Epigenetic and neurological effects and safety of high-dose nicotinamide in patients with Friedreich's ataxia: an exploratory, open-label, dose-escalation study

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Summary

Background Friedreich's ataxia is a progressive degenerative disorder caused by deficiency of the frataxin protein. Expanded GAA repeats within intron 1 of the *frataxin* (*FXN*) gene lead to its heterochromatinisation and transcriptional silencing. Preclinical studies have shown that the histone deacetylase inhibitor nicotinamide (vitamin B3) can remodel the pathological heterochromatin and upregulate expression of *FXN*. We aimed to assess the epigenetic and neurological effects and safety of high-dose nicotinamide in patients with Friedreich's ataxia.

Methods In this exploratory, open-label, dose-escalation study in the UK, male and female patients (aged 18 years or older) with Friedreich's ataxia were given single doses (phase 1) and repeated daily doses of 2–8 g oral nicotinamide for 5 days (phase 2) and 8 weeks (phase 3). Doses were gradually escalated during phases 1 and 2, with individual maximum tolerated doses used in phase 3. The primary outcome was the upregulation of frataxin expression. We also assessed the safety and tolerability of nicotinamide, used chromatin immunoprecipitation to investigate changes in chromatin structure at the *FXN* gene locus, and assessed the effect of nicotinamide treatment on clinical scales for ataxia. This study is registered with ClinicalTrials.gov, number NCT01589809.

Findings Nicotinamide was generally well tolerated; the main adverse event was nausea, which in most cases was mild, dose-related, and resolved spontaneously or after dose reduction, use of antinausea drugs, or both. Phase 1 showed a dose-response relation for proportional change in frataxin protein concentration from baseline to 8 h post-dose, which increased with increasing dose ($p=0\cdot0004$). Bayesian analysis predicted that 3·8 g would result in a 1·5-times increase and 7·5 g in a doubling of frataxin protein concentration. Phases 2 and 3 showed that daily dosing at 3·5–6 g resulted in a sustained and significant ($p<0\cdot0001$) upregulation of frataxin expression, which was accompanied by a reduction in heterochromatin modifications at the *FXN* locus. Clinical measures showed no significant changes.

Interpretation Nicotinamide was associated with a sustained improvement in frataxin concentrations towards those seen in asymptomatic carriers during 8 weeks of daily dosing. Further investigation of the long-term clinical benefits of nicotinamide and its ability to ameliorate frataxin deficiency in Friedreich's ataxia is warranted.

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Introduction

Friedreich's ataxia is the most common inherited ataxia in the white population, affecting between 1 in 30 000 and 1 in 50 000 people.[1,2] It usually presents in childhood with a relentlessly progressive, predominantly sensory ataxia and dysarthria, and is associated with deafness, visual impairment, diabetes, and hypertrophic cardiomyopathy (a frequent cause of premature mortality).[3] No effective disease-modifying treatment exists, leaving many patients severely disabled by adulthood.

In 97% of cases, Friedreich's ataxia is caused by the pathological expansion of a GAA triplet repeat within the first intron of both alleles of the *frataxin* (*FXN*) gene, which results in partial silencing of the gene, leading to frataxin protein deficiency.[4,5] Most clinical trials so far have focused on ameliorating the downstream effects of frataxin deficiency, with little success in modifying the natural history of the disease.[6] Mariotti and colleagues[7] assessed the use of erythropoietin to upregulate frataxin, but did not report a significant effect. In this study, we focus on a novel treatment aimed at correcting the primary defect, *FXN* gene silencing.

We previously showed[8] that expanded GAA repeats can induce gene silencing in vivo. This mechanism resembles the archetypal epigenetic silencing known as position effect variegation, in which abnormal proximity of a gene to highly condensed DNA (heterochromatin) results in the stochastic silencing of the gene in a proportion of cells that would normally express it. Indeed, *FXN* acquires

several hallmarks of heterochromatin when silenced by a pathological GAA-repeat expansion.[9–11]

A key feature of heterochromatin is the presence of methylation (me) at aminoacid position 9 of the histone H3 tail (H3K9me3) that protrudes from the basic subunit of chromatin, the nucleosome.[12,13] This methylation was shown to be deposited by a powerful genetic modifier of position effect variegation, the histone methyltransferase SUV39H.[14,15] Subsequently other histone methyltransferases with this activity have been discovered.[16,17] H3K9me3 can be regarded as part of the histone or epigenetic code, which is then read by another powerful genetic modifier of position effect variegation, heterochromatin protein 1 (HP1). HP1 not only recognises and binds to H3K9me3, but can also recruit SUV39H, providing a mechanism for the propagation of heterochromatin along the DNA.[14,15] Chromatin immunoprecipitation in cells from model systems and primary cells from patients with Friedreich's ataxia showed an increase in H3K9me3 at the *FXN* locus and a reduction in acetylation of histone H3, the marker for active transcription.[9–11] These discoveries have led to the finding that inhibition of histone deacetylases (HDACs) can antagonise heterochromatin-mediated *FXN* gene silencing in cells from patients with Friedreich's ataxia and in mouse models,[9,11,18,19] and the development of a putative model for how such inhibitors might function (appendix p 2).[20]

Nicotinamide (vitamin B3) is a classical class III HDAC inhibitor.[21–24] It has good bioavailability and rapidly penetrates all tissues, readily passing across the blood–brain barrier.[25–28] Nicotinamide has a good safety profile, having been given safely for 5 years at about 3 g (1·2 g/m²) per day to more than 250 individuals in an attempt to prevent diabetes in an at-risk population.[29] However, the safety of nicotinamide in Friedreich's ataxia has not previously been investigated. Our preclinical studies showed that nicotinamide can remodel the heterochromatinised *FXN* locus and significantly upregulate *FXN* expression in primary cells from human beings, cell lines, and a mouse model for Friedreich's ataxia.[9] We aimed to assess whether high-dose nicotinamide could be used to safely upregulate *FXN* expression in patients with Friedreich's ataxia, in an attempt to restore frataxin concentrations towards those in healthy individuals.

Methods

Study design and patients

In this exploratory, open-label, dose-escalation study, participants were recruited via the National Hospital for Neurology and Neurosurgery (London, UK) and the Imperial College Healthcare NHS Trust (London, UK). Male and female patients aged 18 years or older were eligible to participate if they had a documented diagnosis of Friedreich's ataxia based on clinical criteria and a genetically confirmed GAA-repeat expansion on both alleles of the *FXN* gene. Exclusion criteria included hypersensitivity to nicotinamide and serious concurrent medical disorders or illnesses besides Friedreich's ataxia, including clinically significant dysphagia and heart disorders (eg, severe atrial fibrillation or hypertrophic cardiomyopathy). The trial registration page includes a full list of inclusion and exclusion criteria. Participants were screened for eligibility within about 28 days before receiving their first dose of nicotinamide, which was provided by Teofarma (Pavia, Italy) and produced in accordance with good manufacturing practice requirements.

The study was approved by the UK Medicines and Healthcare Products Regulatory Agency (MHRA; EudraCT 2011-002744-27), the Riverside Research Ethics Committee (11/LO/0998), and the Imperial College London Joint Research & Compliance Office. All participants provided written informed consent before any study-related procedures were initiated.

Procedures

The study was open label and divided into three phases. Phase 1 was a supervised, single-dose, dose-escalation phase, taking place over five visits, with a minimum 1-week washout period between each visit. Escalating doses (0, 2, 4, 6, 7, and 8 g) of oral nicotinamide were given on the basis of a prespecified scheme (appendix p 8) and patients were observed for 24 h after dosing. Dose escalation was adjusted or stopped on the basis of tolerability and antinausea treatments were given as needed. In phase 2, participants were given escalating doses for 5 consecutive days, and in phase 3 they received their maximum tolerated dose (if known from phase 2) daily for 8 weeks.

Phase 1 was used to determine the minimum dose at which nicotinamide could safely upregulate the *FXN* gene and to assess its pharmacokinetics and pharmacodynamics over 24 h. Blood samples for the measurement of plasma nicotinamide and *FXN* mRNA and frataxin protein concentrations (from separated peripheral blood mononuclear cells) were collected before treatment and at 2, 4, 8, and 24 h after dosing. Additionally, at the last dosing visit, blood samples were collected before dosing and 8 h after dosing for the measurement of heterochromatin modifications at the *FXN* locus by chromatin immunoprecipitation. The analyses were done at the MRC Clinical Sciences Centre (Hammersmith Hospital, London, UK), as previously described (appendix pp 12–13).[9,30,31] Because of encouraging results from phase 1, the original study protocol was amended to extend the study and include phase 3, at which time the lowest individual maximum tolerated dose identified in phase 1 (3·5g) was specified as the starting dose in phase 2. At this stage the post-hoc clinical scale outcomes were also added to the study. The gap between phases 1 and 2 varied between 3 and 7 months because of the time necessary to plan phase 3 and obtain relevant approvals from the ethics committee and the MHRA.

Phase 2 was used to assess the pharmacokinetics and pharmacodynamics of nicotinamide in relation to the

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For the **trial registration page** see http://www.clinicaltrials.gov/ct2/show/NCT01589809

See **Online** for appendix

upregulation of *FXN* expression, to determine whether this upregulation was associated with changes in chromatin, and to assess the safety and tolerability of nicotinamide after repeated administration over 5 consecutive days. Patients were allocated to either nicotinamide treatment (n=8) or no treatment (n=2) at the discretion of the investigator, as specified in the protocol. The no-treatment control group was intended to allow the identification of any changes in *FXN* expression in the absence of nicotinamide over the treatment period. On the first day of phase 2, patients scheduled for active treatment were given the lowest individual maximum tolerated dose recorded in phase 1 (ie, 3·5 g). On subsequent days the dose was escalated to their individual maximum tolerated dose, but not higher than 4 g on day 2, 5 g on day 3, or 6 g on days 4 and 5. As in phase 1, the dose-escalation schedule was adjusted or stopped on the basis of tolerability and antinausea treatments were given in the event of excessive nausea or vomiting. Blood samples for the measurement of nicotinamide plasma concentration, *FXN* mRNA expression, and frataxin protein concentration were collected each day before dosing and 2, 4, and 8 h after dosing.

Exploratory (post-hoc) neurological and speech dysarthria assessments were done and blood samples for chromatin analysis were taken on day 1 (predose) and day 5 (8 h post-dose). The neurological assessments used were the scale for the assessment and rating of ataxia (SARA)[32] and the spinocerebellar ataxia functional index (SCAFI).[33] Speech dysarthria was assessed with the Speech Intelligibility Test (Communication Disorders Software, Lincoln, NE, USA), a computer-based speech dysarthria assessment. SARA and SCAFI have been validated as sensitive indicators of neurological deterioration in Friedreich's ataxia. The SCAFI score is derived from the scores obtained from three tasks: the 8 m walk score (based on the time taken to walk 8 m); the nine-hole peg test score (based on the time taken to insert and remove pegs from a board); and the PATA score (based on the number of times the patient can say "pata" in 10 s). The Speech Intelligibility Test determines how easy it is to understand speech by recording the patient reciting set phrases and relies on the judgment of the investigator. Additionally, patients and their carers were asked to record their impressions about patients' activities of daily living using part 2 of the FARS scale at the same study timepoints as the neurological assessments.[34,35] Increases in SARA or activities of daily living scores indicate clinical deterioration, as do decreases in SCAFI or Speech Intelligibility Test scores.

On completion of phase 2, patients entered immediately into phase 3 of the study, continuing daily treatment with nicotinamide at their maximum tolerated dose for a period of 8 weeks. Patients who were scheduled to receive no drug in phase 2 were gradually escalated to 3 g of nicotinamide within 1 week and then to their maximum tolerated dose (but not higher than 6 g). Patients were instructed to take their daily nicotinamide dose at home except when they returned to the investigational unit once a week for safety assessment and predose blood sampling to measure nicotinamide plasma concentration, *FXN* mRNA expression, and frataxin protein concentration. Neurological (SARA and SCAFI scales), speech dysarthria, and activities of daily living assessments were done twice

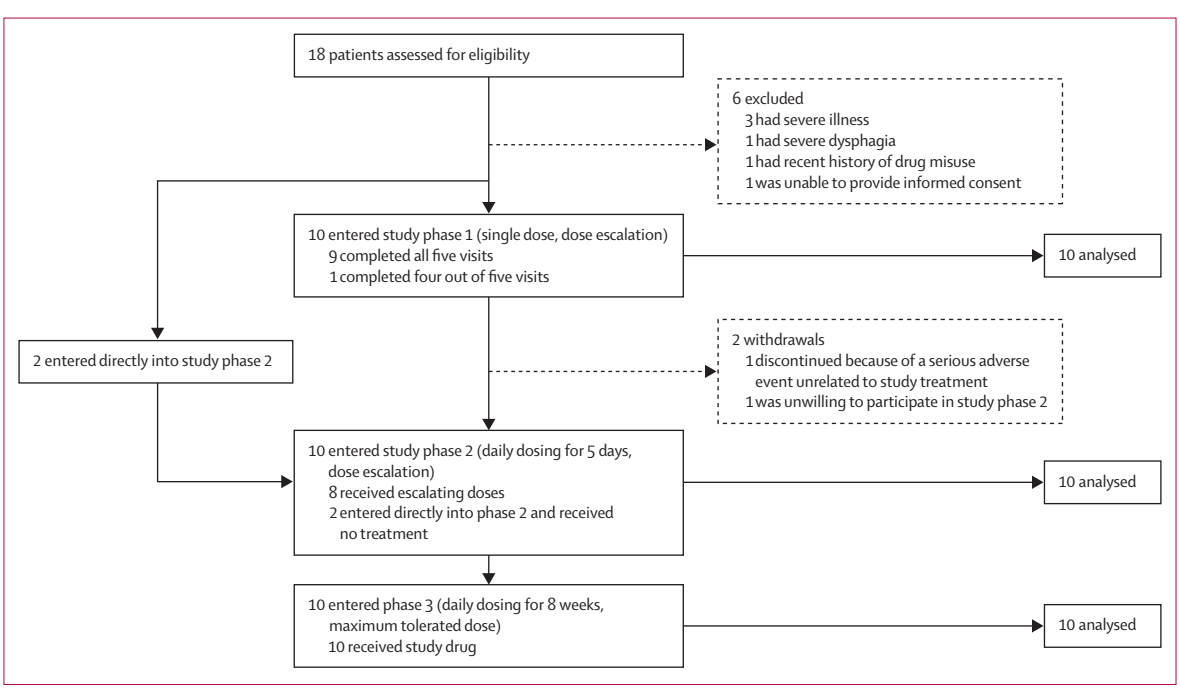

Figure 1: **Trial profile**

per month. Additionally, patients were provided with weekly diaries to be completed daily for the entire duration of phase 3, until the end-of-study visit. Patients were asked to record the time of nicotinamide dose and number of tablets taken, missing doses, adverse events, and any concomitant drugs used. The end-of-dosing follow-up visit was scheduled to take place 1–3 weeks after the final dose of nicotinamide was given.

Statistical analysis

We calculated mean values for *FXN* mRNA expression and frataxin protein concentration, expressed as proportional change (simple ratio) relative to baseline at screening, by study day during phases 2 and 3 (up to and including week 8). We used a non-parametric regression to fit a trend line to the mean values, with 95% CIs derived by bootstrapping. Additionally, we used the non-parametric version of the Hotelling test[36] (with a χ^2 approximation) to test the null hypothesis of no increase in frataxin protein concentration relative to baseline.

We analysed the proportional change in frataxin protein concentration from baseline data in phase 1 using a repeated-measures linear regression model to examine the relation between relative change and dose, taking into account the association between repeated measurements for individual patients. For the purpose of selecting the doses to be used in phase 2, we also analysed these data using a Bayesian dose-response model[37] to generate predicted values of doses likely to yield relative increases in frataxin concentration of 1·5 and 2·0 times.

To assess changes in chromatin, we analysed enrichment for H3K9me3 and H3 panacetylation using a two-tailed paired Student's *t* test in Microsoft Excel for Mac 2011 (version 14.3.5). In phase 3, comparison of clinical scores between baseline and week 8 was also done using a two-tailed paired Student's *t* test in the same software.

For all cases in which data were missing, no imputation was done and the missing datapoints were omitted from the analysis. The only exception to this approach was for the non-parametric Hotelling test, wherein missing data for changes in frataxin protein concentration were imputed by setting the missing value equal to the null hypothesis value of 1·0.

Analyses of pharmacokinetics and pharmacodynamics for phases 2 and 3 were done on an intention-to-treat basis. Data from all patients in the treatment group were analysed irrespective of the dose they received.

Statistical analyses other than Student's *t* tests were done with SAS version 9.3, R version 3.0.1, or OPENBUGS version 3.2.2.

The study is registered at ClinicalTrials.gov, number NCT01589809.

Role of the funding source

The funders of the study had no role in data collection, data analysis, data interpretation, or writing of the report, although Ataxia UK had some input into the study design, particularly in the early stages. All authors had access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 18 patients screened between June 8, 2012, and June 24, 2013, ten met the eligibility criteria and were enrolled into phase 1 of the study, with follow-up for phase 1 taking place between June, 2012, and October, 2012 (figure 1). Nine participants completed phase 1 and one completed four out of five visits, but withdrew before the final visit because she found the hospital environment stressful. All remaining patients were offered the opportunity to continue to the multiple-dose phases of the study (phases 2 and 3), but one was excluded as a safety precaution because of an episode of

	Phase 1 (n=10)	Phases 2 and 3 (n=10)
Women	6	7
Age	33 (23–54)	33 (19–54)
Ethnic origin		
White (British or Irish)	9	9
Asian (Bangladeshi)	1	1
SARA at baseline	16·3 (5·0–31·5)	16·3 (5·0–31·0)
Age at onset (years)	17·9 (8·1)	21·7 (9·5)
Onset after age 25 years	2	4
Years of disease	15·4 (6·7)	14·0 (8·0)

Data n, mean (SD), or median (range). SARA=scale for the assessment and rating of ataxia.

***Table 1*: Baseline demographic and clinical characteristics**

	Dose						Total number of episodes
	0 g	2 g	3·5 g	4 g	6 g	7–8 g	
Nausea							14 (24%)*
Mild	0	0	0	1	4	2	7
Moderate	0	0	1	1	5	0	7
Headache							11 (19%)*
Mild	0	0	0	2	1	1	4
Moderate	0	0	0	1	3	1	5
Severe	0	0	0	0	2	0	2
Vomiting							8 (14%)*
Mild	0	0	0	2	3	1	6
Moderate	0	0	0	0	1	0	1
Severe	0	0	0	0	1	0	1
Fatigue							4 (7%)*
Mild	0	0	0	1	1	1	3
Moderate	0	0	0	1	0	0	1
Fever	1	0	0	2	0	0	3 (5%)*
Presyncope	0	0	0	1	1	1	3 (5%)*
Hypotension	0	1	0	0	1	0	2 (3%)*

Adverse events that occurred only once are not shown. *Data are total number of episodes (percentage of total adverse events reported).

***Table 2*: Most common adverse events during study phase 1, by dose**

atrial tachycardia associated with a respiratory infection that occurred 2 months after completion of phase 1 and before the start of phase 2; this event was similar to a previous episode that predated the study. Two additional patients (also recruited during the screening period, which continued until sufficient patients for each phase were enrolled) were enrolled directly into phase 2, and were allocated to receive no treatment during this phase. These two patients were used as a control group to allow us to determine the effect of the hospital environment on frataxin concentrations and the other measurements. All 10 patients completed phase 2 and continued into phase 3, which took place between January, 2013, and August, 2013, and during which all patients received treatment. Data from all completed patients were analysed. Table 1 summarises the demographic and baseline clinical characteristics of the participants (including baseline disease severity as measured by SARA).

	Number of events	Number of participants affected (n=10)
Nausea	58	10
Headache	15	5
Lightheadedness	13	6
Vomit	7	5
Hypersomnia	7	1
Fatigue	6	3
Diarrhoea	6	4
Raised alanine transaminase	5	3
Fall	4	3
Anorexia	3	3
Raised aspartate aminotransferase	3	2
Migraine	3	1
Dizziness	2	1
Sore throat	2	1
Retching	2	1
Cold	2	2
Fever	2	2
Infections	2	2
Cough	2	2
Anaemia	2	2
Flu-like symptoms	2	1

Adverse events that occurred only once are not shown.

Table 3: **Most common adverse events during study phases 2 and 3**

	3 g	3·5 g	4 g	5 g	6 g	Total
Mild nausea	4	2	44	0	0	50
Moderate nausea	1	0	4	2	0	7
Severe nausea	0	0	0	0	1	1
Mild vomiting	0	1	1	1	0	3
Moderate vomiting	0	0	0	1	0	1
Severe vomiting	0	0	2	0	1	3
Mild LFT abnormality	0	0	2	0	3	5
Moderate LFT abnormality	0	0	1	0	1	2
Severe LFT abnormality	0	0	0	0	1	1

LFT=liver function tests.

Table 4: **Nausea, vomiting, and abnormal LFT results during study phases 2 and 3, by dose**

Nicotinamide was generally well tolerated. The main adverse event was nausea, which in most cases was mild, dose-related, and resolved either spontaneously or after dose reduction, use of antinausea drugs, or both. The dose escalation showed an increase in adverse events as the dose increased, thereby allowing identification of a maximum tolerated dose for each patient. Increases in abnormal results from liver function tests in three out of ten patients occurred only when taking high doses—in two cases these effects were mild to moderate and self-limiting and in one case they were severe, but all resolved with reduction of the nicotinamide dose (tables 2–4, appendix pp 9–10). No clinically significant changes in vital signs or physical findings were seen, and no serious adverse events occurred while patients were taking nicotinamide. The only adverse events in the no-treatment group (phase 2) were related to the common cold (nasal congestion, cough, and sore throat), which occurred in one patient.

In phase 1, we noted a dose-response relation for the mean proportional change in frataxin protein concentration at 8 h over the range of 2–8 g nicotinamide given as a single oral dose ($p=0·0004$; appendix p 3). Frataxin concentrations returned to baseline by 24 h. Predicted doses from the Bayesian model were 3·8 g for a 1·5-times increase in frataxin concentration, and 7·5 g for a 2·0-times increase.

The pharmacokinetic analysis population included all participants who had received at least one dose of nicotinamide (n=12). In phase 1, nicotinamide reached a dose-dependent mean peak plasma concentration at 1–3 h (appendix p 4). Plasma concentrations subsequently declined with an apparent half-life (estimated from a Bayesian one-compartment model) of 12·1 h, which is similar to the pharmacokinetics of nicotinamide reported by other investigators.[28,38,39] In phase 2, repeated administration of nicotinamide of 3·5–6 g resulted in a dose-dependent increase in nicotinamide plasma peak and trough concentrations (figure 2), with steady-state plasma concentrations from week 2 onwards in phase 3 (figure 3).

Further analysis of the data from phase 1 provided insight into the mechanism by which nicotinamide might upregulate *FXN* expression. Plasma nicotinamide concentration reached its peak value first (2 h), followed by *FXN* mRNA expression (4 h), and then by frataxin protein concentration (8 h; appendix pp 4–5). This finding is consistent with the hypothesis that nicotinamide acts on gene transcription. After decay of nicotinamide, *FXN* mRNA expression fell more rapidly than frataxin protein

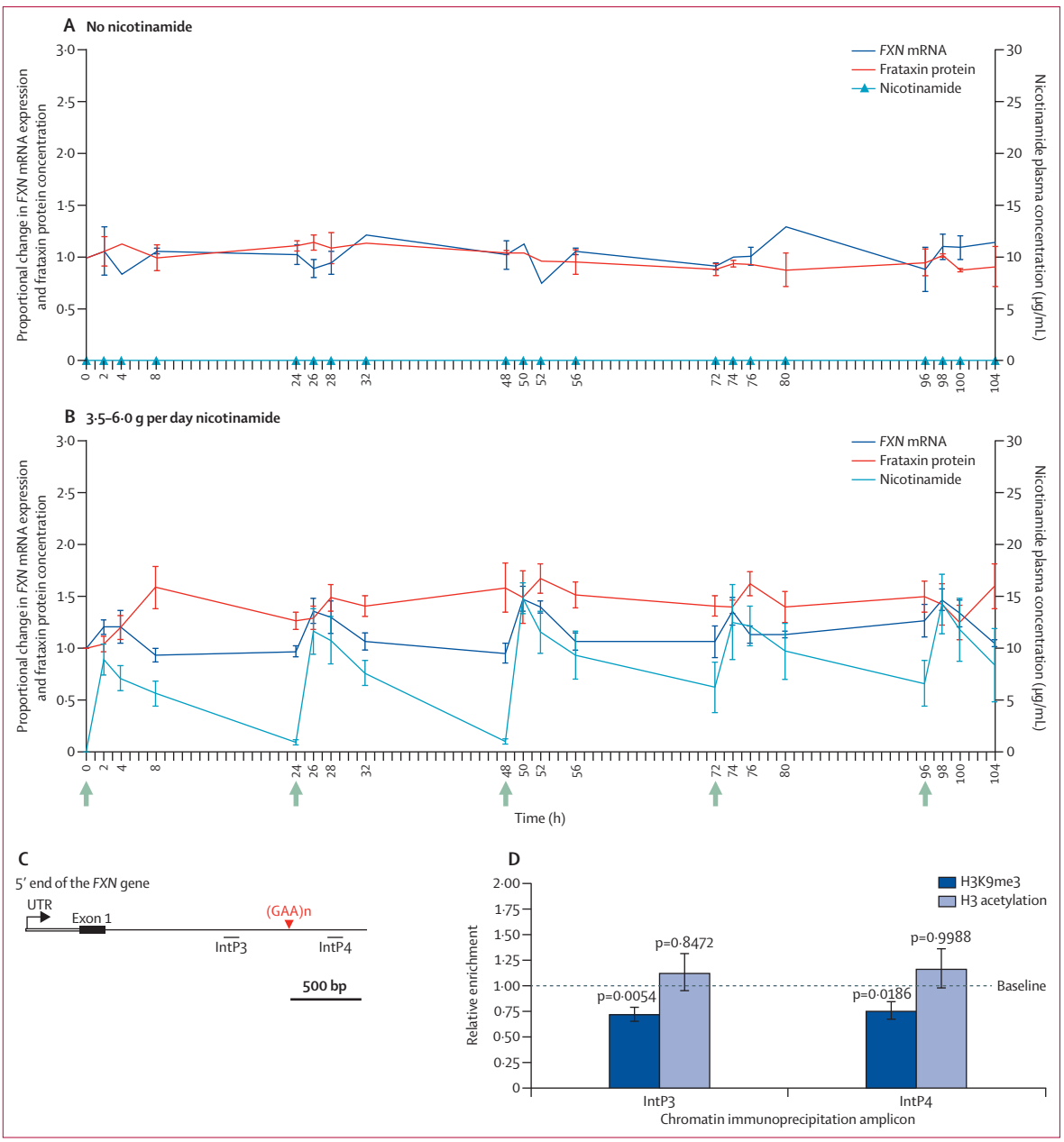

Figure 2: **Pharmacokinetics and pharmacodynamics of daily doses of nicotinamide over 5 days (study phase 2)**
Data are mean values for nicotinamide plasma concentrations and proportional changes in *FXN* mRNA expression and frataxin protein concentration in peripheral blood mononuclear cells (simple ratio relative to baseline) for no nicotinamide (A; n=2, error bars show range) and doses of 3·5–6 g per day (B; n=8, error bars show SEs) over 5 days. Green arrows indicate the time of dosing. Data for individual patients are shown in the appendix (pp 6–7). (C, D) The chromatin immunoprecipitation assay used antibodies against heterochromatic (inactive) H3K9me3 and the euchromatic (active) H3 acetylation histone marks, and the DNA obtained was analysed by real-time PCR with primers for intron P3 (IntP3) and intron P4 (IntP4) flanking the (GAA)n repeats in intron 1 of the *FXN* gene. (D) Data are mean values of relative enrichment (simple ratio relative to baseline). Error bars show SEs (n=8). UTR=untranslated region.

concentration, in line with the fact that the half-life of the protein is higher than that of the mRNA.[40] A similar pattern was seen after multiple dosing in phase 2 (figure 2).

To further investigate the mechanism of *FXN* upregulation, we used chromatin immunoprecipitation on peripheral blood mononuclear cells before and after nicotinamide treatment, which showed a significant reduction in the characteristic heterochromatin modification H3K9me3 in the regions flanking the *FXN* GAA-repeat expansion (upstream p=0·0054; downstream p=0·0186), accompanied by a non-significant increase in acetylation (upstream p=0·8472; downstream p=0·9988) of histone H3 (figure 2). Taken together, these results are consistent with the original hypothesis that nicotinamide

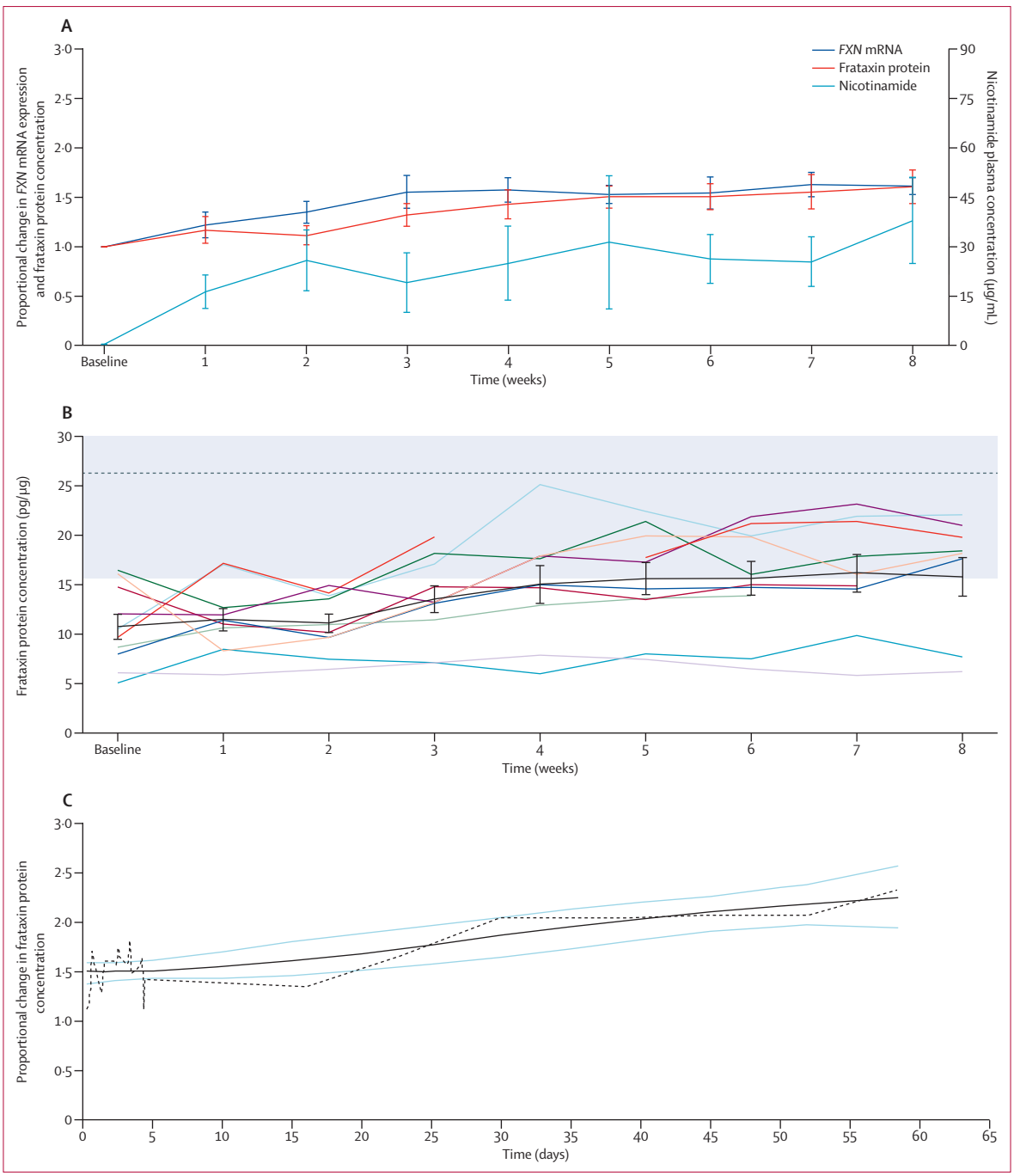

***Figure 3*: The effect of nicotinamide on *FXN* mRNA expression and frataxin protein concentration over 8 weeks (study phase 3) and a non-parametric regression model of this effect (study phases 2 and 3 combined)**

(A) Pharmacokinetics and pharmacodynamics of daily dosing with nicotinamide over 8 weeks (study phase 3). Data are mean values for nicotinamide plasma concentrations and proportional changes in *FXN* mRNA expression and frataxin protein concentration in peripheral blood mononuclear cells (simple ratio relative to predose baseline) for doses of 3·5–6 g per day (n=10, error bars show SEs). (B) Spaghetti plot of data for the concentration of frataxin protein in peripheral blood mononuclear cells by individual patients over 8 weeks (study phase 3; absolute mRNA data for individual patients are shown in the appendix [p 7]). Each coloured line indicates the data from one patient; the thick black line indicates the mean for each datapoint (error bars show SEs). The grey zone indicates the range of frataxin protein expression seen in asymptomatic Friedreich's ataxia carriers and the dashed line indicates the average for this population.[30] (C) Non-parametric regression model for the proportional changes in frataxin protein concentration in peripheral blood mononuclear cells (simple ratio relative to baseline at screening) with daily nicotinamide treatment over 60 days (study phases 2 and 3). The dotted line indicates the mean of the raw data. The solid black line indicates the fitted non-parametric trend line, and the blue lines indicate the 95% CI of the regression trend line, generated by bootstrapping. The graph shows a sustained and significant (p<0·0001) increase in frataxin protein, with the lower bound of the CI above the null value of 1·0 at all timepoints.

can antagonise the pathological heterochromatinisation at the *FXN* gene in patients with Friedreich's ataxia, thereby facilitating restoration of *FXN* expression.

Taking into account tolerability results from phase 1, patients were prescribed between 3·5 g and 6 g nicotinamide daily in phases 2 and 3. We noted a sustained and significant (p<0·0001) increase in frataxin protein concentration during both phases (figure 3C), with the lower bound of the 95% CI remaining above the null value of 1·0 at all timepoints. However, the response was variable, with two of the ten patients showing little change in frataxin concentration (figure 3B).

No significant effects were seen on the clinical scale ratings for disease progression over study phases 2 and 3, although a slight, non-significant improvement was seen in the more subjective assessment of activities of daily living (table 5).

	Difference from baseline (95% CI)	p value
SARA	−0·4 (−2·2 to 1·5)	0·69
SCAFI	0·1 (−0·1 to 0·3)	0·31
8 m walk	−0·1 (−0·2 to 0·1)	0·18
Nine-hole peg test	0·1 (0·0 to 0·3)	0·10
PATA	0·1 (−0·2 to 0·5)	0·39
SIT	−0·1 (−0·1 to 0·0)	0·15
ADL	−4·1 (−9·0 to 0·7)	0·07

Data are mean differences in scores between the start of phase 2 and the end of phase 3 (week 8), with associated p values. SARA=scale for the assessment and rating of ataxia. SCAFI=spinocerebellar ataxia functional index. SIT=Speech Intelligibility Test. ADL=activities of daily living.

Table 5: **Clinical scale ratings for disease progression (study phases 2 and 3)**

Discussion

Our results showed a significant and sustained upregulation of frataxin protein in most patients over an 8-week period of daily dosing with nicotinamide. Thus, the present study is one of the first clinical studies to establish the proof-of-concept of a treatment aimed at halting the progression of Friedreich's ataxia by restoring the expression of *FXN* using an HDAC inhibitor (panel).

Our chromatin immunoprecipitation results showed that nicotinamide treatment led to a significant reduction in the heterochromatin modification H3K9me3 and a non-significant increase in H3 acetylation in the regions flanking the *FXN* GAA-repeat expansion. Obviously it was not possible to analyse these effects directly in the tissues affected in Friedreich's ataxia. However, these data are consistent with results obtained from the cerebellum of mice carrying a human *FXN* gene with a GAA-repeat expansion[9] and support the hypothesis that nicotinamide is acting at the chromatin level to antagonise heterochromatin. Interestingly, nicotinamide was previously shown not only to inhibit deacetylation of histones, but also deacetylation of the histone methyltransferase SUV39H,[24] thereby inhibiting its activity, and provide an additional putative mechanism by which nicotinamide inhibits heterochromatin formation at the *FXN* gene.

Our study represents one of the first attempts to assess an epigenetic therapeutic approach for a disease other than cancer, introducing a potential novel use for a widely available drug in doses that were previously shown to be well tolerated by healthy individuals for a long period.[26,29] Diseases other than Friedreich's ataxia caused by similar mechanisms could also be amenable to such a therapeutic approach—eg, other repeat-induced diseases and those caused by mutations in the regulatory elements of genes. Interestingly, nicotinamide has been investigated in the past for its neuroprotective role in the modulation of cellular energy metabolism together with coenzyme Q_{10} in Parkinson's disease,[41] suggesting a possible additional therapeutic benefit in Friedreich's ataxia.

Panel: Research in context

Systematic review

Our initial finding[8] that GAA-triplet repeat expansions, which cause Friedreich's ataxia by silencing the frataxin (*FXN*) gene, could induce heterochromatin-mediated gene silencing in transgenic mice led us to the hypothesis that epigenetic modifiers could provide a novel therapeutic approach for Friedreich's ataxia. Subsequently, several groups confirmed that the *FXN* gene in patients with Friedreich's ataxia was heterochromatinised,[9–11] providing support for our hypothesis. Preclinical studies[9,11,18] have shown that HDAC inhibitors can partly restore frataxin expression towards asymptomatic levels. We searched PubMed for articles published in any language between March 8, 1996, and Dec 10, 2013, using combinations of the search terms: "histone deacetylase inhibitor", "HDAC inhibitor", "nicotinamide", "vitamin B3", "Friedreich's ataxia", "heterochromatin", "epigenetics", and "clinical trial". We identified no reports of clinical studies investigating the use of histone deacetylase (HDAC) inhibitors in patients with Friedreich's ataxia. The author of an extensive review of clinical trials for Friedreich's ataxia[6] similarly did not identify any such studies. Our previous finding[9] that the HDAC inhibitor nicotinamide (vitamin B3) could partly restore expression of the *FXN* gene in model systems led us to investigate whether a similar upregulation of *FXN* could be achieved in patients.

Interpretation

In this exploratory study, we have identified safe and well-tolerated doses of nicotinamide, which when given to patients with Friedreich's ataxia led to partial reversal of the abnormal heterochromatinisation of the *FXN* gene and restoration of frataxin concentration towards asymptomatic levels. These findings provide a proof-of-concept that such an approach might provide a means of restoring frataxin concentrations in the long term, which could prevent deterioration in Friedreich's ataxia. This study is not adequate to support the use of nicotinamide as a treatment for Friedreich's ataxia, but our findings suggest that further studies of its long-term clinical efficacy are warranted.

The doses of nicotinamide used in this study are consistent with previous data about nicotinamide safety and tolerability,[26] but are much higher than the recommended daily vitamin B3 requirement. However, the drug was well tolerated when given for an 8-week period with nausea as the main adverse reaction; this was readily controlled with the use of antiemetics and by modification of the dose. Dose-related increases in abnormal liver function test results should be taken into account when deciding on doses for longer studies.

Despite inherent variability between individuals and some changes in dose in response to adverse events, our results showed a significant upregulation in frataxin protein concentrations. However, two patients seemed to be relative non-responders. Interestingly, these patients had the lowest frataxin protein concentrations at baseline of the ten patients who took part in phase 3. One possible explanation is that the *FXN* gene is so strongly repressed in these patients that it is refractory to the effect of nicotinamide. Among the eight patients who responded to nicotinamide, absolute concentrations of frataxin protein ranged between 17·66 and 22·14 pg per µg of of total protein extract from peripheral blood mononuclear cells at week 8. These concentrations are within the range previously reported for Friedreich's ataxia carriers (15·5–50·6 pg/µg; mean 26·5 pg/µg [SD 7·1]),[30] whereas the range for healthy individuals is higher (25·3–55·9 pg/µg; mean 38·6 pg/µg [7·6]). Since carriers are asymptomatic, this finding raises the possibility that sustained correction of frataxin deficiency in patients could be beneficial and modify disease progression. The effect of restoration of frataxin in Friedreich's ataxia is currently unknown. Several treatment strategies that are believed to address the causes of neurodegenerative diseases suggest diverse outcomes. For example, replacing MECP2 in RETT syndrome[42] could result in reversal of the disease phenotype, whereas in Alzheimer's disease removal of plaques is believed by some researchers not to be disease modifying.[43]

The clinical scale assessments were post-hoc and exploratory, to assess disease progression or improvement and the association between frataxin concentration and clinical outcomes. The rate of neurological decline in Friedreich's ataxia is so slow[6] that any preventive effect of nicotinamide would be unlikely to be identified from the objective clinical rating scores over the short duration of this study. Although some short studies have shown improvements in clinical rating scales,[6,44,45] albeit with different drugs, timescales, and clinical scales, this study did not show improvements. Such scales might not be sensitive enough to capture small changes. However, such changes might have been picked up by the more subjective activities of daily living survey, with the caveats that this is an open-label study (making such assessment subject to bias) and that the change in this score was not significant. Increasing frataxin expression towards asymptomatic concentrations might be expected to prevent further deterioration, but such an effect could only be captured by a longer and larger study.[6]

Our results have shown that nicotinamide is rapidly absorbed after oral administration and is generally well tolerated in patients with Friedreich's ataxia after repeated daily dosing for 8 weeks. Moreover, consistent with preclinical findings, frataxin protein concentrations in peripheral blood mononuclear cells are increased towards those seen in asymptomatic carriers. This preliminary study is not adequate to support the use of nicotinamide as a treatment for Friedreich's ataxia, but trials to establish safety and clinical efficacy over a sufficient timescale to measure clinical decline are warranted.

Contributors

RF was the chief investigator, had the initial idea for the study, designed the study (with help from VL, NL, and CY), and helped to write the report. PG provided advice about the design of the study. VL, CY, and RF discussed and helped to interpret the results. VL helped to do the clinical assessments, supervised clinical data acquisition, and helped to write the report. CY did the frataxin protein and *FXN* mRNA measurements, made the figures, and helped to write the report. SA, AR, NL, PG, and MHP helped to recruit the participants and discussed and reviewed the report. NL was the clinical project manager and helped with the study design, patient scheduling, and analysis of the Speech Intelligibility Test results. SA did most of the clinical assessments (together with TM), analysed the clinical data (with the help of LH), helped to write the report, and helped with the frataxin protein measurements. CY, TN, PPL, PKC, KTT, MM, and AR prepared samples for chromatin analysis. TN did most of the chromatin analysis with help from PPL, CY, and PKC. JL and SP did all the measurements of nicotinamide plasma concentrations. JL helped to write the report. LH provided expert statistical advice, did the pharmacokinetic modelling and the main analyses of the changes in frataxin concentrations, and helped to write the report. All the authors reviewed the report before submission.

Declaration of interests

We declare that we have no competing interests.

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