Title of study:

A randomised, placebo-controlled, double-blind, double-dummy, four-way crossover, single-centre study to investigate the effects of 2 mg and 10 mg intravenously administered NRL972 on the QT_C interval in healthy volunteers

Investigators:

D Bell

Study centres:

Belfast, Northern Ireland

Publications (references):

Not applicable

Study period ('first patient in' to 'last patient out'):	**Phase of development:**
7 May 2008 to 13 August 2008	I

Objectives:

Primary objective:

To investigate the effects of intravenous doses of 2 mg and 10 mg NRL972 on QT_C interval in healthy volunteers, in comparison with placebo.

Secondary objectives:

1. To quantify any effect on QT_C interval in relation to the maximum observed concentration (C_{max}) and time of occurrence of C_{max} relative to dosing (t_{max}) values after administration of intravenous doses of 2 mg and 10 mg NRL972 to healthy volunteers.
2. To compare the pharmacokinetic (PK) profile, including dose-dependency, following the intravenous administration of 2 mg and 10 mg NRL972.
3. To assess the safety and tolerability of intravenous doses of 2 mg and 10 mg NRL972 in healthy volunteers.

Methods:

This was a randomised, placebo and positive-controlled, double-blind, double-dummy, four-way crossover, single-centre study to investigate the effects of 2 mg and 10 mg intravenously administered NRL972 on the QT_C interval in healthy volunteers.

Subjects were consented and screened for eligibility within 28 days of entering the study. The following screening assessments were conducted: laboratory tests (including virology), serum follicle-stimulating hormone test (post-menopausal females), beta human chorionic gonadotrophin (β-hCG) pregnancy test (females of child-bearing potential), physical examination, electrocardiogram (ECG), and vital signs. Demography and medical background of the subjects were also collected, including details of concomitant medications. Adverse event (AE) data were collected from the time of consent. Subjects who fulfilled the eligibility criteria and were able to comply with the study restrictions returned to the centre on day –1 for scheduled assessments, including urine drug and alcohol screen, vital signs, and physical examination. The study consisted of four study periods and each subject was to receive all treatments, with a minimum washout period of 72 hours, in a randomised order. On day 1 of each study period, each subject received both an intravenous and an oral dose (intravenous 2 or 10 mg NRL972 or saline and either oral placebo or 400 mg moxifloxacin) in a randomised order.

Blood samples were obtained for the determination of NRL972 and moxifloxacin concentrations and for the analysis of PK variables one hour before dosing and at the following times after dosing: 2.5, 5, 7.5, 10, 15, 30, and 45 minutes, and 1, 2, 4, and 12 hours.

ECGs were collected for each subject by attaching a 24-hour ambulatory device approximately 90 minutes before dosing and subsequently throughout the dosing day. ECGs for evaluation were recorded at the following time points: –1 and –0.5 hours before dosing, at 2.5, 5, 10, 15, 30, and 45 minutes, and at 1, 2, 3, 4, 8, and 12 hours post-dose.

At least 72 hours after completion of the last study period, subjects had the scheduled end-of-study assessments: physical examination, laboratory safety tests, pregnancy test, vital signs, ECG, and recording of AEs.

Number of patients:	**Planned:** 48	**Analysed:** 48 (safety and ITT population) 43 (PP population)

Diagnosis and main criteria for inclusion:

Inclusion criteria:

1. Healthy adult male or female volunteers (as determined by medical history, physical examination, laboratory test values, vital signs, and ECGs at screening) aged 18–50 years.
2. Non-smokers for at least 3 months prior to screening.
3. Body Mass Index (BMI) ≥ 18 and ≤ 30 kg/m^2.
4. Able and willing to receive intravenous and oral treatments.
5. Able to voluntarily provide written informed consent to participate in the study.
6. Must have understood the purposes and risks of the study and agreed to follow the restrictions and schedule of procedures as defined in the protocol.
7. Female volunteers were to have been postmenopausal (for at least one year), surgically sterile, abstinent, or if sexually active, be using an effective method of contraception (such as prescription oral contraceptives, contraceptive injections, intrauterine device, double-barrier method, contraceptive patch, male partner sterilisation) before entry into the study and throughout the study's duration; women of childbearing potential were to have had a negative serum β-hCG pregnancy test at screening.
8. Females who were using contraceptives must have been on a stable dose for at least 3 months and agreed to not initiate new, or alter their current use, of contraceptives until 30 days after the completion of the study.
9. Male volunteers were to have used contraception with their partners throughout the study and for 30 days after completion of the study.
10. Must have been willing to consent to have data entered into The Over Volunteering Prevention System.
11. The volunteer's primary care physician was to have been confirmed that there was nothing in their medical history that would preclude their enrolment into this clinical study.

Exclusion Criteria:

12. Positive for HIV, hepatitis B, or hepatitis C as demonstrated by the results of testing at screening.
13. History or presence of any significant cardiovascular, pulmonary, hepatic, renal, haematological, gastrointestinal (GI), endocrine, immunological, dermatological, neurological, or psychiatric disease.
14. ECG abnormalities (clinically significant, in the opinion of the Investigator), repeated demonstration of a QT_C interval >450 ms or any vital sign abnormalities.
15. History of additional risk factors for torsades de pointes (e.g. heart failure, hypokalaemia, and family history of long QT Syndrome or sudden unexplained cardiac death).
16. Pregnant or lactating females.
17. Current or history of drug or alcohol abuse or a positive drugs of abuse test at screening or check in.
18. Tobacco smoking.
19. History of an allergic reaction to moxifloxacin or other study drugs.
20. Use of any drugs known to induce ECG abnormalities (at the discretion of the Investigator), including any medication that is known to prolong the QT/QT_C interval.
21. Volunteers who, in the opinion of the Investigator, are unsuitable to participate in the study.
22. Participation in a clinical drug study during the 90 days preceding the initial dose in this study.
23. Any significant illness during the 4 weeks preceding entry into this study.
24. Donation of blood or blood products within 90 days prior to study drug administration, or at anytime during the study, except as required by this protocol.
25. Consumption of alcoholic beverages within 48 hours prior to the first dose. Abstinence is required until completion of the post-study physical examination.

26. Consumption of caffeine or xanthine-containing drinks or products within 24 hours of each dosing.
27. Use of any concomitant medication, including over-the-counter items and non-steroidal anti-inflammatory drugs with the exception of paracetamol and approved contraceptives, within 14 days prior to study drug administration until the end of the study.
28. In the case of sexually active volunteers, the use of unreliable forms of contraception during the study period and 30 days post study.
29. Presence of clinically relevant bradycardia, history of symptomatic arrhythmias.
30. History of tendon diseases or disorders related to quinolone treatment.
31. Contraindication to receiving moxifloxacin or similar drugs.

Test product, dose and mode of administration; batch numbers:

Subjects received all four treatments in a randomised, double-blind manner with a minimum washout of 72 hours between periods. Subjects received both an intravenous and an oral dose during each treatment arm.

The four treatments were:

Treatment	Intravenous	Oral
A: Therapeutic dose	NRL972, 2 mg dose	Placebo
B: Supratherapeutic dose	NRL972, 10 mg dose	Placebo
C: Placebo	Saline placebo, 0.9%	Placebo
D: Moxifloxacin (positive control)	Saline placebo, 0.9%	Moxifloxacin 400 mg

Study drug batch numbers are given below:

NRL972, batch number: NORp004, expiry date: 11 July 2008

Moxifloxacin 400 mg tablets, batch number: BXF26U, expiry date: September 2012

Placebo 400 mg tablets, batch number: D07247, retest date: 31 July 2008

Placebo IV infusion, Sodium Chloride 0.9%, batch number: 7401C13, expiry date: February 2010

Duration of treatment:

Approximately 4 weeks (the final dose of investigational medicinal product was administered on 08 July 2008).

Reference therapy, dose and mode of administration; batch numbers:

Moxifloxacin (400 mg tablets) was the positive control and 0.9% saline IV and oral placebo were administered as placebo control.

Criteria for evaluation:

Efficacy:

There were no assessments of efficacy

ECG measurements

A 24 hour ambulatory device was attached to the subject approximately 90 minutes before dosing and was used throughout the dosing day. ECGs for evaluation were recorded at the following time points: –1 and –0.5 hours (prior to dosing), 2.5, 5, 10, 15, 30 and 45 minutes, and 1, 2, 3, 4, 8 and 12 hours post-dose. Biomedical Systems SA/NV calculated the QT interval and other ECG variables at these time points from the device's output according to their SOPs. ECG baseline for each treatment was calculated as the average of three recordings.

Pharmacokinetics

For each treatment period, a 6 mL blood sample was obtained within 60 minutes pre-dose and at: 2.5, 5, 7.5, 10, 15, 30, and 45 minutes, and 1, 2, 4, 12 hours after study drug administration for the determination of NRL972 and moxifloxacin concentrations and for analysis of the PK variables.

The following PK variables were calculated for NRL972:

$C_{(t_Z)}$	Concentration at the time point with the last quantifiable serum concentration (time: t_Z)
$C_{(t_Z)}'$	Concentration at the time point with the last quantifiable serum concentration (time: t_Z) fitted according to the apparent terminal log-linear disposition slope λ_Z
C_{max}	Maximum observed concentration
t_{max}	Time of occurrence of C_{max} relative to dosing
$T_{½}$	Apparent terminal disposition half-life = $\ln(2)/\lambda_Z$
AUC	Area under the time course of the serum concentrations according to the combined linear/log-linear trapezoidal rule

$AUC_{(0-t_z)}$	AUC up to the last quantifiable serum concentration
$AUC_{(t_z-\infty)}$	AUC extrapolated beyond $t_Z = C(t_Z)'/\lambda_Z$
$AUC_{(0-\infty)}$	AUC (extrapolated to infinity)
MRT	Mean residence time
CL	Clearance
CL/BW	Clearance per kg body weight
k_{el}	The apparent first-order terminal elimination rate constant

The following PK variables were calculated for moxifloxacin:

C_{max}

t_{max}

$AUC_{(0-t_z)}$

Safety:

Safety was assessed by recording AEs, vital signs, 12-lead electrocardiogram (ECG), physical examination, and clinical laboratory tests.

Statistical methods:

Based on a within-subject standard deviation of 9 ms, and making the reasonable assumption that ECGs at successive time points were correlated with a correlation coefficient of 0.5, at least 40 subjects for 90% power to exclude a difference of 10 ms were needed. A sample size of 48 however was considered a conservative estimate based on historical sample size calculation for these studies by regulatory authorities. All analyses were performed using Stata 9.2 (Stata Corporation, College Station, Texas).

The primary endpoint was the change from baseline in QT_C interval using Fridericia's correction at the time at which there is the greatest mean difference between NRL972 and placebo. The primary QT_C analysis used Fridericia's adjustment (QT_CF). Analyses were also done using Bazett's (QT_CB) and individual adjustments (QT_CI) using linear and non-linear regression techniques. The formula for individual adjustments was derived from data collected following placebo treatment.

To conclude that NRL972 does not prolong the QT interval, the upper bound of the one-sided 95% confidence interval (CI) for the difference in QT_CF change from baseline between NRL972 and placebo at the time at which there was the greatest mean difference between NRL972 and placebo was to have been less than 10 ms.

The relationship of PK variables to QT_C results was determined. In addition, the relationship of the PK variables calculated for the two doses was determined by calculating the ratio of geometric means for C_{max}, $AUC_{(0-t_z)}$, and $AUC_{(0-\infty)}$ between the 10 mg and 2 mg doses and their 95% CIs. The ratios and CIs were to be calculated from an appropriate linear regression model on the log-transformed data, taking account of the within-subject comparisons.

Post-hoc analyses that were not pre-planned in the SAP were added at the request of the Sponsor after the results had been analysed. These analyses determined PK variables for dose normalised 10 mg NRL972 and t-tests for PK variables with raw and natural log transformed data, comparing 2 mg NRL972 with 10 mg NRL972 and 2 mg NRL972 with dose normalised 10 mg NRL972.

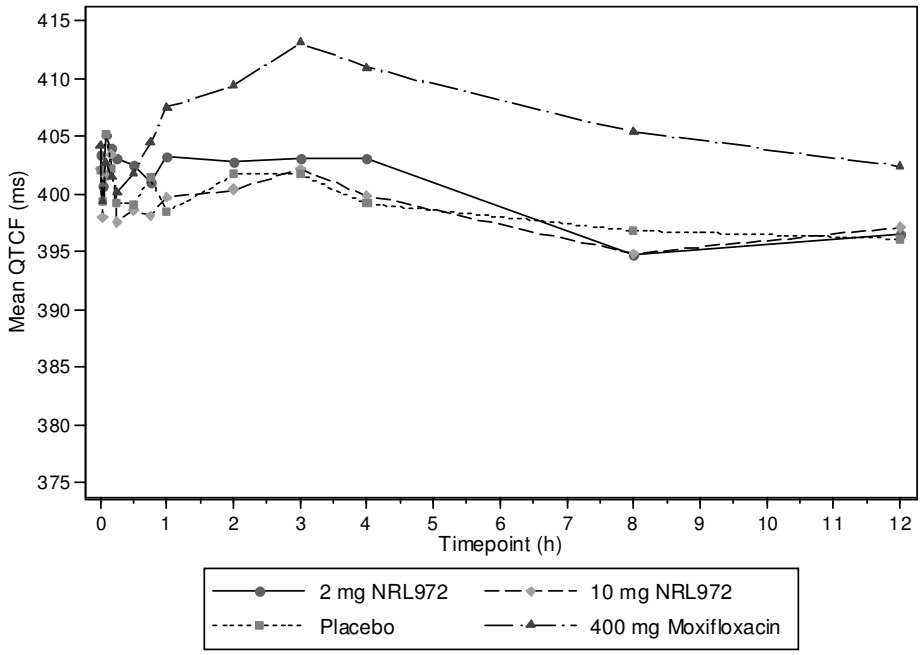
Safety and demographic data are presented descriptively.

Summary/conclusions:

Pharmacodynamic and pharmacokinetic results:

Holter ECG results

The mean QT_CF values from 0–12 hours, by treatment, are shown in the graph below.



The QT_CF interval began to increase at approximately 45 minutes after moxifloxacin administration.

The mean change from baseline in QT_CF values over time was similar for both doses of NRL972 and placebo. The results of the statistical analyses comparing treatments with placebo are show in the table below.

Comparator	Difference (ms)	95% CI (ms)	p
2 mg NRL972*	0.86	–2.57, 4.29	0.622
10 mg NRL972*	1.49	–1.93, 4.92	0.393
400 mg moxifloxacin**	9.38	5.8, 12.96	< 0.001

*at 10 minutes; **at 3 hours.

The difference between NRL972 and placebo was not statistically significant at either dose.

However, the upper limit of 95% CI for each dose of NRL972 treatment was not greater than 10 ms. The difference between moxifloxacin and placebo was statistically significant and the upper limit of the 95% CI was greater than 10 ms (12.96 ms). This is very similar to the result of another study that used moxifloxacin to establish assay sensitivity. In that study, moxifloxacin prolonged QT_CF by 9.77 ms 3 hours after moxifloxacin administration. Therefore, moxifloxacin worked effectively as a positive control.

The results for QT_C, QT_CB, and QT_CI showed similar time profiles after dosing with both doses of NRL972 and placebo. There was a statistically significant effect of moxifloxacin on QT_C, QT_CB, and QT_CI.

There were no obvious effects of any of the treatments on heart rate, PR interval, or QRS interval.

Pharmacokinetic results

The calculated PK variables for NRL972 are shown in the table below.

Variable	Statistic	2 mg NRL972	10 mg NRL972	Dose normalised 10 mg NRL972
$AUC_{(0-\infty)}$ (ng/mL.h)	GM	119.93	655.46	131.09
	CV%	25.06	21.75	21.75
$AUC_{(0-t_z)}$ (ng/mL.h)	GM	118.26	639.35	127.87
	CV%	25.70	22.58	22.58
$AUC_{(t_z-\infty)}$ (ng/mL.h)	GM	2.69	5.11	1.02
	CV%	41.00	62.20	62.20
$C_{(t_z)}$ (ng/mL)	Mean (SD)	14.92 (4.9)	16.92 (4.93)	3.38 (0.99)
CL (mL/min)	Mean (SD)	285.93 (69.24)	260.17 (56.73)	-
CL/BW (mL/(kg.min)	Mean (SD)	4.06 (0.95)	3.74 (0.84)	-
C_{max} (ng/mL)	GM	567.25	2935.51	587.10
	CV%	15.55	16.37	16.37
k_{el} (/h)	Mean (SD)	4.47 (0.91)	2.44 (1.19)	-
MRT (min)	Mean (SD)	13.76 (2.16)	17.23 (3.70)	-
$C_{(t_z)}$' (ng/mL)	Mean (SD)	12.57 (5.12)	11.63 (3.81)	2.33 (0.76)
$T_{½}$ (min)	Mean (SD)	9.63 (1.66)	21.83 (12.06)	-
t_{max} (min)	Median	2.50	2.50	-
	Min–max	2.5–2.5	2.50–2.50	-

CV, coefficient of variation; GM, geometric mean; Min, minimum; Max, maximum; -, not applicable

The results of the statistical analyses, showing the ratio of geometric means of PK variables comparing both doses of NRL972, are given in the table below.

Variable	Test treatment	Reference treatment	Test value	Reference value	Estimate	95% CI
$AUC_{(0-\infty)}$ (ng/mL.h)	10 mg NRL	2 mg NRL	119.93	655.46	5.435	5.2, 5.68
$AUC_{(0-t_z)}$ (ng/mL.h)	10 mg NRL	2 mg NRL	118.26	639.35	5.426	5.17, 5.7
C_{max} (ng/mL)	10 mg NRL	2 mg NRL	567.25	2935.51	5.155	4.99, 5.33

The C_{max} was proportional to the dose of NRL972. An increase in the dose of a factor of 5 led to an increase in the C_{max} by a factor of approximately 5.

For AUC_t and AUC_{inf} an increase in the dose of a factor of 5 led to an increase in the $AUC_{(0-t_z)}$ and $AUC_{(0-\infty)}$ by a factor of 5.4. Although this difference was statistically significant, it is unlikely to be of clinical significance.

Post-hoc t-test analysis showed that CL was significantly slower and $t_{1/2}$ significantly higher with 10 mg NRL972 compared with 2 mg NRL972 ($p < 0.01$).

The calculated PK variables for moxifloxacin are shown in the table below.

Variable	Statistic	400 mg moxifloxacin
$AUC_{(0-t_z)}$ (ng/mL.h)	GM	24279.6
	CV%	23.33
t_{max} (min)	Median	120
	Min–max	30–240
C_{max} (ng/mL)	GM	3018.5
	CV%	21.8

CV, coefficient of variation; GM, geometric mean; Min, minimum; Max, maximum.

Relationship between pharmacokinetics and pharmacodynamics

The mean predicted values for the delta-delta QT_CF ($\Delta\Delta QT_CF$) and the $\log_{10}$ plasma concentration for both drugs are shown in the table below. $\Delta\Delta QT_CF$ is the difference between NRL972 and placebo in the change from baseline.

Population	Test treatment	Mean $\log_{10}$ C_{max} (ng/mL)	Mean predicted $\Delta\Delta QT_CF$ (ms)	Lower 95% CI	Upper 95% CI
PP	2 mg NRL972	2.754	–1.194	–3.638	1.250
PP	10 mg NRL972	3.468	–1.711	–4.727	1.305
PP	400 mg moxi-floxacin	3.479	6.885	3.344	10.426

The statistical models show that for a ten-fold increase in the mean plasma NRL972 concentration there was a change in the mean placebo-corrected change from baseline in QT_CF of –0.73 ms (95% CI: –2.1, 0.62). This effect was not statistically significant (p=0.289). For moxifloxacin, for a ten-fold increase in the mean plasma concentration, there was a change in the mean placebo-corrected change from baseline in QT_CF of 8.1 ms (95% CI: 4.7, 11.5) and this was statistically significant ($p<0.001$).

Safety results:

There were no deaths and no SAEs during the course of the study. Forty two AEs were reported in 22 subjects. None of the AEs led to withdrawal from the study. One subject (008) did not complete study period 2 owing to an AE (tonsillitis), which resolved. He did not complete study periods 3 and 4 and did not return for the post study physical examination, therefore formal lost to follow up procedures were completed. There were more AEs in the placebo group (14 AEs) compared with the other treatment groups (2 mg NRL972, 8 AEs; 10 mg NRL972, 11 AEs; Moxifloxacin, 5 AEs). All of the AEs were mild to moderate in severity. Six AEs reported by three subjects were possibly related to study medication and all occurred in the placebo group. Most AEs were unrelated to study medication. The most frequently occurring AEs were dizziness, headache, nausea, and venous puncture site haematoma. In addition, there were no clinically relevant effects of study medication on vital signs or laboratory parameters.

Conclusions:

Neither dose of NRL972 induced clinically or statistically significant QT_C prolongation in healthy volunteers compared with placebo. Moxifloxacin produced a statistically significant QT_CF prolongation of 9.38 ms compared with placebo 3 hours after dosing.

CL and CL/BW were significantly slower and $t_{1/2}$ significantly higher with 10 mg NRL972 compared with 2 mg based on the extended detection of NRL972 at later sampling time points. The C_{max} occurred at the same time point for both doses of NRL972 and was dose proportional. For AUC_t and AUC_{inf} an increase in the dose of a factor of 5 led to an increase in the $AUC_{(0-t_z)}$ and $AUC_{(0-\infty)}$ by a factor of 5.4. This difference was statistically significant, but unlikely to be clinically significant.

Both doses of NRL972 were well tolerated; there were no clinically relevant AEs attributable to the investigational clinical trial medication. There were no limiting noteworthy findings upon physical examination, vital signs or ECG, and there were no noteworthy findings or changes in the clinical laboratory test results.

This thorough QT_C study indicates that NRL972 has no threshold pharmacological effect on cardiac repolarisation, as detected by QT_C prolongation, when administered either at a therapeutic (2 mg) or supratherapeutic (10 mg) dose. These results indicate it is not necessary to intensively study the effect of NRL972 on the QT_C interval during the development of NRL972.

Date of report: 23 July 2009