

# **Efficacy and safety of once-daily nitisinone for patients with** alkaptonuria (SONIA 2): an international, multicentre, open-label, randomised controlled trial

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## Summary

Background Alkaptonuria is a rare, genetic, multisystem disease characterised by the accumulation of homogentisic acid (HGA). No HGA-lowering therapy has been approved to date. The aim of SONIA 2 was to investigate the efficacy and safety of once-daily nitisinone for reducing HGA excretion in patients with alkaptonuria and to evaluate whether nitisinone has a clinical benefit.

Methods SONIA 2 was a 4-year, open-label, evaluator-blind, randomised, no treatment controlled, parallel-group study done at three sites in the UK, France, and Slovakia. Patients aged 25 years or older with confirmed alkaptonuria and any clinical disease manifestations were randomly assigned (1:1) to receive either oral nitisinone 10 mg daily or no treatment. Patients could not be masked to treatment due to colour changes in the urine, but the study was evaluator-blinded as far as possible. The primary endpoint was daily urinary HGA excretion (u-HGA<sub>34</sub>) after 12 months. Clinical evaluation Alkaptonuria Severity Score Index (cAKUSSI) score was assessed at 12, 24, 36, and 48 months. Efficacy variables were analysed in all randomly assigned patients with a valid u-HGA<sub>24</sub> measurement at baseline. Safety variables were analysed in all randomly assigned patients. The study was registered at ClinicalTrials.gov (NCT01916382).

Findings Between May 7, 2014, and Feb 16, 2015, 139 patients were screened, of whom 138 were included in the study, with 69 patients randomly assigned to each group. 55 patients in the nitisinone group and 53 in the control group completed the study. u-HGA<sub>24</sub> at 12 months was significantly decreased by 99.7% in the nitisinone group compared with the control group (adjusted geometric mean ratio of nitisinone/control 0.003 [95% CI 0.003 to 0.004], p<0.0001). At 48 months, the increase in cAKUSSI score from baseline was significantly lower in the nitisinone group compared with the control group (adjusted mean difference -8.6 points [-16.0 to -1.2], p=0.023). 400 adverse events occurred in 59 (86%) patients in the nitisinone group and 284 events occurred in 57 (83%) patients in the control group. No treatment-related deaths occurred.

Interpretation Nitisinone 10 mg daily was well tolerated and effective in reducing urinary excretion of HGA. Nitisinone decreased ochronosis and improved clinical signs, indicating a slower disease progression.

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# Introduction

Alkaptonuria (OMIM 203500) is a rare, serious, autosomal recessive multisystem disorder1 affecting approximately one in every 250000 to 1 million people.<sup>2</sup> The disease was described in a paper by Archibald E Garrod in 1902,3 in which Mendel's laws of inheritance were applied in human disease for the first time. However, no pharmacological treatment for alkaptonuria exists. Genetic deficiency of homogentisate dioxygenase activity results in accumulation of homogentisic acid (HGA; appendix p 10). HGA is then progressively deposited as a yellow or dark pigment in connective tissue, rendering these tissues more rigid and eventually brittle and prone to degradation, a process called ochronosis.45 As the causal agent of alkaptonuria, HGA could represent a suitable surrogate for a clinically meaningful endpoint in clinical trials. This endpoint was also suggested by the European Medicines Agency, during scientific advice provided to our group before starting our clinical programme. Degradation of ochronotic tissue is the main cause of multisystem involvement; various phenotypes of the disease exist, characterised by severe

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# **Research in context**

# Evidence before this study

We searched MEDLINE for publications up to July 22, 2020 using the terms "nitisinone", "alkaptonuria", and "outcomes". No language restrictions were applied. Our search identified only one previous long-term clinical study that has evaluated the effect of nitisinone on alkaptonuria disease progression. In addition, because alkaptonuria is a rare disease, personal contact with researchers and clinicians in the field enables us to confidently state that there has been only one previous outcomes trial using nitisinone in alkaptonuria. The US National Institutes of Health (NIH) nitisinone outcomes study, which included 20 patients treated with nitisinone and 20 control patients, employed an improvement in the lateral rotation of the hip as the endpoint to evaluate efficacy of 2 mg nitisinone daily over 3 years. The result for this endpoint was deemed to be inconclusive. Three short-term studies, two in the NIH and one in Liverpool, UK, have reported the metabolic efficacy of nitisinone in terms of lowering homogentisic acid (HGA). An assessment of the use of nitisinone 2 mg daily offlabel in the National AKU Centre in Liverpool, funded by NHS England Highly Specialised Services, showed metabolic benefit, arrest of ochronosis, and slower progression of alkaptonuria disease, but this was an audit of a service rather than a research study.

premature spondyloarthritis, lithiasis, cardiac valve disease, fractures, muscle and tendon ruptures, and osteopenia.<sup>67</sup> Alkaptonuria therapy primarily consists of palliative analgesia and arthroplasty.

Alkaptonuria is a disorder of tyrosine metabolism similar to hereditary tyrosinaemia type 1 (OMIM 276700). Hereditary tyrosinaemia type 1 is characterised by a deficiency of fumarylacetoacetate hydrolase, resulting in early liver and kidney disease and death in childhood if untreated.<sup>8,9</sup> Nitisinone is an inhibitor of 4-hydroxyphenylpyruvate dioxygenase and has been used in hereditary tyrosinaemia type 1 since 1991.8 Because activity of 4-hydroxyphenylpyruvate dioxygenase leads to formation of homogentisic acid (appendix p 10), nitisinone was hypothesised in the late 1990s to be a potential treatment for alkaptonuria.<sup>10</sup> Following initial research of nitisinone for treatment of alkaptonuria,<sup>1,11</sup> a 3-year clinical trial was done by the US National Institutes of Health (NIH),12 comparing a nitisinone-treated patient group receiving a 2-mg daily dose (n=20) with an untreated patient group (n=20). Although nitisinone showed excellent biochemical efficacy, the trial was inconclusive.12

Despite this setback, research into the use of nitisinone in alkaptonuria has continued. In addition, nitisinone 2 mg daily has been reimbursed for use in the UK National Alkaptonuria Centre (NAC) since 2012, and a 2018 study of NAC data described positive outcomes for nitisinone in its metabolic and non-metabolic effects.<sup>13,14</sup> Although the off-label use of nitisinone in the UK NAC allows

#### Added value of this study

We did an international, multicentre, randomised, controlled, evaluator-blind study to evaluate the efficacy of nitisinone 10 mg daily for alkaptonuria, with 69 patients treated with nitisinone and 69 control patients. One of the study outcomes was the effect of nitisinone on the change in alkaptonuria severity score index, a composite disease score, over 4 years. The power of the study was much increased by the use of this composite score, and we were able to show for the first time in a randomised study that nitisinone decreased the progression of alkaptonuria. The data from the control group over 4 years has further improved our understanding of the natural history of alkaptonuria.

# Implications of all the available evidence

Alkaptonuria is a rare and serious disease, for which there is no approved pharmacological treatment. The metabolic benefit of nitisinone in reducing HGA has been shown previously. The findings of SONIA 2 indicate that nitisinone 10 mg daily could reduce urine and serum HGA in patients with alkaptonuria, reduce ochronosis, and slow disease progression. Regulatory approval is now urgently required for the use of nitisinone in alkaptonuria, followed by enabled access to nitisinone for patients. J Szamosi Msc); Nordic Bioscience, Herlev, Denmark (F Genovese PhD); Institute of Clinical and Translational Research, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia (H Glasova PhD, R Imrich DSc, A Zatkova PhD); Institute of Pharmacology and Clinical Pharmacology, Slovak Medical University, Bratislava, Slovakia (H Glasova); Cudos, Hoofddorp, Netherlands (A K Hall MBBS): National Institute of Rheumatic Diseases, Piešťany, Slovakia (R Imrich, O Lukáčová PhD V Mlynáriková PhD, Prof J Rovenský DSc, I Sedláková PhD, R Stančík PhD, E Vrtíková MD, E Záňová PhD); School of Sport and Exercise Science, Liverpool John Moores University, Liverpool. UK (Prof J C Jarvis PhD); PSR Group, Hoofddorp, Netherlands (D Laan MSc. C van Kan MSc): and AKU Society, Cambridge, UK (C Scott BA, N Sireau PhD) Correspondence to:

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high-quality data to be collected in a protocolised manner, the drug is used in a service capacity and not in a controlled clinical trial.

In designing the SONIA 2 study, it was assumed that the NIH trial did not succeed because of the small number of patients recruited, an insufficient duration given the slowly progressive nature of alkaptonuria, an incomplete understanding of the natural history, and the use of a single and possibly unreliable outcome measure in this multifaceted disease. An identification campaign to maximise patient recruitment for a new trial was subsequently done in the UK and the rest of Europe.15 A better understanding of the natural history of the disease and its modification by nitisinone was shown in a mouse alkaptonuria model.<sup>16-18</sup> Careful phenotyping of the disease in a cohort of untreated individuals with alkaptonuria resulted in a composite score, termed the Alkaptonuria Severity Score Index (AKUSSI), which is a useful tool in researching a multifaceted condition with a variable phenotype.<sup>19,20</sup> In addition, a new clinical trial of nitisinone was considered, with a higher number of patients and a longer duration. The dose used in the inconclusive NIH trial was based on the experience of administering nitisinone to two patients with alkaptonuria;10 furthermore, the European Medicines Agency suggested finding a dose that normalises HGA, and therefore the issue of optimal dose was revisited in a dose-response study, SONIA 1.21 In SONIA 1, the 8 mg daily dose of nitisinone resulted in a mean reduction of

24-h urinary HGA excretion (u-HGA<sub>24</sub>) of 98.8%, with a clear dose response and less variability compared with the other doses studied (1 mg, 2 mg, and 4 mg). An increase in tyrosine levels was seen at all doses but the doseresponse relationship was less clear than that for HGA, with no tyrosine-related adverse events seen at any dose. Because the 8 mg dose of nitisinone resulted in u-HGA<sub>24</sub> close to normal values, a dose of 10 mg daily, which was achieved with an available capsule strength, was selected for the new trial, SONIA 2. The primary objective of SONIA 2 was to investigate the efficacy of nitisinone in reducing u-HGA<sub>44</sub> in patients with alkaptonuria after 12 months. Secondary objectives were to evaluate the sustained control of urinary and serum HGA up to 48 months, to evaluate the effect on clinical parameters, and to assess the safety of nitisinone in alkaptonuria.

# Methods

## Study design

SONIA 2 was a 4-year, open-label, evaluator-blind, multicentre, randomised, no treatment controlled, parallelgroup study to investigate the efficacy and safety of nitisinone for patients with alkaptonuria. The study was done at three investigational sites: Royal Liverpool University Hospital, Liverpool, UK; Hôpital Necker-Enfants Malades, Paris, France; and National Institute of Rheumatic Diseases, Piešťany, Slovakia. Due to challenges in recruitment, 19 patients from Jordan were also included in the study. These patients, of white ethnicity, were treated and followed up at the study site in Slovakia. Independent ethics committees at each centre approved the study. The study protocol is provided in the appendix (pp 97–175).

## Participants

Eligible participants were aged 25 years or older, with a confirmed diagnosis of alkaptonuria and any clinical manifestation in addition to increased HGA. Inclusion and exclusion criteria are described in the appendix (p 3). All patients provided written informed consent prior to inclusion.

## Randomisation and masking

Patients were randomly assigned (1:1) to receive nitisinone or no treatment. The randomisation was stratified by study centre and age (≤55 years and >55 years) and was done using randomly permuted blocks (four patients per block) within each study centre and age stratum. The study statistician created a program to randomly assign the patients to the two treatment groups using SAS version 9.3. The randomisation was centrally implemented in the electronic case report form system.

Masking is not possible in a study with nitisinone in alkaptonuria because one of the signs of the disease is that the urine darkens due to oxidation of excreted HGA. Patients can therefore easily notice if they are receiving active drug or not. Therefore, the control group received no placebo treatment. Instead, the study was evaluator-blinded as far as possible. Assessments that did not require direct contact between the evaluator and the patient (such as evaluation of images) were masked during the entire study. The masked evaluators were experts in their respective fields and never met the patients. Other assessments were made by objective measurements, such as that of bone density. However, we recognise that reporting of subjective assessments could have introduced bias for some of the secondary endpoints, such as pain and quality-of-life assessments and reporting of adverse events.

## Procedures

Oral nitisinone (Orfadin, Swedish Orphan Biovitrum, Stockholm, Sweden) 10 mg daily was administered in the treated group. The control group did not receive the study drug.

Nitisinone was withdrawn in patients who developed signs of ocular tyrosine-related adverse events. If feasible, once the symptoms had resolved (minimum 2 months after temporary withdrawal), nitisinone was reintroduced at a lower dose (2 mg daily). Alternatively, the patient was withdrawn from the study. If ocular tyrosine-related symptoms reappeared on the lower dose, nitisinone was permanently withdrawn and the patient was monitored until the symptoms resolved.

There were no restrictions regarding concomitant medications. Patients in both groups could freely use analgesics, anti-inflammatory drugs, and other drugs as needed to treat symptoms of alkaptonuria.

Patients visited study sites at 3 months, and then at 12, 24, 36, and 48 months; a close-out telephone call was made at month 49. A questionnaire, completed by patients, collected safety information at 6, 18, 30, and 42 months.

The assessments done at each visit are described in the appendix (pp 28–31). These assessments included collection of u-HGA<sub>24</sub> for HGA and creatinine determination, fasting acidified serum for HGA, tyrosine, and creatinine, medical history and physical examination, and a wide range of clinical outcome measures, including range-of-motion tests and quality-of-life assessments.<sup>13,14</sup> All items included in the AKUSSI were assessed at baseline and yearly thereafter (appendix pp 28–29).<sup>13,19,20</sup> Two types of AKUSSI were assessed: the clinical evaluation AKUSSI (cAKUSSI) and a modified AKUSSI (mAKUSSI; the same as cAKUSSI but without pigmentation features).

At each visit, adverse events and laboratory values were recorded. Adverse events included clinically significant signs and symptoms and abnormal test findings (eg, laboratory analysis results, vital signs, or electrocardiogram [ECG]) that the investigator considered clinically significant or that led to a medical or surgical intervention, including withdrawal of nitisinone or discontinuation from the study.

## Outcomes

The primary endpoint was u-HGA<sub>24</sub> in patients with alkaptonuria after 12 months. A formal interim analysis

was planned when all patients completed 12 months of treatment. This analysis included the complete set of efficacy and safety data up to 12 months, thus including the final analysis of the primary endpoint. The purpose was to evaluate if data demonstrated results suitable for a regulatory application already at that stage, even though the study was to continue for another 3 years to collect more complete efficacy and safety data. The study design is summarised in the appendix (p 11).

Secondary endpoints were u-HGA<sub>24</sub> at months 3, 24, 36, and 48; changes from baseline in cAKUSSI, mAKUSSI, and individual cAKUSSI items at 12, 24, 36, and 48 months; pre-dose serum HGA (sHGA) at 3, 12, 24, 36, and 48 months; changes from baseline in quality of life measured by SF-36, range of motion in the joints and spine, other predefined rheumatology assessments (pp 97–175), Health Assessment Questionnaire, and Knee injury and Osteoarthritis Outcome Score at 12, 24, 36, and 48 months; change from baseline in ear cartilage pigmentation at 48 months; and adverse events, serum concentration of tyrosine, clinical chemistry and haemotology, vital signs, ECG, and corneal eye assessments.

All adverse events during the study were coded using the Medical Dictionary for Regulatory Activities (version 16.0).

Exploratory endpoints were the effects of nitisinone on inflammatory biomarkers, biomarkers of bone, cartilage, and cardiovascular damage, metabolites of tyrosine (other than HGA), and metabolic pathways in patients with alkaptonuria (metabolomics); the effect of nitisinone on spine and joint disease as assessed by MRI and knee radiographs; phenotype–genotype correlations; and digital image analysis of photographs, x-rays, and scans as a measure of disease progression of alkaptonuria.

# Statistical analysis

Only a small number of participants would be needed to detect a statistically significant effect on the primary endpoint, u-HGA<sub>24</sub>. Therefore, the sample size was based on the AKUSSI score, to allow the possibility of establishing an effect on a clinical endpoint. Using data from a cross-sectional study of alkaptonuria using AKUSSI<sup>13,14,20</sup> and follow-up data, it was assumed that if nitisinone reduced the mean increase in AKUSSI over the 4-year period to 4 points, compared with 8 points in the control group, and taking the SD of the increase to be 8, then a sample size of 64 per group was required for a two-sided *t* test with 80% power for a significance level of 0.05. With an estimated 10% drop-out rate, a sample size of 70 per group was required (140 patients in total).

The full analysis set was used for the analysis of efficacy variables, including all randomly assigned patients who had a valid u-HGA<sub>24</sub> measurement at baseline. The safety analysis set was used for the analysis of safety variables, including all randomly assigned patients.

A longitudinal model (mixed model for repeated measures [MMRM]) with an underlying normal distribution was fitted for the analysis of the primary endpoint. An unstructured covariance matrix was used along with a restricted maximum likelihood method, and the degrees of freedom were estimated using the Kenwood-Rogers method. Treatment, site, age category, visit, and treatment by visit interaction were added as fixed factors in the model together with the baseline log(u-HGA<sub>24</sub>) value as a covariate and with subject-within-site included as a random factor. The analysis was done using log(u-HGA<sub>24</sub>) as the dependent variable. Model-based point estimates and associated two-sided 95% CIs were calculated. u-HGA<sub>24</sub> at months 3, 24, 36, and 48 was analysed using the same MMRM model as in the primary endpoint analysis.

For continuous secondary endpoints, the same statistical model as in the primary endpoint analysis was used, with





	Control (n=69)	Nitisinone (n=69)	Total (n=138)		
Age, years	47.6 (10.1)	49.0 (11.3)	48·3 (10·7)		
Bodyweight, kg	74.1 (15.6)	74.8 (14.8)	74·4 (15·1)		
Height, cm	167 (9.5)	166 (9·2)	167 (9.4)		
Sex					
Female	29 (42%)	24 (35%)	53 (38%)		
Male	40 (58%)	45 (65%)	85 (62%)		
Race					
White	67 (97%)	67 (97%)	134 (97%)		
Black	0	1(1%)	1(1%)		
Asian	2 (3%)	1(1%)	3 (2%)		
Study centre					
Liverpool, UK	21 (30%)	20 (29%)	41 (30%)		
Piešťany, Slovakia	32 (46%)	33 (48%)	65 (47%)		
Paris, France	16 (23%)	16 (23%)	32 (23%)		
Data are mean (SD) or n (%).					

the exception that these analyses were done on the original scale without transformation. However, for sHGA and serum tyrosine (s-Tyr), log transformation was used. Ordinal secondary endpoints were modelled using a generalised estimating equations approach, whereas count data were modelled using an MMRM with an underlying Poisson distribution.

The incidence of adverse events was summarised in frequency tables. The changes in safety laboratory parameters from baseline to all post-baseline visits were summarised by treatment group and visit using descriptive statistics. These included serum concentration of clinical chemistry, haematology, vital signs, ECG, and corneal eye assessments.

All statistical analyses were done with SAS (version 9.3). Two-sided 95% CIs corresponding to a two-sided 5% level of significance were used throughout the analyses. All relevant study data were tabulated with descriptive statistics, including mean, SD, SEM, median, minimum and maximum for the continuous variables, and frequencies and proportions for the categorical variables. Both absolute values and changes from baseline were tabulated, if feasible. No allowance for multiplicity was made.



HGA=homogentisic acid. u-HGA<sub>24</sub>=daily urinary HGA excretion.

A data monitoring committee was assigned to safeguard the interests of study participants and to continuously monitor the safety of the patients in the study.

The study was registered at ClinicalTrials.gov (NCT01916382).

# Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

#### Results

Between May 7, 2014, and Feb 16, 2015, 139 patients were screened, of whom 138 were included in the study, with 69 patients randomly assigned to each of the two study groups. The first patient was randomised on May 7, 2014 and the last patient's last visit was Feb 15, 2019. SONIA 2 was funded by the European Commission with a strict time limit for its completion. Therefore, as the number of recruited patients was deemed sufficient at the end of the recruitment period, recruitment was ended after 138 patients were included (139 screened), to meet these timelines. Of these, 108 patients completed the study. All 138 patients (69 per group) were included in the analysis according to the groups to which they were assigned. The main reason for discontinuation in the control group was withdrawal of consent (n=10), whereas adverse events (n=9) were the most common reason for discontinuation in the nitisinone group (figure 1).

Demographic data and baseline characteristics in the two groups were well balanced (table 1). The majority of the patients (134 [97%] of 138) were white. There were more male patients in the nitisinone-treated group (45 [65%] of 69) than in the control group (40 [58%] of 69). The mean age was slightly lower in the control group than in the nitisinone group (47.6 years [SD 10.1] *vs* 49.0 years [11.3]).

u-HGA<sub>24</sub> was statistically significantly decreased in the nitisinone-treated group compared with the control group at all visits after baseline (figure 2A, table 2). These findings were consistent irrespective of age, sex, or study site. At month 12, the time of evaluation of the primary endpoint, the adjusted mean u-HGA<sub>24</sub> had statistically significantly decreased by 99.7% in the nitisinone group compared with the control group (adjusted geometric mean ratio of nitisinone/control 0.003 [95% CI 0.003–0.004], p<0.0001).

At baseline, mean sHGA was similar for the two study groups (figure 2B, table 2). At month 12, the adjusted geometric mean sHGA in the nitisinone group had statistically significantly decreased by 98.8% compared with the control group (adjusted geometric mean ratio of nitisinone/control 0.01 [95% CI 0.01-0.02]; p<0.0001). At each visit after baseline, sHGA was statistically significantly lower in the nitisinone group compared with the control group (p<0.0001).

	Baseline Month		Month 12	nth 12		Month 48	
	Control	Nitisinone	Control	Nitisinone	Control	Nitisinone	
HGA							
υ-HGA <sub>24</sub> , μmol							
Mean (SD)	35 394 (13869)	35 019 (13124)	26444 (10397)	179 (398)	33 207 (10160)	1569 (6220)	
Adjusted geometric mean, ratio nitisinone/ control (95% CI)		NA		0.003 (0.003 to 0.004)		0.005 (0.003 to 0.008)	
Serum HGA, mmol/L							
Mean (SD)	28.26 (8.66)	30.35 (10.98)	28.93 (13.04)	0.71 (1.63)	37.08 (21.03)	2.80 (7.33)	
Adjusted geometric mean, ratio nitisinone/ control (95% CI)		NA		0·01 (0·01 to 0·02)		0.02 (0.02 to 0.03)	
AKUSSI							
cAKUSSI score							
Mean (SD)	80.5 (33.4)	87.0 (34.2)	80.1 (34.7)	84.5 (33.7)	95.6 (36.0)	93.7 (37.8)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		-2·5 (-5·7 to 0·7)		-8.6 (-16.0 to -1.2)	
mAKUSSI score							
Mean (SD)	54.1 (24.9)	56.7 (26.7)	54.8 (25.7)	57.5 (26.8)	66.7 (29.7)	66.1 (31.1)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		-0·5 (-2·5 to 1·6)		-3·6 (-9·6 to 2·4)	
Individual AKUSSI items							
Eye ochronosis							
Mean (SD)	14.1 (9.6)	17.3 (9.2)	14.7 (9.0)	16.8 (9.5)	16.4 (9.5)	16.5 (9.3)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		-0·8 (-1·9 to 0·3)		-2·5 (-3·9 to -1·0)	
Ear ochronosis							
Mean (SD)	3.9 (2.9)	4.1 (2.9)	4.0 (2.8)	4·1 (2·9)	4.0 (2.8)	4.0 (2.9)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		-0·2 (-0·4 to 0·0)		-0·5 (-0·9 to -0·1)	
Bone mineral density, T-sco	ore						
Mean (SD)	-1.26 (0.98)	-1.3 (1.2)	-1.28 (0.98)	-1.39 (1.14)	-1.41 (0.81)	-1.22 (1.17)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		-0.09 (-0.18 to -0.01)		0·14 (0·00 to 0·28)	
Aortic velocity, m/s							
Mean (SD)	1.6 (0.6)	1.8 (0.8)	1.6 (0.6)	1.8 (0.8)	1.7 (0.6)	1.8 (0.8)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		-0·009 (-0·092 to 0·075)		-0·030 (-0·149 to 0·089)	
Joint pain							
Mean (SD)	4.6 (3.3)	4.8 (3.0)	4.0 (3.1)	3.5 (2.7)	4.2 (3.3)	3.8 (2.7)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		-0·9 (-1·6 to -0·1)		-0·7 (-1·6 to 0·1)	
Number of joints with oste	oarticular disease						
Mean (SD)	6.7 (3.2)	6.1 (3.1)	6.7 (3.2)	6.4 (3.2)	9.1 (3.3)	8.5 (3.6)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		0·0 (-0·1·to 0·2)		-0·1 (-1·3 to 1·1)	

	Baseline		Month 12		Month 48	Month 48	
	Control	Nitisinone	Control	Nitisinone	Control	Nitisinone	
(Continued from previous page)							
Spinal pain score							
Mean (SD)	2.3 (1.2)	2.3 (1.3)	2.0 (1.2)	1.9 (1.3)	2.2 (1.4)	1.7 (1.3)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		-0·2 (-0·5·to 0·2)		-0.5 (-0.9 to 0.0)	
Number of spinal regions with osteoarticular disease							
Mean (SD)	3.0 (2.1)	3.4 (2.1)	3.0 (2.1)	3.4 (2.2)	3.5 (2.1)	3.7 (2.0)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		0·0 (-0·3·to 0·4)		-0·1 (-0·4 to 0·3)	
Kyphosis, Cobb angles							
Mean (SD)	35.2 (10.2)	36.4 (10.6)	35.2 (9.2)	37.1 (10.7)	37.2 (7.8)	39.5 (9.7)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		0·9 (-0·3·to 2·1)		1·0 (-0·8 to 2·7)	
Scoliosis, Cobb angles							
Mean (SD)	10.5 (5.4)	10.8 (5.2)	10.5 (4.9)	10.7 (4.5)	11.8 (6.6)	12.1 (5.2)	
Adjusted mean difference, nitisinone vs control (95% CI)		NA		0.0 (-0.6.to 0.6)		-0·1 (-1·6 to 1·4)	
HGA=homogentisic acid. u-HGA <sub>34</sub> =daily urinary HGA excretion. NA=not applicable. AKUSSI=Alkaptonuria Severity Score Index. cAKUSSI=clinical evaluation AKUSSI. mAKUSSI=modified AKUSSI, without pigmentation features.							

Table 2: HGA and other continuous efficacy variables in AKUSSI

The 12-month analysis of the secondary efficacy endpoints did not support a regulatory authority application for the new indication (despite the primary endpoint supporting the application).

At baseline, cAKUSSI was slightly higher in the nitisinone group than in the control group (table 2). In the control group, cAKUSSI increased from baseline to month 48, whereas the increase was smaller in the nitisinone group. The difference between the two groups in the change from baseline to month 48 was statistically significant (adjusted mean difference -8.6 points [95% CI -16.0 to -1.2], p=0.023). The adjusted mean increase was 15.1 points in the control group and 6.7 points in the nitisinone group, over the duration of the study (figure 3A, appendix pp 28–29).

At month 48, the difference between the two groups in change in mAKUSSI from baseline was not statistically significant (adjusted mean difference  $-3 \cdot 6$  [-9.6 to 2.4], p=0.23; table 2). However, a continuous increase in mAKUSSI was seen in the control group from baseline to month 48, whereas a slower increase was seen in the nitisinone group (table 2, figure 3B).

Regarding individual AKUSSI items, statistically significant differences between the two treatment groups were observed at month 48, and for some variables also from earlier time points, for eye pigmentation (table 2, appendix p 12), ear pigmentation (appendix p 13), osteopenia of the hip (T-scores for bone density; appendix p 14), and the number of spinal regions with pain (appendix p 18). For the number of joints with pain, a statistically significant difference in favour of nitisinone was observed at month 12 (adjusted mean difference -0.9 [95% CI -1.6 to -0.1], p=0.023; table 2, appendix p 17). Numerically, the difference between the groups was similar at subsequent visits and at month 48 (adjusted mean difference -0.7 [-1.6 to 0.1], p=0.10). For the number of fractures (appendix p 15) and the number of tendon, ligament, and muscle ruptures (appendix p 16), the difference between groups increased from baseline to month 48, suggesting a lower rate of disease progression in the nitisinone group, but these results did not reach statistical significance.

Consistent patterns towards better outcome in the nitisinone group compared with the control group were also observed for quality of life (SF-36; appendix p 19), self-evaluated transition in SF-36 (appendix p 36), and range of motion of the joints (appendix p 20). No notable difference between the treatment groups was observed for any of the other variables.

In the nitisinone group, 400 adverse events occurred, with 59 (86%) of 69 patients experiencing at least one event (table 3). In the control group, 284 adverse events occurred, with 57 (83%) of 69 patients experiencing at least one event. The most common adverse events reported (54 events in 31 patients in the nitisinone group; 53 events in 24 patients in the control group) were within the system organ class of musculoskeletal and connective tissue disorder (mostly manifestations of alkaptonuria;

appendix p 38). The second most common system organ class was infections and infestations, with a higher incidence in the nitisinone group (56 events in 27 patients) than in the control group (24 events in 11 patients; appendix p 39). Pneumonia and bronchitis were more commonly reported in the nitisinone group (11 events in nine patients) than in the control group (one event in one patient; appendix p 39). No other clear patterns were observed (appendix pp 37–82). Eye disorders was the third most common system organ class, reported for 25 patients (65 events) in the nitisinone group (appendix p 40).

The incidence of adverse events was  $2 \cdot 1$  per 10 patient years in the control group and  $2 \cdot 3$  in the nitisinone group (table 3). The incidence of eye-related adverse events was  $0 \cdot 3$  per 10 patient years in the control group and  $1 \cdot 0$  per 10 patient years in the nitisinone group (appendix pp 40–41).

There were two deaths in the study, one due to heart failure and the other due to myocardial infarction; both occurred in patients who received nitisinone (table 3). Neither of the events was considered to be related to nitisinone treatment.

53 patients experienced at least one serious adverse event during the study (52 serious adverse events in 26 patients in the control group; 57 serious adverse events in 27 patients in the nitisinone group; table 3). None of these events was considered by the investigator to be related to nitisinone (appendix pp 38–48). The system organ class of musculoskeletal and connective tissue disorders had the highest number of serious adverse events (appendix pp 38–39), most of which were related to joint replacements, fractures, and other manifestations of alkaptonuria.

The majority of adverse events in the eye disorders class in the nitisinone group, such as keratopathy (nine patients), eye pain (eight patients), dry eye (six patients), increased lacrimation (four patients), ocular hyperaemia (four patients), and eye irritation (three patients), were considered to be related to the increased levels of tyrosine caused by nitisinone treatment (appendix pp 37–48).

Nine (13%) of 69 patients in the nitisinone group developed tyrosine-related keratopathy in one or both eyes confirmed by slit-lamp examination (appendix p 37). One additional patient, who could not come for a followup visit, was withdrawn due to suspected keratopathy based on convincing ocular symptoms. Of the nine keratopathy patients confirmed by slit-lamp examination, eight had other eye symptoms, such as pain, blurred vision, or other signs. One patient reported no symptoms before keratopathy was seen by slit-lamp at a pre-planned visit. In these nine patients with keratopathy, complete resolution was shown at a follow-up visit at least 2 months after nitisinone withdrawal. Eight patients restarted nitisinone at a dose of 2 mg per day after the recovery; five of these patients had recurrent symptoms and three



Figure 3: cAKUSSI (A) and mAKUSSI (B) scores

AKUSSI=Alkaptonuria Severity Score Index. cAKUSSI=clinical evaluation AKUSSI. mAKUSSI=modified AKUSSI, without pigmentation features.

were still asymptomatic at the end of the study (table 3, appendix p 37).

As expected, serum tyrosine concentrations were greater than 500  $\mu$ mol/L in all patients who received nitisinone. At month 12, the median value was 925  $\mu$ mol/L, with a range from 563  $\mu$ mol/L to 1530  $\mu$ mol/L. Decreasing the dose in those who switched from 10 mg to 2 mg nitisinone after keratopathy had little effect on serum tyrosine, with all patients still having concentrations greater than 500  $\mu$ mol/L (appendix pp 21, 37).

## Discussion

The direct cause of morbidity in alkaptonuria is HGA accumulation, resulting from genetic homogentisate dioxygenase deficiency.<sup>22</sup> HGA is therefore a surrogate for a clinically meaningful endpoint in clinical trials. In SONIA 2, nitisinone treatment decreased both u-HGA<sub>24</sub> and s-HGA, with mean values at month 12 decreasing by

	Control (n=69)		Nitisinone (n=69)	
	Number	Incidence per 10 patient years	Number	Incidence per 10 patient years
Patients with at least one adverse event	57 (83%)	2.1	59 (86%)	2.3
Adverse events	284		400	
Patients with at least one serious adverse event	26 (38%)	1.0	27 (39%)	1.0
Serious adverse events	52		57	
Patients with at least one study drug- related adverse event*	NA	NA	18 (26%)	0.7
Study drug-related adverse events*	NA		48	
Deaths	0	0.0	2 (3%)	0.1
Patients with adverse events leading to study discontinuation	1(1%)	0.0	9 (13%)	0.3
Patients with adverse events leading to dose reduction	NA	NA	8 (12%)	0.3

Data are number of events or n (%) unless indicated otherwise. NA=not applicable. \*Adverse event was judged to be related to the study drug by the investigator.

#### Table 3: Adverse events

more than 98% compared with the control group for both variables.

The difference between the groups in change in pigmentation (ie, ochronosis), which is the fundamental pathophysiological process in alkaptonuria, was statistically significant. This finding indicates that treatment with nitisinone arrested the ochronosis process in the eye and reversed it in the ear, by decreasing the accumulation of HGA. The crucial importance of ochronosis in alkaptonuria was highlighted in a review from 2019.<sup>22</sup> In SONIA 2, reversal of the disease process in the ear was seen soon after starting nitisinone, and continued throughout the study duration. Although reversal of ochronosis in the ear was observed, the decrease in pigmentation was not total, and it is not clear whether a longer follow-up period would have shown more depigmentation.

A weighted composite score, cAKUSSI, was used in SONIA 2, as for previously published data in alkaptonuria.<sup>13</sup> This score was because it would have been difficult to have a sufficiently large number of patients to show a difference in a single endpoint, such as lateral rotation of the hip as employed in the NIH trial,<sup>12</sup> given the rare nature of alkaptonuria and the heterogeneous phenotypic severity. In SONIA 2, baseline cAKUSSI scores were higher in the nitisinone group than in the control group. A possible explanation is that the nitisinone group was older, with a difference in median age of three years, and contained more male patients, who have been shown to experience a more severe disease.<sup>19,20</sup>

In SONIA 2, a statistically significant effect (difference between the treatment groups in change from baseline) on cAKUSSI was seen. The cAKUSSI consists of clinically meaningful outcomes, such as fractures, ruptures, and joint replacements, among others. The adjusted mean increase in score was  $15 \cdot 1$  in the control group and  $6 \cdot 7$  in the nitisinone group over the duration of the study, a reduction of almost 56%. This reduction is equivalent to a difference of two joint replacements or one fracture or rupture, if the difference occurred in a single feature of cAKUSSI rather than across all the features. There were fewer ruptures in the nitisinone group than in the control group, consistent with the decrease in observed ochronosis scores. Also, there were were fewer fractures in the nitisinone group than in the control group, but neither finding reached statistical significance. There was a statistically significant difference in bone mineral density between the treatment groups over the study period, in favour of nitisinone, although the clinical significance of this result is currently uncertain. Previous investigations have shown that stable or increased bone mineral density after bone-strengthening therapy is associated with fracture protection.23-25

Amelioration of pain is a crucial and constant requirement in patients with alkaptonuria. In this regard, the significant decrease in pain from baseline, both in joints and spine, in patients treated with nitisinone is important. The difference in change from baseline at month 48 between treatment groups was statistically significant only for the spine, but a positive treatment effect was also suggested for joint pain. The difference in pain between the control and treatment groups could explain the beneficial difference in SF-36 and active range of motion between the two groups.

A larger number of adverse events were reported in the nitisinone group than in the control group, partly due to more reports of infections and infestations, eye disorders, and weight gain. There is no obvious explanation for the higher number of infections and infestations and no known mechanism by which nitisinone could increase infections. This finding has not been observed in previous experience with nitisinone in hereditary tyrosinaemia type 1. Tyrosine-related eye disorders were not unexpected, considering that the patients were not actively managed on a truly low-protein diet and that patients in the nitisinone group had serum tyrosine concentrations considerably higher than 500 µmol/L. Due to study logistics, serum tyrosine was not measured at the time of keratopathy and was measured only during study site visits. In fact, the majority of patients in the nitisinone group (59 [86%] of 69) did not develop tyrosine-related symptoms, despite having very high serum tyrosine. Also, all patients who developed keratopathies did so during the first 3 years of the study. During year 4, there were no new cases.

In patients with keratopathies, lowering the nitisinone dose to 2 mg per day resulted in only minor decreases in serum tyrosine, which was consistent with results from a previously reported dose-response study,<sup>26</sup> and recurrent keratopathies were seen in several of those patients. No direct relationship between tyrosine levels and occurrence of these events could be seen. The ocular tyrosine concentrations are likely to be a causal factor for keratopathy, rather than those in the serum.

All patients were asked to reduce their protein intake. Decreasing dietary protein could have led to consumption of a diet containing more carbohydrates and fat, which could explain the weight gain seen in the nitisinone group, given that these patients were probably more likely to make the dietary change than those in the control group, because they were made aware of the risk of developing tyrosine-related ocular symptoms. In patients who develop keratopathies, plasma tyrosine levels should be monitored. A diet restricted in tyrosine and phenylalanine should be implemented to keep the plasma tyrosine level below 500 µmol/L, through active dietetic management in routine clinical practice. Such a strict diet, which was not possible to implement in SONIA 2, requires amino acid supplements, free of tyrosine and phenylalanine. In addition, nitisinone should be temporarily discontinued and reintroduced only when the keratopathy has resolved.

There were some limitations in SONIA 2. The inability to blind patients to nitisinone led to a trial design that could have affected the results of some subjective variables, including possibly leading to an under-reporting of adverse events in the control group. Morbid events, such as fractures and ruptures, were studied in an unselected population. For example, fracture intervention trials have traditionally been carried out in homogenous populations that have osteoporosis at recruitment; in SONIA 2, only some participants had osteopenia at recruitment, affecting the statistical significance of outcomes.27 The age of patients varied from 26 years to older than 70 years, with a large variation in disease severity. More dropouts occurred than anticipated due to patients with disabilities and poor mobility having to travel long distances to attend the study. It was especially hard to motivate the control patients to attend the final visit at month 48. Furthermore, continuous monitoring of serum tyrosine or a very strict dietetic management of expected tyrosinaemia was not possible because of patients being dispersed all over Europe and Jordan; such a measure could have possibly reduced dropouts due to keratopathies in the nitisinone group. In addition, a longer trial could have provided further insights for this slowly progressive disease; however, a longer duration was not logistically feasible.

In conclusion, we have shown that nitisinone 10 mg daily offers a biochemical cure for alkaptonuria, as shown by marked decreases in urine and serum HGA. This is the first randomised trial to show that nitisinone also reverses the ochronotic process as shown by a reduction in ear pigment, and it reduces the rate of disease progression as shown by a lower cAKUSSI score in the nitisinone group.

#### Contributors

LRR, NS, and JAG pioneered the idea for SONIA 2, secured funding, and managed the study. AKH finalised SONIA 2 logistics, wrote the protocol, and served as a medical monitor. ASD, ATH, JHH, AMM, BPN and ES did the metabolic analyses. DB, FG, AS, and AZ did the biomarker and genetic analyses. MB, NL, HB, MF, RF, MK, EL, AM, ST, SV, CW, and EW assisted in the conduct of the study and advised on the investigations, assessments, and processes. J-BA and KHLQS assisted with the conduct of the study in Paris. HG, RI, OL, VM, JR, JSe, RS, EV, and EZ assisted with the conduct of the study in Piešťany. JPD assisted in the conduct of the study. EEP and TFC planned and performed all statistic aspects of the study. TFC contributed to study design; EEP took over as the main trial statistician after TFC retired. DL and CvK managed and coordinated the clinical trial. BO, MR, and JSz contributed to the study design and interpretation of the results. AB contributed to interpretation of the results. JCJ and NPR edited the manuscript and contributed to planning and securing funding for the study. CS was responsible for study conduct. All authors contributed to drafting the manuscript and gave final approval of the manuscript.

#### Declaration of interests

LRR reports grants from the European Commission. AB reports personal fees from Swedish Orphan Biovitrum. FG reports grants from the EU, and is an employee and stock owner of Nordic Bioscience. AKH reports grants from the European Commission and is a shareholder of Cudos and PSR Group. BO, MR, and JSz are employees and shareholders of Swedish Orphan Biovitrum. CS and NS report that the AKU Society received a £10000 grant from Swedish Orphan Biovitrum towards organising an alkaptonuria patient workshop. All other authors declare no competing interests.

#### Data sharing

Data access will be granted in response to qualified research requests. All deidentified individual participant data, for patients with separate consent signed for this purpose, can be made available to researchers. Data will be shared on the basis of the scientific merit of the proposal (ie, the proposal should be scientifically sound, ethical, and have the potential to contribute to the advancement of public health) and the feasibility of the research proposal (ie, the requesting research team must be scientifically qualified and have the resources to conduct the proposed project). The data files would exclude data dictionaries that require user licenses. Data could be made available following finalised regulatory authority review and at the end of any data exclusivity periods, and ending 36 months after the regulatory authority review decision has been received or until the corresponding author is able to fulfil this obligation, whichever is earlier. Furthermore, the study protocol and statistical analysis plan can be made available. Proposals should be directed to j.a.gallagher@liverpool.ac.uk. Data requestors will need to sign a data access agreement.

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