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First-in-Human Single-Ascending-Dose, Multiple-Dose, and Food Interaction Studies of NRD.E1, an Innovative Nonopioid Therapy for Painful Diabetic Peripheral Neuropathy

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Abstract

Painful diabetic peripheral neuropathy is characterized by burning, stabbing, or electric shock–type pain, which severely impacts day-to-day functioning and quality of life. Here, we report the results of 3 phase I studies with NRD135S.E1 (referred to as NRD.E1), a new, orally available chemical entity, presently developed for the treatment of painful diabetic peripheral neuropathy. The first study was a first-in-human, randomized, placebo-controlled, single-ascending-dose study, where NRD.E1 was administered to healthy male subjects in single dosages ranging from 300 to 1200 mg. The second study was a randomized, placebo-controlled multiple-dose study, where healthy male subjects received 300 mg of NRD.E1 once daily for 5 consecutive days. The third study was an open-label food interaction study in healthy men and women following a crossover design, where NRD.E1 was administered under fed and fasted conditions at 40 mg. The studies revealed dose-dependent absorption, increased exposure to NRD.E1 when administered with food, and no relevant accumulation after once-daily administration. All 3 phase I studies consistently showed rapid absorption of orally administered NRD.E1 followed by fast elimination, mainly via metabolization (glucuronidation), and small secondary increases in plasma concentrations. NRD.E1 was well tolerated, with no subject discontinuation due to treatment-emergent adverse events in any study.

Keywords

food effect, multiple-dose, nonopioid, NRD.E1, NRD135S.E1, painful diabetic peripheral neuropathy, PDPN, pharmacokinetics, phase 1, single-ascending-dose, tolerability

Peripheral neuropathy is a persistent condition encompassing a broad range of disorders presenting with nervous system damage and dysfunction.[1] The most common form of neuropathy is diabetic neuropathy, which develops in approximately half of all patients with diabetes. A common subtype of diabetic neuropathy, accounting for up to 25% of cases, is painful diabetic peripheral neuropathy (PDPN).[2,3] The symptoms of PDPN interfere with day-to-day functioning, quality of life, and sleep and cause anxiety and weakening.[4–6] The clinical management of patients with PDPN remains a major challenge since most available drugs fail to achieve a relevant pain reduction or are often poorly tolerated.[3,7–9] Furthermore, many therapies for patients with PDPN, such as treatment with tricyclic antidepressants or classical opioids, are prescribed off-label and can be associated with risk of abuse, physical dependence, and withdrawal symptoms upon discontinuation.[10–12] A

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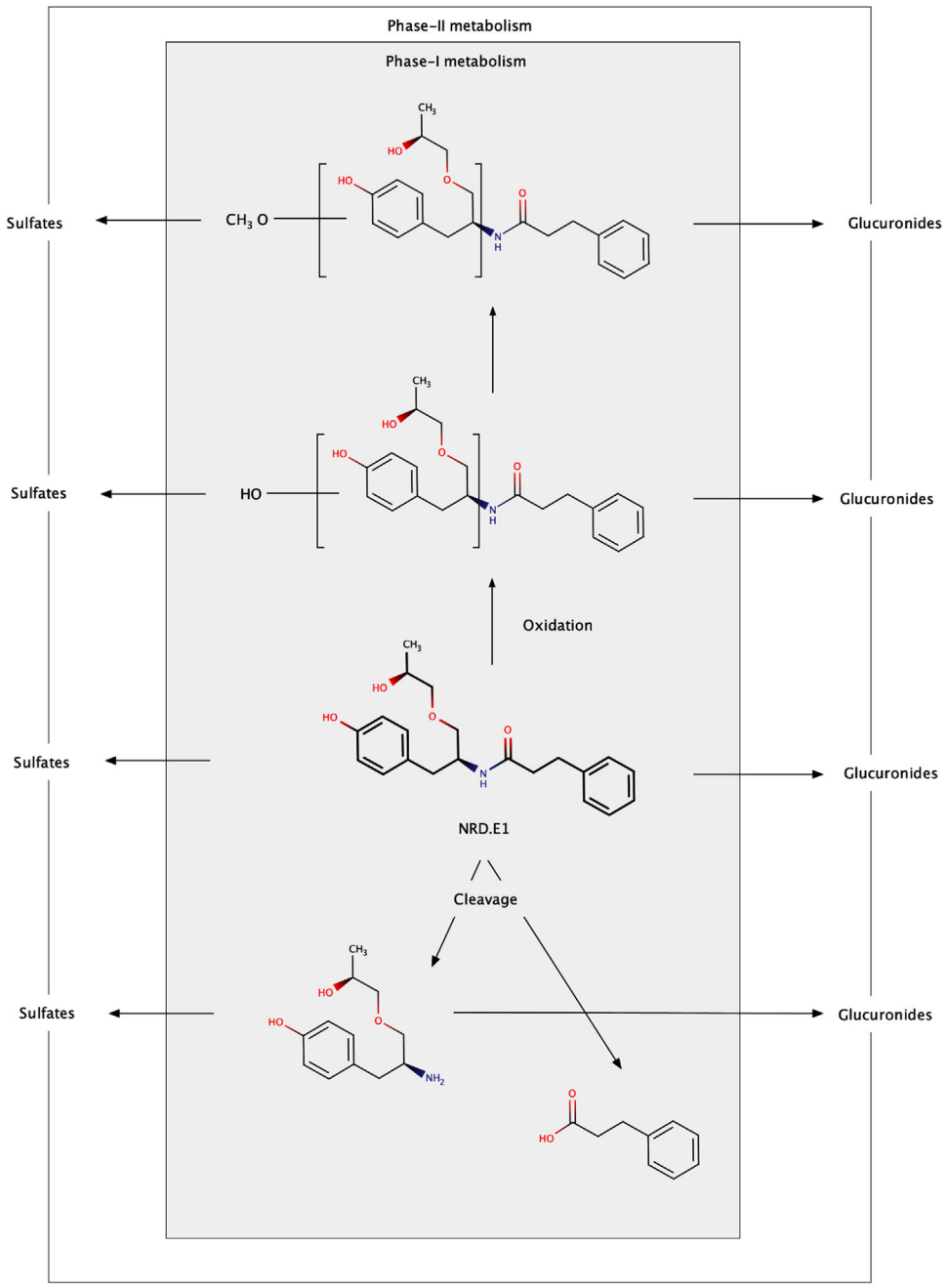

Figure 1. Metabolism of NRD.E1 ($C_{21}H_{27}NO_4$, 357.44 g/mol). Putative structures from LC-HRAMS and -MS/MS experiments. LC, liquid chromatography; HRAMS, high-resolution accurate mass spectrometry; MS/MS, tandem mass spectrometry.

new chemical entity, NRD135S.E1 (referred to as NRD.E1), is currently being developed as a nonopioid, orally available small molecule (Figure 1) with promising therapeutic potential for the treatment of PDPN.[13]

NRD.E1 is a chiral molecule with 2 stereogenic centers. The drug substance included in the drug product is the stereoisomer with (S,S)-configuration. Solubility testing revealed that NRD.E1 is a lipophilic molecule (logD 2.44, calculated using ACD lab Software[14]), which is freely soluble in ethanol (334 mg/mL) and methanol (208 mg/mL); slightly soluble in acetonitrile (9.01 mg/mL) and ethyl acetate (7.13 mg/mL); only very slightly soluble in water (0.42 mg/mL), methyl tert-butyl ether (0.71 mg/mL), phosphate buffer pH 7.4 (0.33 mg/mL), and simulated gastric fluid (0.33 mg/mL); and insoluble in simulated intestinal fluid and acid phthalate buffer pH 3.0.

Investigation of the crystalline structure of NRD.E1 revealed 2 different polymorphic forms. Polymorph 1 is

thermodynamically less stable than polymorph 2 and converts into the more stable form in the presence of water.

In nonclinical (rat and mouse) models of acute and chronic pain such as Chung's spinal nerve ligation model (compression-induced neuropathic pain)[15] and streptozotocin-induced painful neuropathy models,[16] NRD.E1 showed dose-dependent, antinociceptive effects. The exact mechanism of action is currently not fully elucidated, yet the compound does not appear to act directly through receptors known to be associated with pain and abuse, including opioid receptors. Importantly, orally administered NRD.E1 did not accumulate after repeated dosing in rats and dogs; it showed rapid absorption, with bioavailability ranging from 11% to 17% in rats to about 40% in dogs. Intravenously administered NRD.E1 was rapidly eliminated with half-lives around 2 to 5 hours. It was extensively metabolized in rats and dogs; the recovered unchanged drug accounted for only 0.10%, 0.38%, and 0.64% in feces, bile, and urine, respectively. In total, 34 different metabolites, most of them at trace levels, have tentatively been identified in nonclinical settings, with the main metabolites in plasma being the cleavage products of an NRD.E1 glucuronide and of NRD.E1 itself; oxidation appeared to be a minor pathway in the rat (Figure 1). Results from studies in human microsomes with specific chemical inhibitors, focusing on Phase-I metabolic pathways, showed that oxidation reactions of NRD.E1 were mainly driven by cytochrome P450 3A4 with no relevant contributions from other isozymes.

To date, the clinical development program of NRD.E1 for PDPN comprises 3 completed phase I studies in healthy volunteers and 1 phase II dose-finding study in patients with PDPN (ClinicalTrials.gov NCT02345291).[13] Here, we report the results of the phase I first-in-human single-ascending-dose, multiple-dose, and food interaction studies to characterize the pharmacokinetics (PK), safety and tolerability of NRD.E1 in healthy subjects as well as to select the dose for the upcoming clinical program in PDPN.

Methods and Subjects

The study protocols and informed consent forms for all 3 studies reported here were reviewed and approved by the relevant regulatory authorities and ethics committees as per local regulations (for the single-ascending-dose study: Ethics [Helsinki] Committee, Tel Aviv, Israel, and Israel Ministry of Health, Jerusalem, Israel; for the multiple-dose study: Ethics [Helsinki] Committee, Tel Aviv, Israel; for the food interaction study: Ethics Committee of the Landesärztekammer Thueringen and BfArm, Bonn, Germany). All studies were conducted in accordance with the Declaration of Helsinki, the International Council for Harmonisation Guidelines for Good Clinical Practice and national requirements. All participants provided written informed consent before study entry.

Different oral formulations and/or drug products of NRD.E1 were used in the 3 phase I studies. In the single-ascending-dose study, subjects received an oral suspension of NRD.E1; in the multiple-dose study, hard gelatin capsules manufactured by Quay Pharmaceuticals LTD (Quay, Deeside, UK); and in the food interaction study, hard gelatin capsules manufactured by HWI development GmbH (HWI, Appenweier, Germany). The single-ascending-dose and the multiple-dose study used polymorph 1 of NRD.E1 (manufactured by Regis Technologies, Morton Grove, Illinois), whereas the food interaction study used the more stable polymorph 2 (manufactured by Dr. Reddy's Laboratories, Hyderabad, India).

Single-Ascending-Dose and Multiple-Dose Studies

Both studies were designed as single-center, randomized, double-blind, placebo-controlled studies and conducted at Tel Aviv Sourasky Medical Center, Israel. The primary objectives were to evaluate safety and tolerability and, at the same time, to determine the PK of NRD.E1. The secondary objectives of the single-ascending-dose study included the determination of the maximum tolerated dose (MTD) and dose-limiting toxicities of NRD.E1.

Eligible subjects of the single-ascending-dose study were treated in 1 of 4 sequential cohorts (8 subjects/cohort, 6 on NRD.E1, 2 on placebo) and received a single oral dose of 300, 600, 900, or 1200 mg NRD.E1, or matching placebo, in suspension after at least a 10-hour fast (water allowed up to 1 hour before dosing). After dosing, subjects continued fasting (water allowed from 1 hour after dosing) until 4 hours after dosing, at which time a standardized light breakfast was provided. Meals were also provided 8 and 12 hours after dosing. Blood samples (8 mL each) were withdrawn within 1 hour before dosing as well as at 10, 30, and 45 minutes; and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after dosing. Urine was collected before dosing on Day 1 and over 0- to 6-, 6- to 12-, and 12- to 24-hour intervals after dosing.

Eligible subjects of the multiple-dose study received oral doses of 300 mg of NRD125S.E1 (4 capsules of 75 mg) or placebo in a 3:1 randomization (12 subjects, 9 on NRD.E1 and 3 on placebo) once daily over 5 consecutive days after at least a 10-hour fast before next-day dosing (water allowed up to 1 hour before dosing). After drug administration, subjects continued fasting for 4 hours when they received a standardized light breakfast. Meals were also provided 8 and 12 hours after dosing. Blood samples were withdrawn within 1 hour before dosing on days 1 and 5,

and trough samples on days 3 and 4 were taken within 15 minutes before dosing. Serial blood samples for PK analysis on Days 1 and 5 were drawn at 10, 30, and 45 minutes; and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after dosing.

The study populations comprised healthy, nonsmoking males aged 18 to 44 and 18 to 45 years for the single-ascending-dose and the multiple-dose study, respectively. Main inclusion criteria were body mass index, 19.0 to 30.0 kg/m^2; body weight, 65 to 85 kg; blood pressure (BP) and heart rate (HR) within normal limits (systolic BP, 90-140 mm Hg; diastolic BP, 50-90 mm Hg; HR, 60-100 bpm); electrocardiogram (ECG) with no clinically significant abnormalities (PR interval, 120-200 milliseconds; QRS interval, <120 milliseconds; corrected QT interval, <440 milliseconds); no clinically significant abnormalities in hematology, blood chemistry, or urinalysis laboratory tests; and no known history of alcohol or drug abuse. The main exclusion criteria were history or ongoing symptoms of any gastrointestinal disorder; history of intestinal surgery; history of significant medical disorder, which in the investigator's judgment contraindicated administration of the study medication; use of any prescription or over-the-counter medications, vitamins, or herbal or dietary supplements within 14 days before the anticipated first study dose (paracetamol or ibuprofen for symptomatic relief of pain was allowed until 24 hours before study treatment); acute illness within 48 hours before the first dose; and participation in a clinical trial with drugs within 3 months before dosing.

Food Interaction Study

The study was designed as a single-center, open-label, randomized (order of treatments), 2-period, 2-sequence, single dose (one 40 mg capsule) crossover trial and conducted at SocraTec R&D GmbH (Erfurt, Germany). After an overnight fasting period of at least 10 hours (water allowed up to 1 hour before dosing), subjects either received the capsule in the fasted state or received a high-fat, high-calorie meal (≈150 kcal from protein, 250 kcal from carbohydrate, and 500-600 kcal from fat) before taking the capsule. Subjects received further standardized meals 4, 7, and 12 hours after study drug administration. The primary objective was to evaluate equivalence of NRD.E1 administration under fasted versus fed conditions by use of area under the concentration–time curve (AUC) from time 0 to infinity ($AUC_{0\text{-inf}}$), AUC from time 0 to the last quantifiable concentration ($AUC_{0\text{-t}}$), and maximum concentration (C_{max}). Secondary objectives were to further characterize the PK of NRD.E1 in plasma after oral single-dose administration under fed and fasted conditions and to evaluate safety and tolerability aspects of NRD.E1. Exploratory objectives were to screen for the presence of NRD.E1-derived metabolites, to assess their relative abundance and PK parameters, and to compare the identified metabolite profile with data collected from nonclinical toxicology studies conducted in rats and dogs.

Eligible subjects were randomly assigned to 1 of the 2 possible treatment sequences (8 subjects/sequence) with a balanced distribution of sequences to both sexes according to a Latin square (fasted or fed). The first treatment under a fasted or fed condition was followed by an at least 6-day treatment-free washout period before assignment to the alternate prandial condition and receipt of another 40-mg capsule of NRD.E1. Blood samples (4 mL each) were withdrawn within 1.0 hour before dosing as well as at 5, 10, 15, 20, 30, 45, 60, 75, and 90 minutes; and 2, 3, 4, 6, 8, 12, 16, 24, 36, and 48 hours after dosing.

The study population comprised both female and male healthy nonsmoking subjects aged 18 to 60 years. Age matching was performed for both sexes according to the following criteria: median of both female and male subjects ±10 years, lower limit ±5 years, and upper limit ±5 years. Main inclusion criteria were body mass index 18.5 to 30.0 kg/m^2 and a good state of health. Women had to be permanently sterile or in the postmenopausal state. The main exclusion criteria were existing cardiac, hematologic, hepatic, renal, and/or gastrointestinal diseases, which might interfere with the safety or tolerability, absorption, and/or disposition of the active ingredient; systolic BP outside 90 to 139 mm Hg, diastolic BP outside 60 to 89 mm Hg, HR outside 50 to 90 bpm, QTc interval >450 milliseconds for men and >470 milliseconds for women, clinically relevant abnormalities in a 12-lead ECG, laboratory values out of normal range unless the deviation from normal was judged as not relevant for the study by the investigator, and administration of any investigational medicinal product within the past 3 months or having participated in more than 4 investigational drug studies within 1 year before individual enrollment of the subject.

Sample Processing and Analysis

For all PK studies, plasma concentrations of NRD.E1 were determined using either protein precipitation or liquid-liquid extraction methods with subsequent tandem mass spectrometric measurement. The internal standards were either a chemical analog of the compound in initial studies or a $13C_3$-labeled isotope standard in later studies. The lower limit of quantification (LLOQ) was 10 μg/L for the single-ascending-dose and multiple-dose studies (to account for the higher levels of NRD.E1 encountered due to the doses [up to 1200 mg/day] administered in these studies) and 0.05 mg/mL for the food interaction study (to account

for the lower levels of NRD.E1 encountered due to the dosage [40 mg/day] administered in this study).

In the metabolism investigations, protein precipitation extraction was used for all studies to avoid losses through incomplete recovery. Metabolites were identified by high-resolution accurate mass spectrometry (MS) and expert interpretation of the product ion spectra. The semiquantitative metabolic profiling comparison between toxicology and human samples was done by single-ion monitoring MS on all known metabolite masses with $13C_3$-NRD.E1 as internal standard.

All analytical methods are detailed in Table 1.

The PK data obtained in the single-ascending-dose and the multiple-dose studies were used to estimate the concentrations that may have been achieved at 2 effective doses in the phase II dose-finding study[13] (all 3 studies conducted with an active pharmaceutical ingredient [API] consisting of polymorph 1). These putative concentrations were put in perspective of concentrations and results of the food interaction study (conducted with a capsule containing polymorph 2 [current capsule]) to estimate an effective and safe dose for future trials when the current capsule formulation is administered.

Safety Assessments

The safety of NRD.E1 was assessed in all 3 studies by continuously monitoring adverse events and evaluating several parameters including vital signs, clinical laboratory assessments, ECGs, and physical examinations at predefined time points. As part of a post hoc ECG safety analysis of the single-ascending-dose study, effects of increasing doses of NRD.E1 on ECGs in healthy male subjects were evaluated by a core ECG laboratory. In this study, single 12-lead ECGs were recorded at screening, on the dosing day within 1 hour before dosing and at 1, 2, 4, 12, and 24 hours after dosing and at the end-of-study visit (7-10 days after dosing day). ECGs were measured and a qualitative interpretation of abnormalities was performed by a cardiologist in a blinded fashion.

In line with recent literature,[17] emphasis was put on the outcome of the concentration-response analysis. Model development started with the prespecified mixed linear model as detailed by Garnett et al[18] considering ΔQTcF (Fridericia's cube-root corrected QT; QTcF [milliseconds] = QT [milliseconds]/RR[s]$^{1/2}$) as dependent variable. Placebo data were considered for the analysis with concentration values set to 0. Moreover, concentrations below the lower limit of quantification (LLOQ), before the first measurable concentration or after the last measurable concentration, were also set to 0. When embedded between 2 measurable concentrations, a concentration below LLOQ was considered as missing.

The fixed-effect parameters of the prespecified model were intercept, slope of NRD.E1 concentrations, influence of baseline (centered on mean) on intercept, treatment-specific intercept (0 = NRD.E1, 1 = placebo), and nominal time as categorical variable. Subject-specific random effects were added on intercept and slope parameters with an unstructured covariance matrix.

$$\Delta QTcF_{ijk} = (\theta_0 + \eta_{0,i}) + TRT_j + (\theta_1 + \eta_{1,i})C_{ijk} + \theta_3 * Time + \theta_2(QTcF_{ijk=0} - QTcF_0) + \varepsilon_{ijk} \quad (1)$$

where $\Delta QTcF_{ijk}$ = change from baseline in Fridericia corrected interval (QTcF) for subject i in treatment j at time k; θ_0 = population mean intercept in the absence of a treatment effect; $\eta_{0,i}$ = random effect associated with the intercept term θ_0; TRT_j = treatment (0 = active drug, 1 = placebo); θ_1 = population mean slope of the assumed linear association between concentration and $\Delta QTcF_{ijk}$; $\eta_{1,i}$ = random effect associated with the slope θ_1; C_{ijk} = concentration for subject i in treatment j and time k; θ_3 = fixed effect associated with time k; and θ_2 = fixed effect associated with baseline $QTcF_{ijk=0}$; and $QTcF_0$ = overall mean of $QTcF_{ij0}$, that is, the mean of all the baseline (= time 0) QTcF values. Changes from baseline were computed as $\Delta Y_{ij} = Y_{ijk}$ (subject i, day j, time k)- Y_i (subject i, baseline).

The ΔΔQTcF and their 2-sided 90%CIs were calculated at each NRD.E1 dose's C_{max} (geometric mean) as follows:

$$\Delta\Delta QTcF_n = (TRT_0 - TRT_1) + \theta_1 * C_{\max,n} \quad (2)$$

where $\Delta\Delta QTcF_n$ = placebo-corrected change from baseline in QTcF at dose n; TRT = treatment (0 = active drug, 1 = placebo); θ_1 = population mean slope of the assumed linear association between concentration and $\Delta QTcF_{ijk}$; and $C_{max,n} = C_{max}$ at dose n.

No additional covariate investigation was performed in order to avoid unnecessary model-building steps, and nonsignificant fixed parameters were not removed from the model, unless they caused nonconvergence or parameter estimation issues. Random effects not supported by the model were removed as they could have resulted in nonconvergence.

Statistical Methods and Analysis Sets

Two analysis sets were defined for the single-ascending-dose and the multiple-dose study: the safety analysis set, including all subjects who received the study drug, and the PK set, including all subjects who received NRD.E1 and had sufficient plasma concentrations of NRD.E1 to estimate PK parameters.

For the single-ascending-dose study, mean concentrations, mean dose-normalized concentrations, and

Table 1. Sample Preparation and Analytical Methods for the Quantification of NRD.E1 and Identification of Metabolites

	NRD.E1 Quantification in SAD and MD Study	NRD.E1 Quantification in FI Study	Metabolic Profiling in MD Study	Metabolic Profiling in FI Study
Internal Standard (IS)	NRD175S (S)-N-(1-(2-hydroxyethoxy)-3-(4-hyroxyphenyl)propan-2-yl)-3-phenylpropanamide	$[^{13}C]_3$ labeled NRD.E1	Propranolol	$[^{13}C]_3$ labeled NRD.E1
Sample preparation	100 μL plasma + 20 μL IS + 1000 μL AcN Vortex, centrifuge (10 min, 13 000*g*, 8°C) 800 μL supernatant evaporated to dryness (stream of nitrogen at 40°C) and reconstituted in 350 μL AcN: water: formic acid 50: 50: 0.1 Vortex, centrifuge (5 min, 5300*g*, room temperature), analyze supernatant	100 μL plasma + 20 μL IS + 400 μL tert-butyl methyl ether Vortex, centrifuge 300 μL supernatants evaporated to dryness (stream of nitrogen at 40°C) and reconstituted in 150 μL of 10mM Ammonium formate brought to pH 3.5 with formic acid/acetonitrile 1/1 Vortex, analyze solution	Plasma + AcN (1/3 v/v) + IS Vortex, centrifuge Supernatants equally pooled across all samples at 2 time points (30 min and 1 h) Urine + equal volume AcN/MeOH (1/1) + IS Vortex, centrifuge Supernatants equally pooled across all samples	300 μL animal plasma + 20 μL IS + 600 μL AcN 90 μL human plasma + 20 μL IS + 180 μL AcN Vortex, centrifuge, analyze supernatant
HPLC	50×2 mm Phenomenex Gemini 110A C_{18}, 3 μm, with guard column (Phenomenex Ltd, Germany), 25°C Mobile phase A = AcN: water: formic acid 10: 90: 0.1 B = AcN: water: formic acid 90:10:0.1 Flow 300 μL/min Gradient (all changes linear): 0 min – 0% B 1.7 min – 100% B 3.8 min – 100% B 3.9 min – 0% B 5 min – end	100×2.1 mm YMC Pro C8, 5 μm (YMC Co. Ltd, Japan), 40°C Mobile phase A = 10 mM ammonium formate pH 3.5 B = AcN Flow 300 μL/min Gradient (all changes linear): 0 min – 35% B 3.5 min – 35% B 3.51 min – 90% B 5 min – 90% B 5.01 min – 35% B 8 min – end	100×2.1 mm Waters Atlantis C18, 3 μm (Waters Corp., Milford, Massachusetts). Mobile phase A = 0.1% acetic acid in water B = 0.1% acetic acid in MeOH/AcN 1:1 Flow 300 μL/min Gradient (all changes linear): 0 min – 0% B 20 min – 30% B 30 min – 40% B 35 min – 100% B 40 min – 100% B, 350 μL/min 40.1 min – 5% B 45 min – 5% B, 300 μL/min 45 min – end	2.1×100 mm Waters Atlantis dC18, 3 μm (Waters Corp, Milford, Massachusetts) Mobile phase A = 0.1% acetic acid in water B = 0.1% acetic acid in MeOH/AcN 1:1 Flow: 300 μL/min Gradient (all changes linear): 0 min – 0% B 20 min – 30% B 30 min – 60% B 35 min – 100% B 40 min – 100% B, 350 μL/min 40.1 min – 5% B, 350 μL/min 43.1 min – 5% B, 350 μL/min 45 min – 5% B 45 min – end
MS	Micromass Quattro Ultima triple quadrupole (Waters Corp., Milford, Massachusetts) in positive electrospray mode using MRM MS/MS	Sciex API-4000 triple quadrupole (AB Sciex LLC, Framingham, Massachusetts) in positive ion electrospray mode using MRM MS/MS	Thermo Instruments hybrid LTQ-Orbitrap XL (Thermo Fisher Scientific Inc., Waltham, Massachusetts) in positive ion electrospray mode	Sciex API-4000 triple quadrupole (AB Sciex LLC, Framingham, Massachusetts) in both positive and negative ion electrospray mode

(*Continued*)

Table 1. (Continued)

	NRD.E1 Quantification in SAD and MD Study	NRD.E1 Quantification in FI Study	Metabolic Profiling in MD Study	Metabolic Profiling in FI Study
	Transitions were m/z 358-282 (NRD.E1) and 344-282 (IS)	Transitions were m/z 358-282 (NRD135S) and 361-285 (IS)	Mass spectral Resolution R = 60 000 Survey scan range 120-950 Th Automatic gain control settings at supplier default	Samples analyzed using 4 targeted single-ion-monitoring methods in both polarities for detecting and quantifying signals and 2 narrowband scan methods in both polarities for the confirmation of molecular signals. In the 3 positive ion methods, a total of 21 m/z values for all known metabolites were acquired and in the negative ion method, 7 m/z values for the acidic structures (sulfates and glucuronides)
Data processing	Peak area ratios using Waters MassLynx and QuanLynx software (Waters Corp., Milford, Massachusetts), using a $1/X^2$ weighted linear regression calibration	Peak area ratios using Analyst 1.5.2 (AB Sciex LLC, Framingham, Massachusetts), using a $1/X^2$ weighted linear regression calibration	Data dependent MS/MS analysis to automatically acquire MS^n spectra of metabolites. LC-MS data files were screened using a list of accurate mass extracted ion currents for putative metabolic conversions and by comparing predose and postdose total ion or base peak currents. Detected signals were verified and documented using targeted MS/MS scans	Manual evaluation by overlaying chromatograms of samples from control vs dosed subjects, comparison between species and with the relative retention times of the metabolic profiling in the MD study. For the quantitative comparison of each metabolite, peak area ratios obtained from animal and human samples were compared
Calibration	Range: 10-2000 mg/mL LLOQ: 10 mg/mL Precision and accuracy determined with 6 replicate analyses per standard solution of 10, 30, 150, 1000, and 1500 mg/mL Intrabatch accuracy: 99%-106% Inter-batch accuracy: 100%-105% Intrabatch precision better than ±4%, interbatch precision better than ±5% RSD	Range: 0.05-50 mg/mL. It was shown that samples up to 500 mg/mL could be analyzed with sufficient accuracy and precision by 1:10 dilution with blank plasma LLOQ: 0.05 mg/mL Intra-batch accuracy: 90%-98% Interbatch accuracy: 92%-94% Intra-batch precision: 1.1%-7.6% Interbatch precision: 1.6%-6.0%	none	none

AcN, acetonitrile; FI, food interaction; HPLC, high-performance liquid chromatography; IS, internal standard; MD, multiple-dose; MeOH, methanol; MRM, multiple ion reaction monitoring; MS, mass spectroscopy; R, mass spectral resolution M/ΔM (full width at half height); RSD, relative standard deviation; SAD, single-ascending-dose.

PK parameters were grouped and summarized per cohort. For the multiple-dose study, calculated PK parameters and mean concentrations across individual subjects were summarized per time point (Days 1 and 5).

Three analysis sets were defined for the food interaction study: the full analysis set, including all randomized subjects, the safety analysis set, including all subjects who received the study drug, and the per-protocol set, including all randomized subjects who finished the clinical trial without major protocol deviations. Calculated PK parameters and mean concentrations were summarized per condition (fed vs fasted).

For the single-ascending-dose and the multiple-dose studies, PK analyses were performed using PKSolver 2.0 software[19] that has been validated against the Statistical Analysis System (SAS) software (SAS Institute, Cary, North Carolina) and STATA/SE (STATACorp LP, College Station, Texas). For the food interaction study, all kinetic parameters were determined model-independently for each treatment using Phoenix WinNonlin software (Certara Pharmaceuticals, Princeton, New Jersey). SAS software was used for the validation of the analysis of concentration data.

Clinical data were summarized and reported using SAS software. No interim analyses were planned or performed.

Results

Patient Disposition and Baseline Characteristics

In the single-ascending-dose study, 32 male subjects were enrolled in total; 24 subjects received NRD.E1, and 8 subjects received placebo. Mean age (25.3-27.5 years) was comparable across the different cohorts (Table S1). All 32 enrolled subjects were included in the safety analysis set and the 24 subjects treated with NRD.E1 formed the PK set.

In the multiple-dose study, 9 male subjects were treated with NRD.E1 and 3 subjects received placebo. Mean age of the NRD.E1 and the placebo group were comparable (25.2 vs 24.1 years; Table S1). All 12 subjects were included in the safety analysis set, and the 9 NRD.E1-treated subjects were included in the PK set.

In the food interaction study, age matching of the 8 men and 8 women fulfilled the set criteria (mean age, 52.9 years [men, 50.9 years; women, 54.9 years]; Table S1). All 16 enrolled subjects were included in the full analysis set, safety analysis set, and per-protocol set. Since regression for estimation of the terminal elimination rate constant (λ_z) was not possible for 2 subjects, the statistical evaluation of PK parameters dependent on λ_z was performed for the remaining 14 subjects only.

No subject was prematurely discontinued from any of the studies.

Pharmacokinetic Evaluation

Overall, all 3 studies showed rapid absorption of NRD.E1 into the systemic circulation followed by a rapid initial decline of plasma concentrations. After the initial decline, slower elimination was observed, and in some subjects, NRD.E1 plasma levels showed a transient, slight increase (Figures 2–4).

Single-Ascending-Dose Study. Across single doses of 300 to 1200 mg of NRD.E1, mean time to C_{max} (t_{max}) and elimination half-life ($t_{1/2}$; in time window 1-3 hours) were in narrow ranges of 0.38 to 0.54 and 0.52-0.62 hour, respectively (Table 2 and Table S2). In some subjects, a secondary increase in plasma NRD.E1 concentration was observed after ≥6 hours after dosing (Figure 2), with the magnitude of the secondary peak ranging from 0.4% to 0.9% of the primary peak concentrations across the different cohorts.

Increases in mean C_{max} and time 0 to the last measurement time point with a concentration value above the LLOQ, calculated by means of the linear up/log down method ($AUC_{0\text{-}t}$) were dose dependent but overproportional. $AUC_{0\text{-}inf}$ was not calculated due to unreliable values for terminal $t_{1/2}$ and λ_z. However, plasma NRD.E1 concentrations of many subjects declined below LLOQ during the study period so that extrapolation to infinity would have only minimally contributed to the total $AUC_{0\text{-}inf}$.

Only a small fraction of the orally administered NRD.E1 (0.15%-0.36%) was eliminated unchanged in the urine. The main metabolite of NRD.E1 observed in urine collected from 0 to 6 hours and in plasma 30 minutes and 1 hour after dosing was a glucuronide of the parent compound. Other metabolites that were detected at significant levels in urine were absent or present in only small quantities in plasma. Overall, 22 metabolites were detected in samples from the study.

Multiple-Dose Study. NRD.E1 was given as capsules and mean t_{max} was achieved about 1 hour later (1.53 hours on days 1 and 5) than in the single-ascending-dose study where NRD.E1 was given as suspension. The decay of plasma concentrations appeared to progress at a slower pace as shown by the longer $t_{1/2}$ observed compared to the single-ascending-dose study (Table 3 and Table S3).

NRD.E1 accumulated only slightly upon multiple dosing. C_{max} and $AUC_{0\text{-}t}$ were 23.9% and 31.0%, respectively, higher on Day 5 than after the first dose on Day 1, and mean plasma trough concentrations on days 2, 3, and 4 remained at similar low mean (standard deviation [SD]) levels of 2.03 (6.09) μg/L, 3.19 (6.64) μg/L, and 1.15 (3.44) μg/L, respectively. Of note, due to

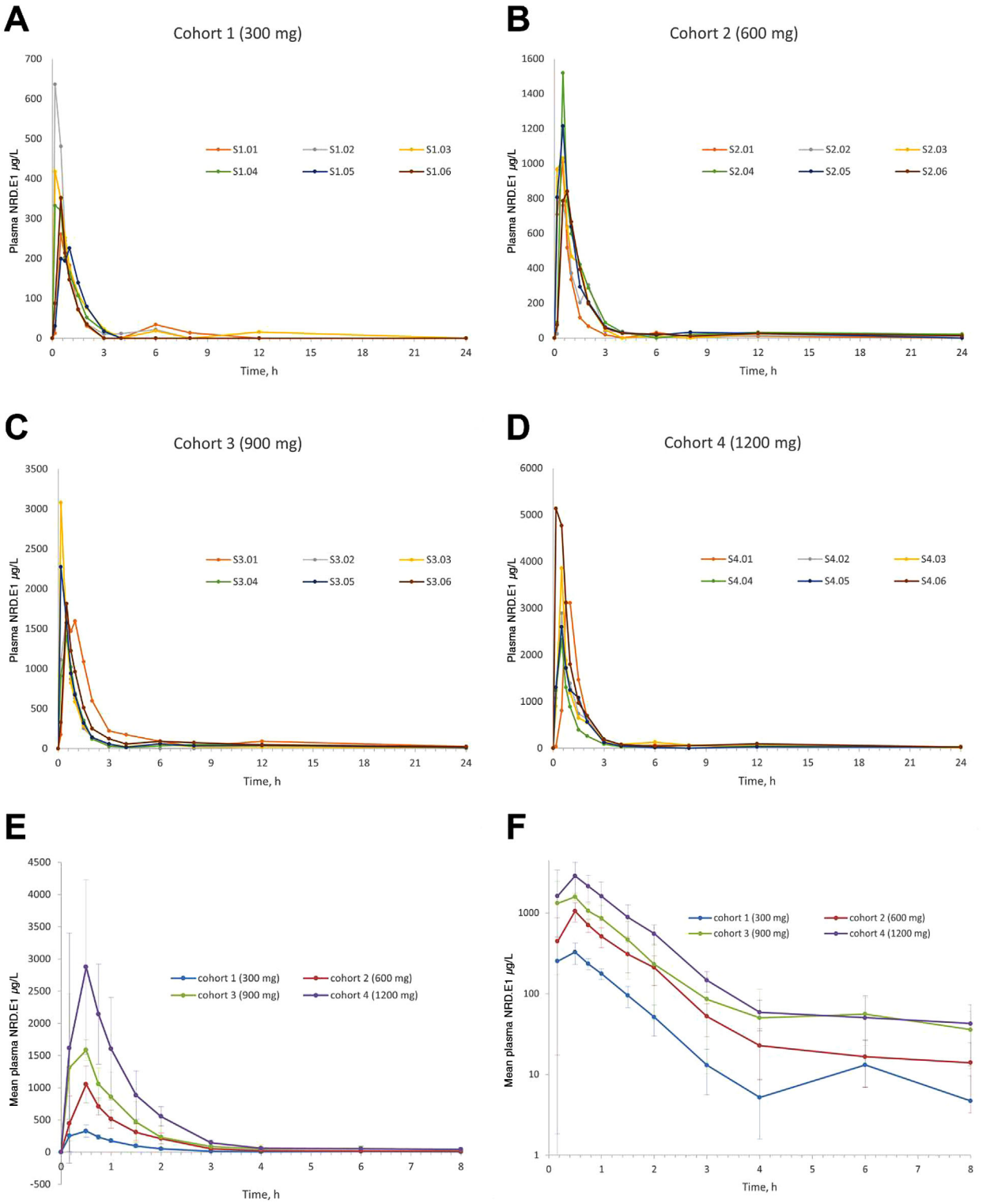

Figure 2. Plasma NRD.E1 concentration over time in the single-ascending-dose study. (A) Cohort 1, 300 mg; (B) cohort 2, 600 mg; (C) cohort 3, 900 mg; (D) cohort 4, 1200 mg. Each line represents the data recorded for 1 individual subject (S1.01-S4.06). (E) Linear scale of mean (SD) NRD.E1 plasma concentrations of the different cohorts over the first 8 hours after dosing. (F) Logarithmic scale of mean (standard deviation) NRD.E1 plasma concentrations of the different cohorts over the first 8 hours after dosing. Shown is the data of the pharmacokinetic set.

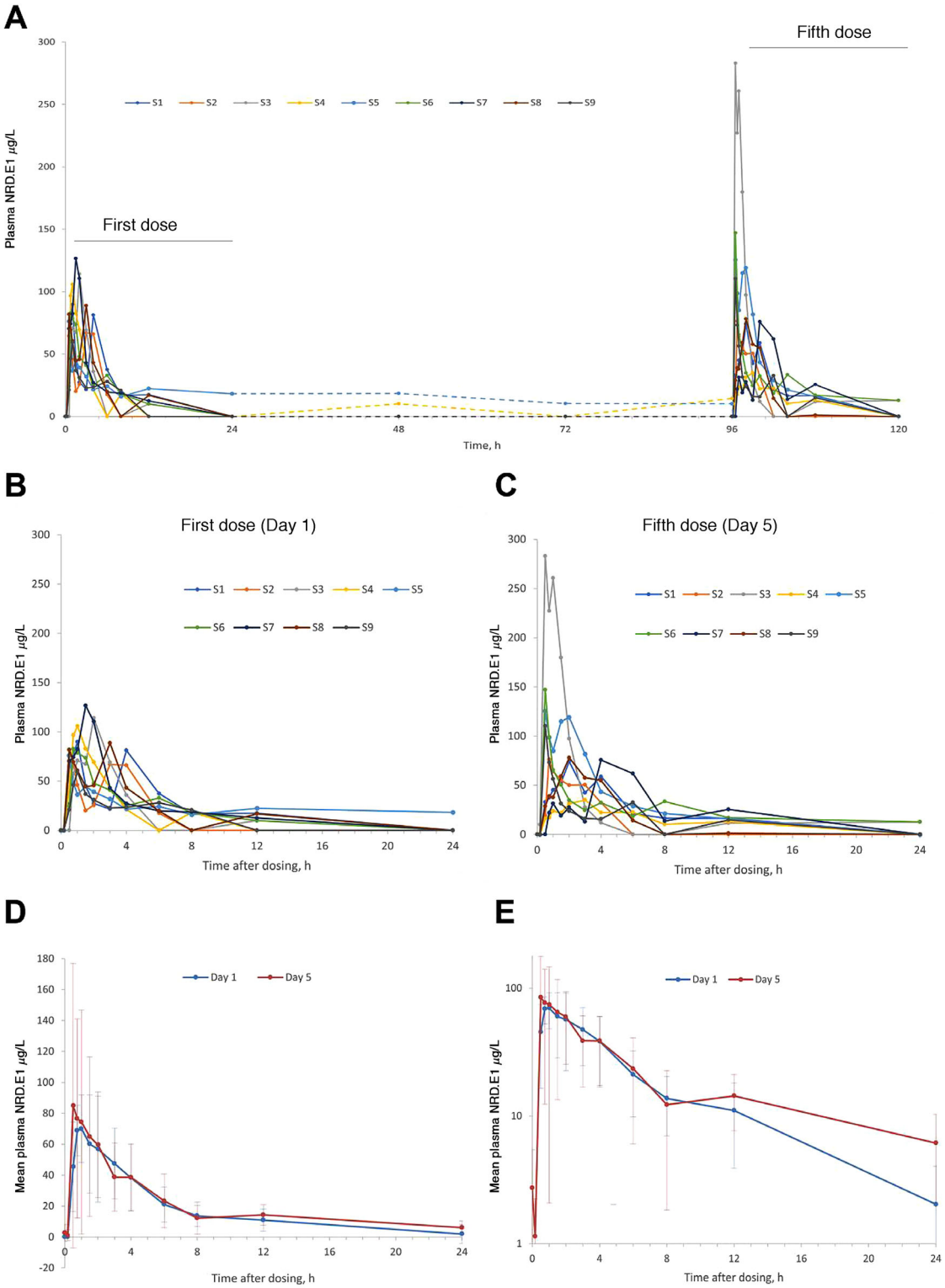

Figure 3. Plasma NRD.E1 concentration over time in the multiple-dose study. (A) Plasma concentrations of NRD.E1 in individual healthy subjects (S1-S9) recorded within the first 120 hours after first dosing. Solid lines reflect time course of concentrations during 24 hours after the first and fifth dosing. Dotted lines connect trough levels recorded before second to fifth dosing. (B) Plasma concentrations of NRD.E1 in individual healthy subjects (S1-S9) recorded within the first 24 hours after first dosing. (C) Plasma concentrations of NRD.E1 in individual healthy subjects (S1-S9) within the first 24 hours after the fifth dosing. (D) Linear scale of mean (standard deviation [SD]) NRD.E1 plasma concentrations over the first 24 hours after dosing on Day 1 (blue) and Day 5 (red). (E) Logarithmic scale of mean (SD) NRD.E1 plasma concentrations over the first 24 hours after dosing on Day 1 (blue) and Day 5 (red). Shown are the data of the pharmacokinetic set.

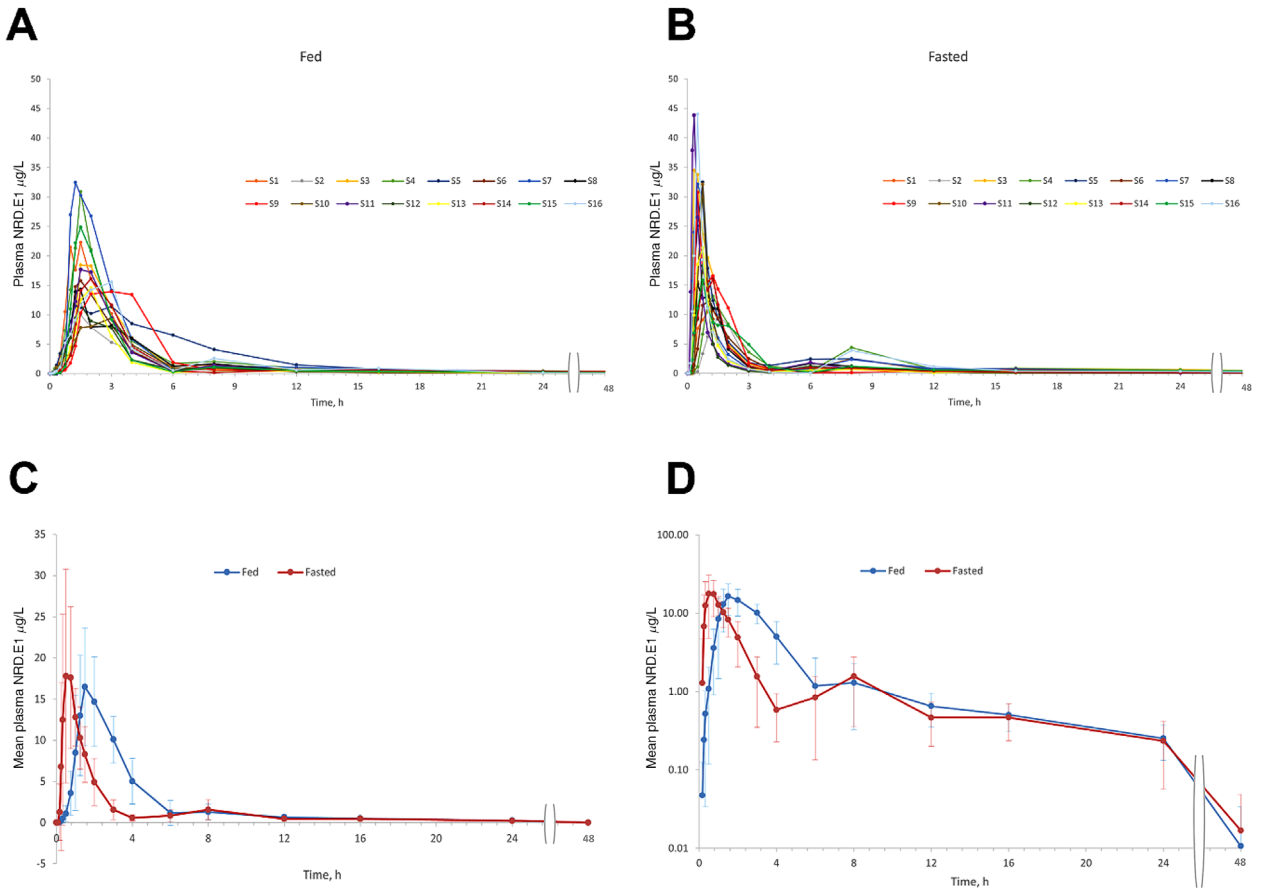

Figure 4. Plasma NRD.E1 concentration over time in the food interaction study. (A) Plasma concentration of NRD.E1 in individual healthy subjects (S1-S16) recorded within the first 48 hours after dosing under fed conditions. (B) Plasma concentration of NRD.E1 in individual healthy subjects (S1-S16) recorded within the first 48 hours after dosing under fasted conditions. (C) Linear scale of mean (standard deviation [SD]) NRD.E1 plasma concentrations recorded over the first 48 hours after dosing under fed (blue) and fasted (red) conditions. (D) Logarithmic scale of mean (SD) NRD.E1 plasma concentrations recorded over the first 48 hours after dosing under fed (blue) and fasted (red) conditions. Shown are the data of the full analysis set.

variable kinetics, the calculated λ_z was not sufficiently reliable so that no extrapolation to obtain $AUC_{0\text{-inf}}$ was performed.

Food Interaction Study. Fed vs fasted status was associated with slightly slower absorption (median t_{max}, 1.50 vs 0.75 hour) and higher exposure (geometric mean $AUC_{0\text{-inf}}$, 54.6 vs 39.4 μg • h/L; Table 4 and Table S4). Similar to the single-ascending-dose and the multiple-dose study, a minor, secondary peak was observed at 8 hours after dosing in both fed and fasted subjects (Figure 4). The magnitude of the secondary peak reached 0.7% and 5.5% of primary peak concentrations under fed and fasted conditions, respectively.

Among women, geometric mean C_{max} values after fed and fasted administration were nearly identical (17.9 vs 17.6 μg/L), whereas among men, 40% lower values were observed after fed compared to fasted administration (14.9 μg/L vs 25.7 μg/L). Overall, the extent of exposure was higher in women than men (arithmetic mean ± SD $AUC_{0\text{-t}}$: 60.4 ± 18.0 [women] vs 46.2 ± 11.5 [men] μg • h/L after fed conditions, arithmetic mean ± SD $AUC_{0\text{-t}}$: 40.8 ± 13.3 [women] vs 33.8 ± 8.79 [men] μg • h/L after fasted conditions).

Since regression for estimation of λ_z was not possible for 2 subjects, $t_{1/2}$, $AUC_{0\text{-inf,}}$ and apparent oral clearance were determined for only 14 subjects. Subjects with incomplete data sets in 1 of the administration arms were excluded from the statistical assessment of equivalence.

Similar to the single-ascending-dose study, MS analysis revealed that glucuronides were the main metabolites found in plasma, with 2 specific glucuronide conjugates accounting for close to 80% of the total MS signal. No oxidation product, neither in free nor conjugated form, reached a level of 10% of the total MS signal. No acyl-glucuronides were formed, nor could glutathione adducts be found.

Table 2. Summary of Pharmacokinetic Parameters of the Single-Ascending-Dose Study

Parameter, Unit	Cohort 1 (300 mg)	Cohort 2 (600 mg)	Cohort 3 (900 mg)	Cohort 4 (1200 mg)
t_{max}, h				
N	6	6	6	6
Median (min-max)	0.34 (0.17-0.75)	0.50 (0.50-0.75)	0.50 (0.17-0.50)	0.50 (0.17-1.0)
C_{max}, μg/L				
N	6	6	6	6
Mean ± SD (CI)	382 ± 135 (240-524)	1060 ± 275 (774-1350)	1980 ± 612 (1337-2622)	3320 ± 1030 (2240-4400)
CV, %	35.5	25.9	30.9	31.1
AUC_{0-t}, μg • h/L				
N	6	6	6	6
Mean ± SD (CI)	481 ± 150 (324-639)	1520 ± 335 (1170-1870)	2630 ± 807 (1780-3480)	4270 ± 1280 (2930-5610)
CV, %	31.2	22.0	30.7	29.9
$t_{1/2}$ 1-3 h, h				
N	6	6	6	6
Mean ± SD (CI)	0.52 ± 0.11 (0.40-0.64)	0.60 ± 0.08 (0.52-0.69)	0.56 ± 0.11 (0.45-0.68)	0.62 ± 0.12 (0.50-0.74)
CV, %	22.0	12.6	19.2	18.8
$t_{1/2}$ terminal, h				
N	2	6	6	6
Mean ± SD (CI)	7.2 ± 0.21 (5.3-9.0)	17.3 ± 10.6 (6.2-28.5)	8.6 ± 3.1 (5.4-12)	33 ± 42 (NA-77)
CV, %	2.86	61.4	35.7	128
CL/F, L/h				
N	6	6	6	6
Mean ± SD (CI)	679 ± 220 (449-910)	412 ± 99.1 (308-516)	366 ± 97.1 (264-467)	300 ± 82.2 (214-387)
CV, %	32.3	24.0	26.5	27.4

AUC_{0-t}, area under the plasma concentration–time curve from time 0 to the last measurement time point with a concentration value above the lower limit of quantitation, calculated by means of the linear up/log down method; CI, confidence interval (95%); CL/F, apparent oral clearance; C_{max}, maximum concentration in plasma, directly taken from measured concentration values; CV, coefficient of variation; min, minimum; max, maximum; N = number of subjects; SD, standard deviation; $t_{1/2}$ (1-3 h), apparent elimination half-life calculated in time window 1-3 hours; $t_{1/2}$ terminal, apparent terminal elimination half-life based on last two time points with measurable values; t_{max}, time to reach maximum concentration.

Table 3. Summary of Pharmacokinetic Parameters of the Multiple-Dose Study

Parameter, Unit	Day 1	Day 5
t_{max}, h		
N	9	9
Median (min-max)	1.0 (0.50-3.0)	0.75 (0.50-4.0)
C_{max}, μg/L		
N	9	9
Mean ± SD (CI)	90.1 ± 22.1 (73.1-107)	112 ± 72.3 (56.2-167)
CV, %	24.5	64.7%
AUC_{0-t}, μg • h/L		
N	9	9
Mean ± SD (CI)	371 ± 85.4 (306-437)	486 ± 153 (369-604)
CV, %	23.0	31.4
$t_{1/2}$ terminal, h		
N	9	9
Mean ± SD (CI)	5.0 ± 5.3 (0.95-9.1)	7.0 ± 3.6 (4.2-9.7)
CV, %	105	51.9
CL/F, L/h		
N	9	9
Mean ± SD (CI)	842 ± 172 (710-975)	694 ± 294 (469-920)
CV, %	20.5	42.3

AUC_{0-t}, area under the plasma concentration–time curve from time 0 to the last measurement time point with a concentration value above the lower limit of quantitation, calculated by means of the linear up/log down method; CI, confidence interval (95%); CL/F, apparent oral clearance; C_{max}, maximum concentration in plasma, directly taken from measured concentration values; CV, coefficient of variation; min, minimum; max, maximum; N = number of subjects; SD, standard deviation; $t_{1/2}$ terminal, apparent terminal elimination half-life; t_{max}, time to reach maximum concentration.

Table 4. Summary of Pharmacokinetic Parameters of the Food Interaction Study

Parameter, Unit	Fed	Fasted
t_{max}, h		
N	16	16
Median (min-max)	1.5 (1.3-3.0)	0.75 (0.33-1.5)
C_{max}, μg/L		
N	16	16
Mean ± SD (CI)	17.5 ± 6.89 (13.8-21.1)	23.9 ± 11.7 (17.7-30.1)
CV, %	39.4	48.8
t_{lag}, h		
N	16	16
Mean ± SD (CI)	0.18 ± 0.087 (0.13-0.22)	0.15 ± 0.080 (0.10-0.19)
CV, %	49.5	55.2
AUC_{0-t}, μg • h/L		
N	16	16
Mean ± SD (CI)	53.3 ± 16.3 (44.6-62.0)	37.3 ± 11.5 (31.2-43.4)
CV, %	30.6	30.7
AUC_{0-inf}, μg • h/L		
N	14	14
Mean ± SD (CI)	56.8 ± 16.0 (47.5-66.0)	41.3 ± 12.7 (33.9-48.6)
CV, %	28.2	30.8
$t_{1/2}$, terminal, h		
N	14	14
Mean ± SD (CI)	8.2 ± 3.6 (6.1-10)	10 ± 8.3 (5.4-15)
CV, %	43.7	81.5
CL/F, L/h		
N	14	14
Mean ± SD (CI)	761 ± 224 (632-890)	1060 ± 319 (876-1240)
CV, %	29.5	30.1

AUC_{0-t}, area under the plasma concentration–time curve from time 0 to the last measurement time point with a concentration value above the lower limit of quantitation, calculated by means of the linear up/log down method; AUC_{0-inf}, area under the plasma concentration–time curve from time 0 extrapolated to infinity; CI, confidence interval (90%); CL/F, apparent oral clearance C_{max}, maximum concentration in plasma, directly taken from measured concentration values; CV, coefficient of variation; min, minimum; max, maximum; N = number of subjects; SD, standard deviation; $t_{1/2}$ terminal, apparent terminal elimination half-life; t_{max}, time to reach maximum concentration; t_{lag}, delay between time of dosing and time of appearance of concentration.

Safety and Tolerability Aspects

Overall, 49 healthy subjects received at least 1 dose of NRD.E1. Thereof, 24 subjects were exposed to a single dose of the drug, 16 subjects were exposed to 2 single dosages of NRD.E1, and 9 subjects were exposed to 5 dosages of the study drug given at 5 consecutive days. No serious adverse events, deaths, marked laboratory abnormalities, clinically relevant changes in mean and median values of biochemistry variables or vital signs, or discontinuations due to AEs were reported in any of the 3 studies.

Single-Ascending-Dose Study. Overall, treatment-emergent adverse events (TEAEs) were reported for 6 of 24 (25.0%) of NRD.E1- and 2 of 8 (25.0%) placebo-treated subjects. All TEAEs were mild, resolved, and none was reported in more than 1 subject or assessed by the investigator as related to the study drug (Table S5). As the occurrence of AEs was not more frequent with increasing NRD.E1 dose, and none of the safety findings were considered dose-limiting, the MTD was not identified in this study.

In none of the subjects, a QTcF value that exceeded 500 milliseconds or a change from baseline in this parameter that exceeded 60 milliseconds was recorded. There was a total of 3 subjects with a change from baseline between 30 and 60 milliseconds in QTcF, 2 of them in the 900-mg dose group and 1 in the 1200-mg dose group. There were no relevant changes for mean and median HR, QRS, and PR after single-ascending-dose administration of NRD.E1, and no relevant changes were evident from the categorical analysis or from the analysis of morphological ECG abnormalities. In addition, all abnormalities recorded in the study were considered as clinically not relevant.

An analysis of central tendency did not indicate that single-ascending-dose administration of NRD.E1 caused a prolongation of QTcF. Placebo- and baseline-corrected values (ΔΔQTcF) increased and decreased

without a clear time-dependent pattern and in different ways among dose groups. The maximum increases (90%CI) in ΔΔQTcF were 8.5 (–5.6, 22.6; at end-of-study) milliseconds in the 300-mg dose group, 2.1 (–12.0, 16.3; at $t = 24$ hours) milliseconds in the 600-mg dose group, 10.8 (–3.5, 25.1; at $t = 2$ hours) milliseconds in the 900-mg dose group, and 17.7 (3.4, 32.0; at end-of-study) milliseconds in the 1200-mg dose group.

The concentration-response modeling approach did not indicate an effect of NRD.E1 on ΔΔQTcF within the limitations (small subject numbers, single-paper ECG recordings, and no time-matched baseline) of the present study.

Overall, it can be concluded that the current data do not indicate that oral administration of NRD.E1 causes a QTcF prolongation.

Multiple-Dose Study. Overall, TEAEs were reported for 2 of 9 (22.2%) NRD.E1-treated patients and for none of the 3 placebo-treated patients (Table S5). Headache was reported 3 times and was considered study drug related in 1 of the 3 occasions. All identified TEAEs were mild, and no safety finding was considered dose limiting.

Food Interaction Study. Frequency and intensity of TEAEs were greater under fasted than under fed conditions (Table S5). In total, TEAEs were reported for 13 subjects under fasted conditions (drug related in 10) and for 6 subjects under fed conditions (drug related in 4). The most frequently reported TEAEs were headache (7 subjects: 6 fasted and 2 fed), BP increased (5 subjects: 4 fasted and 2 fed) and sinus bradycardia (4 subjects: 2 fasted and 2 fed). All other TEAEs were reported in ≤ 2 subjects only. Most of the TEAEs were of mild severity (12 subjects: 10 fasted and 6 fed). Moderate TEAEs were reported in 4 subjects (3 fasted [sinus bradycardia, BP increased, back pain, headache], 1 fed [BP increased]) and severe TEAEs in 2 subjects (both fasted [BP increased]).

No clinically relevant changes were observed in arithmetic mean and median values of the ECG parameters measured during the 8 hours after dosing compared to the values recorded prior dosing under either fasted or fed conditions.

Discussion

The 3 phase I studies of NRD.E1 in healthy volunteers (first-in-human single-ascending-dose study, multiple-dose study, food interaction study) evaluating PK parameters at doses from 40 mg up to 1200 mg consistently showed rapid absorption of orally administered NRD.E1 followed by fast elimination of parent compound, mainly via metabolization, in particular glucuronidation. In line with the rapid elimination, multiple once-daily doses of 300 mg NRD.E1 over 5 days showed no relevant accumulation (23.9% increase in C_{max} after the fifth vs the first dose) and low trough levels (1-3.5% of mean C_{max}). When administered to fed subjects, absorption rate was slightly reduced, yet exposure to NRD.E1 was increased compared to fasted subjects. This observation indicates higher bioavailability of NRD.E1 when administered with food. Data of the food interaction study further showed a 20% to 30% greater extent of exposure in women compared to men. However, this variation is not considered to be of clinical relevance.

When analyzing the data of the single-dose phase I studies (single-ascending-dose and food interaction studies) with respect to mechanisms contributing to elimination, the metabolic processes of NRD.E1 can be reconstructed. First of all, the putative structures of the metabolites found in urine and plasma provide evidence that metabolism of NRD.E1 proceeds predominantly via glucuronidation and sulfation. Minor metabolites were products of amide hydrolysis, monooxygenation, methylation, and phase II metabolism thereof. No phase I metabolites could be found in human samples. Comparison of the metabolite profile identified in the food interaction study with the respective profiles determined in nonclinical animal studies revealed that all human metabolites of NRD.E1 were also previously found in rat and dog toxicology studies. Given the complete coverage of human metabolites and their overall lower concentrations compared to studies conducted with rats or dogs, the combined use of these 2 animal species represents a suitable model to predict the human safety of NRD.E1 and its associated metabolites. An observation allowing for further reconstruction of the metabolic properties of NRD.E1 in humans is the occurrence of the conserved secondary increases of plasma concentrations $\approx$4 to 8 hours after dosing in the 3 phase I studies. Overall, secondary peaks in the plasma concentration vs time curves were less prominent in cohorts 2 to 4 (600-1200 mg) of the single-ascending-dose study as compared to cohort 1 (300 mg) and the multiple-dose and the food interaction studies. Secondary peaks can be indicative of enterohepatic circulation (EHC), often seen with drugs undergoing metabolic conjugation processes. EHC describes the process of excretion of a metabolic product into bile followed by cleavage by intestinal bacteria and reabsorption of the parent compound back into the systemic circulation.[20,21] However, such fluctuations can also occur in the absence of EHC and be caused by redistribution of the drug after transient changes in protein binding.[22] Such changes have been observed particularly with highly bound drugs and, in the case of diazepam, have been traced back to fatty acid–induced variations in the

free drug fraction.[23] As NRD.E1, with a free fraction of 31.3%, is not extensively bound, such a redistribution effect appears to be a remote possibility only to explain the irregularities seen in concentration-time profiles.

Investigation of drug exposure after the single-ascending-dose study showed dose-dependent, but overproportional increases in C_{max}, AUC, and urinary excretion, possibly due to a saturable first-pass effect. However, such an effect was observed at high doses of 300 to 1200 mg and is not expected to affect the therapeutic dosage of NRD.E1 identified in the phase II dose-finding study NCT02345291, conducted in patients with PDPN.

With the different drug formulations used in the single-ascending-dose and the multiple-dose studies, expected differences in absorption were observed, with the capsules showing greater t_{max} values compared to the suspension.

Variability in absorption and disposition parameters was comparable across the doses in the single-ascending-dose study (coefficient of variation [CV] of 13-35% for C_{max} and AUC_{0-t}). A similar intersubject variability was also observed for AUC values in the food interaction study; however, the C_{max} in this study, particularly when the drug was given without food, showed increased variability (CV fasted, 49%; fed, 39%). This may partly reflect interstudy differences, but may also be due to the fact that in this study NRD.E1 was administered in a capsule formulation and as the more stable polymorph. In the multiple-dose study, the 300-mg dose was administered in the form of 4 capsules of 75 mg each, and more variability in parameters reflecting rate of absorption was to be expected. Also in this study, exposure (AUC_{0-t}) showed a variability across subjects comparable to that in the other studies (CV first dose, 23%; last dose, 31%). Difficulties in estimating the terminal slope of the concentration-time profiles due to secondary peaks translated into larger variabilities in the estimates of $t_{1/2}$ in all studies.

Different drug products of NRD.E1 (differences in polymorphic form, formulation) were used in the different studies. Whereas the single-ascending-dose study, the multiple-dose study, and a phase II dose-finding study (NCT02345291) were conducted with an API consisting of polymorph 1, the food interaction study and all planned future studies were or will be conducted with a hard gelatin capsule with an API consisting of polymorph 2 (current capsule). Hence, accurate elaboration of the doses to be selected for future phase II studies with NRD.E1 is important. Data from the completed phase II dose-finding study revealed efficacy and good tolerability at dose levels of 40 and 150 mg given once daily for 3 weeks. As no concentration data from the phase II dose-finding study were available, the concentrations likely to be achieved at these 2 effective doses were estimated by extrapolation from the data collected in the phase I studies.

The conclusions from these extrapolations were that a daily dose of 80 mg NRD.E1 given as the current capsule is expected to produce at the minimum an AUC and C_{max} equivalent to that estimated to have been achieved with the 40-mg once-daily dose in the phase II dose-finding study. Additionally, administration of an 80-mg once-daily dose of the current capsule formulation, even when given with food, is not expected to exceed the AUC and C_{max} values likely to have been achieved for the 150-mg once-daily dose in the phase II dose-finding study. Consequently, since both the 40- and 150-mg once-daily doses of the former capsules induced a clinically relevant reduction in pain and were well tolerated,[13] an 80-mg once-daily dose of the current capsule can be expected to be safe and to induce the desired therapeutic effect.

In-depth analysis of the safety data suggests that NRD.E1 is well tolerated within the limits of the small-subject study populations. TEAEs were generally mild, resolved without sequelae, and no serious TEAEs leading to the discontinuation of the treatment in any of the 3 herein described studies were registered. In the food interaction study, the reported numbers of TEAEs were greater compared to the single-ascending-dose and the multiple-dose studies, although the NRD.E1 dosage (40 mg) was lower. Potential reasons for the observed discrepancy are (1) the substantially higher mean age of subjects in the food interaction study (52.9 years) compared to subjects in the cohorts of the other 2 studies (24.1-28.2 years) and (2) the open-label design of the food interaction study, which might trigger overreporting of TEAEs and introduce a certain bias toward classification of a TEAE as drug related. Importantly, no clinically relevant changes related to the study drug were observed in mean and median HR, BP, respiration rate, body temperature, hematology and biochemistry parameters, ECG profiles, or physical traits. As no safety finding was considered dose limiting, the MTD could not be determined across the tested doses up to 1200 mg NRD.E1. The overall observed benign safety profile is in line with data from the phase II dose-finding study, which demonstrates good tolerability of NRD.E1 in patients with PDPN.[13]

Conclusion

Overall, the phase I studies presented here show rapid absorption and availability of NRD.E1 without signs of relevant accumulation over multiple administrations as capsules or signs of dose-limiting toxicities; hence, further evaluation of NRD.E1 in phase II studies in patients with PDPN is warranted.

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Conflicts of Interest

E.T. (corresponding author), Sara M., M.R., E.K., and L.H. are current or former employees of Novaremed. T.G. and Stephan M. are consultants for Novaremed AG.

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Supplemental Information

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