse events)		
Adverse events		
Pre-treatment	5 (38.5; 13)	7 (46.7; 14)
On treatment	12 (92.3; 70)	14 (93.3; 58)
Resulting in study discontinuation	0	0
Number of patients (% of total patients)		
Any event		
Injection site bruising	4 (30.8)	5 (33.3)
Injection site pain	3 (23.1)	3 (20.0)
Nausea/vomiting	2 (15.4)	3 (20)
Diarrhea	2 (15.4)	1 (6.7)
Upper respiratory tract infection	2 (15.4)	4
Urinary tract infection	2 (15.4)	1 (6.7)
Fever	1 (7.7)	1 (6.7)
Hypertension	2 (15.4)	3 (20.0)
Edema	2 (15.4)	2
Osteopenia/osteoporosis	1	2 (13.3)
Neutrophilia	0	1 (6.7)
Angina pectoris	0	1 (6.7)
Cardiac arrest	1 (7.7)	0
Cardiac failure	0	1 (6.7)
Chronic obstructive pulmonary disease	1 (7.7)	0
Acidosis	0	1 (6.7)
Hypercalcemia	1 (7.7)	0
Hypercholesterolemia	0	1 (6.7)
Hypertriglyceridemia	0	1 (6.7)
Dry mouth	1 (7.7)	0
Serious adverse events		
On treatment		
Cardiac disorders	1 (7.7)	1 (6.7)
Unspecified surgical and medical procedures	0	1 (6.7)
Off treatment		
Hospitalization	0	1 (6.7)
Death		
Post-treatment cardiac arrest	1 (7.7)	0

appearance, there is little doubt that AKL-0707 caused a consistent anabolic effect, which makes the apparently lacking effect on protein synthesis, breakdown, or net protein balance in leucine kinetics studies difficult to explain. Previous experience with rhGH administration in patients with end-stage renal disease showed an improved net protein balance and increased protein synthesis in subjects with increased baseline ^{13}C-leucine oxidation indicative of catabolism.[7] However, in our patients ^{13}C-leucine oxidation rates at baseline were markedly lower than those previously observed in CKD patients.[26] It has been argued that in stable chronic illnesses, whole-body protein turnover may be down-regulated by an adaptive metabolic response to a lower steady state. Furthermore, the normalized protein homeostasis in our patients might be attributable to the careful correction of metabolic acidosis, which was required before participation in the study. It has been shown that correction of metabolic acidosis mitigates whole-body protein degradation in patients with CKD.[27]

In addition, the kinetic study was carried out in the fasting state, a condition characterized by low endogenous insulin and high cortisol release. The sensitivity to detect anabolic effects of GHRH might have been higher in the postfeeding state. In addition, regional kinetic studies, measuring turnover in leg or arm muscle, might be more sensitive than studies of whole-body turnover. However, as regional kinetic studies need to cannulate a set of major arteries and veins, we opted against this more invasive approach because of safety considerations. Finally, the apparent lack of stimulation of net protein synthesis as assessed by leucine kinetics despite the obvious net accretion of lean body mass in the AKL-0707 group might reflect differences in effect dynamics. In GH-deficient adults, rhGH therapy suppressed leucine oxidation at 2 weeks; while this early response was predictive of the FFM increase and FM decrease at 12 weeks, leucine oxidation had already returned to baseline levels and was not correlated with the change in body composition observed at the 12-week time point.[28]

AKL-0707 was very well tolerated; the number and nature of AEs observed were consistent with the underlying disease without a pattern distinguishing the treatments from each other. As hypertension due to fluid retention is a common early side effect of rhGH treatment in CKD, it was reassuring that blood pressure remained constant in AKL-0707 treated patients.

In conclusion, in this proof-of-concept phase II study of AKL-0707 GHRH super-agonist in malnourished patients with CKD, we demonstrated that AKL-0707 is safe and tolerable and improves nutritional status and body composition even within a short treatment period. In individual patients with advanced CKD, reduced blood urea nitrogen accumulation due to increased muscle protein accretion might help to delay the need for renal replacement therapy. Follow-up studies with longer treatment duration and larger sample sizes will be required to determine whether the gain of lean body mass and attenuation of uremia will help stabilizing patient well-being in pre-dialysis CKD, and positively affect the physical functional status in these often frail patients. In addition, it remains to be seen whether the stimulation of endogenous pulsatile GH secretion by GHRH analogs will confer a clinical benefit over direct exogenous administration of rhGH.

MATERIALS AND METHODS

Study participants

Men and women aged over 40 years with clinically stable CKD stage 4 or 5 (glomerular filtration rate 10–30 ml/min per 1.73 m^2) and malnutrition of any severity (defined by serum albumin $\leqslant$40 g/l, body mass index $\leqslant$23 mg/m^2, or nonpurposeful $>$5% loss of body weight in the previous 6 months), and who had given written informed consent were eligible to participate in the study. Concomitant erythropoietin therapy was allowed if dose was unchanged for the previous 8 weeks. Exclusion criteria were severe fluid overload, severe anemia, uncontrolled metabolic acidosis, rapidly progressive renal failure, history of neoplasia, glucocorticoid treatment within the previous 4 weeks, active systemic inflammatory

or infectious disease, congestive heart failure, active liver disease or clinically relevant abnormal laboratory results, hypothyroidism, history of drug abuse, or treatment with centrally acting drugs. Pregnant or lactating women were excluded.

The study was conducted at three sites in Poland in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. The protocol was approved by the central ethics committee at the Silesian University of Medicine (Katowice, Poland).

Study design, blinding, randomization, and interventions

Patients were randomly assigned to receive two daily subcutaneous injections of either 1 mg of AKL-0707 or placebo for 28 days. Randomization was carried out with random permuted blocks of four subjects. Supplies were numbered serially so that randomization was achieved by assigning the subject to the next available number in the sequence. Patients, all study personnel, and those assessing the outcome were blinded, and the allocation sequence was concealed until data base closure and issuance of final statistical report. Treatment compliance was 100% as the investigational drug was administered by a nurse.

After the initial screening visit, approval of participation and randomization, subjects attended the clinic for five study visits at 7-day intervals, plus for a follow-up assessment 14 days after the last dose. The trial protocol and patient flow is summarized in Figure 1. A physical and anthropometric examination, BIA, and blood tests were conducted at every visit. On days 0 and 28/29, subjects underwent a whole-body DXA scan, 24-h GH profiling, leucine kinetics studies, urine tests, and SGA. DXA-derived FFM, FM, and bone mineral content were recorded for the total body, arms, legs, and trunk. AEs and concomitant medications were collected throughout the study.

Study objectives, hypothesis, and outcome measures

The primary objective was to determine the effect of treatment on protein turnover as assessed by ^{13}C leucine kinetics. Secondary objectives were to determine the effect of AKL-0707 on endogenous time-integrated 24-h GH secretion, circulating IGF-1, FFM and FM, biochemical parameters of nutritional, and metabolic state (serum albumin, pre-albumin, transferrin, leptin, adiponectin, glycosylated hemoglobin, fasting glucose, insulin, lipids), spontaneous nutrient intake (3-day dietary protocols), and safety and tolerability as assessed by AE collection, physical examination, vital signs, routine laboratory safety screening, and monitoring for human anti-drug antibodies. The working hypothesis was that AKL-0707 would break resistance to endogenous GH and reverse muscle wasting by decreasing irreversible protein degradation.

Subjective Global Assessment

Subjective Global Assessment was completed at baseline, on day 28 and again on day 42, and included a review of the medical history (weight and weight change, dietary intake, gastrointestinal symptoms, disease state, and subject's functional status), a physical examination for negative changes in body composition (loss of subcutaneous fat or muscle wasting), and signs of edema or ascites.[29] After evaluation, the subject was classified as well nourished, mild to moderately malnourished, or severely malnourished by the physicians.

GH deconvolution analysis

Growth hormone concentration time series were analyzed using a recently developed automated deconvolution method, which was mathematically verified by direct statistical proof and empirically validated using hypothalamo-pituitary sampling and simulated pulsatile time series.[30,31] Deconvolution parameters comprised basal secretion ($\beta 0$), two half-lives ($\alpha 1$, $\alpha 2$), secretory-burst mass ($\eta 0$, $\eta 1$), random effects on burst mass (σA), procedural/measurement error ($\sigma \varepsilon$), and a three-parameter flexible γ-secretory-burst waveform ($\beta 1$, $\beta 2$, $\beta 3$). Parameters (and units) included frequency (number of bursts per total sampling period, lambda of Weibull's distribution), regularity of inter-pulse intervals (unitless gamma of Weibull), fast and slow half-lives (min), basal and pulsatile secretion rates (concentration units per session), mass secreted per burst (concentration units), and waveform shape (mode, or time delay to maximal secretion after objectively estimated burst onset, min).

Leucine kinetics

Fasting leucine kinetics were measured on days 0 and 29 using a primed-constant infusion technique during substrate and isotropic steady state.[32] The priming dose consisted of 4 μmol/kg of L-[1-^{13}C] leucine and 0.11 mg/kg of $NaH^{13}CO_3$ administered at a sustained infusion rate for 4 h (Cambridge Isotope Laboratories, Andover, MA, USA). Venous blood and breath samples were collected before isotope administration and at 180, 195, 210, 225, and 240 min after the start of infusion. ^{13}C-leucine and ^{13}C-ketoisocaproate enrichment in the plasma were quantified by gas chromatography-mass spectrometry. Plasma leucine was converted to the heptafluorobutyrl,*n*-propyl-ester and analyzed by negative chemical ionization, whereas ketoisocaproate was converted to the trimethylsilyl-quinoxalinol derivative and analyzed by electron ionization. Breath CO_2 was quantified by isotope ratio mass spectrometry by monitoring masses 44 and 45. All analyses and calculations were performed by Metabolic Solutions (Nashua, NH, USA).

Blood testing

Blood sampling for GH secretion profile commenced on day 0 (baseline) by collecting samples at 20-min intervals for 20 h, followed by 10-min intervals for 4 h. The first dose of investigational product was administered after GH sampling. On day 28, samples were collected pre-dose, at 10-min intervals for the first 4 h and at 20-min intervals thereafter for a further 20 h.

Total IGF-1, IGFBP-1, IGFBP-3, and insulin were measured weekly by a central laboratory using commercial kits and human anti-drug antibodies by LAB Research (Laval, QC, Canada). All other blood and urine chemistry analyses were performed locally.

Statistical analysis and sample size

All hypothesis tests were conducted with a two-sided significance level of $\alpha = 0.05$. All CIs were 95%. Numerical data with one data point after baseline were analyzed using ANCOVA (analysis of covariance) with the baseline value as covariate and treatment and site as factors. If the assumption of (log) normality was questionable, nonparametric ANCOVA with the baseline value as covariate was used instead. If there were several data points after baseline, ANOVA (analysis of variance) for repeated measures was the method of choice. The ANOVA model included baseline as covariate, site, treatment, and visit and treatment by visit interaction term as factors. The final analysis method was selected as part of blind data review.

The study was designed to show the superiority of AKL-0707 to placebo on protein turnover as assessed by leucine oxidation rate. In a previous study in GH-deficient adults treated with GH

replacement, the s.d. of the leucine oxidation rate varied between 9 and 17 μmol/kg.[33] At this level of variability, 13 subjects per group are required to detect a difference in means of 15 μmol/kg (that is, ~1 s.d.) at a power of 80% and an error probability of 5%. Anticipating an overall dropout rate of ~20%, a total of 32 subjects were planned to be randomized.

DISCLOSURE

PG is a discoverer of AKL-0707. TJ is an executive officer of Akela. HS and SWKK were at the time of study conduct and manuscript preparation employees of Akela. HS owns stock in Akela. FS has been a consultant for development of AKL-0707 for Akela. DAW received funds from Akela to perform leucine kinetics. All the other authors declared no competing interests.

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