# The KHENERGY Study: Safety and Efficacy of KH176 in Mitochondrial m.3243A>G Spectrum Disorders

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**KH176 is a potent intracellular reduction–oxidation-modulating compound developed to treat mitochondrial disease. We studied tolerability, safety, pharmacokinetics, pharmacodynamics, and efficacy of twice daily oral 100 mg KH176 for 28 days in a double-blind, randomized, placebo-controlled, two-way crossover phase IIA study in 18 adult m.3243A>G patients without cardiovascular involvement. Efficacy parameters included clinical and functional outcome measures and biomarkers. The trial was registered within ClinicalTrials.gov (NCT02909400), the European Clinical Trials Database (2016-001696-79), and ISRCTN (43372293) (The KHENERGY study). Twice daily oral 100 mg KH176 was well tolerated and appeared safe. No serious treatment-emergent adverse events were reported. No significant improvements in gait parameters or other outcome measures were obtained, except for a positive effect on alertness and mood, although a coincidence due to multiplicity cannot be ignored. The results of the study provide first data on safety and efficacy of KH176 in patients with mitochondrial disease and will be instrumental in designing future clinical trials.**

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Patients with an m.3243A>G mutation in their mitochondrial genome show chronically progressive, often early fatal, multisystem disorders for which no clinical beneficial treatment is available. KH176 is a new innovative reduction–oxidation modulator that, based on extensive investigations in preclinical studies, has the potential to positively change the clinical burden of MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes) spectrum disorders and other mitochondrial diseases.

### WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The aim of this exploratory phase IIA clinical trial was to evaluate the compound's potential. We studied tolerability, safety, pharmacokinetics, pharmacodynamics, and efficacy.

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ KH176 was well tolerated and appeared safe at the 100-mg twice a day dose regimen.

### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The results of the study obtained will be used to frame upcoming clinical trials with KH176. The final choice on which primary end point to select for a future trial to obtain (conditional) market authorization is made on the basis of the results of all phase II study parameters.

Mitochondrial disease (estimated prevalence, 1 in 4,300 adults[1]) is caused by pathogenic mutations in genes finally encoding for mitochondrial proteins of the different enzyme complexes of the oxidative phosphorylation system.[2,3] Such mutations have a major impact on cellular functioning, with high energy dependent tissues and organs, like the brain and skeletal muscle, being

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especially prone to malfunctioning.[4] Among these mutations, the mitochondrial *MT-TL1* transfer RNA$^{Leu(UUR)}$ 3243A>G nucleotide change is the most prevalent.[1] The estimated number of affected patients in the United States, Europe, and Japan approximates 50,000. Carriers of the m.3243A>G mutation show a wide spectrum of multisystem signs and symptoms, including stroke-like episodes, migraine, depression, sensorineural hearing loss, diabetes mellitus, cardiomyopathy, myopathy, and gait disturbance.[5–7] The phenotype varies among patients: although some carriers of the mutation remain asymptomatic (dormant carriers), others die in childhood from neurological or cardiac complications of the disease.[5,8,9] The patient's disease expression and progression is difficult to predict and can only be partially explained by the presence of heteroplasmy, the balance between wild-type and mutated mitochondrial DNA, within cells and tissues.[5,8,10]

Because cellular reduction–oxidation (redox) imbalance has shown to play a key role in the pathogenesis of many of the clinical manifestations of mitochondrial diseases,[11,12] many studies and clinical trials have attempted to manipulate reactive oxygen species levels in cells, animals, or patients with mitochondrial disease.[13,14] Idebenone, a short-chain benzoquinone, was shown to have clinically relevant benefit for patients with Leber hereditary optic neuropathy (estimated prevalence, 1:65,000[13,15]) and is currently approved for the treatment of Leber hereditary optic neuropathy by the European Medicines Agency under "exceptional circumstances." The care for patients with all other mitochondrial diseases is mostly supportive.

Khondrion, a Netherlands-based pharmaceutical company, developed the potent intracellular redox-modulating agent KH 176 (or ((S)-6-hydroxy-2,5,7,8-tetramethyl-N-((R)-piperidin-3-yl) chroman-2-carboxamide hydrochloride; patent WO2014011047 A1)[16]). KH176 is considered to be a prodrug being active similar to its metabolite KH176 m. Preclinical studies in patient-derived fibroblasts with a wide variability of genetic backgrounds showed that KH176 had a protective effect against redox perturbation in cells. In complex I deficient knockout mice (*Ndufs4*$^{-/-}$), KH176 retained brain microstructural coherence in the external capsule in *Ndufs4*$^{(-/-)}$ mice and normalized the increased lipid peroxidation in this area and the cerebral cortex.[17] Furthermore, KH176 treatment was able to significantly improve rotarod and gait performance and reduced the degeneration of retinal ganglion cells in *Ndufs4*$^{(-/-)}$ mice.[17] A phase I clinical trial in healthy volunteers deemed that KH176 is well tolerated up to single doses of 800 mg and multiple doses of 400 mg b.i.d. and had a pharmacokinetic profile supportive of a twice daily (b.i.d.) dosage. At blood concentrations >1,000 ng/ml, KH176 caused changes in cardiac electrophysiology, including prolonged QTc interval and changes in T-wave morphology.[18]

In the current double-blind, randomized, placebo-controlled, single-center, two-way crossover phase IIA study, we aim to evaluate the tolerability, safety, and pharmacokinetics of KH176 in patients with m.3242A>G related mitochondrial disease and explore the effects of treatment with KH176 for 4 weeks on clinical signs and symptoms and biomarkers of mitochondrial disease.

## RESULTS

Between September and December 2016, 21 patients were enrolled. Nineteen entered the first treatment period and were randomly assigned treatment ($n$ = 10 KH176, and $n$ = 9 placebo first). The last patient last visit was on May 23rd, 2017. **Figure 1** shows the trial profile: 195 patients were assessed for eligibility, 50 patients were eligible for entering the clinical trial, and 27 patients were screened. Finally, 20 patients were randomized. One patient was immediately excluded because of a preexistent Mobitz I atrioventricular block, which was only discovered on the Holter registration at the first day of treatment. Treatment was discontinued for one patient in the second week of the second period because of nonsustained ventricular tachycardia. This patient received placebo treatment in that period and still completed the clinical outcome assessments. Nineteen patients were assessed for the end points at baseline and outcome for both treatment periods, of which 18 were included in the statistical analysis.

**Table 1** summarizes the baseline characteristics of these 18 patients. A total of 6 males and 12 females were included in the per-protocol analysis. Average age was 44 (range, 21–61) years. The most important symptoms included myopathy, exercise intolerance, and fatigue. Only two patients reported gait instability. Six patients had diabetes, of which five received insulin treatment.

Twice daily oral 100-mg KH176 was well tolerated. No serious adverse events were reported. The total number of treatment-emergent adverse events was 35 in the KH176 arm and 51 in the placebo arm (**Table 2**). On the basis of the findings from the phase I study, cardiac repolarization, which is directly related to KH176 plasma concentrations, at higher plasma concentrations was identified as an important potential risk.

The peak plasma concentration of KH176 ranged from 125–351 ng/ml (geomean, 227 ng/mI; coefficient of variation (CV) %, 26.4; Table S1), which was well beneath the predefined safety threshold of 1,000 ng/ml. A 7-day Holter registration revealed a statistically significant lower average heart rate compared with placebo (difference, −2.1 (95% confidence interval (CI), −3.5 to −0.57); $P$ = 0.0098) and a lower maximum heart rate (difference, −5.9 (95% CI, −10.0 to −1.9); $P$ = 0.0063), without a significant difference in minimum heart rate, resulting in a significant lower basic rhythm of total beats per minute during the active treatment period ($P$ = 0.0036).

There were no differences in the corrected QT time, with both Fridericia correction and Bazett, as indicated in **Figure 2**. The maximum difference in the change from baseline compared with placebo of the Bazett-corrected QTcB interval was 10.68 milliseconds (95% CI, 2.58–18.78 milliseconds), which occurred 4 hours after dose on day 3 and coincided with the time to maximum concentration ($T_{max}$) of the pharmacokinetics of KH176 in plasma ($T_{max}$ = 2 hours). However, the same timepoint at day 21 did not show a difference compared with placebo (active median change from baseline, −7.0 milliseconds (minimum–maximum, −48 to 17 milliseconds); placebo median change from baseline, −7.5 milliseconds (minimum–maximum, −41 to 17 milliseconds)), indicating the possibility of a chance finding at day 3. Pharmacokinetics followed the same pattern as was previously observed in healthy male volunteers (**Figure 3**, Table S2 ), with a $T_{max}$

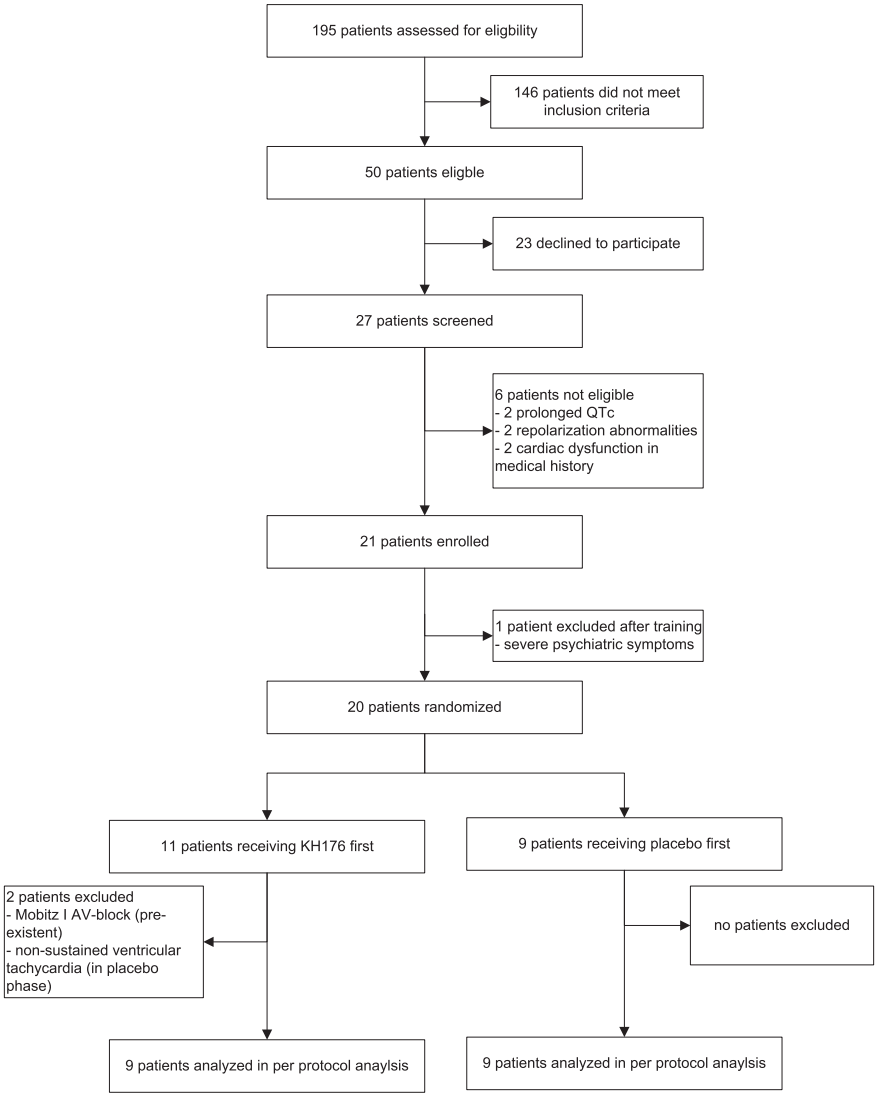


**Figure 1** Trial profile. AV, atrioventricular.

for KH176 of 1.73 hours (geometric mean; CV%, 52.6), a maximum plasma concentration of KH176 of 404 ng/ml (geometric mean; CV%, 23.8), and an area under the curve for one dosing interval of 3,340 h.ng/ml (geometric mean; CV%, 27.9).

**Table 3** summarizes the results of the clinical and functional end points. No significant difference of KH176 compared with placebo was observed on step length and variability in step time and step width, as measured with the Gaitrite system. No significant differences were observed between KH176 and placebo for all other clinical and functional outcomes, including the 6-minute walking test, 6-minute mastication test, maximal grip strength, the 3-second sit to stand test, accelerometry, spirometry, the Newcastle Mitochondrial Disease Adult Scale, and the Checklist Individual Strength (**Table 3**; Table S2). The Test of Attentional Performance showed a positive effect/trend for alertness in test conditions without and with alarm (effect size, −2.2 (95% CI, −4.1 to −0.38; $P = 0.025$ and $P = 0.06$, respectively).

The Beck Depression Inventory showed statistical differences between the KH176 and placebo treatment period on the total score (effect size, −2.9 (95% CI, −5.7 to −0.13); $P = 0.04$) and the affective subscale (effect size, −1.1 (95% CI, −1.7 to −0.4); $P = 0.004$). This observation was supported by a positive trend in the Hospital Anxiety and Depression Scale (effect size, −1.9 (95% CI, −3.6 to −0.2); $P = 0.03$). At the end-of-study visits, 12 of 18 patients predicted correctly during which treatment period they believed to have received KH176.

At group level, none of the biomarkers (glutathione (GSH), glutathione disulfide (GSSG), fibroblast growth factor 21 (FGF21), growth and differentiation factor 15 (GDF15), or peroxiredoxin 1 (PRDX1)) showed significant differences between the active and the placebo arm.

## DISCUSSION

Herein, we report the results of a randomized, double-blind, crossover phase IIA exploratory study of KH176 in patients harboring the m.3243A>G mutation with different percentages of heteroplasmy. This is the first clinical trial with KH176 in patients with multisystem mitochondrial disease. In 18 evaluable patients, this

**Table 1 Baseline characteristics of participants (per-protocol set)**

| Characteristics | KH176 first (*n* = 9) | Placebo first (*n* = 9) | Total (*n* = 18) |
|---|---|---|---|
| Age, years | 44 (21–54) | 43 (22–61) | 44 (21–61) |
| Height, cm | 172 (163–186) | 165 (150–179) | 169 (150–186) |
| Weight, kg | 71 (53–94) | 66 (51–79) | 68 (51–94) |
| BMI, kg/m$^2$ | 24 (20–30) | 24 (22–27) | 24 (20–30) |
| m.3243A>G heteroplasmy (urinary epithelial cells), % | 51 (22–77) | 73 (34–86) | 61 (22–86) |
| m.3243A>G heteroplasmy (leukocytes), % | 13 (1–29) | 24 (6–48) | 18 (1–48) |
| NMDAS score at baseline of first treatment period | 16 (4–24) | 19 (9–32) | 17 (4–32) |
| Section I: current function | 9 (0–15) | 10 (3–14) | 9 (0–15) |
| Section II: system-specific involvement | 4 (1–9) | 6 (0–13) | 5 (0–13) |
| Section III: current clinical assessment | 2 (0–6) | 3 (1–6) | 2 (0–6) |
| No. of patients with following conditions | | | |
| Myopathy | 9 | 7 | 15 |
| Exercise intolerance | 9 | 8 | 17 |
| Fatigue | 9 | 8 | 17 |
| Diabetes mellitus | 2 | 4 | 6 |
| Hearing loss | 3 | 3 | 6 |
| Gait instability | 1 | 1 | 2 |
| Gastrointestinal complaints | 5 | 4 | 9 |
| Migraine | 2 | 1 | 3 |

Data are given as mean (range) or number.
BMI, body mass index; NMDAS, Newcastle Mitochondrial Disease Adult Scale.

phase IIA trial revealed that 100-mg KH176 b.i.d. was well tolerated, showed a favorable safety profile, and had a pharmacokinetic profile comparable with that of healthy male volunteers in the previous phase I trial.[17]

The total number of adverse events and those at head level group term were lower in the active group compared with the placebo group.

The study is exploratory by nature, and given the uncertainties in putative treatment responses and outcomes, no formal primary end point was defined for which the study could be powered and no statistical hypothesis was set for this proof-of-concept study. Because the study was exploratory by nature, we chose not to correct for multiplicity, taking the risk that the large number of end points measured could have resulted in positive conclusions on the basis of chance. The end points chosen included a set of clinical parameters known to be affected in patients with mitochondrial disease. In recent studies, more than half of the patients harboring the m.3243A>G mutation were found to have trouble maintaining balance when walking. Others demonstrated that these patients had an abnormal gait pattern, as quantified by gait analysis.[19–22] For this reason, and the observation that gait improved during KH176 treatment in a mitochondrial disease mouse model, gait was chosen as a primary end point.[17,19,20] This 28-day phase IIA study showed no improvement in gait parameters. This can be explained by the fact that 16 of 18 patients participating in this study already had a normal gait at baseline. None of the other motor functional outcome measures showed any statistically significant improvement in this relatively short-duration study.

On the basis of the phase I study results, stringent safety criteria were applied, including the exclusion of patients with cardiac abnormalities and the use of medication known to interfere with cytochrome P450 (CYP) 3A4 and P-glycoprotein (PgP). This has led to the inclusion of patients with a relatively mild clinical phenotype. Furthermore, patients were only treated for 28 days, as dictated by the available preclinical regulatory toxicology data. Nevertheless and of interest, we detected some differences between the active and the placebo period.

Improvements were observed in the overall mental health status, measured with the Beck Depression Index, especially its affective subscale; the Hospital Anxiety and Depression Scale, especially its depression subscale; and the Test of Attentional Performance. Although a putative issue with multiplicity is recognized, the fact that both mood-related indexes appear to respond to treatment is considered of interest for a planned phase IIB clinical trial.

Because depressive symptoms are prevalent in patients with mitochondrial disease,[6,7,21–23] a reduction of the mitochondrial disease-related depressive symptoms, as observed, may be of clinical importance. This improvement in mood was accompanied by a higher level of alertness, which was evident for some patients because they were more alert and comfortable when driving at night or able to watch television without falling asleep. At the

**Table 2 Adverse events by body system (safety population)**

| | | KH176 (*N* = 19) | | Placebo (*N* = 20) | | Overall (*N* = 20) | |
|---|---|---|---|---|---|---|---|
| System organ class | Preferred term | *N* (%) | nTEAEs | *N* (%) | nTEAEs | *N* (%) | nTEAEs |
| Any TEAE | | 16 (84.2) | 35 | 17 (85.0) | 51 | 19 (95.0) | 86 |
| Cardiac disorders | | 3 (15.8) | 4 | 5 (25.0) | 9 | 7 (35.0) | 13 |
| | Palpitations | 1 (5.3) | 1 | 2 (10.0) | 3 | 3 (15.0) | 4 |
| | Rhythm idioventricular | 0 (0.0) | 0 | 1 (5.0) | 1 | 1 (5.0) | 1 |
| | Sinus arrhythmia | 0 (0.0) | 0 | 1 (5.0) | 1 | 1 (5.0) | 1 |
| | Sinus tachycardia | 1 (5.3) | 1 | 0 (0.0) | 0 | 1 (5.0) | 1 |
| | Supraventricular extrasystoles | 1 (5.3) | 1 | 0 (0.0) | 0 | 1 (5.0) | 1 |
| | Supraventricular tachycardia | 1 (5.3) | 1 | 0 (0.0) | 0 | 1 (5.0) | 1 |
| | Tachycardia | 0 (0.0) | 0 | 1 (5.0) | 1 | 1 (5.0) | 1 |
| | Ventricular extrasystoles | 0 (0.0) | 0 | 2 (10.0) | 2 | 2 (10.0) | 2 |
| | Ventricular tachycardia | 0 (0.0) | 0 | 1 (5.0) | 1 | 1 (5.0) | 1 |
| Eye disorders | | 0 (0.0) | 0 | 1 (5.0) | 1 | 1 (5.0) | 1 |
| | Eye movement disorder | 0 (0.0) | 0 | 1 (5.0) | 1 | 1 (5.0) | 1 |
| Gastrointestinal disorders | | 3 (15.8) | 4 | 7 (35.0) | 10 | 9 (45.0) | 14 |
| | Abdominal discomfort | 0 (0.0) | 0 | 1 (5.0) | 1 | 1 (5.0) | 1 |
| | Abdominal pain | 0 (0.0) | 0 | 2 (10.0) | 2 | 2 (10.0) | 2 |
| | Abdominal pain upper | 1 (5.3) | 1 | 0 (0.0) | 0 | 1 (5.0) | 1 |
| | Constipation | 1 (5.3) | 1 | 1 (5.0) | 1 | 2 (10.0) | 2 |
| | Diarrhea | 0 (0.0) | 0 | 2 (10.0) | 2 | 2 (10.0) | 2 |
| | Dyspepsia | 0 (0.0) | 0 | 1 (5.0) | 1 | 1 (5.0) | 1 |
| | Nausea | 1 (5.3) | 1 | 0 (0.0) | 0 | 1 (5.0) | 1 |
| | Tooth disorder | 0 (0.0) | 0 | 1 (5.0) | 1 | 1 (5.0) | 1 |
| | Vomiting | 1 (5.3) | 1 | 2 (10.0) | 2 | 3 (15.0) | 3 |
| General disorders and administration site conditions | | 1 (5.3) | 2 | 6 (30.0) | 9 | 7 (35.0) | 11 |
| | Condition aggravated | 0 (0.0) | 0 | 1 (5.0) | 1 | 1 (5.0) | 1 |
| | Fatigue | 0 (0.0) | 0 | 3 (15.0) | 4 | 3 (15.0) | 4 |
| | Feeling abnormal | 1 (5.3) | 1 | 1 (5.0) | 1 | 2 (10.0) | 2 |
| | Gait disturbance | 1 (5.3) | 1 | 0 (0.0) | 0 | 1 (5.0) | 1 |
| | Influenza-like illness | 0 (0.0) | 0 | 2 (10.0) | 2 | 2 (10.0) | 2 |

nTEAE, Total number of treatment emergent adverse events.

end-of-study visit, when both the investigators and patients were still blind, 12 of 18 patients predicted correctly during which treatment period they believed to have received KH176. The mechanism for the improvement in depressive symptoms and alertness—if confirmed in studies with a larger sample size—is currently unknown. Hypothetically, the improvement may be secondary to other treatment effects of KH176 reported by the patients in this study, such as the improvement of migraine headache, the reduction of muscle complaints, and more energy. Clinical trials of longer duration are needed to confirm a true treatment effect on mood-related outcomes and to understand whether the improved mood is a primary effect of KH176 or secondary to improvement of other mitochondrial disease-related symptoms.

Pharmacokinetic profiles of both KH176 and its active metabolite KH176 m in patients with the m.3243A>G mutation were in accordance with those found in the phase I study, albeit with a slightly higher (≈20%) KH176 exposure and a slightly lower (20%) exposure to KH176 m.[18] Because KH176 is known to prolong the QTcF interval at doses of 800 and 2,000 mg,[18] intensive cardiac monitoring at the intensive care unit during the first 3 days of each treatment arm, continuous Holter monitoring, and weekly visits to the clinics were adopted. None of the patients encountered study drug-related cardiac safety issues in the KH176 treatment period. There were no changes in the corrected QT time, with both Fridericia and Bazett correction. All absolute QT times remained within the normal range for women and men, and

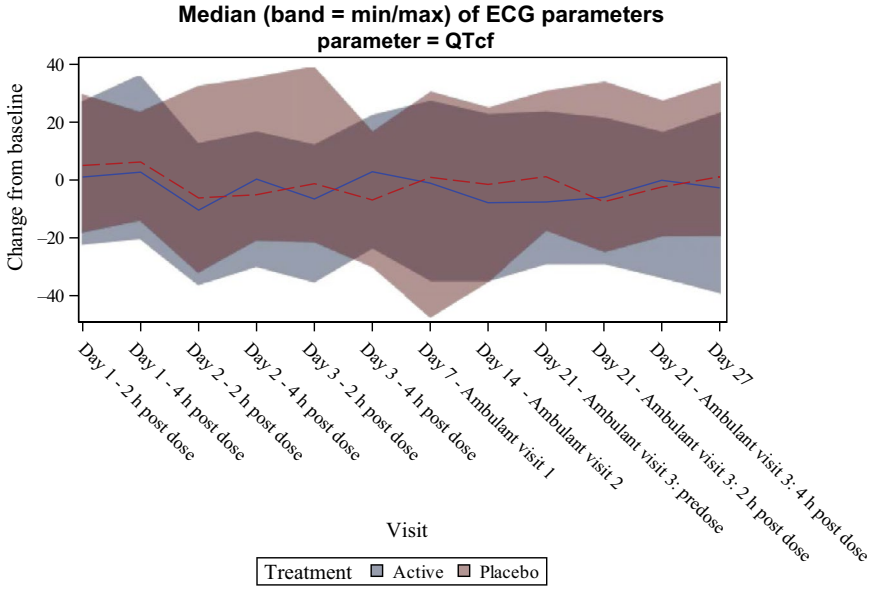


**Figure 2** ECG, electrocardiogram; max, maximum; min, minimum; QTcf, Change in Fridericia-corrected QT during the KH176 treatment period.

no KH176-associated rhythm disturbances were observed in this study.

The maximum change from baseline of the Bazett-corrected QTcB interval occurred 4 hours postdose on day 3 and coincided with the $T_{max}$ of the pharmacokinetics of KH176 in plasma ($T_{max}$ = 2 hours). However, given the lack of relevant differences at other timepoints, including a 4-hour postdose electrocardiogram (ECG) at day 21, on which the same plasma concentrations were present, we consider this as a coincidental finding.

No differences in FGF21, GDF15, and PRDX1 concentrations were observed. FGF21 and GDF15 are markers for muscle-manifesting mitochondrial disease,[24] but there were no indicators for general mitochondrial disease severity.[25,26]

Although this study was exploratory in nature, the scientific rigor of a randomized, double-blind, crossover trial allowed us to compare changes within the same patients receiving active and placebo treatment. Moreover, patients were able to report on their experiences on both treatments. For safety reasons, we only included patients without cardiac abnormalities. Because many patients with the m.3243A>G mutation experience cardiac abnormalities[27–29] and cardiac signs correlate with general disease severity,[28] only mildly affected patients were eligible for the current study. Because most patients had a normal score on the functional baseline measurements included in this study, a ceiling effect likely prohibited the possibility of finding a beneficial treatment response. Indeed, the fact that response on many outcomes seems related to disease severity is suggestive, and the effects of KH176 on motor function need further exploration.

In conclusion, the current study provides first information on the tolerability and safety of KH176 in patients with mitochondrial disease attributable to the m.3243A>G mutation. Future pivotal (phase IIB and/or phase III) trials should confirm the potential effects of KH176 in patients with mitochondrial disease.

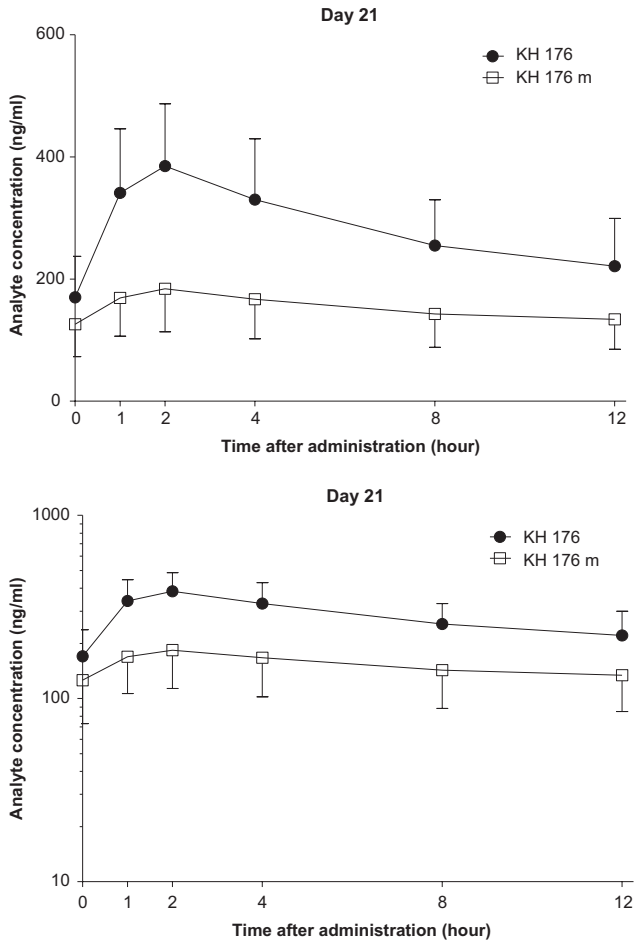


**Figure 3** Mean (SD) plasma concentration–time profiles of KH176 and its metabolite KH176 m on day 21 after administration of 100 mg KH176 twice daily for 28 days on linear and semilogarithmic scales.

**Table 3 Clinical and functional outcomes**

| | Baseline KH176 period | | Comparison KH176-Placebo | |
|---|---|---|---|---|
| Variable | Mean ± SD | Median (range) | Estimate treatment effect (95% CI) | *P* value |
| Gait pre–6-minute walking test | | | | |
| Velocity, cm/second | 116.9 ± 11.3 | 114.7 (88.0–139.9) | 0.7 (−4.5 to 6.0) | 0.77 |
| Cadence, steps/minute | 112.8 ± 6.9 | 113.5 (95.7–123.1) | −1.0 (−3.7 to 1.6) | 0.43 |
| Step length right, cm | 62.1 ± 5.6 | 61.7 (47.7–72.3) | 0.8 (−0.9 to 2.6) | 0.32 |
| Step length left, cm | 62.6 ± 5.8 | 62.5 (46.1–74.5) | 1.1 (−0.8 to 2.9) | 0.25 |
| Step time right, seconds | 0.54 ± 0.04 | 0.54 (0.49–0.62) | 0.00 (−0.01 to 0.02) | 0.63 |
| Step time left, seconds | 0.53 ± 0.04 | 0.53 (0.49–0.64) | 0.01 (−0.01 to 0.02) | 0.37 |
| Stride length right, cm | 124.5 ± 10.9 | 124.9 (94.3–145.3) | 2.0 (−1.5 to 5.5) | 0.24 |
| Stride length left, cm | 125.1 ± 11.3 | 124.7 (93.8–147.2) | 1.9 (−1.7 to 5.6) | 0.28 |
| Step length variability right, cm | 2.16 ± 0.70 | 2.13 (1.09–3.84) | 0.08 (−0.26 to 0.42) | 0.63 |
| Step length variability left, cm | 2.17 ± 0.94 | 2.04 (0.53–4.30) | 0.11 (−0.30 to 0.53) | 0.57 |
| Step time variability right, seconds | 0.017 ± 0.006 | 0.015 (0.009–0.034) | 0.001 (−0.002 to 0.004) | 0.40 |
| Step time variability left, seconds | 0.016 ± 0.005 | 0.015 (0.009–0.032) | 0.000 (−0.004 to 0.003) | 0.76 |
| Stride length variability right, cm | 3.37 ± 1.35 | 3.08 (1.46–6.34) | 0.01 (−0.61 to 0.62) | 0.98 |
| Stride length variability left, cm | 3.67 ± 1.56 | 3.36 (0.95–6.47) | 0.07 (−0.45 to 0.58) | 0.78 |
| Gait post–6-minute walking test | | | | |
| Velocity, cm/second | 118.6 ± 11.9 | 117.7 (90.9–144.9) | 1.4 (−3.1 to 5.9) | 0.53 |
| Cadence, steps/minute | 113.5 ± 6.2 | 114.8 (98.3–122.8) | 2.3 (−1.2 to 5.7) | 0.19 |
| Step length right, cm | 62.8 ± 6.1 | 62.9 (48.1–73.1) | −0.8 (−2.6 to 1.0) | 0.37 |
| Step length left, cm | 62.7 ± 6.2 | 62.4 (46.2–74.8) | 0.5 (−1.0 to 2.1) | 0.46 |
| Step time right, seconds | 0.53 ± 0.03 | 0.53 (0.49–0.60) | −0.01 (0.03 to 0.01) | 0.21 |
| Step time left, seconds | 0.53 ± 0.03 | 0.52 (0.49–0.62) | 0.00 (−0.02 to 0.01) | 0.73 |
| Stride length right, cm | 125.6 ± 12.1 | 123.7 (94.4–147.6) | 0.1 (−3.2 to 3.4) | 0.94 |
| Stride length left, cm | 126.0 ± 12.3 | 125.3 (94.4–148.9) | −0.6 (−3.7 to 2.4) | 0.66 |
| Step length variability right, cm | 2.05 ± 0.50 | 2.14 (1.10–3.04) | 0.06 (−0.44 to 0.55) | 0.81 |
| Step length variability left, cm | 2.09 ± 0.58 | 1.87 (1.47–3.26) | 0.19 (−0.65 to 1.02) | 0.64 |
| Step time variability right, seconds | 0.015 ± 0.004 | 0.016 (0.010–0.021) | −0.001 (−0.004 to 0.001) | 0.38 |
| Step time variability left, seconds | 0.015 ± 0.006 | 0.013 (0.009–0.030) | −0.002 (−0.005 to 0.000) | 0.090 |
| Stride length variability right, cm | 3.09 ± 1.11 | 2.84 (1.11–5.41) | 0.32 (−0.66 to 1.30) | 0.50 |
| Stride length variability left, cm | 3.14 ± 0.81 | 3.06 (1.33–4.81) | 0.33 (−0.61 to 1.27) | 0.47 |
| Newcastle Mitochondrial Disease Adult Scale score | 17.4 ± 8.0 | 17 (4–36) | 0.4 (−1.2 to 2.0) | 0.63 |
| Section I | 9.8 ± 5.0 | 10 (0–20) | −0.7 (−2.1 to 0.7) | 0.32 |
| Section II | 4.9 ± 4.0 | 5 (0–11) | 0.5 (−0.4 to 1.3) | 0.24 |
| Section III | 2.7 ± 1.9 | 3 (0–6) | 0.6 (0.0 to 1.2) | 0.06 |
| Sit–stand test, *n* of standings | 13.2 ± 4.2 | 12.5 (7–23) | −0.4 (−1.3 to 0.5) | 0.40 |
| Grip strength right, kg | 23.9 ± 10.9 | 23.5 (0–44) | 0.0 (−2.4 to 2.4) | 0.99 |
| Grip strength left, kg | 23.5 ± 10.0 | 22.0 (1–40) | −1.2 (−3.0 to 0.6) | 0.17 |
| 6-Minute mastication test, *n* of chewings | 483.8 ± 125.3 | 458.0 (233–717) | −9.1 (−44.0 to 25.8) | 0.59 |
| 6-Minute walk test, m | 477.5 ± 60.2 | 464.0 (390–608) | −14.0 (−29.2 to 1.2) | 0.069 |
| Spirometry | | | | |
| FVC, % of predicted | 103.2 ± 11.8 | 102.0 (86–135) | 0.2 (−2.8 to 3.3) | 0.88 |
| FEV1, % of predicted | 96.2 ± 10.0 | 95.0 (83–126) | 2.2 (−0.8 to 5.2) | 0.13 |
| PEF, L/second | 8.1 ± 2.1 | 8.0 (5.2–13.7) | −0.4 (−0.8 to 0.1) | 0.090 |
| MIP, kPa | 7.0 ± 2.0 | 7.2 (3.2–11.7) | −0.3 (−1.0 to 0.5) | 0.47 |

(*Continues*)

**Table 3 (continued)**

| Variable | Baseline KH176 period | | Comparison KH176-Placebo | |
|---|---|---|---|---|
| | Mean ± SD | Median (range) | Estimate treatment effect (95% CI) | *P* value |
| MEP, kPa | 11.3 ± 3.2 | 11.1 (7.0–20.5) | −0.9 (−2.0 to 0.3) | 0.13 |
| QoL (SF-36) score | 601.3 ± 153.4 | 604.0 (340–819) | 0.1 (−50.9 to 51.1) | 0.99 |
| Physical functioning | 66.9 ± 21.6 | 70.0 (15–100) | 0.3 (−4.2 to 4.9) | 0.88 |
| Physical role | 54.2 ± 43.9 | 62.5 (0–100) | −14.3 (−32.0 to 3.4) | 0.11 |
| Emotional role | 88.9 ± 28.0 | 100.0 (0–100) | −0.1 (−17.0 to 16.7) | 0.99 |
| Emotional well-being | 78.9 ± 14.2 | 80.0 (48–100) | 3.8 (−1.5 to 9.1) | 0.14 |
| Energy/fatigue | 46.1 ± 18.0 | 52.5 (10–75) | 2.1 (−4.8 to 9.1) | 0.53 |
| Social functioning | 70.1 ± 20.2 | 68.8 (38–100) | −7.5 (−17.7 to 2.6) | 0.13 |
| Pain | 61.3 ± 26.7 | 68.4 (22–100) | 5.8 (−1.8 to 13.5) | 0.12 |
| General health | 39.2 ± 20.7 | 35.0 (15–95) | 5.6 (−1.8 to 13.0) | 0.13 |
| Hospital Anxiety and Depression Scale score | 9.2 ± 6.7 | 7.0 (0–22) | −1.9 (−3.5 to 0.2) | **0.03** |
| Depression scale | 5.3 ± 4.3 | 4.5 (0–13) | −1.1 (−2.3 to 0.2) | 0.091 |
| Fear scale | 4.3 ± 3.3 | 4.0 (0–11) | −0.7 (−2.0 to 0.6) | 0.26 |
| Beck Depression Inventory | 9.28 ± 6.00 | 9.0 (0–22) | −2.90 (−5.67 to 0.13) | **0.042** |
| Affective scale | 1.4 ± 1.0 | 2.0 (0–3) | −1.1 (−1.7 to 0.4) | **0.0038** |
| Cognitive scale | 2.1 ± 2.3 | 1.0 (0–7) | −0.6 (−1.4 to 0.2) | 0.15 |
| Somatic scale | 5.4 ± 3.8 | 6.0 (0–12) | −1.0 (−2.8 to 0.8) | 0.24 |
| Checklist Individual Strength score | 85.2 ± 19.6 | 82.5 (55–118) | −4.7 (−14.6 to 5.2) | 0.33 |
| Subjective fatigue | 39.0 ± 9.7 | 37.0 (22–54) | −3.0 (−8.1 to 2.0) | 0.22 |
| Concentration | 19.2 ± 7.0 | 19.0 (8–31) | −0.5 (−2.9 to 2.0) | 0.68 |
| Motivation | 15.9 ± 4.8 | 17.0 (8–25) | −0.3 (−2.9 to 2.4) | 0.83 |
| Physical activity | 11.1 ± 3.6 | 10.5 (7–17) | −0.9 (−3.4 to 1.6) | 0.45 |
| Test of Attentional Performance | | | | |
| Alertness with alarm | 39.2 ± 6.4 | 38.0 (32–60) | −3.4 (−6.8 to 0.1) | 0.056 |
| Alertness without alarm | 40.0 ± 6.9 | 38.0 32–62) | −2.2 (−4.1 to 0.3) | **0.025** |
| Mental flexibility | 58.4 ± 11.2 | 59.0 (39–79) | 2.0 (−3.3 to 7.3) | 0.44 |
| Biomarkers | | | | |
| GSH, µmol/l | 1,234 ± 160 | 1,200 (970–1,660) | 16 (−145 to 178) | 0.83 |
| GSSG, µmol/l | 0.69 ± 0.24 | 0.60 (0.32–1.13) | 0.02 (−0.12 to 0.16) | 0.80 |
| Ratio GSH/GSSG | 1,967 ± 593 | 1,980 (956–3,000) | −119 (−387 to 149) | 0.36 |
| FGF21, pg/ml | 313 ± 243 | 199 (129–926) | −8 (−67 to 50) | 0.77 |
| GDF15, pg/ml | 1,359 ± 787 | 1,260 (540–3,894) | −11 (−180 to 157) | 0.89 |
| PRDX1, ng/ml | 22 ± 19 | 14 (8–80) | −7 (−19 to 5) | 0.22 |
| Accelerometer | | | | |
| Total activity, minutes per week | | | 39.5 (−71.1 to 150.2) | 0.46 |
| Sedentary activity, % | | | −0.06 (−2.22 to 2.11) | 0.96 |
| Light activity, % | | | 0.39 (−0.27 to 1.05) | 0.23 |
| Moderate activity, % | | | −0.06 (−1.71 to 1.60) | 0.94 |
| Vigorous activity, % | | | 0.11 (−0.05 to 0.27) | 0.16 |
| Sleep quantity, minutes in 1 week | | | −37 (−100 to 26) | 0.23 |
| Sleep efficiency, % | | | −0.9 (−4.9 to 3.0) | 0.62 |
| Goal Attainment Scale score | | | 0.11 (−0.69 to 0.91) | 0.77 |

Bold *P* values are significant.
CI, confidence interval; FEV1, forced expiratory volume in first second; FGF21, fibroblast growth factor 21; FVC, forced vital capacity; GDF15, growth and differentiation factor 15; GSH, glutathione; GSSG, glutathione disulfide; MEP, maximum expiratory pressure; MIP, maximum inspiratory pressure; PEF, peak expiratory flow; PRDX1, peroxiredoxin 1; QoL, quality of life; SF-36, 36-Item Short Form Health Survey.

## METHODS

### Study design and participants

This is a double-blind, randomized, placebo-controlled, two-way, crossover phase IIA study primarily designed to assess safety, tolerability, pharmacokinetics, and pharmacodynamics. The study is exploratory by nature, and no statistical hypothesis has been set for this proof-of-concept study. The sample size was based on empirical considerations. Patients were recruited from the Radboud Center for Mitochondrial Medicine (RCMM) at the Radboud University Medical Center in the Netherlands, where the study was also conducted.

Male and female participants were eligible if they were aged ≥18 years at screening, had a confirmed mitochondrial DNA transfer $RNA^{Leu(UUR)}$ m.3243A>G mutation with a heteroplasmy percentage ≥20% in urinary epithelial cells, had a body mass index between 18 and 30 $kg/m^2$, and had clinical evidence of mitochondrial disease with preserved cardiac functioning. Patients with chronic progressive ophthalmoplegia only and patients with motor abnormalities other than related to the mitochondrial disease were not eligible. Full inclusion and exclusion criteria are given in Table S3. The use of vitamins, antioxidant supplements, medication negatively influencing mitochondrial functioning, and any strong inhibitors or inducers of cytochrome P450 enzymes or medication known to affect cardiac repolarization was prohibited during the study. Because KH176 is a substrate for CYP3A4 and the efflux pump PgP, concomitant drugs known to inhibit CYP3A4 and PgP, which might increase plasma concentrations of KH176, were listed in the exclusion criteria.

This study was performed in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and the Declaration of Helsinki (2013). Ethical approval was obtained from the Ethics Committee of the Arnhem-Nijmegen region, the Netherlands (NL57767.091.16). All patients provided written informed consent before the screening procedures. The trial was registered with ClinicalTrials.gov (NCT02909400), the European Clinical Trials Database (2016-001696-79), and ISRCTN (43372293).

### Randomization and masking

Patients were randomized using an externally produced computer-generated list of random numbers to receive 28 days of KH176 and subsequently placebo, or vice versa. For safety reasons, patients were randomized per block of two, with no stratification. The randomization code was generated by an external pharmacy (Pharmavize, Mariakerke, Belgium). The randomization list was kept strictly confidential for all RCMM investigators and the study sponsor. Only the bioanalysis laboratory for assessment of plasma concentrations of KH176 and KH176 m (ABL, Assen, The Netherlands) received an open randomization list to avoid unnecessary measurements in the placebo group. In case of emergency procedures, sealed emergency envelopes were present at the intensive care unit and at Khondrion BV. An independent monitor (PSR, Hoofddorp, The Netherlands) checked the seal of these envelopes at every visit. Last patient last visit took place on May 23, 2017, which was followed by data lock (October 5, 2017) and the unblinding of the randomization code, consecutively.

Both KH176 and placebo were delivered to the clinic in individually labelled light-protected glass vials with identical appearance and taste masking, according to a randomized list provided by Pharmavize. All participants, investigators, coordinating staff, and site assessors were blinded to the treatment group assignment for the full duration of the study.

### Procedures

During a 28-day dosing period, all patients received 100 mg KH176 (Khondrion BV, Nijmegen, The Netherlands; manufactured at Pharmavize) or placebo (bitrex powder/mannitol) b.i.d. Both treatments were provided as a powder, to be dissolved in saline in 10-ml bottles that were used to ingest the solution orally.

Clinical baseline assessments were performed on days −2 and −1 before the start of the intervention on day 1. The outcome assessments consisted of the exact same set of measurements and were performed on the last 2 days (days 27 and 28) of the intervention period. Both baseline and outcome assessments were identical for both treatment periods. To minimize the influence of a possible training effect on the comparability of both treatment periods, a training session consisting of several clinical assessments was conducted at least 1 week before the baseline measurements of the first treatment period. The primary functional end points were step length and variability in step time and step width (Gaitrite, Franklin, NJ). Secondary efficacy measurements included both clinical and functional end points.[30]

Plasma concentration analysis of KH176 (ABL) was performed 1, 2, and 4 hours postdose on days 1 and 21 and predose on days 1, 7, 14, 21, and 27. Between the two treatment periods, a washout period of at least 28 days was implemented to prevent pharmacokinetic (half-life in healthy male volunteers, 9 hours) and pharmacodynamic carry-over effects between the two treatment periods.

Blood for pharmacodynamic analyses was drawn at days −1 and 28 for both periods. A portion of the blood samples was deproteinized and derivatized with N-ethylmaleimide. The resulting supernatant was used to measure the GSH and GSSG concentrations by liquid chromatography–tandem mass spectrometry and to calculate the ratio of GSH/GSSG. Enzyme-linked immunosorbent assays were executed, according to manufacturer instructions, to measure FGF21 (Biovendor, MT-Diagnostics, Netherlands BV), GDF15 (R&D R&D Biosystems, Bio-Techne Ltd., Abingdon, UK), and PRDX1 (Abnova, Taipei, Taiwan.).

### Safety

Safety was measured by the number of adverse and serious adverse events, coded using the Medical Dictionary for Regulatory Activities, version 11.0 (https://www.meddra.org/).

During the first 3 days of dosing (days 1–3), patients were admitted to the intensive care unit for continuous vital sign and ECG cardiac monitoring. Baseline continuous cardiac monitoring was obtained from day −2 until at least day 7. The weekly safety assessments at the outpatient clinic included physical examination, safety laboratory measurements, and ECGs. Interim safety analyses consisted of continuous ECG monitoring on the first 3 days of treatment and measurements of plasma concentrations of KH176 on the first day of treatment (analyzed by ABL). The latter was reported blindly to investigators: safety threshold KH176 blood concentration ≥1,000 ng/ml (yes/no). The drug was discontinued in case of an absolute Fridericia-corrected QTc value >500 milliseconds or a change from baseline >60 milliseconds, torsade de pointes (nonsustained) ventricular tachycardia on the ECG, or clinically relevant changes in T-wave morphology.

At least 2 weeks after the last dosing of the second treatment period, an end-of study safety visit was scheduled, including an echocardiogram and a detailed interview of the patients' own experiences of the investigative product.

### Efficacy

Because parameters for gait deem to be a common denominator for the vast majority of patients with the m.3243A>G mutation,[22,23] gait parameters (step length and variability in step time and step width, as measured with the Gaitrite system) were defined as primary outcome measures.

The secondary objective was to explore the effect of KH176 on both clinical and functional end points, including, but not limited to, validated

questionnaires to measure mood, depressive complaints, and quality of life. At the end-of-study visit, participants were asked in which period they believed to having received active treatment and to provide an explanation for their prediction.

### Statistical analysis

No sample size calculation was performed for this exploratory phase IIA study. The two-way crossover study enabled intraparticipant comparison between the change from baseline to outcome in the KH176 and the placebo treatment period. Efficacy was analyzed on the per-protocol analysis set ($N = 18$, per protocol was defined as all-treated subjects, with complete assessments, who did not violate the protocol in a way that might affect the evaluation of the effect of the study drug(s) on the primary end point (i.e., without major protocol violations)) using mixed-effects modeling, allowing evaluation of sequence, carry-over, and baseline effects.

Safety was analyzed on all patients who had undergone at least one dose and one safety assessment. Pharmacokinetic analysis was performed with Phoenix WinNonlin V.6.3 (Certara, Princeton, NJ); pharmacodynamics and safety and tolerability end points were summarized descriptively.

Pharmacokinetic analysis was performed under responsibility of CDTS Consulting BV (Etten-Leur, The Netherlands); all other analyses were performed using Statistical Analysis System (SAS software, version 9.4; SAS Institute Inc., Cary, NC) by Arlenda (Mont-St-Guibert, Belgium).

External good clinical practice monitoring ensuring good clinical practice compliance was performed by an external party (PSR). Data safety monitoring was performed on an ongoing basis, with a decision point for intensive care unit discharge, by a committee consisting of the principal investigator, Khondrion's chief marketing officer, intensivist (P.P.), and cardiologist (R.B.). The trial was registered on ClinicalTrials.gov (NCT02909400), the European Clinical Trials Database (2016-001696-79), and ISRCTN (43372293).

### Role of funding source

The sponsor designed the study in collaboration with members of the RCMM. The sponsor did not engage in data collection. The data analysis, data interpretation, and writing of the manuscript were performed by the RCMM team; and data validity was approved by Khondrion BV. The corresponding author had full access to all data of the study and had final responsibility for the decision to submit for publication.

## SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cptjournal.com).

**Table S1.** Pharmacokinetics of KH176.
**Table S2.** Outcome measures.
**Table S3.** Full inclusion and exclusion criteria.

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### CONFLICT OF INTEREST

E.S. is the chief marketing officer, J.B. is the chief operating officer, and J.S. is the chief executive officer of Khondrion BV. All other authors report no disclosures.

### AUTHOR CONTRIBUTIONS

M.C.H.J. and S.K. wrote the manuscript. M.C.H.J., S.K, P.P., E.S., R.B., J.G., J.B., J.S., and C.V. designed the research. M.C.H.J., P.H., R.B., P.d.L., P.P., and S.K. performed the research. M.C.H.J., S.K., P.H., R.B., E.S., J.S., J.B., and C.V. analyzed the data.



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