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Mechanistic early phase clinical pharmacology studies with disease-modifying drugs for neurodegenerative disorders

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CHAPTER 3

SAFETY, PHARMACOKINETICS AND TARGET ENGAGEMENT OF NOVEL RIPKI INHIBITOR SAR443060 (DNL747) FOR NEURODEGENERATIVE DISORDERS: RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND PHASE I/IB STUDIES IN HEALTHY SUBJECTS AND PATIENTS

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ABSTRACT

RIPK1 is a master regulator of inflammatory signaling and cell death and increased RIPK1 activity is observed in human diseases, including Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). RIPK1 inhibition has been shown to protect against cell death in a range of preclinical cellular and animal models of diseases. SAR443060 (previously DNL747) is a selective, orally bioavailable, CNS-penetrant, small-molecule, reversible inhibitor of RIPK1. In three early-stage clinical trials in healthy subjects and patients with AD or ALS (NCT03757325 and NCT03757351), SAR443060 distributed into the cerebrospinal fluid (CSF) after oral administration and demonstrated robust peripheral target engagement as measured by a reduction in phosphorylation of RIPK1 at serine 166 (pRIPK1) in human peripheral blood mononuclear cells (PBMCs) compared to baseline. RIPK1 inhibition was generally safe and well tolerated in healthy volunteers and patients with AD or ALS. Taken together, the distribution into the CSF after oral administration, the peripheral proof-of-mechanism, and the safety profile of RIPK1 inhibition to date, suggest that therapeutic modulation of RIPK1 in the CNS is possible, conferring potential therapeutic promise for AD and ALS, as well as other neurodegenerative conditions. However, SAR443060 development was discontinued due to long-term nonclinical toxicology findings, although these nonclinical toxicology signals were not observed in the short duration dosing in any of the three early stage clinical trials. The dose-limiting toxicities observed for SAR443060 preclinically have not been reported for other RIPK1-inhibitors, suggesting that these toxicities are compound-specific (related to SAR443060) rather than RIPK1 pathway-specific.

INTRODUCTION

Receptor-interacting serine/threonine protein kinase 1 (RIPK1) is an intracellular protein that regulates between pro-survival NF- κ B signaling and cell-death in response to inflammatory and pro-death stimuli,¹ and RIPK1 activation has been implicated in autoimmune, inflammatory and neurodegenerative diseases. RIPK1 activation most notably occurs via tumor necrosis factor alpha (TNF- α) signaling through TNF receptor 1 (TNFR1).² Upon activation, RIPK1 initiates a complex signaling cascade that triggers cytokine release, microglial activation, and RIPK1-dependant apoptosis or under apoptosis-deficient conditions a regulated form of necrotic cell death known as 'necroptosis'.²⁻⁴ RIPK1 activation and necroptosis have been demonstrated in post-mortem tis-

sue samples from patients with neurodegenerative conditions,⁵⁻⁷ and RIPK1 inhibition has been shown to protect against necroptotic cell death *in vitro* across a range of cell death models.⁸⁻¹³ In animal models of diseases ranging from ulcerative colitis to multiple sclerosis (MS), RIPK1 pathway inhibition protects against necroptotic cell death and also prevents the occurrence of pathologic findings.^{6,12-21} These preclinical findings suggest that inhibition of RIPK1 could be beneficial in many different chronic diseases.^{5-7,12,14,22,23}

RIPK1 inhibitors that do not penetrate the central nervous system (CNS), GSK2982772 and SAR443122 (DNL758), are currently in early stage clinical development for inflammatory diseases.²⁴⁻²⁶ A CNS-penetrant inhibitor of RIPK1 may have the potential to modify the course of neurodegenerative diseases like MS, Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS).^{23,27}

The results of 3 early-phase, placebo-controlled, clinical studies with SAR443060 (previously DNL747) are presented here. SAR443060 is a selective, orally bioavailable, CNS-penetrant, small-molecule reversible inhibitor of RIPK1. Presented results include assessment of safety, pharmacokinetics, and target engagement of SAR443060 following;

- 1 A first-in-human (FIH) single and multiple ascending dose study in healthy subjects;
- 2 A cross-over study in patients with AD;
- 3 A cross-over study in patients with ALS which was followed by an open-label long-term extension (OLE).

PRECLINICAL PHARMACOLOGY AND TOXICOLOGY

In vitro target engagement and dose-response

Reduction of phosphorylation of RIPK1 at serine 166 (pRIPK1) in human peripheral blood mononuclear cells (PBMCs) is considered a reliable biomarker for target engagement assessment and for the translation of human dose projection.^{6,8,28} *In vitro*, SAR443060 blocks TNF- α -induced phosphorylation of RIPK1 in PBMCs from healthy human donors (N=4) with a geometric mean 50% maximum inhibitory concentration (IC₅₀) of 3.9 nM (*Figure 1*). When corrected for human plasma protein binding (87%), this IC₅₀ corresponds to a total SAR443060 plasma concentration of approximately 0.03 μ M. Based on preclinical models demonstrating that RIPK1 inhibition in astrocytes and microglia can attenuate neurodegeneration and disease progression, it was hypothesized that maintaining high levels of RIPK1 inhibition for the duration of each dosing interval could translate to slowing the clinical progression of AD and ALS.¹⁴

Preclinical safety

Based on 28-day Good Laboratory Practice (GLP) toxicity studies in the most sensitive species with pharmacological relevance (cynomolgus monkey), the original no observed adverse effect level (NOAEL) for SAR443060 was established at 200 mg/kg/d (mean AUC_{0-24h} of 254 $\mu M \cdot h$ and mean C_{max} of 29.2 μM). Protein binding of SAR443060 is similar across species (data on file). The key toxicity findings in the 28-day monkey study at 1000 mg/kg/d (AUC_{0-24h} of 548 $\mu M \cdot h$) included adverse effects to the immune system (lymph nodes, bone marrow, and spleen) and skin. A subsequent 3-month toxicity study in monkeys, running in parallel to the first-in-human study (*Section Methods*), identified additional serious toxicities that were considered to be immune-mediated, including thrombocytopenia, anemia, and bleeding at doses of ≥ 40 mg/kg/day (20 mg/kg BID). As a result, the NOAEL was reduced from 200 mg/kg/day to 20 mg/kg/day (mean AUC_{0-24h} of 24.8 $\mu M \cdot h$ and mean C_{max} of 2.57 μM) which led to a dose reduction in the subsequent phase 1B patient studies to keep a 3-4X margin to the new NOAEL (*Section Treatments Administered*). During an extended 9-month GLP toxicity study in monkeys, anemia was also observed at 20 mg/kg/day (10 mg/kg BID) and the NOAEL was further adjusted down ~ 3 -fold to 6 mg/kg/day. Three-month GLP toxicity studies in Sprague-Dawley rats showed good tolerability of SAR443060 to the highest doses tested at 1000 mg/kg/day (mean AUC_{0-24h} of 251 $mM \cdot h$ and 1040 $mM \cdot h$ in males and females, respectively).

METHODS

All clinical studies were conducted in accordance with the International Conference for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use, Good Clinical Practice (GCP), and the principles of the Declaration of Helsinki. The protocols and all study materials were approved by independent ethics committees (ECs)/institutional review boards (IRBs), and all subjects provided their written informed consent before participation.

Study designs and randomization

FIRST-IN-HUMAN STUDY The randomized, double-blind, placebo-controlled FIH study was conducted at a single site in the Netherlands (PRA Health Sciences, Groningen) in healthy male and female subjects, aged 18-55 years, between March and October 2018. Women had to be of nonchildbearing

potential (sterilized or postmenopausal). The study consisted of two parts: a single ascending dose (SAD) PART A and a multiple ascending dose (MAD) PART B (*Figure 2A*). PART A used a sequential ascending dose cohorts design with 4 cohorts of 8 subjects randomized 3:1 SAR443060 to placebo, to evaluate the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of single ascending doses of SAR443060. The effect of food on the PK of SAR443060 was evaluated with a fixed sequence crossover food assessment in COHORTS A2 and A4. In PERIOD 1 the investigational medicinal product (IMP) was administered under fasted conditions and in PERIOD 2 after a high-fat, high-caloric breakfast in the same 8 subjects, with at least one-week wash-out between the 2 periods (*Figure 2A*).

PART B evaluated the safety, tolerability, PK, and PD of multiple ascending doses of SAR443060 administered twice daily (BID) for 14 days in 3 cohorts (*Figure 2A*) of 10 subjects randomized 4:1 SAR443060 to placebo. Cerebrospinal fluid (CSF) was sampled via lumbar punctures (LP) predose and after 12 days of dosing.

STUDIES IN PATIENTS WITH AD OR ALS Two multicenter, randomized, double-blind, placebo-controlled phase 1B studies were conducted between December 2018 and June 2020 in the Netherlands and the United States: one in patients with AD and one in patients with ALS (ClinicalTrials.gov identifiers: NCT03757325 and NCT03757351). Both studies used a similar cross-over design consisting of two 28-day treatment periods, separated by 14 days of wash-out, to evaluate the safety, tolerability, PK and PD of SAR443060 in at least 16 and up to 26 patients in each study (*Figure 2B*). Subjects were randomized 1:1 to receive either active or placebo treatment in the first treatment period and then switched to the opposite treatment assignment in the second treatment period. For the AD study, inclusion criteria included AD diagnosis per NIA-AA guidelines,²⁹ a Mini-Mental State Examination (MMSE) score of 16-26 points inclusive, an age of 55 to 85 years with a body mass index (BMI) between 18 to 35 kg/m^2 , a Clinical Dementia Rating (CDR) score of 0.5-1.0, and a historical confirmatory amyloid positron emission tomography (PET) scan or positive CSF Amyloid β ($A\beta$)₄₂ test (documented history or CSF sampling at screening).

Participants in the ALS study were male or female patients of non-child-bearing potential aged between 21 and 80 years, with laboratory-supported probable, probable, or definite ALS according to the revised El Escorial criteria,³⁰ less than 3 years since symptom onset, a BMI between 18 and 35 kg/m^2 , and a forced vital capacity (FVC) $>50\%$ of predicted.

For both patient studies use of prescription medications had to be stable for ≥ 1 month prior to screening and throughout the study. Moderate to strong cytochrome P450 3A (CYP3A) inducers or inhibitors, as well as antiplatelet and anticoagulation medications, apart from daily aspirin < 100 mg, were not allowed. Patients were confined to the clinical research unit for 3 days at the start and end of each treatment period and returned to the clinical unit for weekly outpatient safety visits and to obtain study medication that was administered at home. CSF was sampled pre-dose in treatment PERIOD 1 and at the end of each treatment period. Safety follow-ups were conducted 1 and 2 weeks after completion of the second treatment period.

The cross-over design was selected to facilitate within-subject analysis of SAR443060-dependent biomarker changes. The sample size in both studies was based on sample size calculations for selected exploratory biomarker endpoints.

Treatments administered

FIRST-IN-HUMAN STUDY Ascending doses of 100-400 mg of SAR443060 in capsules as a spray-dried nanosuspension (SDN) formulation or a micronized drug substance (MDS) formulation or placebo were administered as single dose in PART A and BID for 14 days in PART B (Figure 2A). Based on the predicted human exposure, a starting dose of 100 mg was selected for the FIH study (expected to result in $> 90\%$ peak RIPK1 inhibition) to enable characterization of the PD response over a dose range that encompassed the anticipated therapeutic range. This starting dose was 45-fold below the original 200 mg/kg/d NOAEL (Section Preclinical pharmacology and toxicology) when scaled to a human equivalent dose based on body surface area, and $> 90\%$ RIPK1 inhibition was previously found to be well tolerated with another CNS-penetrant RIPK1 inhibitor.²⁸ Emerging safety data and available PK data from preceding cohorts were reviewed prior to dose escalation, and all cohorts used a sentinel design.

STUDIES IN PATIENTS WITH AD OR ALS A dose of 200 mg BID was initially selected for both patient studies based on adequate safety, tolerability, PK, and target engagement of SAR443060 in the previous FIH study. However, as the nonclinical NOAEL was significantly reduced following the 3-month toxicity study in monkeys (Section Preclinical pharmacology and toxicology), lower clinical doses were implemented via protocol amendments to maintain plasma concentrations 3-4X below the new NOAEL of 20 mg/kg/day (mean C_{max} of 2.57 μ M). Patients in both studies were hence administered SAR443060 SDN 50 mg or matching placebo approximately every 12 hours (BID) in each treatment period for 28 days, followed by a final morning dose on the 29th day.

Preliminary PK/PD modeling based on the FIH study data suggested that doses at or above 50 mg BID could provide $> 80\%$ inhibition of pRIPK1 throughout a BID dosing interval. This dose was expected to allow exploration of potential biomarker effects in patients with neurodegenerative diseases and provided adequate safety margins from toxicities seen in the 3-month monkey study.

Safety, pharmacokinetic and pharmacodynamic assessments

Safety and tolerability outcome measures for all 3 studies consisted of incidence and severity of adverse events (AEs), incidence of clinical laboratory abnormalities (hematology, chemistry, coagulation, and urinalysis), vital signs, electrocardiograms (ECGs), physical examinations (including lymph nodes, skin, and mucosa), and suicidal risk monitoring via the Columbia-Suicide Severity Rating Scale (C-SSRS, except for FIH PART A). Based on the preclinical toxicology studies (Section Preclinical safety), cutaneous or mucosal changes, lymphadenopathy, anemia, thrombocytopenia, and bleeding, petechiae, purpura, or ecchymoses were defined as AEs of special interest (AESI). PK outcomes comprised the measurement of the concentration of SAR443060 in plasma, urine (FIH PART A only) and CSF (except for FIH PART A) using a validated liquid chromatography-tandem mass spectrometry method (PRA Bioanalytical Laboratory, the Netherlands) with a lower limit of quantification (LLOQ) of 0.00247 μ M. A standard set of plasma PK parameters were estimated using noncompartmental analysis, including maximum plasma concentration observed (C_{max}), time to reach C_{max} (T_{max}), half-life ($T_{1/2}$), area under the concentration time curve (AUC), and accumulation ratios (R_{ac}), as well as the CSF-to-unbound plasma ratio as an indication for CNS-penetration. Reduction of pRIPK1 levels in PBMCs was used to measure inhibition of RIPK1 kinase as a pharmacodynamic marker of peripheral drug target engagement, similar as described previously but without *ex vivo* stimulation.²⁸

A digital clock-drawing test (DTCLOCKTM) was included in the AD study as an exploratory endpoint to gain experience with this measure in the AD population. The Amyotrophic Lateral Sclerosis Functional Rating Scale Revised (ALSFRS-R) was used as an exploratory clinical endpoint in the ALS study. Both studies were not adequately powered for these exploratory endpoints.

Statistical analyses

No formal hypothesis testing was performed for these exploratory studies. All PK, PD, and safety data were listed, all data were summarized in tabular and/or graphical form, and descriptive statistics were given, as appropriate, using Statistical Analysis Software (SAS[®]) version 9.4 or higher. AEs were coded using the Medical Dictionary for Regulatory Activities (MEDDRA).

Noncompartmental PK analysis was performed on individual plasma and urine concentration data using PHOENIX® WINNONLIN® (Version 8.1). The PK parameters were analyzed for dose proportionality using a power model approach or analysis of variance (ANOVA) model as appropriate.

ALS open-label extension

An open label extension with SAR443060 50 mg BID up to 12 months was available for patients completing the ALS study in the Netherlands (Figure 2B). Routine safety assessments continued on a biweekly (up to month 6) and monthly (up to month 9) basis with a final planned visit after 12 months. The ALS OLE study part was prematurely terminated in June 2020 (9 months after first subject dosed), due to the sponsor's decision to stop the development of SAR443060.

RESULTS

Demographics and baseline characteristics

FIRST-IN-HUMAN STUDY Fifty-six healthy male and female subjects, between 18 and 55 years of age and with a BMI between 19.6 and 31.8 kg/m², were included in the study. In PART A, due to recruitment challenges, only 7 subjects of the planned 8 subjects were included in COHORTS A2, A3 and A4, leading to a total of 21 subjects that received a single dose of 100-400 mg SAR443060 and 8 subjects that received placebo. In PART B, only 7 subjects were included in COHORT B3 as the goals of the study had been reached. This resulted in 21 subjects receiving 100-400 mg SAR443060 BID for 14 days and 6 subjects receiving placebo in PART B (Figure 2A). One subject was withdrawn during the study (COHORT A2 after completion of PERIOD 1), due to a medical history of eczema which made the subject ineligible for the study and 55 (98.2%) subjects completed the study as per protocol (Figure 2A). Baseline characteristics (Supplemental Table S1) were similar across cohorts and treatments.

STUDIES IN PATIENTS WITH AD OR ALS In the AD study a total of 16 (100.0%) patients completed treatment PERIOD 1 and a total of 15 (93.8%) patients completed treatment PERIOD 2 (Figure 2B). One patient discontinued from the study due to AD progression during administration of placebo in PERIOD 2. Patient demographic and baseline characteristics (Supplemental Table S1), were comparable between both treatment sequences, except that patients randomized to the placebo/SAR443060 treatment sequence were on average older (73.4 vs 68.4 years), had a higher proportion of male patients (75%

vs 50%), and a higher BMI (29.4 vs 24.1 kg/m²) compared to subjects in the SAR443060/placebo treatment sequence, although none of these comparisons were evaluated statistically.

Treatment compliance was high ($\geq 90\%$) in all but 1 (6.3%) patient, who received $<80\%$ of the placebo doses and discontinued the study due to the reason explained above. All 16 patients are included in the safety and PK analysis and all 15 subjects that completed both treatment periods are included in the PD analysis (Figure 2B). In the ALS study, only 15 of the planned 16 patients were included due to recruitment challenges. Eight patients were allocated to the treatment sequence placebo/SAR443060 and 7 patients were allocated to the treatment sequence SAR443060/placebo. The demographic and baseline characteristics (Supplemental Table S1) were balanced between the 2 cross-over sequences. All 15 randomized patients had high treatment compliance ($\geq 90\%$). The first patient in the ALS study (sequence SAR443060/placebo) was enrolled at the original planned dose of 200 mg BID and completed 21 days in the first treatment period. This patient decided to forgo the rest of treatment PERIOD 1 without withdrawing from the study, and subsequently completed treatment PERIOD 2 (50 mg BID). No other patients were given the dose of 200 mg BID in this study. One patient in sequence SAR443060/placebo discontinued the study at the end of treatment PERIOD 2 due to ALS disease progression. All 15 patients were included in the safety analysis. One patient was excluded from the PK and PD analysis due to a protocol violation: this patient had stopped taking edaravone during the study (Figure 2B).

ALS OPEN-LABEL EXTENSION After completion of the double-blind ALS study, 8 patients enrolled in the ALS OLE study (Figure 2B, Supplemental Table S1). Two patients withdrew from the study prematurely due to disease progression and the other 6 patients were treated until the OLE study was terminated by the sponsor. One subject had an IMP interruption during the OLE study due to unrelated ECG abnormalities diagnosed as coronary heart disease by cardiologist consult. Duration of exposure to SAR443060 in the OLE ranged from 66 to 268 days (mean 165.5 \pm 82.3 days). All 8 patients were included in the OLE safety analysis (Figure 2B).

Safety and tolerability

In all three clinical studies there were no SAR443060-related deaths reported or serious AEs (SAEs) and no subject withdrawals due to AEs (Supplemental Table S2). The safety profile and the nature of the adverse events were comparable between the SAR443060 and placebo.

FIRST-IN-HUMAN STUDY All treatment emergent AES (TEAEs) in the FIH study were rated as mild or moderate in severity (*Supplemental Table S2*), and there were no AESIS. The most frequently reported TEAEs in PART A were medical device (ECG-electrodes) site irritation (12.1%), catheter site related reaction (6.1%), headache (6.1%), diarrhea (6.1%), and nasopharyngitis (6.1%). In PART B, the most frequently reported TEAEs were procedural pain (LP procedures) (25.0%), post-LP syndrome (15.6%), skin irritation (7.8%), catheter site related reaction (6.3%), and nausea (4.7%). No clinically significant changes from baseline or trends were observed in the clinical laboratory results, vital signs, 12-lead ECGs, physical examinations, or the C-SSRS.

STUDIES IN PATIENTS WITH AD OR ALS In the AD study there was 1 SAE of vomiting following placebo administration in the second treatment period, which required hospitalization, and was considered not related to the IMP by the investigator. There were no SAEs in the double-blind part of the ALS study (*Supplemental Table S2*). In the AD study a total of 7 AESIS were reported in 5 subjects: 2 AESIS during SAR443060 administration (mild, asymptomatic, and self-limiting anemia in 2 subjects) and 5 in subjects on placebo (mild anemia (2 subjects), thrombocytopenia (1 subject), epistaxis (1 subject), and moderate urticaria (1 subject)). All AESIS in the placebo period occurred prior to SAR443060 exposure. Two patients in the ALS study reported AESIS: seborrheic dermatitis (placebo treatment) and erythema (SAR443060 treatment). Both AESIS were of mild intensity, and only the event of erythema was considered related to the IMP by the investigator.

In the AD trial, the most common TEAEs observed in subjects during treatment with SAR443060 and in greater frequency than with placebo were confusion, headache, and procedural pain, each reported in 2 (12.5%) subjects. For the ALS trial these were headache and rhinitis, each reported in 3 (20.0%) patients. Both TEAEs of confusion following SAR443060 administration in the AD study occurred during inpatient stays and were assessed by the investigator as not related to the study drug, but likely a result from chronic AD.

In both the AD and ALS studies, other than the above reported anemias and low platelet count, there were no other clinically significant laboratory abnormalities, changes in vital signs, 12-lead ECGs, physical and neurological examinations, or changes in the C-SSRS.

ALS OPEN-LABEL EXTENSION During the ALS OLE part, one patient (12.5%) reported a treatment emergent SAE (hospitalization due to pneumonia

aspiration) and one patient reported a severe TEAE (worsening of ALS), both not considered related to SAR443060 by the investigator (*Supplemental Table S2*). Four patients (50.0%) reported 5 AESIS related to skin and subcutaneous tissue disorders (seborrheic dermatitis (2x), atopic dermatitis, contact dermatitis and post inflammatory pigmentation change) and 1 patient (12.5%) experienced 2 AESIS: increased alanine and aspartate aminotransferase (ALT and AST). The increases in ALT and AST were assessed to be related to riluzole based on a de- and rechallenge with riluzole, and the skin and subcutaneous tissue findings were not considered related to SAR443060 by the investigator after consultation with a dermatologist.

Pharmacokinetics

FIRST-IN-HUMAN STUDY Following administration of single doses of 100-400 mg SAR443060 SDN in the fasted state, SAR443060 concentrations had a median T_{max} after 2 to 4 hours. Total exposure increased dose-proportionally, while the increase of C_{max} was found to be less than dose proportional (*Figure 3A, Table 1*). There was a 7% decrease in geometric mean C_{max} and an increase of 10% in geometric mean AUC_{0-inf} after administration of 100 mg SAR443060 SDN in fed versus fasted conditions.

A 200 mg single dose of SAR443060 MDS had a median T_{max} after 4 hours, and there was a 125% increase in geometric mean C_{max} and an increase in geometric mean AUC_{0-inf} of 13% in fed versus fasted conditions (*Figure 3A*). Geometric mean values for $T_{1/2}$ ranged from 9.7 to 11.4 hours with no clear dependence on dose, formulation or fed state (*Table 1*).

A comparison of the oral exposure of 200 mg SAR443060 MDS and SDN formulations indicated that the C_{max} of SAR443060 MDS was 61% (CI; 47-78%) of the C_{max} for SDN. The geometric mean AUC_{0-inf} after 200 mg SAR443060 MDS was 27% higher than after 200 mg SDN with a range CIs outside the 80-125% bioequivalence interval (*Figure 3A*).

During the multiple-dose period, there was an accumulation in AUC_{0-tau} of SAR443060 SDN, with an accumulation ratio (R_{ac}) ranging from 2.19 after 100 mg to 1.45 after 400 mg SAR443060, with steady state being reached at or before DAY 4 (*Figure 3C, Supplemental Figure S1C*). On DAY 14 of the MAD dosing period no evidence of deviation from dose proportionality was found for both C_{max} and AUC_{0-tau} . Geometric mean CSF concentrations of SAR443060 increased dose proportional (*Table 1*).

Excretion of SAR443060 in urine was very limited and not dependent on dose, with mean $FE_{urine} < 0.1\%$ for all doses.

STUDIES IN PATIENTS WITH AD OR ALS After multiple dose administrations of 50 mg BID (end of treatment day 29 of PERIOD 1 or 2), the mean SAR443060 plasma concentrations increased over time and peaked at 1-1.5 hours (T_{max}) and declined gradually over 24 hours post-dose in a biphasic manner. For the total PK Population, the mean C_{max} and AUC_{0-12} were 0.670 μM and 3.62 $\mu\text{M} \cdot \text{h}$, respectively, in the AD study and 0.638 μM and 3.12 $\mu\text{M} \cdot \text{h}$ in the ALS study (Table 2). Mean $T_{1/2}$ was 19.0 hours in the AD study and 14.2 hours in the ALS study. The mean R_{ac} at steady state for C_{max} was 1.77 in the AD study and 1.11 in the ALS study, while the mean R_{ac} for AUC was 2.25 and 1.48, respectively (Table 2). SAR443060 displayed a mean CSF-to-unbound plasma concentration ratio of 1.35 in the AD study and 1.00 in the ALS study.

PHARMACODYNAMICS – TARGET ENGAGEMENT

FIRST-IN-HUMAN STUDY SAR443060 demonstrated >90% median peripheral pRIPK1 inhibition in PBMCs at all dose levels tested. The duration that inhibition remained >90% increased with increasing doses: ≥ 4 hours following 100 mg, ≥ 8 hours following 200 mg, and for approximately 24 hours following a 400 mg single fasted dose of SAR443060 SDN (Figure 4A). In the multiple dose cohorts, median pRIPK1 inhibition was 79.9%, 93.7%, and 95.4% at 12 hours after the first dose on DAY 1 (trough concentration) for doses of 100 mg, 200 mg, and 400 mg respectively. Twelve hours after the last dose of SAR443060 on DAY 14, median pRIPK1 inhibition was 90.4%, 96.3%, and 96.1% following BID doses of 100 mg, 200 mg, and 400 mg respectively (Figure 4B).

STUDIES IN PATIENTS WITH AD OR ALS In the AD study, median (CI) percentage inhibition of pRIPK1 in PBMCs compared to baseline at steady state dosing (DAY 29) was 93.98% (CI: 95.4, 92.55, N=15) at 2 hours post dose (around T_{max}) and diminished over time to 81.83% (CI: 85.62, 78.03, N=15) at 12 hours post-dose (trough) (Figure 5A). In the ALS study, this was 92.34% (CI: 95.75, 68.11, N=14) at 2 hours post dose and diminished overtime to 65.92% (CI: 79.3, 45.39, N=14) at 12 hours post-dose (Figure 5B).

No statistically significant differences were seen with the DCTCLOCK™ for the AD study, or the ALSFRS-R clinical rating scale for the ALS study.

DISCUSSION

The results from these randomized, placebo-controlled, phase 1 and 1B clinical studies indicate that treatment with the RIPK1-inhibitor SAR443060

for up to 28 days is well tolerated with similar PK and target engagement response across healthy subjects and patients with AD or ALS. Although AEs of dermatological findings, mild anemia and thrombocytopenia were observed during the study, none were severe or considered related to the IMP. All the hematological findings resolved spontaneously without intervention. While the OLE study in ALS patients did not identify any clear relevant safety concerns, no robust conclusion regarding the long-term safety of SAR443060 administration can be derived due to the limited sample size and early termination of the OLE trial.

SAR443060 administration demonstrated a dose-dependent effect on peripheral pRIPK1 inhibition in PBMCs from healthy subjects and patients with AD and patients with ALS. Administration of doses of 100-400 mg BID in healthy subjects led to robust target engagement $\geq 90\%$ at steady state trough for all dose levels tested. However, due to dose-limiting toxicity findings in a parallel GLP 3-month preclinical study, the dose level for the AD and ALS patient studies was adjusted from 200 mg to 50 mg BID. This 50 mg BID dose level resulted in peripheral inhibition of pRIPK1 in PBMCs compared to baseline of 81.83% in the AD study and 65.92% in the ALS study, at steady state trough. We could not find any significant changes in DCTCLOCK™ for the AD study, or the ALSFRS-R clinical rating scale for the ALS study, and the study was not powered for the number and duration to allow detection of clinical change.

While it is not possible to measure pRIPK1-inhibition directly at the target site (astrocytes and microglia) in human subjects with neurodegenerative conditions, the combination of CSF-distribution and robust peripheral target engagement demonstrated in these studies with SAR443060 offers an encouraging proof-of-mechanism that therapeutic modulation of RIPK1 in the CNS may be possible.³¹

Although the long-term preclinical toxicities were not observed in the short-duration SAR443060 clinical studies, SAR443060 development was discontinued due to the potential risk of these findings from the non-clinical studies. The dose-limiting toxicities observed preclinically for SAR443060 have not been reported for other RIPK1-inhibitors with dosing periods of up to 84 days,^{24,25} suggesting that these are compound specific and not common to RIPK1-pathway inhibition. Recently, it was announced that SAR443820 (DNL788), a CNS-penetrant back-up compound for SAR443060, successfully completed first in human studies and a phase 2 study (HIMALAYA) in ALS patients is expected to commence in the first quarter of 2022.³²

Table 1 Summary statistics of pharmacokinetic parameters for SAR443060 in the FIH study (DNLI-D-0001).

Treatment	C _{max} (μM)	T _{max} (h)	AUC _{O-12} (μM · h)	AUC _{O-inf} (μM · h)	AUC _{O-tau} (μM · h)	AUC R _{ac}	T _{1/2} (h) ¹	CSF (μM) ²
SINGLE DOSE								
100 mg SDN (N=5)	0.663 (0.315-1.82)	4.03 (0.53-4.05)	2.94 (1.62-7.42)	4.75 (2.25-11.6)	-	-	10.0 (8.81-12.2)	-
200 mg SDN fasted (N=5)	1.02 (0.950-1.12)	2.12 (1.03-4.07)	5.25 (4.23-6.76)	9.26 (6.56-15.5)	-	-	11.4 (8.11-15.6)	-
100 mg SDN fed (N=4)	0.615 (0.399-0.838)	4.00 (4.00-4.00)	3.38 (2.40-4.95)	5.21 (3.50-8.28)	-	-	9.79 (8.85-10.3)	-
400 mg SDN (N=5)	1.53 (1.11-1.91)	4.03 (2.07-4.03)	9.84 (9.03-11.5)	17.8 (14.1-24.5)	-	-	9.71 (7.08-12.6)	-
200 mg MDS fasted (N=5)	0.624 (0.460-0.895)	4.05 (1.03-4.05)	4.61 (3.41-6.56)	11.8 (7.68-14.3)	-	-	8.99 (7.71-12.4)	-
200 mg MDS fed (N=5)	1.40 (0.564-2.93)	4.00 (2.02-12.03)	7.77 (4.16-14.1)	13.3 (9.09-19.1)	-	-	9.96 (7.73-15.0)	-
MULTIPLE DOSE								
100 mg SDN BID day 1 (N=8)	0.580 (0.346-1.04)	1.28 (0.50-4.05)	2.85 (1.96-3.98)	-	-	-	-	-
100 mg SDN BID day 14 (N=8)	0.966 (0.685-1.42)	1.52 (1.05-4.07)	-	-	6.24 (4.55-8.62)	2.19 (1.74-3.07)	14.2 (10.3-20.3)	0.103 (0.0711-0.157)
200 mg SDN BID day 1 (N=8)	1.07 (0.617-2.18)	2.09 (1.03-4.07)	5.34 (3.41-7.41)	-	-	-	-	-
200 mg SDN BID day 14 (N=8)	1.59 (1.06-2.47)	2.03 (1.03-4.03)	-	-	9.50 (5.50-12.8)	1.78 (1.03-2.84)	13.5 (8.38-20.5)	0.159 (0.0793-0.267)
400 mg SDN BID day 1 (N=5)	2.22 (1.44-2.90)	2.25 (1.50-4.08)	13.2 (8.94-17.2)	-	-	-	-	-
400 mg SDN BID day 14 (N=5)	3.10 (2.09-4.53)	2.05 (1.50-4.05)	-	-	19.1 (10.1-33.9)	1.45 (1.12-2.43)	12.1 (9.51-18.9)	0.234 (0.141-0.285)

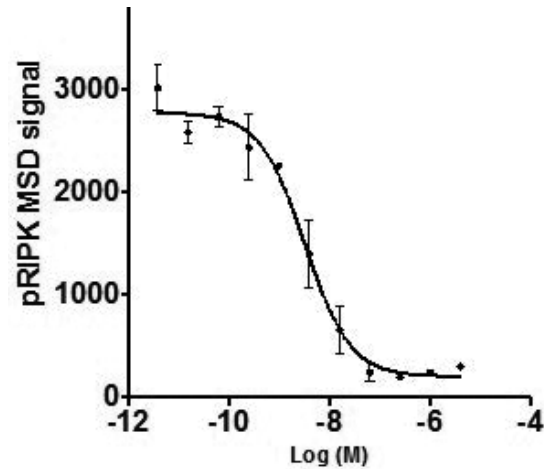
Values are presented as geometric mean (minimum-maximum), except for T_{max} where the median (range) is presented. BID=twice daily /CSF=cerebrospinal fluid /MDS=micronized drug substance /N=number of subjects receiving study medication /SDN=spray-dried nanosuspension. /1) There is uncertainty in the calculation of the geometric mean half-life for all dose levels as the sampling period was less than 5 times the half-life which is too short for a reliable half-life calculation. /2) CSF samples were collected 4 hours postdose on DAY 12.

Table 2 Summary statistics of plasma pharmacokinetic parameters for SAR443060 in the AD and ALS patient studies.

Treatment	C _{max} (μM)	C _{max} R _{ac}	T _{max} (h)	AUC _{O-12} (μM · h)	AUC R _{ac}	T _{1/2} (h)	CSF-to- unbound plasma ratio
DNLI-D-0002: PHASE 1B STUDY IN AD PATIENTS							
50 mg BID single dose (SOT)	0.427 (0.222)	-	1.72 (0.5; 8.0)	1.64 (0.626)	-	-	-
50 mg BID multiple dose (EOT)	0.670 (0.281)	1.77 (0.737)	1.50 (1.0; 4.2)	3.62 (1.48)	2.25 (0.598)	19.0 (16.25)	1.35 (0.538)
DNLI-D-0003: PHASE 1B STUDY IN ALS PATIENTS							
50 mg BID single dose (SOT) ¹	0.581 (0.405)	-	1.05 (0.47; 4.00)	2.11 (1.89)	-	-	-
50 mg BID multiple dose (EOT)	0.638 (0.267)	1.31 (0.540)	1.25 (0.50; 4.52)	3.12 (1.20)	1.85 (0.325)	14.2 (5.18)	1.00 (0.256)

Values are presented as mean (standard deviation), except for T_{max} where the median (range) is presented. BID=twice daily /CSF=cerebrospinal fluid /EOT=end of treatment (DAY 28) /N=number of subjects receiving study medication /SOT=start of treatment (DAY 1). As there were only 2 patients on G-TUBE administration in the ALS OLE study, PK parameters were not analyzed for G-TUBE administration. /1) The first patient who received 200 mg twice daily for 21 days is included in the descriptive statistics of the PK parameters after the 1st administration. As a result, SOT C_{max} and AUC_{O-12} for the ALS study are likely an overestimated and AUC R_{ac} underestimated.

Figure 1 SAR443060 inhibition of RIPK1 serine 166 autophosphorylation in human PBMCs. Human peripheral blood mononuclear cell (PBMCs) from 4 healthy donors were thawed and resuspended in Roswell Park Memorial Institute (RPMI) complete medium. Cells were incubated with a range of concentrations of SAR443060 and then stimulated with a combination of TNF- α , SM-164, and ZVAD-FMK (TSZ). Two hours later, cells were lysed, and phosphorylated receptor-interacting serine/threonine-protein kinase (pRIPK1) was detected by a plate-based immunoassay on the Meso Scale Discovery (MSD) platform. Increasing concentrations of SAR443060 (3.8 pM–4 μ M) reduced the phosphorylation of RIPK1 at SER166 in stimulated human PBMCs in a concentration-dependent manner with a geometric mean IC₅₀ value of 3.9 nM. Sample dose-response curve is from 1 donor. At each concentration tested, the mean and SD of the pS166 RIPK1 signal were calculated from the technical duplicate. Error bars are SDs.



CAPTION FIGURE 2 (NEXT PAGE) >

a. 1) One subject was withdrawn during the study (COHORT A2 after completion of PERIOD 1), due to a medical history of eczema which made the subject ineligible for the study. b. 1) One patient discontinued during administration of placebo in PERIOD 2 of the AD study due to disease progression and was not included in the PD analysis. 2) The first patient in the ALS study was enrolled at the original dose of 200 mg BID and completed 21 days in the first treatment period. This patient decided to forgo the rest of treatment PERIOD 1 without withdrawing from the study, and subsequently completed the treatment PERIOD 2 and the FFU visits. 3) One patient in the ALS study discontinued during administration of placebo in PERIOD 2 due to disease progression. 4) One patient in the ALS study was excluded from the pharmacodynamic and pharmacokinetic analysis

as this patient had stopped taking edaravone 9 days prior to administration of SAR443060 in PERIOD 1 (protocol deviation). 5) Two subjects withdrew from the ALS OLE study prematurely due to disease progression. 6) For the ALS OLE study, the COVID-19 pandemic prevented the pre-planned collection of several CSF and blood samples from patients. Furthermore, the OLE study was terminated early by Sponsor decision. As a result, there was not enough PK and PD data from the OLE available for analysis. AD = Alzheimer's disease/ALS = amyotrophic lateral sclerosis/BID = twice daily/ FIH = first-in-human/MAD = multiple ascending dose/MDS = micronized drug substance/OLE = open-label extension/PD = pharmacodynamics/PK = pharmacokinetics/SAD = single ascending dose;SDN = spray-dried nanosuspension.

Figure 2 Study designs, randomization and analysis populations for the completed SAR443060 phase 1 and phase 1B clinical program. A. Phase 1 first-in-human study in healthy volunteers. B. Phase 1B studies in patients with AD or ALS.

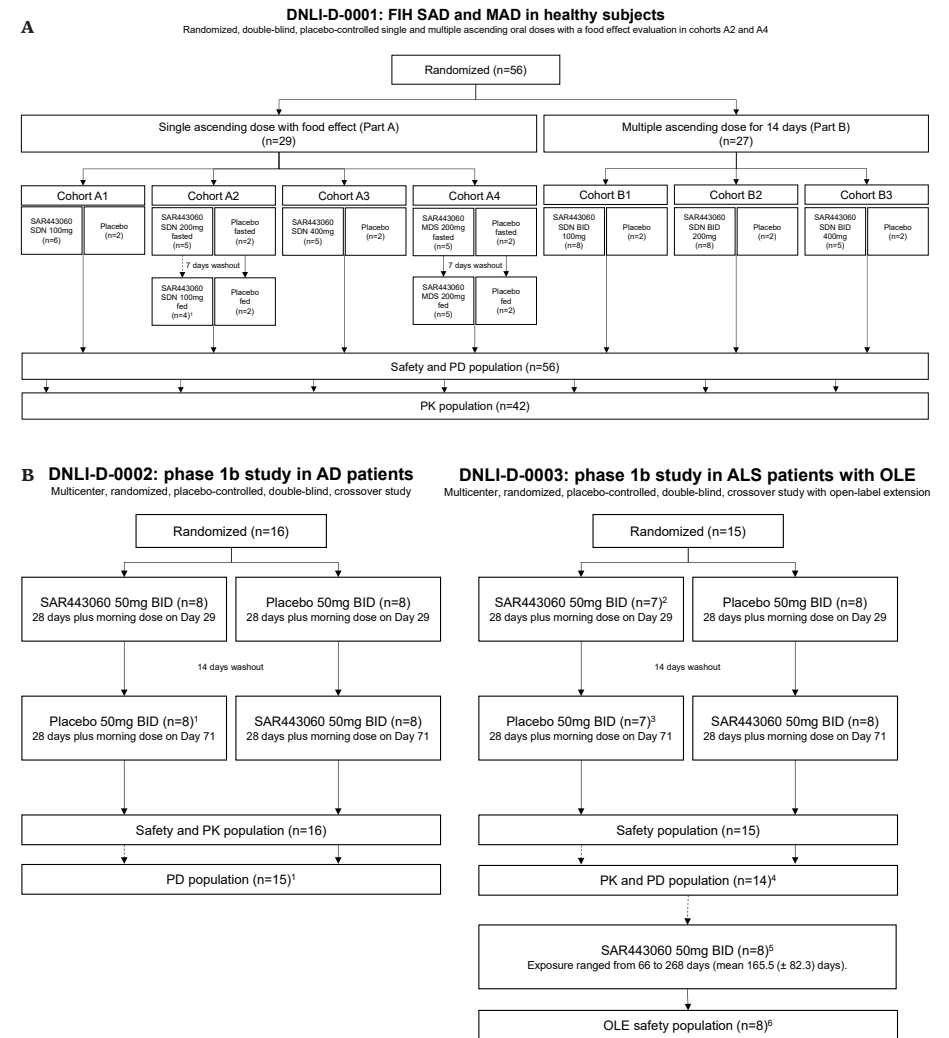


Figure 3 SAR443060 geometric mean plasma concentration-time profiles in healthy subjects. A. After administration of a single dose of the SDN and MDS formulations in fasted conditions and after a high-fat breakfast on a semi-logarithmic scale. B. DAY 1 and DAY 14 overlay for administration of twice-daily dosing (BID) on a semi-logarithmic scale. C. DAY 1 and DAY 14 full PK plasma concentration-time profiles and DAY 4, 7 and 11 predose (trough) concentrations during administration of twice-daily dosing (BID) on a semi-logarithmic scale. Mean (\pm SD) PK plasma concentration-time profiles on a linear scale per cohort are available in *Supplemental Figure S1*.

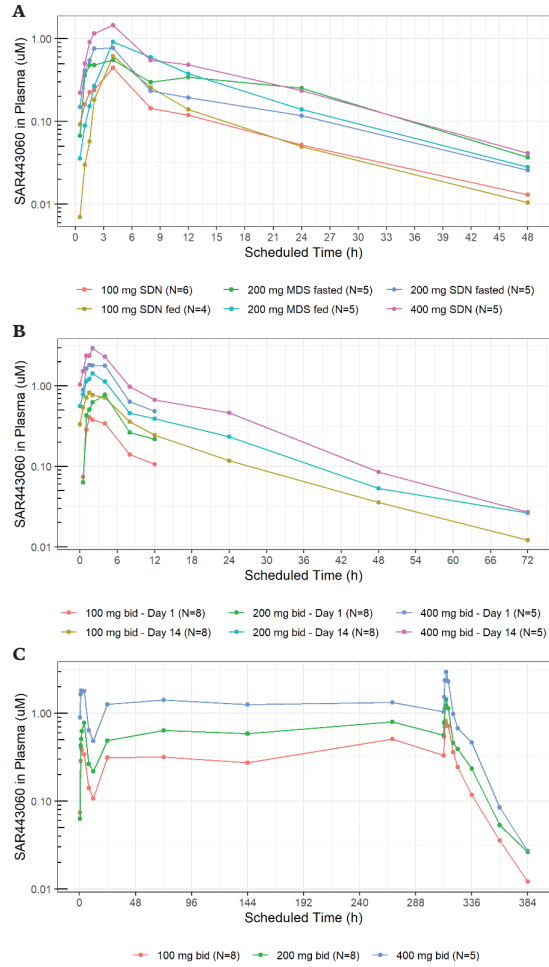


Figure 4 Median percentage of PRIPK1 inhibition compared to baseline after SAR443060 and placebo administration in healthy subjects.

A. After single ascending doses and placebo up to 48 hours post dose. B. After ascending BID doses and placebo up to 48 hours post the last dose on DAY 14. Error bars represent inter quartile range (IQR). X-axis states the study days (D) and hours (HR) for each sampling timepoint. D1.0HR represents predose (baseline) measurement and D1.2HR the first measurement 2 hours post dose on DAY 1. Timepoints on the X-axis are not equally spaced in time.

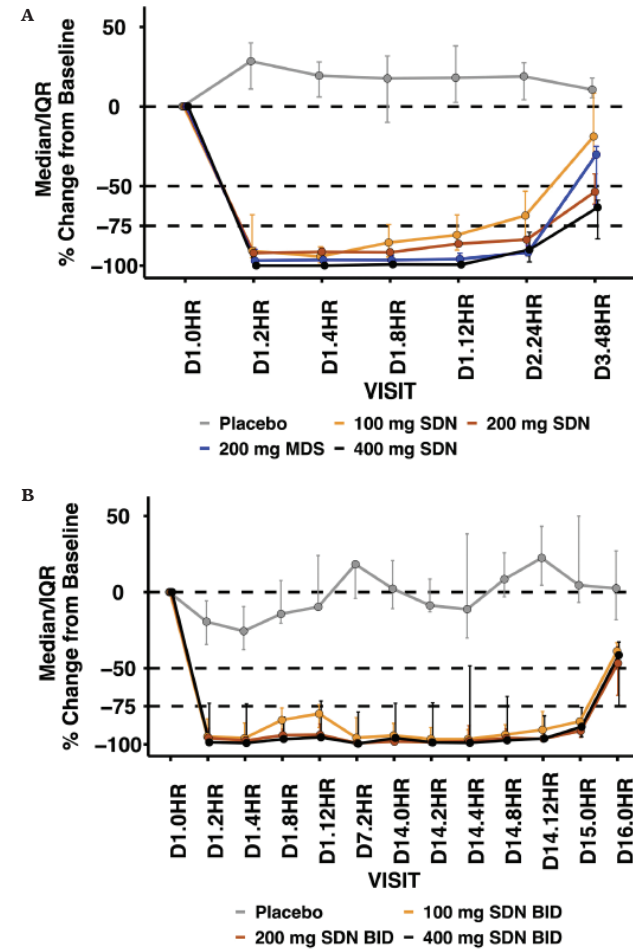


Figure 5 Median percentage of pRIPK1 inhibition compared to baseline after SAR443060 and placebo administration in patients with AD (A) or ALS (B). SAR443060 50 mg or matching placebo was administered approximately every 12 hours (BID) in each treatment period for 28 days, followed by a final morning dose on the 29th day. Error bars represent inter quartile range (IQR). D1.0HR represents predose (baseline) measurement and D1.2HR the first measurement 2 hours post dose on DAY 1. Timepoints on the X-axis are not equally spaced in time.

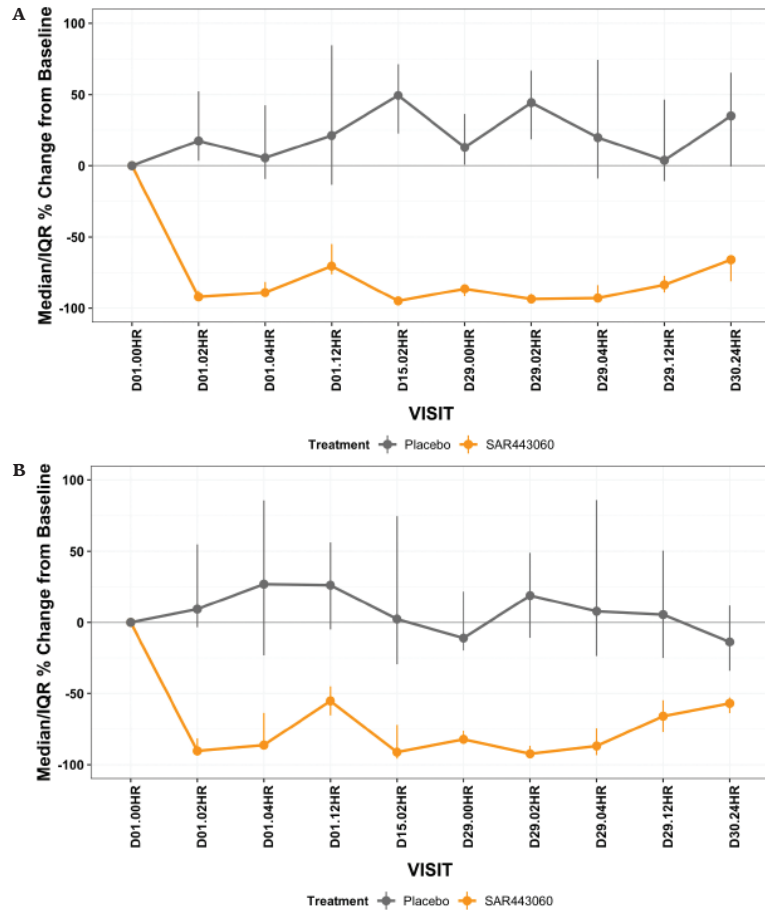


Table S1 Subject baseline characteristics at screening for the SAR443060 phase 1 and phase 1B clinical studies.

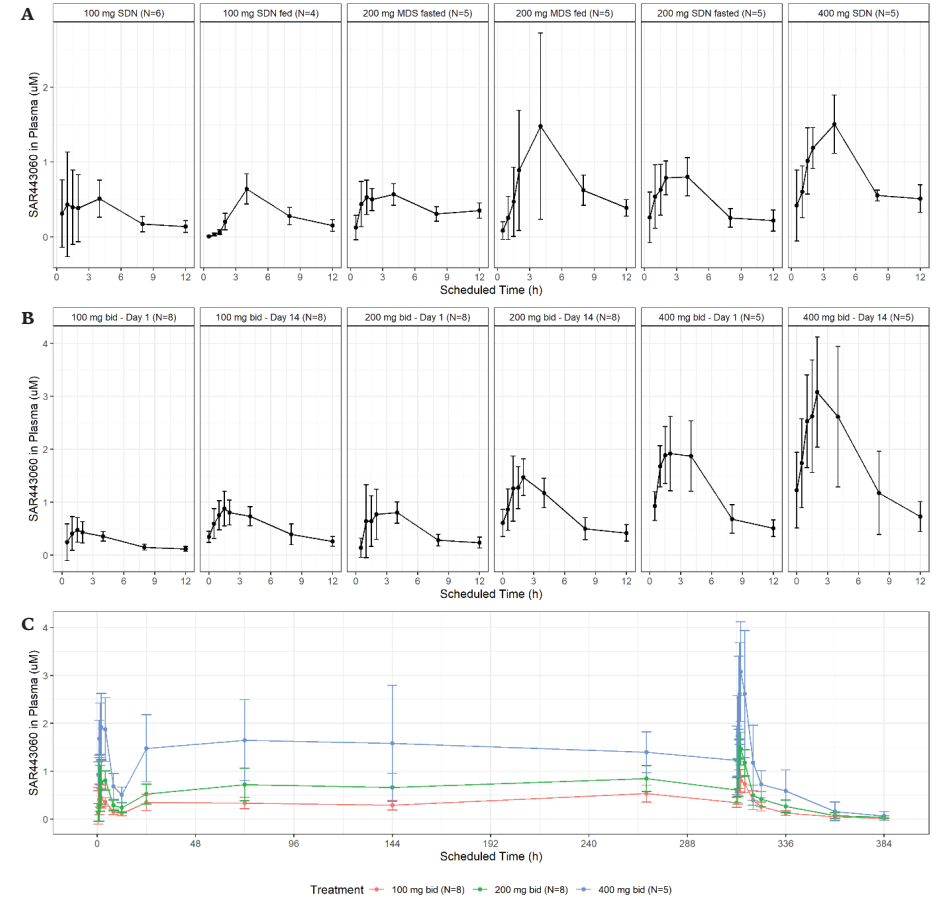
Characteristic	DNLI-D-0001: FIH SAD in healthy subjects	DNLI-D-0001: FIH MAD in healthy subjects	DNLI-D-0002: phase 1B study in AD patients	DNLI-D-0003: phase 1B study in ALS patients	DNLI-D-0003: phase 1B OLE in ALS patients
Total subjects (N)	29	27	16	15	8
Age, years, mean (SD)	31 (11)	35 (11)	70.9 (7.7)	57.5 (6.8)	59.9 (7.0)
Gender, male, N (%)	25 (86%)	25 (93%)	10 (63%)	12 (80%)	7 (75%)
BMI, kg/m ² , mean (SD)	24.6 (3.0)	25.4 (2.9)	26.8 (3.8)	26.7 (2.4)	26.6 (2.2)
Race, N (%)					
White	21 (72%)	21 (78%)	15 (94%)	15 (100)	8 (100%)
Asian	1 (3%)	1 (4%)	-	-	-
Black or African American	2 (7%)	1 (4%)	-	-	-
Other	1 (3%)	1 (4%)	1 (6%)	-	-
Mixed	4 (14%)	3 (11%)	-	-	-
Baseline MMSE, mean (SD)	N/A	N/A	20.6 (2.8)	N/A	N/A
Baseline CDR, N (%)					
0-5	N/A	N/A	7 (43.8)	N/A	N/A
1-10	N/A	N/A	9 (56.3)	N/A	N/A
ALS disease duration at screening, months, mean (SD)	N/A	N/A	N/A	9.05 (4.58)	-
Disease medication use at baseline	N/A	N/A	8 (50%) ¹	15 (100%) ²	8 (100%)

¹ Including donepezil, galantamine, rivastigmine, and memantine /² Most frequently reported medications by therapeutic class (>30%) in both treatment groups were other nervous system drugs, including riluzole or edaravone (100%), vitamins (53.3%) and analgesics (33.3%).

Table S2 Summary of treatment-emergent AEs for the SAR443060 phase 1 and phase 1B clinical studies.

	DNLI-D-0001: FIH SAD in healthy subjects		DNLI-D-0001: FIH MAD in healthy subjects		DNLI-D-0002: phase 1B study in AD patients		DNLI-D-0003: phase 1B study in ALS patients		DNLI- D-0003: phase 1B OLE in ALS patients
	Placebo (N=8)	SAR443060 (N=21)	Placebo (N=6)	SAR443060 (N=21)	Placebo (N=16)	SAR443060 (N=16)	Placebo (N=15)	SAR443060 (N=15)	SAR443060 (N=8)
Subject reporting ≥ 1 AE, N (%)	3 (37.5%)	13 (61.9%)	6 (100.0%)	19 (90.4%)	11 (68.8%)	12 (75.0%)	11 (73.3%)	12 (80.0%)	8 (100.0%)
Number of TEAEs, N	13	25	11	53	27	36	27	21	43
Number of mild TEAEs, N (%)	13 (100.0%)	25 (100.0%)	9 (81.8%)	48 (90.6%)	21 (81.5%)	27 (75.0%)	23 (85.2%)	18 (85.7%)	33 (76.7%)
Number of moderate TEAEs, N (%)	0	0	2 (18.2%)	5 (9.4%)	5 (18.5%)	9 (25.0%)	4 (14.8%)	3 (14.3%)	9 (20.9%)
Subjects with severe TEAE, N (%)	0	0	0	0	0	0	0	0	1 (12.5%)
Subjects with any treatment emergent SAE, N (%)	0	0	0	0	1 (6.3%)	0	0	0	1 (12.5%)
Subjects with any TEAE leading to death, N (%)	0	0	0	0	0	0	0	0	0
Subjects with any TEAE leading to study discontinuation, N (%)	0	0	0	0	0	0	0	0	0
Subjects with any TEAE of special interest (AESI), N (%)	0	0	0	0	2 (12.5%)	3 (18.8%)	1 (6.7%)	1 (6.7%)	6 (62.5%)

Figure S1 SAR443060 mean (\pm SD) plasma concentration-time profiles in healthy subjects. A. After administration of a single dose of the SDN and MDS formulations in fasted conditions and after a high-fat breakfast. B. After administration of a multiple doses (BID) of the SDN formulations in fasted conditions on DAY 1 and DAY 14. C. DAY 1 and DAY 14 full PK plasma concentration-time profiles and DAY 4, 7 and 11 predose (trough) concentrations during administration of twice-daily dosing (BID) on a linear scale.



REFERENCES

- 1 Ting AT, Pimentel-Muñoz FX, Seed B. RIP mediates tumor necrosis factor receptor 1 activation of NF-κB but not Fas/APO-1-initiated apoptosis. *EMBO Journal*. 1996;15(22):6189-6196. Doi:10.1002/j.1460-2075.1996.tb01007.x
- 2 Ofengeim D, Yuan J. Regulation of RIP1 kinase signalling at the crossroads of inflammation and cell death. *Nature Reviews Molecular Cell Biology*. 2013;14(11):727-736. Doi:10.1038/nrm3683
- 3 Degterev A, Huang Z, Boyce M, et al. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nature Chemical Biology*. 2005;1(2):112-119. Doi:10.1038/nchembio711
- 4 Degterev A, Hitomi J, Germscheid M, et al. Identification of RIP1 kinase as a specific cellular target of necrostatins. *Nature Chemical Biology*. 2008;4(5):313-321. Doi:10.1038/nchembio.83
- 5 Re DB, Le Verche V, Yu C, et al. Necroptosis drives motor neuron death in models of both sporadic and familial ALS. *Neuron*. 2014;81(5):1001-1008. Doi:10.1016/j.neuron.2014.01.011
- 6 Najjar M, Suebsuwong C, Ray SS, et al. Structure guided design of potent and selective ponatinib-based hybrid inhibitors for RIPK1. *Cell Reports*. 2015;10(11):1850-1860. Doi:10.1016/j.celrep.2015.02.052
- 7 Qiu X, Klausen C, Cheng JC, Leung PCK. CD40 ligand induces RIP1-dependent, necroptosis-like cell death in low-grade serous but not serous borderline ovarian tumor cells. *Cell Death and Disease*. 2015;6(8):e1864-e1864. Doi:10.1038/cddis.2015.229
- 8 Cougnoux A, Cluzeau C, Mitra S, et al. Necroptosis in niemann-pick disease, type C1: A potential therapeutic target. *Cell Death and Disease*. 2016;7(3). Doi:10.1038/cddis.2016.16
- 9 Smith CCT, Davidson SM, Lim SY, Simpkin JC, Hothersall JS, Yellon DM. Necrostatin: A potentially novel cardioprotective agent? *Cardiovascular Drugs and Therapy*. 2007;21(4):227-233. Doi:10.1007/s10557-007-6035-1
- 10 Lukens JR, Vogel P, Johnson GR, et al. RIP1-driven autoinflammation targets IL-1α independently of inflammasomes and RIP3. *Nature*. 2013;498(7453):224-227. Doi:10.1038/nature12174
- 11 Liu M, Wu W, Li H, et al. Necroptosis, a novel type of programmed cell death, contributes to early neural cells damage after spinal cord injury in adult mice. *Journal of Spinal Cord Medicine*. 2015;38(6):745-753. Doi:10.1179/2045772314Y.0000000224
- 12 Ofengeim D, Ito Y, Najafov A, et al. Activation of necroptosis in multiple sclerosis. *Cell Reports*. 2015;10(11):1836-1849. Doi:10.1016/j.celrep.2015.02.051
- 13 Su X, Wang H, Kang D, et al. Necrostatin-1 Ameliorates Intracerebral Hemorrhage-Induced Brain Injury in Mice Through Inhibiting RIP1/RIP3 Pathway. *Neurochemical Research*. 2015;40(4):643-650. Doi:10.1007/s11064-014-1510-0
- 14 Yin B, Xu Y, Wei RL, He F, Luo BY, Wang JY. Inhibition of receptor-interacting protein 3 upregulation and nuclear translocation involved in Necrostatin-1 protection against hippocampal neuronal programmed necrosis induced by ischemia/reperfusion injury. *Brain Research*. 2015;1609(1):63-71. Doi:10.1016/j.brainres.2015.03.024
- 15 Viringipurampeer IA, Metcalfe AL, Bashar AE, et al. NLRP3 inflammasome activation drives bystander cone photoreceptor cell death in a P23H rhodopsin model of retinal degeneration. *Human Molecular Genetics*. 2016;25(8):1501-1516. Doi:10.1093/hmg/ddw029
- 16 Mifflin L, Hu Z, Dufort C, et al. A RIPK1-regulated inflammatory microglial state in amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2021;118(13). Doi:10.1073/PNAS.2025102118
- 17 Ito Y, Ofengeim D, Najafov A, et al. RIPK1 mediates axonal degeneration by promoting inflammation and necroptosis in ALS. *Science*. 2016;353(6299):603-608. Doi:10.1126/science.aaf6803
- 18 Caccamo A, Branca C, Piras IS, et al. Necroptosis activation in Alzheimer's disease. *Nature Neuroscience*. 2017;20(9):1236-1246. Doi:10.1038/nn.4608
- 19 Ofengeim D, Mazzitelli S, Ito Y, et al. RIPK1 mediates a disease-associated microglial response in Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2017;114(41):E8788-E8797. Doi:10.1073/pnas.1714175114
- 20 Mifflin L, Ofengeim D, Yuan J. Receptor-interacting protein kinase 1 (RIPK1) as a therapeutic target. *Nature Reviews Drug Discovery*. Published online July 15, 2020. Doi:10.1038/s41573-020-0071-y
- 21 Zelic M, Pontarelli F, Woodworth L, et al. RIPK1 activation mediates neuroinflammation and disease progression in multiple sclerosis. *Cell reports*. 2021;35(6):109112. Doi:10.1016/j.celrep.2021.109112
- 22 Weisel K, Berger S, Thorn K, et al. A randomized, placebo-controlled experimental medicine study of RIPK1 inhibitor GSK2982772 in patients with moderate to severe rheumatoid arthritis. *Arthritis Research and Therapy*. 2021;23(1):1-12. Doi:10.1186/s13075-021-02468-0
- 23 Weisel K, Scott NE, Tompson DJ, et al. Randomized clinical study of safety, pharmacokinetics, and pharmacodynamics of RIPK1 inhibitor GSK2982772 in healthy volunteers. *Pharmacology Research and Perspectives*. 2017;5(6). Doi:10.1002/prp2.365
- 24 Yuan J, Amin P, Ofengeim D. Necroptosis and RIPK1-mediated neuroinflammation in CNS diseases. *Nature Reviews Neuroscience*. 2019;20(1):19-33. Doi:10.1038/s41583-018-0093-1
- 25 Mifflin L, Ofengeim D, Yuan J. Receptor-interacting protein kinase 1 (RIPK1) as a therapeutic target. *Nature Reviews Drug Discovery*. 2020;19(8):553-571. Doi:10.1038/s41573-020-0071-y
- 26 Grievink HW, Heuberger JAAC, Huang F, et al. DNL104, a Centrally Penetrant RIPK1 Inhibitor, Inhibits RIP1 Kinase Phosphorylation in a Randomized Phase I Ascending Dose Study in Healthy Volunteers. *Clinical Pharmacology and Therapeutics*. 2020;107(2):406-414. Doi:10.1002/cpt.1615
- 27 Shen J, Swift B, Mamelok R, Pine S, Sinclair J, Attar M. Design and Conduct Considerations for First-in-Human Trials. *Clinical and Translational Science*. 2019;12(1):6-19. Doi:10.1111/cts.12582
- 28 Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's and Dementia*. 2011;7(3):280-292. Doi:10.1016/j.jalz.2011.03.003
- 29 Ludolph A, Drory V, Hardiman O, et al. A revision of the El Escorial criteria - 2015. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*. 2015;16(5-6):291-292. Doi:10.3109/21678421.2015.1049183
- 30 A Study to Evaluate the Benefit and Safety of GSK2982772 in Moderate to Severe Psoriasis Participants - Full Text View - ClinicalTrials.gov. Accessed May 22, 2021. <https://clinicaltrials.gov/ct2/show/NCT04316585>
- 31 Vissers MFJM, Heuberger JAAC, Groeneveld GJ. Targeting for success: Demonstrating proof-of-concept with mechanistic early phase clinical pharmacology studies for disease-modification in neurodegenerative disorders. *International Journal of Molecular Sciences*. 2021;22(4):1-33. Doi:10.3390/ijms22041615
- 32 Denali Therapeutics Announces Positive Clinical Results and Regulatory Progress for Development Programs in Amyotrophic Lateral Sclerosis (ALS). Accessed Jan 20, 2022. <https://www.globenewswire.com/en/news-release/2021/10/06/2309876/0/en/Denali-Therapeutics-Announces-Positive-Clinical-Results-and-Regulatory-Progress-for-Development-Programs-in-Amyotrophic-Lateral-Sclerosis-ALS.html>