Comparing nitisinone 2 mg and 10 mg in the treatment of alkaptonuria—An approach using statistical modelling

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Abstract
Background: Outcomes from studies employing nitisinone 10 mg and 2 mg in alkaptonuria were compared.

Patients and methods: Sixty-nine patients in each of the nitisinone (10 mg daily) and controls of suitability of nitisinone in alkaptonuria 2 (SONIA 2), as well as 37 and 23 in nitisinone (2 mg daily) and control cohorts at the National Alkaptonuria Centre (NAC), respectively, were followed up for 4 years. Severity of alkaptonuria (AKU) was assessed by the AKU Severity Score Index (AKUSSI). 24-h urine homogentisic acid (uHGA24), serum HGA (sHGA), serum tyrosine (sTYR) and serum nitisinone (sNIT) were also analysed at each time point. Dietetic support was used in the NAC, but not in SONIA 2. Safety outcomes were also compared. All statistical analyses were post hoc.

Results: The slope of the AKUSSI was 0.55, 0.19, 0.30, and 0.06 per month in the control NAC, nitisinone NAC, control SONIA 2, and nitisinone SONIA 2 cohorts, respectively. The intersection of the slopes on the x-axis was −132, −411, −295, and −1460 months, respectively. The control and nitisinone slope comparisons were statistically significant both in the NAC (p < 0.001) and in SONIA 2 (p < 0.001). Corneal keratopathy occurred in 3 and 10 patients in the NAC and SONIA 2, respectively.

Discussion: The nitisinone 10 mg dose decreased disease progression more than the 2 mg dose although the incidence of corneal keratopathy was 14.5% and 4.9%, respectively.

Conclusion: Nitisinone 10 mg decreased urine and serum HGA, increased serum tyrosine, and decreased disease progression more than 2 mg. Low-protein dietetic support may be needed to mitigate tyrosinaemia following nitisinone.
INTRODUCTION

Alkaptonuria (AKU; OMIM#203500) is an autosomal recessive genetic disorder characterised by deficiency of homogentisate dioxygenase activity (EC:1.13.11.5), with a prevalence of around 1 in 250,000 in most non-consanguineous societies. The inability to fully metabolise ingested dietary phenylalanine and tyrosine results in accumulation of homogentisic acid (HGA). The increase in circulating HGA is directly causal in the disease process, known as ochronosis, in which a melanin-like HGA pigment is deposited in connective tissues including cartilage, causing these tissues to break down.

Manifestations of AKU include premature arthritis, cardiac valve disease, fractures, and ruptures of muscle and tendon. Significant amounts of accumulating HGA is excreted in the urine and can lead not only to dark urine but also to renal stones.

Until recently, there was a lack of HGA-lowering disease-modifying therapy. In 1998, it was suggested that nitisinone, already in use as treatment of hereditary tyrosinaemia type 1 (HT-1; OMIM 276700), could also decrease HGA. Nitisinone inhibits the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD) (EC:1.13.11.27), thereby decreasing the accumulation of HGA. Ochronotic pigment develops from HGA; therefore, a decrease in HGA could decrease pigment, and consequently the morbidity of AKU. Initial studies while not carrying out a full dose–response study of nitisinone in AKU arrived at an oral dose of 2 mg daily, adopted for the first nitisinone interventional study.

2 METHODS

2.1 Patients

2.1.1 The NAC cohort

Patients with confirmed AKU, through documented increase in urine HGA (uHGA), attended the RLUH
between 2007 and 2020. Attendance between 2007 and 2011, a single non-interventional visit (VR), was part of a research study (Natural History study in alkaptonuria; UK Research Ethics Committee Number 07/Q1002/111). Some of these patients constitute the control group. The NAC provides a comprehensive service including off-label nitisinone. No patient received nitisinone prior to 2012. Patients who started attending the NAC after June 2012 are only included in the nitisinone group. The visits were designated as V0, V1, V2, V3, and V4, to denote baseline, 12, 24, 36, and 48 months in the study. The plan of the visits and numbers of patients are shown in Figure S1A, and this is, along with ethics and consent considerations, are described in more detail in the Supplementary Material. V0 was the baseline visit, and the duration between VR and V0 varied among control patients as the data were collected as part of providing a service. The 4-year nitisinone data from the NAC were chosen to allow a direct comparison with SONIA 2 which had a duration of 4 years.

2.1.2 | The SONIA 2 study cohort

SONIA 2 was a 4-year, open-label, evaluator-blinded, multicentre, randomised, no-treatment controlled, parallel-group study described further in the Supplementary Material (Figure S1B). The study sites were Liverpool (UK), Paris (France), and Piešťany (Slovakia). The aim was to recruit 140 patients, 70 in control and 70 randomised to nitisinone 10 mg, aged 25 years or older, with diagnosed AKU and any clinical manifestation in addition to increased HGA. The visits were designated as V0, V3, V12, V24, V36, and V48, to denote baseline, 3, 12, 24, 36, and 48 months in the study; this was named differently from NAC due to the extra 3-month visit. Patients who developed keratopathy were put on 2 mg nitisinone after temporary withdrawal and recovery. Independent ethics committees at each centre approved the study. All patients provided written informed consent prior to inclusion in the NAC and SONIA 2 cohorts.

2.1.3 | Assessment at every visit

The AKUSSI is a semi-quantitative composite score that collects data from a large number of assessments to calculate the severity of AKU, which, by not relying on a single disease feature, increases the likelihood of detecting a treatment effect. The AKUSSI items include the morbid features assessed by a variety of assessments including historical data and investigations. The NAC and SONIA 2 both employed the AKUSSI to determine the effect of nitisinone on disease progression.

2.1.4 | Management of tyrosinaemia following nitisinone therapy in the NAC and SONIA 2

At the NAC, a specialist dietician managed the increase in serum tyrosine, to defined serum tyrosine thresholds (Table S1), employing 7-day food diaries and blood spot serum tyrosine remote monitoring. The aim was to keep serum tyrosine (sTYR) below 500 μmol/L. In SONIA 2, the diet of patients was not actively managed, apart from providing information sheets regarding eating a lower protein diet. Details of how corneal keratopathy was managed in terms of assessment and nitisinone treatment are described in the Supplementary Material.

2.1.5 | Chemical and data analyses

In both studies, measurements of 24-huHGA24 and fasting serum for HGA (sHGA), tyrosine (sTYR), and nitisinone (sNIT) were carried out. Other assessments included a range of clinical outcome measures (AKUSSI), safety assessment, and other procedures as described elsewhere. There were minor differences in the AKUSSI scoring in the NAC and the SONIA 2 cohorts (Table S2A,B). However, the AKUSSI scoring of control and nitisinone comparisons within each of these two cohorts were identical.

2.1.6 | Statistical analysis

Continuous variables for metabolites uHGA24, sHGA, sTYR, and sNIT were compared using ANOVA with Tukey–Kramer test for multiple comparisons using Instat GraphPad 3. Two-sided 95% confidence intervals corresponding to a two-sided 5% level of significance were used throughout the analyses. SONIA 2 analyses described here were post hoc.

The progression of the AKU disease was calculated using linear regression of AKUSSI versus time to obtain the average slope of the change in AKUSSI. Models also included centred variables for age and gender to account for any effect of these demographics. The analyses were conducted for each of the four data sets separately. Backwards extrapolation based on the model slope parameters were performed to estimate the point in past
time at which AKUSSI is zero, that is, a hypothetical point just before disease features could have started. In terms of the analysis of slopes and intersection, data are presented in terms of medians (interquartile range). All analyses were conducted using a \( p < 0.05 \) to determine statistical significance, using R (Version 4.0).

2.1.7 | Role of the funding source

The funding sources were not involved in the study design, collection, analysis and interpretation of data, the writing of the manuscript, or in the decision to submit the manuscript for publication.

3 | RESULTS

3.1 | Baseline demographic and other parameters in the NAC and SONIA 2

The comparison of changes in uHGA\(_{24}\), sHGA, sTYR, sNIT, and AKUSSI, within the controls and the nitisinone groups, as well as between the controls and the nitisinone groups, both in the NAC and the SONIA 2, can be found in previous publications.\(^{11,12}\) However, for ease of access, relevant tables and figures containing these data are included in the Supplementary Material, but will not be discussed here, as the focus is on the direct comparison of the SONIA 2 and the NAC. In the NAC nitisinone group, there were 37 patients, 23 men and 14 women, with mean (±SD) age of 46.8 ± 14.5 years (Table 1). In the NAC control group, there were 23 patients, 15 men and 8 women, with a mean (±SD) age of 47.4 ± 14.8 years (Table 1). In the SONIA 2 nitisinone group, there were 69 patients, 45 men and 24 women; with mean (SD) age of 49.0 ± 11.3 years. The SONIA 2 control group consisted of 69 patients, 40 men and 29 women, with a mean age of 47.7 ± 10.2 years. uHGA\(_{24}\), sHGA, and sTYR tended to be higher in men than in women both in the NAC and SONIA 2.

3.2 | Changes in uHGA\(_{24}\) at follow-up in the NAC and SONIA 2

Changes in uHGA\(_{24}\) in the nitisinone and control groups in the NAC and SONIA 2 are shown in Figure S2A–D (Tables S3 and S4). Direct comparisons of baseline, 1-, 2-, 3-, and 4-year visits between SONIA 2 and NAC in the nitisinone group are shown in Table 2. uHGA\(_{24}\) was significantly higher at baseline but lower at the 1 year during nitisinone therapy in SONIA 2 compared with NAC.

3.3 | Changes in sHGA at follow-up in the NAC and SONIA 2

Changes in sHGA in the nitisinone and control groups in the NAC and SONIA 2 are shown in Figure S3A–D (Tables S3 and S4). Direct comparisons of baseline, 1-, 2-, 3-, and 4-year visits between SONIA 2 and NAC in the nitisinone group are shown in Table S2. sHGA was significantly lower at 1, 2, 3 and 4 years following nitisinone in SONIA 2 compared with NAC.

The percentage change against baseline (V0) was calculated for visits V12, V24, V36, and V48 in SONIA 2 and directly compared against corresponding values in the NAC namely, V1, V2, V3, and V4 (Table 3); these showed significantly greater lowering of uHGA\(_{24}\) by nitisinone for V12, V24, and V48 in the SONIA 2.

3.4 | Changes in sTYR at follow-up in the NAC and SONIA 2

Changes in sTYR in the nitisinone and control groups in the NAC and SONIA 2 are shown in Figure S4A–D (Tables S3 and S4). Direct comparisons of baseline, 1-, 2-, 3-, and 4-year visits between SONIA 2 and NAC in the nitisinone group are shown in Table 2. sTYR was significantly higher at 1 and 2 years following nitisinone in SONIA 2 compared with NAC.

The percentage change against baseline (V0) was calculated for visits V12, V24, V36, and V48 in SONIA 2 and directly compared against corresponding values in the NAC namely, V1, V2, V3, and V4 (Table 3); these showed significantly greater lowering of sHGA by nitisinone for V12, V24, and V48 in SONIA 2.

3.5 | Levels of sNIT at follow-up in the NAC and SONIA 2

Levels of sNIT in the nitisinone groups in the NAC and SONIA 2 are shown in Figure S5A,B. Direct comparisons of the 1-, 2-, 3-, and 4-year visits between SONIA 2 and NAC in the nitisinone group are shown in Table 2. sNIT was significantly higher at all time points following nitisinone in SONIA 2 compared with NAC. Despite
| TABLE 1 | Baseline demographic and related variables in the NAC and SONIA 2 studies—Mean (SD) |
|----------|-----------------------------------------------------------------------------------|
|          | Control group                                                                     | Nitisinone group                                                                     |
|          | All    | Male                  | Female                  | All    | Male                  | Female                  |
| National Alkaptonuria Centre (NAC) | | | | | | |
| Numbers of patients                     | 23 | 15 | 8 | 37 | 23 | 14 |
| Age (years)                              | 47.4 (14.8) | 44.3 (14.8) | 53.4 (13.7) | 46.8 (14.5) | 42.8 (12.9) | 53.4 (15.1) |
| Weight (kg)                              | 75.1 (18) | 83.1 (16.2) | 60.1 (10) | 74.4 (14) | 80.4 (11.2) | 64.6 (12.8) |
| Body mass index (kg/M^2)                 | 27 (5.2) | 28.4 (5.4) | 24.6 (4.1) | 26.9 (4.1) | 27.2 (3.6) | 26.4 (4.9) |
| uHGA_{24} (μmol/day)                     | 25 230 (13 973) | 30 013 (14 807) | 17 259 (9610) | 21 845 (9557) | 24 502 (10 361) | 17 669 (6464) |
| sHGA (μmol/L)                            | 28.3 (15.2) | 35.1 (13.9) | 17.0 (10.8) | 26.2 (13.1) | 26.1 (10) | 26.5 (17.6) |
| sTYR (μmol/L)                            | 51.4 (15.4) | 59.2 (14.4) | 38.3 (2.3) | 53.9 (43.7) | 59.4 (53.3) | 44.6 (16.9) |
| Number of patients with keratopathy      | 0 | 0 | 0 | 3 | 3 | 0 |
| SONIA 2                                   | | | | | | |
| Numbers of patients                      | 69 | 40 | 29 | 69 | 45 | 24 |
| Age (years)                              | 47.7 (10.2) | 48.1 (9.9) | 47 (10.7) | 49 (11.3) | 47.4 (11.9) | 51.9 (9.6) |
| Weight (kg)                              | 74.1 (15.6) | 80.4 (13.3) | 65.6 (14.6) | 74.8 (14.8) | 79.2 (12.6) | 66.3 (15.1) |
| Body mass index (kg/M^2)                 | 26.4 (4.6) | 27 (4.1) | 25.5 (5.2) | 26.9 (4.4) | 27.3 (4.2) | 26.2 (4.7) |
| uHGA_{24} (μmol/day)                     | 35 394 (13 868) | 38 740 (12 282) | 30 778 (14 797) | 35 019 (13 124) | 37 149 (12 583) | 31 024 (13 447) |
| sHGA (μmol/L)                            | 28.3 (8.7) | 29.1 (7.7) | 27.1 (9.8) | 30.3 (11) | 31.7 (11.2) | 27.9 (10.4) |
| sTYR (μmol/L)                            | 64.5 (15.5) | 69.4 (15.3) | 57.8 (13.1) | 65.3 (14.8) | 67 (13.7) | 62.2 (16.6) |
| Number of patients with keratopathy      | 0 | 0 | 0 | 10 | 8 | 2 |

**Abbreviations:** NAC, National Alkaptonuria Centre; sHGA, serum homogentisic acid; SONIA 2, suitability of nitisinone in alkaptonuria 2; sTYR, serum tyrosine; uHGA_{24}, 24-h urine homogentisic acid.
**TABLE 2** Direct comparison following nitisinone of uHGA24, sHGA, and sTYR concentrations at 12-, 24-, 36-, and 48-month visits in SONIA 2 and NAC—Mean (SD)

| Measurements | Visits | Cohorts | Baseline | 12 months | 24 months | 36 months | 48 months |
|--------------|--------|---------|----------|-----------|-----------|-----------|-----------|
| uHGA24 (μmol/day) | SONIA 2 | 35 019 (13 124)*** | 181 (401)*** | 990 (3870) | 1640 (6365) | 1542 (6166) |
| | NAC | 21 845 (9557) | 1249 (946) | 1328 (1187) | 1153 (847) | 1218 (978) |
| sHGA (μmol/L) | SONIA 2 | 30.3 (11.0) | 0.74 (1.67)*** | 2.07 (6.55)* | 2.49 (6.36)** | 2.23 (5.99)** |
| | NAC | 26.2 (13.1) | 4.33 (1.65) | 4.42 (2.93) | 3.47 (0.97) | 3.86 (2.2) |
| sTYR (μmol/L) | SONIA 2 | 65.3 (14.8) | 918 (205)*** | 884 (230)*** | 894 (285) | 875 (305) |
| | NAC | 53.9 (43.7) | 707 (153) | 701 (211) | 799 (156) | 782 (181) |
| sNIT (μmol/L) | SONIA 2 | 4.34 (1.86)*** | 5.04 (2.67)*** | 5.28 (2.74)*** | 5.91 (3.92)*** |
| | NAC | 1.26 (0.64) | 0.92 (0.41) | 1.02 (0.34) | 1.03 (0.67) |

Note: *p < 0.05; **p < 0.01; ***p < 0.001 for SONIA 2 versus NAC comparisons.

Abbreviations: NAC, National Alkaptonuria Centre; sHGA, serum homogentisic acid; SONIA 2, suitability of nitisinone in alkaptonuria 2; sTYR, serum tyrosine; uHGA24, 24-h urine homogentisic acid.

**TABLE 3** Direct comparison of percentage change in uHGA24, sHGA, and sTYR at 12-, 24-, 36-, and 48-month visits in SONIA 2 and NAC—Mean (SD)

| Measurements | Visits | Cohorts | 12 month | 24 month | 36 month | 48 month |
|--------------|--------|---------|----------|-----------|-----------|-----------|
| uHGA24 (μmol/day) | SONIA 2 | −99.5 (0.89)*** | −98.3 (5.8)*** | −97.1 (13.0) | −97.7 (8.7)a |
| | NAC | −94.0 (4.9) | −93.7 (6.0) | −94.6 (3.8) | −94.3 (4.0) |
| sHGA (μmol/L) | SONIA 2 | −97.5 (5.3)*** | −92.7 (23.7)** | −89.4 (31.0) | −91.5 (21.2)a |
| | NAC | −80.6 (9.5) | 80.2 (18.5) | −84.3 (10.2) | −80.9 (16.1) |

Note: *p < 0.05; **p < 0.01; ***p < 0.001 for SONIA 2 versus NAC comparisons.

Abbreviations: NAC, National Alkaptonuria Centre; sHGA, serum homogentisic acid; SONIA 2, suitability of nitisinone in alkaptonuria 2; sTYR, serum tyrosine; uHGA24, 24-h urine homogentisic acid.

**TABLE 4** The mean (SD) of the slopes and intersection on the x-axis of the control and nitisinone groups in the NAC and SONIA 2 are shown

| Measurements | NAC control | NAC nitisinone 2 mg | SONIA 2 control | SONIA 2 nitisinone 10 mg |
|--------------|-------------|---------------------|----------------|-------------------------|
| Mean (SD) age at baseline (years) | 47.4 (14.8) | 46.8 (14.5) | 47.7 (10.2) | 49.0 (11.3) |
| Mean (SD) AKUSSI score at baseline | 72.9 (42.3) | 76.1 (37.8) | 80.5 (33.4) | 87.0 (34.2) |
| Slope AKUSSI score per month—mean (SEM) | | | | |
| Unadjusted | 0.55 (0.15) | 0.19 (0.074)* | 0.30 (0.06) | 0.06 (0.065)* |
| Age | 0 (0.006) | 0 (0.003) | 0.05 (0.12) | 0.18 (0.126) |
| Sex | 0 (0.192) | 0.07 (0.099) | 0 (0.006) | −0.01 (0.006) |
| X-axis intersection—years (months) | −11.0 (−132) | −34.25 (−411) | −24.58 (−295) | −121.67 (−1460) |
| Disease onset in control or delayed to age using age of onset in nitisinone group (years) | 36.4 | 70.65 | 23.12 | 144.79 |
| Nitisinone-induced delay compared to control (years) | 23.25 | 97.09 |

Note: The delay due to 2 mg and 10 mg nitisinone is shown. Data adjusted for age and sex are also shown. *NAC control versus NIT 2 mg p < 0.001 and SONIA 2 Control versus NIT 10 mg p < 0.001.

Abbreviations: AKUSSI, Alkaptonuria Severity Score Index; NAC, National Alkaptonuria Centre; NIT, nitisinone; SONIA 2, suitability of nitisinone in alkaptonuria 2.
higher sNIT at later visits in SONIA 2, sHGA tended to increase over time (Figure S5C,D).

### 3.6 | Slope and intersection of AKUSSI in the NAC and SONIA 2

In the NAC, AKUSSI scores rose on average at a rate of 0.55 per month in the control group, and extrapolating backwards to the x-axis gives an intersection at an average time for an AKUSSI score equal to 0 at −132 months (minus 11 years). In the NAC nitisinone group, AKUSSI scores rose on average at a rate of 0.19 per month, and extrapolating backwards to the x-axis gives an intersection at an average time for an AKUSSI score equal to 0 at −295 months (minus 24.58 years). In SONIA 2 nitisinone group, AKUSSI scores rose on average at a rate of 0.06 per month, and extrapolating backwards to the x-axis gives an intersection at an average time for an AKUSSI score equal to 0 at −1460 months (minus 121.67 years; Figure 1C,D; Table 4).

All models were adjusted for patient age and sex (as centred variables). For no model was there any
evidence of meaningful impact for either age or sex. Adjustment for age and sex in the NAC and SONIA 2 data sets had no notable impact on the slope analyses (Table 4).

### 3.7 Corneal keratopathy in the NAC and SONIA 2

In the NAC, there were three patients, out of a total of 60 patients treated with nitisinone, a frequency of 5%, with corneal keratopathy as shown in Table 5. All three were male. Patient 1 continued with the lower protein diet at 2 mg nitisinone daily. Patient 2 was on 2 mg nitisinone twice weekly and was poorly compliant with the lower protein diet. Patient 3 died from carcinoma of the stomach which came to light at the same time as the keratopathy.

In the SONIA 2, there were 10 patients with keratopathy, with 9 confirmed by slit-lamp examination, consisting of 2 women and 8 men (Table 5); one patient had clinical features of keratopathy unconfirmed by slit-lamp examination due to not returning for this assessment. Of the patients with slit-lamp confirmed keratopathy, resolution following discontinuation was only confirmed in eight patients. Five of the nine confirmed cases were bilateral corneal keratopathies. The age ranged from 29 to 69 years.

| Patient no. | Sex | Age at onset (years) | Dose nitisinone (mg) | Onset keratopathy (months) | Slit-lamp confirmation | sTYR μmol/L a |
|-------------|-----|---------------------|---------------------|---------------------------|------------------------|--------------|
| NAC         |     |                     |                     |                           |                        |              |
| 1           | M   | 25                  | 2                   | 36, B                     | Yes                    | 964          |
| 2           | M   | 21                  | 2                   | 1.5, U                    | Yes                    | 941          |
| 3           | M   | 55                  | 2                   | 3, B                      | No                     | NA           |
| SONIA 2     |     |                     |                     |                           |                        |              |
| 4           | F   | 56                  | 10                  | 3, B                      | Yes                    | 1022         |
| 5           | F   | 69                  | 10                  | 14, U                     | No                     | 1191         |
| 6           | M   | 50                  | 10                  | 12, U                     | Yes                    | 1236         |
| 7           | M   | 39                  | 10                  | 6, B                      | Yes                    | 1118         |
| 8           | M   | 54                  | 10                  | 1, U                      | Yes                    | 816          |
| 9           | M   | 44                  | 10                  | 13, U                     | Yes                    | 1036         |
| 10          | M   | 29                  | 10                  | 36, B                     | Yes                    | 1149         |
| 11          | M   | 41                  | 10                  | 30, U                     | Yes                    | 976          |
| 12          | M   | 51                  | 10                  | 24, U                     | Yes                    | 934          |
| 13          | M   | 43                  | 10                  | 6, U                      | Yes                    | 609          |

**Abbreviations:** B, Bilateral (following nitisinone in months); F, female; M, male; U, unilateral (following nitisinone in months).

*aLast serum tyrosine value before onset of keratopathy or withdrawal of nitisinone; serum tyrosine not always measured at diagnosis of keratopathy.

Overall, across SONIA 2 and the NAC, there were 11 men and 2 women who developed keratopathy. The numbers of patients with keratopathy in SONIA 2 at the Liverpool, Piešťany, and Paris sites were six, three, and one, respectively. The sTYR at the three sites in the nitisinone group was comparable, while the NAC values were lower (Table S5). In the keratopathy patients, most of the sTYR values were high despite such measurements not having been made immediately after the onset of keratopathy.

### 4 DISCUSSION

The current data analysis was carried out to assess the difference between the 2 mg and 10 mg daily doses of nitisinone used clinically in AKU. Prior publications suggest that data on disease progression, metabolic efficacy, and safety outcomes of both the nitisinone 2 mg daily and 10 mg daily are beneficial in AKU, with a greater HGA-lowering effect at increasing doses. The nitisinone 10 mg dose has been approved by the European Medicines Agency for the treatment of adult patients with AKU. Nitisinone was initially developed for use in HT-1, where the daily dose recommended is between 1 to 2 mg/kg body weight, with dietetic restriction of tyrosine and phenylalanine. To manage
tyrosinaemia during nitisinone therapy, tyrosine and phenylalanine-free amino acid supplements are widely used in addition to dietetic protein restriction; however, the efficacy of special amino acid supplements remains unproven as these are unpalatable. During the initial evaluation of nitisinone in AKU, doses such as 0.7 mg daily, 2.8 mg daily,2 2.1 mg,9 and 2 mg10 both as once- or twice-daily administration were used. A formal dose–response of nitisinone in AKU carried out in SONIA 1 showed that an 8 mg dose was more efficacious than 2 mg in HGA-lowering effect, with comparable effects on sTYR and short-term safety.14 The commercially available 10-mg capsule was then used in the SONIA 2 outcomes study.12 A later smaller study confirmed the metabolic superiority of nitisinone 8 mg compared to 2 mg in terms of decreasing the HGA, and like SONIA 1, also that serum tyrosine plateaued despite increasing nitisinone dose.23 One report described the use of 0.2 mg daily dose of nitisinone in a pregnant woman.24

Progressive symptomatic AKU develops after the first two decades in life, as seen in Figure 2. The delay in AKU progression was studied in the NAC and SONIA 2. When one examines the NAC first, the untreated AKUSSI slope intersects the x-axis (the time when it reaches zero) at minus 11 years. Since the baseline age in NAC was 47.4 years, the NAC intersect is at age 36.4 (47.4 – 11) years. In other words, AKUSSI increases from age 36.4 years to reach baseline values at 47.4 years. The NAC nitisinone 2 mg AKUSSI slope intersects the x-axis at minus 34.25 years, a further 23.25 years delay. If nitisinone therapy was started at 36.4 years, when AKUSSI was zero, it would reach baseline AKUSSI values by age 70.65 (47.4 + 23.25) years, an overall delay of 23.25 (70.65 / 47.4) years.

In SONIA 2, the untreated AKUSSI slope intersects the x-axis at minus 24.58 years. Since baseline age in SONIA 2 was 47.7 years, the AKUSSI slope intersect is at age 23.12 (47.7 – 24.58) years. In other words, AKUSSI increases from age 23.12 years to reach baseline values at 47.7 years. The nitisinone 10 mg AKUSSI slope intersects the x-axis at minus 121.67 years. If nitisinone therapy was started at 23.12 years, it would reach baseline AKUSSI values by age 144.79 (23.12 + 121.67) years. The overall delay of AKU disease by 10 mg nitisinone is 97.09 (144.79 / 47.7) years. Disease progression is reduced by 23.25 and 97.09 years on nitisinone 2 mg and 10 mg, respectively, compared to no nitisinone.

Our direct comparisons between matched visits of SONIA 2 and NAC showed greater decreases following nitisinone in uHGA24 in SONIA 2 transiently. Unlike urine, sHGA has a causal role in the morbidity of AKU. In contrast to the transitory difference for uHGA24, the absolute values of sHGA showed significantly lower values following nitisinone in SONIA 2, and the percentage change was greater compared with the NAC, suggesting that 10 mg nitisinone had a greater HGA lowering effect than the 2 mg dose.

Nitisinone lowers HGA but increases circulating and tissue tyrosine levels.25 Direct comparison of sTYR between matched SONIA 2 and NAC visits showed lower levels in the NAC. There were also fewer keratopathies in the NAC, due to active management of the diet and possibly the lower dose. However, data demonstrate that
even a dose as low as 1 mg daily increases sTYR above the target safety levels.14

The 10 mg nitisinone resulted in higher sNIT than the 2 mg dose. What has not been previously reported is the much higher sNIT values in the later visits of SONIA 2 only, but not in the NAC (Figure S5A–D). Nitisinone has been shown to inhibit cytochrome P450 mixed-function oxidases and has been associated with drug interactions as a result;26 we suggest that higher doses of nitisinone may inhibit its own catabolism over time leading to an increase in sNIT concentrations, as it is also catabolised by the cytochrome P450 mixed-function oxidases.27 Despite this increase in sNIT later on in SONIA 2, paradoxically sHGA did not decrease further; nitisinone has been shown to inhibit organic anion transporters and putative mediators of renal HGA excretion and may account for this effect at the higher dose (Figure S5A–D).28

Nitisinone-induced tyrosinaemia causes reversible dendritiform corneal keratopathy. In the NAC, there were three cases of keratopathy, a prevalence of 5%, all in the first 3 years after service commissioning, and it is likely that a dietetic management enabled effective management of the sTYR and mitigated the risk of keratopathy. In SONIA 2, there were 10 cases of keratopathy out of 69 nitisinone-treated patients, a prevalence of 14.5%, where no formal dietetic input was used. Most of the cases of keratopathy exhibited sTYR greater than 900 μmol/L, consistent with previous data.29 Dietetic support following nitisinone in adult AKU patients habituated to consume protein unrestricted in adult AKU patients may minimise the occurrence of keratopathy.

Only two of the 13 patients with keratopathy were women, the reason being unclear. The single case of keratopathy reported in the NIH clinical trial was also male.10 Rats developed corneal keratopathy but not mice, and male rats have higher levels of sTYR compared to female rats following HPPD inhibitor administration, possibly due to sex hormone influence. We suggest that a similar mechanism may explain the male predisposition for keratopathy during nitisinone both in the SONIA 2 and the NAC.30–32

The data comparison carried out here has limitations. The SONIA 2 was a randomised trial while the NAC is a service. Nevertheless, the NAC data set collection was protocol-driven and also overlapped temporally with SONIA 2. SONIA 2 was carried out in Liverpool, Pécsfány, and Paris, while the NAC was only carried out in Liverpool. Similar AKUSSI assessments and outcomes have been collected in these two cohorts, but they were not identical. Diet was managed in the NAC, but not in SONIA 2, and may have influenced some outcomes especially regarding tyrosine and safety. The current analysis is important as it is unlikely that a direct comparison of nitisinone 2 mg and 10 mg on disease progression will be carried out in a clinical study.

In conclusion, nitisinone 10 mg leads to greater decreases in urine and serum HGA, due to greater HPPD inhibition, and greater increases in serum tyrosine, and more profoundly decreases disease progression. It is likely that a lower protein diet would improve safety following nitisinone at the 10 mg dose.

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CONFLICT OF INTERESTS
Nicolas Sireau received payments to the AKU Society to develop a health passport, a patient survey, a patient workshop, a video, a talk at a workshop, and a children's information booklet. Lakshminarayan R. Ranganath received fees for lectures and consultations from Swedish Orphan Biovitrum. Anna M Milan, Andrew T Hughes, Milad Khedr, Brendan P. Norman, Mohammed Alsbou, Richard Imrich, Matthew Gornall, James A. Gallagher, and Richard Jackson declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS
Lakshminarayan R. Ranganath, Nicolas Sireau, and James A. Gallagherpioneered the idea for SONIA 2, secured funding, and managed the study, drafting manuscript, and final approval of the manuscript. Lakshminarayan R. Ranganath and Milad Khedr are clinicians running the NAC service. Anna M. Milan, Andrew T Hughes, and Brendan P. Norman carried out the metabolic analyses, drafting manuscript, and final approval of the manuscript. Milad Khedr and Mohammed Alsbou assisted with conduct of the SONIA 2 study in Liverpool and Pécsfány, respectively, drafting manuscript, and final approval of the manuscript. Assisted with conduct of the SONIA 2 study.
in Paris, drafting manuscript, and final approval of the manuscript. Richard Imrich assisted with conduct of study in Piešťany, drafting manuscript, and final approval of the manuscript. Matthew Gornall and Richard Jackson carried out all statistical aspects including the slope analysis, drafting manuscript, and final approval of the manuscript.

**DATA AVAILABILITY STATEMENT**

SONIA 2 data access will be granted in response to qualified research requests. All de-identified individual participant data, for patients with separate consent signed for this purpose, can be made available to researchers. Data will be shared based on the scientific merit of the proposal, that is, the proposal should be scientifically sound, ethical, and have the potential to contribute to the advancement of public health as well as the feasibility of the research proposal, that is, 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 licences. Data could be made available following finalised regulatory authority review and end of any data exclusivity periods and ending after 36 months or until corresponding author is able to fulfil this obligation whichever is earlier. Further, the study protocol and statistical analysis plan can be made available. Proposals should be directed to j.a.gallagher@liverpool.ac.uk to gain access. Data requestors will need to sign a data access agreement. The data from the NAC can also similarly made available. Requests for data can be made to lrang@liv.ac.uk or milad.khedr@liverpoolft.nhs.uk.

**ETHICS STATEMENT**

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

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**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher's website.
Figure S5. (A–D) sNIT in the nitisinone groups of the NAC (upper left panel, S5A) and SONIA 2 (upper right panel, S5B) is shown. In SONIA 2 (S5B), values at V48 were increased compared to V3 ($p < 0.01$), and V12 ($p < 0.05$), indicating an increase over the 4 years. In SONIA 2, sNIT (lower left panel, S5C) and sHGA (lower right panel, S5D) without baseline values show trend to increase in sHGA in later visits despite increase in sNIT.

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