**Suitability Of Nitisinone In Alkaptonuria 1 (SONIA 1): An international, multicenter, randomized, open-label, no-treatment controlled, parallel-group, dose-response study to investigate the effect of once daily nitisinone on 24-hour urinary homogentisic acid excretion in patients with alkaptonuria after 4 weeks of treatment**

Lakshminarayan R Ranganath, FRCP(Edin), FRCPath[1], Anna M Milan PhD, FRCPath[1], Andrew T Hughes MPhil[1], John J Dutton FIBMS[1], Richard Fitzgerald MRCP[2], Michael C Briggs FRCS[3], Helen Bygott BSc[1], Eftychia E Psarelli MSc[4], Trevor F Cox PhD[4], James A Gallagher PhD[5], Jonathan C Jarvis PhD[6], Christa van Kan[7], Anthony K Hall MBBS, BSc, AKC[8], Dinny Laan MSc[7], Birgitta Olsson MSc[9], Johan Szamosi MSc[9], Mattias Rudebeck MSc, BMedSc[9], Torbjörn Kullenberg MD[9], Arvid Cronlund MSc[9], Lennart Svensson PhD[9], Carin Junestrand DDS[9], Hana Ayoob BA[10], Oliver G Timmis BA[10], Nicolas Sireau PhD[10], Kim-Hanh Le Quan Sang[11], Federica Genovese PhD[12], Daniela Braconi PhD[13], Annalisa Santucci PhD[13], Martina Nemethova MSc[14], Andrea Zatkova PhD[14], Judith McCaffrey MSc[15], Peter Christensen PhD[16], Gordon Ross PhD[16], Richard Imrich MD, PhD[17], Jozef Rovensky[17].

**Affiliations:**

[1]Department of Clinical Biochemistry and Metabolism, Royal Liverpool University Hospital, Prescot Street, Liverpool, L7 8XP, UK

[2]Department of Clinical Pharmacology, Royal Liverpool University Hospital, Prescot Street, Liverpool, L7 8XP, UK

[3]Department of Ophthalmology, Royal Liverpool University Hospital, Prescot Street, Liverpool, L7 8XP, UK

[4]Cancer Research UK Liverpool Cancer Trials Unit, University of Liverpool, Block C, Waterhouse Building, 1-3 Brownlow Street, L69 3GL

[5]Department of Musculoskeletal Biology, University of Liverpool, Liverpool L69 3GE, UK

[6]School of Sport and Exercise Science, Liverpool John Moores University, Liverpool L33AF

[7]PSR group B.V., 2132 HN Hoofddorp, Netherlands

[8]Cudos BV, 2132 HN Hoofddorp, Netherlands

[9] Swedish Orphan Biovitrum AB (publ), SE-11276 Stockholm, Sweden

[10] AKU Society, 66 Devonshire Road, Cambridge, CB 1 2BL, UK

[11]Hôpital Necker-Enfants Malades, 149, rue de Sèvres, 5743 Paris Cedex 15, France

[12]Nordic Bioscience, Herlev Hovedgade 207, DK-2730 Herlev, Denmark

[13]Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, via Fiorentina 1, 53100, Siena, IT

[14]Laboratory of Genetics, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlarska 5, 833 34, Bratislava, Slovakia

[15]Agilent Technologies Ireland, Euro Business Park, Little Island, Cork, Ireland

[16]Agilent Technologies, 5500 Lakeside, Cheadle Royal Business Park, Stockport SK8 3GR

[17]National Institute of Rheumatic Diseases, Nabrezie, Ivana Krasku 4, SK-921 01, Piešťany, Slovakia

**Correspondence:**

Lakshminarayan R Ranganath, FRCP(Edin), FRCPath

Department of Clinical Biochemistry and Metabolism

Royal Liverpool University Hospital

Prescot Street

Liverpool, L7 8XP,

UK

Telephone: 0151 706 4197 FAX: 0151 706 5813 Email: lrang@liv.ac.uk

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**ABSTRACT**

**Background**

Alkaptonuria (AKU) is a serious genetic disease characterized by premature spondyloarthropathy. Homogentisate-lowering therapy is being investigated for AKU. Nitisinone decreases homogentisic acid (HGA) in AKU but the dose-response relationship has not been previously studied.

**Methods**

SONIA 1 was an international, multicenter, randomized, open-label, no-treatment controlled, parallel-group, dose-response study. The primary objective was to investigate the effect of different doses of once daily nitisinone on 24-hour urinary HGA excretion (u-$HGA_{24}$) in patients with AKU after 4 weeks of treatment. Forty patients were randomized into 5 groups of 8 patients each, with groups receiving no treatment or 1, 2, 4 and 8 mg of nitisinone.

**Findings**

A clear dose-response relationship was observed between nitisinone and the urinary excretion of HGA. At 4 weeks, the adjusted mean u-$HGA_{24}$ was 31152, 3846, 1668, 686 and 327 μmol for the no treatment or 1, 2, 4 and 8 mg doses respectively.

For the most efficacious dose, 8 mg daily, this corresponds to a mean reduction of u-$HGA_{24}$ of 98·8% compared to baseline. An increase in tyrosine levels was seen at all doses but the dose-response relationship was less clear than the effect on HGA. Despite tyrosinaemia, there were no safety concerns and no serious adverse events were reported over the 4 weeks of nitisinone therapy.

**Conclusion**

In this study in AKU patients, nitisinone therapy decreased urinary HGA excretion to low levels in a dose-dependent manner and was well tolerated within the studied dose range.

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## INTRODUCTION

Alkaptonuria (AKU) is a serious, autosomal recessive, multisystem disorder [1-3] affecting approximately one in every 250,000 people [4], although some countries such as Slovakia and the Dominican Republic have a higher prevalence rate of around one in 19,000 [5,6]. Morbidity in AKU is caused by increased levels of homogentisic acid (2,5-dihydroxyphenylacetic acid, HGA) due to a deficient enzyme, homogentisate 1,2-dioxygenase (HGD) [7]. Despite efficient urinary excretion of the HGA [4], some of it is oxidized to a melanin-like polymeric pigment via benzoquinone acetic acid (BQA). This pigment polymer is deposited in connective tissues, particularly cartilage, a process termed ochronosis [8], leading especially to severe premature arthritis with an early onset, affecting the spine and synovial joints, large and small 3,4].

Current treatments are limited to palliative analgesia and arthroplasty [9]. Nitisinone , is a competitive inhibitor of the enzyme 4-hydroxyphenyl-pyruvate dioxygenase (HPPD), decreasing the formation of HGA [10,11]. It is hypothesized that if HGA levels are reduced before the onset of overt ochronosis, this might prevent the development of the debilitating features of the disease. While nitisinone has been shown to reduce plasma HGA levels and urinary excretion in patients with AKU [12,13], and in a mouse model of AKU [14], it has not undergone any formal clinical development for AKU although three published investigator-initiated studies have been completed [4,12,13]. The 3-year study by Introne et al showed a significant reduction in urine HGA concentrations by about 95% using a daily dose of 2 mg of nitisinone but no significant effect on clinical parameters [13]. One possible factor for the inconclusive effect on clinical parameters may be the use of a sub-optimal dose.

SONIA 1 is part of the DevelopAKUre program, which has received funding from the European Union Seventh Framework Program (FP7). The objective of the clinical development program is to investigate the possibility of an effective and safe treatment of AKU.

The present study was designed to investigate the relationship between different doses of nitisinone and u-$HGA_{24}$. A separate study of u-$HGA_{24}$ in non-AKU patients was performed in parallel in order to define normal ranges [15].

## METHODS

### Patients

Patients with a well-documented AKU verified by increased urine HGA excretion and who were at least 18 years old were eligible for inclusion in the study. Details of inclusion and exclusion criteria are described in the supplementary appendix (Table S1). In all patients, diagnosis of AKU was confirmed by *HGD* gene mutation identification performed during the study (data not shown).

### Study Design and Intervention

SONIA 1 was a randomized, open-label, parallel-group study with a no-treatment control group. Patients were randomized to receive either 1, 2, 4 or 8 mg nitisinone once daily or no treatment (control). Forty patients were randomized, equally distributed amongst the groups (8 patients per group). The study design is summarized in Figure 1.

The study was open label, since it is not feasible to blind a study with HGA-lowering treatment in AKU. One of the cardinal signs of AKU is urine darkening on standing as HGA is oxidized. Patients could therefore easily know whether they were on nitisinone or not. Furthermore, any personnel involved at the investigative sites who were involved in the processing of urine samples would also be able to see this difference. Since this study used objective assessments of efficacy (changes in HGA levels), the open design was unlikely to have introduced bias. The only subjective reporting in the study was that of adverse events. Patients were requested to maintain stable dietary habits during the 4-week study period in order not to change their dietary protein intake.

### Rationale for dose selection

The choice of doses used in the study was based on current knowledge regarding the HGA-lowering effect of nitisinone. In one study, mean u-$HGA_{24}$ was reduced from 4 g/day to 230 mg/day using a dose of 2·1 mg of nitisinone daily [12]. In a 3-year study, using a dose of 2 mg daily, mean u-$HGA_{24}$ was decreased from 5·1 g/day to values ranging from 113 to 203 mg/day during the course of the study, which on average corresponded to a 95% decrease [13]. We wanted to investigate the effect of nitisinone on HGA at higher doses than the ones used in previous studies. At the same time, we were interested in determining the effect of nitisinone on serum tyrosine levels at a dose lower than 2 mg daily. Therefore, doses of 1, 2, 4 and 8 mg were used in this study. The daily dosing frequency is based on the long half-life of nitisinone [11].

A suspension of nitisinone (Orfadin®) containing 4 mg/mL administered in the morning was used as it allowed easy administration of the selected doses.

**Randomization procedures**

Patients were randomly assigned to one of five groups, in a 1:1:1:1:1 ratio stratified by study centre using randomly permuted blocks. Results for HGA and tyrosine were not accessible to the medical monitors, sponsor personnel, or study site personnel until study completion.

**Prior and concomitant therapy**

Patients were allowed to continue on any chronic medication and any changes during the study were recorded, from the time of the screening and randomization visit until the follow-up telephone call. Patients were not allowed to have used nitisinone within the 60 days prior to randomization.

**Treatment compliance**

Product accountability records were kept by the pharmacy and investigator. All unused IMP was returned to the clinical study sites and measured. The amount consumed was compared to the expected consumption for the randomized dose.

**Chemical measurements**

*Urine sample collection and handling*

At baseline, and weeks 2 and 4, urine was collected over 24 hours into 2·5 L bottles containing 30 mL of 5N $H_2SO_4$ and stored away from bright light in cool conditions. The weight of the collected urine was recorded and used as the volume in the calculations of u-$HGA_{24}$ assuming a density of 1g/mL. An aliquot of the collected urine was frozen and kept at -20ºC until analysis.

*Serum sample collection and handling*

Measurements of serum tyrosine (s-Tyr) and HGA (s-HGA) concentrations were performed at weeks 0, 2 and 4. At each visit one sample was collected pre-dose in fasting patients. Blood samples were collected in non-gel serum tubes. An aliquot of serum was immediately acidified using perchloric acid (10% v/v 5·8M), and kept frozen at -20ºC until analysis.

*Analyses of HGA and tyrosine*

The concentrations of tyrosine and HGA in serum and urine were measured by liquid chromatography tandem mass spectrometry [16,17]. All analyses were performed on an Agilent 6490 Triple Quadrupole mass spectrometer with Jet-Stream® electrospray ionisation (ESI-MS/MS) coupled with an Agilent 1290 infinity UHPLC pump and HTC autosampler. This method incorporates reverse-phase chromatographic separation on an Atlantis C18 column (100mmx3·0mm, 3µm). Initial conditions of 80:20 water:methanol with 0·1% formic acid (v/v) increased linearly to 10:90 over five minutes. Matrix-matched calibration standards and quality controls were utilized with appropriate isotopically labelled internal standards. Quantitation was achieved in multiple reaction mode (MRM) with two product ion transitions for both tyrosine (positive ionisation) and HGA (negative ionisation). Samples were prepared by dilution in a combined internal standard solution (final concentrations of 0·4µmol/L $^{13}C_6$-HGA and 2µmol/L $d_2$-tyrosine in 0·1% formic acid (v/v) in

deionised water). All serum and urine quantitation analysis were performed by the Department of Clinical Biochemistry and Metabolic Medicine at the Royal Liverpool University Hospital (RLUH).

**Endpoints**

The primary endpoint was the u-$HGA_{24}$ in patients with AKU after 4 weeks of nitisinone treatment. Secondary endpoints supporting the primary objective included u-$HGA_{24}$ after 2 weeks, as well as the u-HGA/u-creatinine ratio at Weeks 2 and 4.

Secondary endpoints included the pre-dose serum HGA concentration (s-HGA) and serum tyrosine concentration (s-Tyr) in patients with AKU at Weeks 2 and 4.

**Safety Assessment**

At each visit, adverse events (AEs) and laboratory values were recorded. Routine laboratory processes at each clinical study site were employed to measure biochemistry and haematology profiles. At each visit a corneal slit lamp examination was performed to check for possible corneal toxicity.

**Study oversight**

The study was conducted at two sites, Liverpool (United Kingdom) and Piešťany (Slovakia) from May to October 2013. Data were recorded by investigators at each site, collected, and monitored by the Contract Research Organization PSR Group (Amsterdam, Netherlands). The protocol and amendments were approved by the relevant ethics review boards and national regulatory authorities. Written informed consent was obtained from all patients before any study procedures. An external Data and Safety Monitoring Board was assigned to evaluate the safety data.

**Statistical analysis**

The primary variable u-$HGA_{24}$ at Week 4 was analyzed using a mixed model for repeated measures (MMRM). The model included the study site, treatment group, visit, and the interaction between treatment group and visit as fixed factors and the baseline u-$HGA_{24}$ as a covariate. Model-based least square means and associated

95% confidence intervals for each treatment group were calculated. The analysis was conducted on the full analyses set (FAS). There were no missing data for the primary and secondary variables. Statistical analyses were carried out using SAS v9·3.

### Analysis of safety and tolerability data

*Adverse events*

All adverse events (AEs) during the study were coded using the Medical Dictionary for Regulatory Activities (MedDRA version 16·0). The incidence of adverse events were summarized in frequency tables. The changes in safety laboratory parameters from baseline to all post-baseline visits were summarized by treatment group and visit using descriptive statistics.

### Role of the Funding Source

This study was funded by grants from the European Union Framework Programme 7 which had no direct participation in any aspect of design and conduct of the study, drug supply or reporting.

## RESULTS

### Patients and Study Treatment

15 and 25 patients with AKU from clinical study sites in Liverpool and Piešťany respectively were randomized into 5 groups (no treatment, 1, 2, 4 and 8 mg groups). All randomized patients completed the study. Patient demographics and baseline characteristics were similar across the 5 groups (Table 1). The majority (67·5%) were male, and the mean age for all patients was 47·2 years ranging from 19 to 63 years. 38 were Caucasian and 2 were Asian. Baseline s-HGA, s-Tyr and u-$HGA_{24}$ are shown in Table 2.

There were no protocol deviations that affected the interpretation of the results.

**Table 1. SONIA 1 Demographic and baseline data [Mean (SD)]**

| | | Untreated (N=8) | 1 mg (N=8) | 2 mg (N=8) | 4 mg (N=8) | 8 mg (N=8) | Total (N=40) | P-value* |
|---|---|---|---|---|---|---|---|---|
| Age (years) | | 45·9 (15·3) | 44·4 (10·9) | 43·9 (13·7) | 47·3 (10·7) | 54·4 (7·3) | 47·2 (11·9) | 0·3336 |
| Body weight (kg) | | 71·0 (23·5) | 86·9 (15·9) | 74·6 (10·9) | 76·9 (14·3) | 81·1 (13·7) | 78·1 (16·3) | 0·2038 |
| Height (cm) | | 165·3 (12·1) | 170·6 (7·1) | 167·1 (9·4) | 168·4 (5·9) | 165·9 (6·7) | 167·5 (8·3) | 0·7509 |
| Gender n (%) | Female | 4 (50·0) | 1(12·5) | 3 (37·5) | 3 (37·5) | 2(25·0) | 13(32·5) | 0·7098 |
| | Male | 4 (50·0) | 7(87·5) | 5 (62·5) | 5 (62·5) | 6(75·0) | 27(67·5) | |
| Race n (%) | White | 7 (87·5) | 7(87·5) | 8(100) | 8 (100) | 7(87·5) | 37(92·5) | 1·000 |
| | Asian | 1 (12·5) | 1(12·5) | 0 (0·0) | 0 (0·0) | 0 (0·0) | 2 (5·0) | |
| | Others | 0 (0·0) | 0 (0·0) | 0 (0·0) | 0 (0·0) | 1(12·5) | 1 (2·5) | |

* P-values: Age/ Body Weight/ Height = Wilcoxon rank-sum – Kruskal Wallis, Gender, Race = Fisher's exact test.

**Table 2. Mean (SD) u-HGA$_{24}$, u-HGA/creatinine, s-HGA and s-Tyr in untreated AKU patients and AKU patients receiving nitisinone 1, 2, 4 and 8 mg daily**

| | **Untreated N=8** | **1 mg N=8** | **2 mg N=8** | **4 mg N=8** | **8 mg N=8** |
|---|---|---|---|---|---|
| **u-HGA$_{24}$ (µmol)** | | | | | |
| Baseline | 29836 (5067) | 36757 (14614) | 31440 (7388) | 35453 (13581) | 27354 (5235) |
| Week 4 | 31042 (4603) | 3931 (1707) | 1602 (842) | 734 (430) | 146 (51) |
| **u-HGA/creatinine (µmol/mol)** | | | | | |
| Baseline | 2827 (876) | 2273 (315) | 3074 (318) | 2824 (368) | 2703 (617) |
| Week 4 | 2987 (916) | 246 (85) | 130 (43) | 48·4 (28) | 14·0 (5) |
| **s-HGA (µmol/L)** | | | | | |
| Baseline | 27·5 (8.9) | 28 (11·1) | 30·3 (7·7) | 32·1 (6·6) | 28·3 (7·8) |
| Week 4 | 30·5(12·4) | ND | ND | ND | ND |
| **s-Tyr (µmol/L)** | | | | | |
| Baseline | 54 (15) | 68 (20) | 62 (10) | 60 (9) | 55 (5) |
| Week 4 | 56 (15) | 653 (106) | 715 (171) | 803 (155) | 813 (145) |

ND = not determined (below 3·1 µmol/L); u-HGA$_{24}$ urine HGA excretion over 24 hours; u-HGA/creatinine: urine HGA excretion adjusted per mol of urine creatine; s-HGA: serum HGA in acidified fasting pre-nitisinone sample; s-Tyr: serum tyrosine in fasting sample.

**Primary Endpoint**

*Urinary HGA excretion*

Data for the 2- and 4-week visits are presented in Table 2, and in Figure 2a and b.

The u-HGA$_{24}$ before starting treatment was highly variable, and ranged from 14,443 to 69,503 µmol (corresponding to 2,426 to 11,677 mg) (Table 2).

At Week 4, a clear dose-response relationship between nitisinone and u-HGA$_{24}$ was observed (Table 2). The adjusted means and associated 95% confidence intervals were 31,152 (25,220 to 37,085), 3,846 (3,086 to 4,606), 1,668 (926 to 2,409), 686 (0 to 1,438) and 327 (0 to 1,079) µmol for the untreated, 1, 2, 4, and 8 mg

doses, respectively. From the MMRM, treatment group was statistically significant (p<0·0001), site, visit, site-visit interaction and u-$HGA_{24}$ were not significant (p values: 0·282, 0·609, 0·446, 0·077 respectively). All pairwise comparisons between groups at 4-weeks were statistically significant except for 3mg group v 4mg group and 4mg group v 8mg group (p<0·0001 in all cases except for: 3mg v 4mg, p=0·067; 3mg v 8mg, p=0·014; 4mg v 8mg, p=0·504). A similar pattern was observed for pairwise comparisons at 2-weeks, the only non-significant result being between 4 mg and 8 mg group (p=0·145). Besides being the most effective dose the 8-mg dose also showed the least variability. Similar results were seen already at Week 2. The dose-response relationship can be seen more clearly in Figure 2b, in which the baseline values and data for untreated patients have been omitted. Mean u-$HGA_{24}$ was about 50 and 250 times higher in the 8 and 4-mg nitisinone dose groups respectively than the highest value observed in non-AKU subjects [15].

The u-HGA/u-creatinine ratios at baseline and week 4 are presented in Table 2. They confirm the results seen for the u-$HGA_{24}$ values without creatinine correction, and indicate acceptably complete 24-hour urine collection.

**Secondary endpoint**

*Serum HGA*

Serum HGA was quantifiable in all patients before starting nitisinone treatment. After treatment, s-HGA values were below the lower limit of quantification (LLOQ) (3·1 µmol/L) in 64 % of all samples collected in treated patients (Table 2).

No comparison could be made with s-HGA in non-AKU subjects as all were below the LLOQ in that population [15].

*Serum Tyrosine*

The s-Tyr data, pre-dose and after 4 weeks of treatment, are presented in Table 2 and Figure 3. Mean s-Tyr increased with dose in all patients post-nitisinone. With few exceptions, all nitisinone-treated patients had levels above 500 µmol/L, with the highest observation (1,117 µmol/L) seen for a patient in the 4-mg group.

**Other safety results**

No safety concerns were identified in this 4-week study. Adverse events are summarized in Table S3. There were no SAEs and no event occurred in more than 1 patient, except for back pain that was reported by 2 patients in different dose groups. All events were considered mild, except for back pain in one patient in the 4-mg dose group. No signals from laboratory data were observed. No patient experienced any corneal effects of elevated tyrosine. (Supplementary appendix Tables S3).

## DISCUSSION

There is currently no approved pharmacological therapy for AKU. Treatment therefore relies on palliative analgesia and joint replacement surgery. Current experience with nitisinone is limited to three studies carried out at the National Institutes of Health (NIH), USA [4,12,13]. The last nitisinone study carried out by the NIH, a 3-year outcomes study, was inconclusive for the rheumatological endpoint (hip rotation) [13]. One possible reason for the inconclusive NIH study is that an optimal dose may not have been used. We therefore investigated the HGA-lowering effect of different doses of nitisinone to find a dose that could lower u-$HGA_{24}$ to levels closer to what is seen in normal subjects.

A clear dose-response relationship was observed for the effect of nitisinone on the urinary excretion of HGA. Excretion decreased consistently across the studied dose interval of 1 to 8 mg. In addition, the inter-subject variability decreased with increasing dose.

In a separate study in normal subjects [15], u-$HGA_{24}$ could only be determined in 7 out of 22 individuals. The remaining subjects had urine concentrations of HGA below the limit of quantitation. The highest amount of HGA excreted that was observed in a normal subject was 2·9 µmol/day. Thus, the adjusted mean value in the 8-mg group (327 µmol) was still about 100 times higher than this value, and u-$HGA_{24}$ was not normalized, even with the highest dose. Nevertheless, the 8-mg dose resulted in a mean reduction of u-$HGA_{24}$ of 98·8 % compared to baseline. We estimate that a complete normalization of u-$HGA_{24}$ would correspond to a reduction of approximately 99·99 %.

There is a lack of data correlating levels of serum and urine HGA with the evolution of AKU in patients. Therefore the level of HGA post-nitisinone that would prevent ochronosis if treatment is started sufficiently early, or the level that would arrest or delay ochronosis in humans if treatment is started later in AKU, is currently unknown. Lifetime treatment of AKU mice with nitisinone resulted in an 88% plasma HGA reduction and completely prevented ochronosis [14]. In the absence of such data in humans, it is reasonable to assume that the lower the serum and urine HGA, the more likely it is that ochronosis will be modulated. However, there must be a point at which further HGA reduction is of no relevance. The effect of nitisinone on clinical symptoms and long-term safety needs to be further investigated.

## CONCLUSIONS

Treatment of AKU patients with nitisinone at doses of 1 to 8 mg reduced u-HGA and s-HGA in a dose-dependent manner. The least inter-patient variability was seen in the 8 mg dose group. No safety concerns were raised from this short-term study.

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## CONTRIBUTORS

LRR, NS, AH, JR, JAG, JCJ were responsible for the conception the trial.

LRR, AH, JR, RI, CVK, BO, DL, MR,TC, EP JS contributed to the study design and statistical analyisis

LRR, NS, HA, OT, JR, RI contributed to recruitment of patients .

LRR, RF,MB, HB, JR RI undertook medical procedures.

LRR, AMM, ATH, JJD, JAG, JCJ, JM, PC, GR provided methodological advice or undetook chemical analysis

AZ, MN were responsible for the HGD gene mutation identification

CJ, TK, AC, LS, AZ, MN, FG KHLQS, DB, AS: contributed to project management

LRR drafted the first version of the report

All authors contributed to the interpretation of data, writing and revision of the manuscript

All authors approved the manuscript for publication.

## CONFLICTS OF INTEREST

None of the authors had any conflict of interests except B Olsson, M Rudebeck, J Szamosi, L Svensson, T Kullenberg, A Cronlund, and C Junestrand who are Sobi employees and share holders.

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**Figures and legends**

Figure 1. SONIA 1 Study Design.

The study consisted of two main periods: treatment, and follow-up. After screening, patients were randomized at baseline (1:1:1:1:1) to no treatment (control), and oral daily doses of nitisinone of 1, 2, 4 and 8 mg. The treatment period consisted of 4 weeks, during which study drug was administered. At 6 weeks a follow up telephone call concluded the study. (Abbreviations: S+R = Screening, Baseline and Randomization Visit; F= Final Treatment Visit; T = Telephone Follow-Up Visit)

Figure 2.

2a. Box-plots of 24-hour urinary excretion (µmol/24 hours) of HGA in untreated and nitisinone-treated AKU patients over time.

2b. Box-plots of 24-hour urinary excretion (µmol/24 hours) of HGA in nitisinone-treated AKUpatients at weeks 2 and 4. The greatest reduction in $uHGA_{24}$ with the least inter-patient variability was the 8 mg dose. An outlier has been excluded in the box plot but shown outside the box.

Figure 3. Fasting pre-dose serum concentrations of tyrosine at Week 4 (all patients). There was increased tyrosine in all patients on nitisinone. There was a trend towards higher tyrosine with dose which was statistically significant (p=0.039).