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Sponsor: Sanofi **Drug substance(s):** SAR443060 (DNL747)/ RIPK1INHIBITOR	**Study Identifiers:** NCT03757351, U1111-1241-8315 **Study code:** TDR16536
Title of the study: A multicenter, randomized, placebo-controlled, double-blind, Phase 1b study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of SAR443060 (DNL747) on subjects with amyotrophic lateral sclerosis (TDR16536 [DNLI-D-0003])	
Study centers: 2 centers in the United States, 1 center in the Netherlands	
Study period: Date first participant enrolled: 14/Dec/2018 Date last patient completed the double-blind (DB) part: 10/Mar/2020 Date last patient completed the open-label extension (OLE) part: 18/Jun/2020 Study Status: Terminated due to change in Sponsor's development strategy for DNL747/SAR443060	
Phase of development: Phase 1b	
Objectives: The primary objective of the **DB part** of the study is: • To investigate the safety and tolerability of 28 days of oral doses of SAR443060 (DNL747) in subjects with amyotrophic lateral sclerosis (ALS) The secondary objectives of the **DB part** of the study are: • To characterize the pharmacokinetics (PK) in plasma and cerebrospinal fluid (CSF) following oral doses of SAR443060 (DNL747) • To characterize the PD and target engagement of SAR443060 (DNL747) using an assay for serine 166 phosphorylated receptor-interacting serine/threonine-protein kinase 1 (pSer166-RIPK1) in peripheral blood mononuclear cells (PBMCs) The primary objective of the **OLE part** of the study is: • To investigate the safety and tolerability of up to 12 months of oral and/or gastrostomy feeding tube (G-tube) doses of SAR443060 (DNL747) low dose twice per day (BID) in subjects with ALS	
Methodology: A multicenter, multinational, randomized, placebo-controlled, double-blind, crossover study with repeated doses of SAR443060 (DNL747) in subjects with ALS. This study consisted of a DB part and an OLE part. In the DB part, the patients were randomized 1:1 to receive either SAR443060 (DNL747) or placebo for 28 days plus a morning dose on the following day for the first treatment period and then cross over to the opposite treatment for another 28 days and a morning dose as the second treatment period with a 14-day washout period in between. Patients who completed the DB part through the safety follow-up (SFU) and final follow-up	

(FFU) visits might be eligible to enter the OLE part and receive oral dosing with SAR443060 (DNL747) low dose BID for up to an additional 12 months.

The DB part of this study was completed. However, the OLE part was terminated due to Sponsor's decision to stop the development of the compound SAR443060 (DNL747).

Number of participants (DB part):

Planned: 16 to 26

Randomized: 15

Treated: 15

Number of participants (OLE part):

Treated: 8

Evaluated (DB part):

Efficacy/pharmacodynamics: 14

Safety: 15

Pharmacokinetics: 14

Evaluated (OLE part):

Efficacy/pharmacodynamics: 8

Safety: 8

Pharmacokinetics: 8

Diagnosis and criteria for inclusion in DB part: Male and female patients of non-childbearing potential, with a clinical diagnosis of ALS less than 3 years since symptom onset, aged between 21 and 80 years, body mass index (BMI) between 18 and 35 kg/m^2, and a weight of at least 45.0 kg.

Diagnosis and criteria for inclusion in OLE part: Male and female patients who successfully completed both periods of the DB, crossover part of this study within 12 months of anticipated first dose of the OLE in the Netherlands; with a continued diagnosis of ALS.

Study Product:

Investigational medicinal product(s): SAR443060 (DNL747) and placebo

Formulation: SAR443060 (DNL747) was supplied as powder in capsules. Matching placebo was formulated as powder in capsules.

Route(s) of administration: PO and/or gastrostomy feeding tube (G-tube)

Dose regimen in the **DB part**: A treatment period contained repeated dosing of SAR443060 (DNL747 low dose or matching placebo approximately every 12 hours (BID) for 28 days, followed by a final dose on the morning of the 29th day.

Dose regimen in the **OLE part**: Repeated dosing of SAR443060 (DNL747) low dose BID for up to an additional 12 months.

Duration of treatment:

- **DB part:** Two treatment periods of 29 days each
- **OLE part:** Planned to be 12 months

Duration of observation:

- **DB part:** Approximately 4 months including screening (32 days), the first treatment period (29 days), the washout period (14 days), the second treatment period (29 days) and two follow up visits (14 days)
- **OLE part:** Planned to be approximately 16 months from screening through the OLE FFU, plus any additional time between the DB part and initiation of the OLE.

Criteria for evaluation:

Efficacy/pharmacodynamics:

Phosphorylation of serine 166 of receptor-interacting serine/threonine protein kinase 1 (pSer166-RIPK1) in PBMC lysates obtained from blood were measured to characterize the pharmacodynamics and target engagement of SAR443060 (DNL747). Additional biomarker analyses were performed on serum, plasma, CSF and urine to determine impact of treatment on disease and pathway specific markers.

Safety:

Adverse events (AEs), vital signs, 12-lead electrocardiogram (ECG, automatic reading), clinical laboratory evaluations (hematology, clinical chemistry, urinalysis), physical examinations and responses obtained from the Columbia Suicide Severity Rating Scale (C-SSRS).

Pharmacokinetics:

The following PK parameters were calculated from SAR443060 (DNL747) plasma concentrations using noncompartmental methods: maximum plasma concentration observed (C_{max}), AUC calculated using the trapezoidal method over the dosing interval ($AUC_{0\text{-}tau}$), AUC from time zero to the last measured concentration above the limit of quantification ($AUC_{0\text{-}last}$), time to reach C_{max} (T_{max}), accumulation ratios (Rac) on C_{max} and $AUC_{0\text{-}tau}$ were determined on Day 29, and other parameters, including oral volume of distribution and oral clearance, were determined as appropriate.

Pharmacokinetic/Pharmacodynamics sampling times and bioanalytical methods:

DB part:

Eighteen plasma samples were collected for SAR443060 (DNL747) concentration measurements during the observation for each period.

- Once at 0 hour (pre-dose) and 7 times after the first dose: 0.5, 1, 1.5, 2, 4, 8, 12 hours on Day 1 (or Day 43);
- Once at 0 hour (pre-dose) and 7 times after the first dose: 0.5, 1, 1.5, 2, 4, 8, 12 hours on hours on Day 29 (or Day 71);
- Additional sample on Day 15 (or Day 57) two hours post-dose;
- Additional sample on Day 30 (or Day 72) at 24 hours after the last dose.

Three CSF samples were collected for SAR443060 (DNL747) measurement. One on Day -1 before the first dose, one at 4 hours post-dose at Day 29 and one at 4 hours post-dose at Day 71 to coincide with the 4-h post-dose PK plasma sample at Day 29 of the second Period.

Plasma and CSF concentrations of SAR443060 (DNL747) were measured by validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. (Numbers PRA-NL-SML-2085 and PRA-NL-SML-2087 for plasma and CSF respectively) with a lower limit of quantification (LLOQ) of 0.00247 µM.

Whole blood for PBMC lysate assay and exploratory biomarkers were collected on Day 1, 29, 43 (Day 1 in Period 2), and 71 (Day 29 in Period 2) at pre-dose and at 2, 4, and 12 h post-dose; and on Day 15 and Day 57 (Day 15 in Period 2) at 2 h post-morning dose. Additional samples were collected on Days 30 and 72 (Day 30 in Period 2) at approximately 24 h after the last dose. PBMCs were isolated and the RIPK1 phosphorylation were measured using fit-for purpose assays.

Spot urine samples for exploratory PD analysis were collected on Days 1, 28, 29, 43, 70, and 71 at 1-4 h post-morning dose and baseline 1, baseline 2, SFU, and FFU at approximately the same time of day when possible.

For potential pharmacogenetics analysis, a blood sample for DNA isolation were collected from all patients at Day 1 as the baseline.

OLE part:

Plasma PK samples were collected at the same time as the lumbar puncture (LP) CSF sample. For patients receiving study drug through a G-tube, plasma PK samples were collected on the first day of G-tube administration pre-dose and at 0.5, 1, 1.5, 2, and 4 hours post-dose.

Two CSF samples were planned to be collected at post-dose at Month 3 for PK and PD, and at the extension FFU/extension early discontinuation (EED) visit for PD only.

Whole blood for PBMC lysate assay and exploratory biomarkers were collected pre-dose on days when LPs were planned. Additional samples for PD plasma analyses were collected on Month 6 pre-dose.

Spot urine samples for exploratory PD analysis were collected pre-dose at Month 3 and the extension FFU/EED, the same time as the planned CSF samples.

All the PK and PD samples were analyzed using the same methods as in the DB part.

Statistical methods:

Patient disposition, demographics and patients' characteristics at baseline and disease characteristics were summarized by sequence of the crossover (SAR443060 (DNL747) then Placebo, or Placebo then SAR443060 (DNL747)) and overall.

Safety:

All randomized patients having received any investigational medicinal product (IMP) (SAR443060 (DNL747) or placebo) during the DB part of the study were included in the DB safety population.

The safety evaluation in DB part was based upon the review of individual values (clinically significant abnormalities) and summary tables by treatment group (SAR443060 (DNL747) or Placebo) in the DB safety population.

All patients who received at least one dose of IMP (SAR443060 (DNL747)) during the OLE part of the study were included in the OLE safety population.

The safety evaluation in OLE part was based upon the review of individual values (clinically significant abnormalities) and summary tables overall in the OLE safety population.

Adverse events were coded according to the Medical Dictionary for Regulatory Activities (Version 22.1) and the statistical analyses focused on the AEs occurring during the treatment-emergent AE (TEAE) period, defined as the time interval from the very first IMP administration up to the FFU visit (included) or up to the early discontinuation visit for patients prematurely withdrawn. For the DB part, the TEAE period was divided into 2 TEAE periods, each of ones starting from the first IMP administration in the given treatment period. The reported TEAEs were allocated for the sake of statistical analysis to the TEAE period in which they started.

An overview of the AE profile as well as the number and percentage of patients with TEAEs and with treatment-emergent adverse event of special interest (AESI) were summarized by primary System Organ Classification (SOC) and preferred term (PT).

Potentially clinically significant abnormalities (PCSAs) in clinical laboratory test results (hematology, biochemistry), vital signs, and ECG data observed during the TEAE period were summarized by treatment group or overall using frequency tables.

Pharmacokinetics:

All randomized patients who received at least one dose of SAR443060 (DNL747) and have evaluable PK data in the treatment period during which SAR443060 (DNL747) was administered were included in the DB PK population.

All the patients included in the OLE part of the study who switched to a G-tube for IMP administration were included in the OLE PK population.

In the DB part, noncompartmental PK analyses were performed on individual PK plasma concentration data. Plasma and CSF concentrations of SAR443060 (DNL747) and PK parameters for plasma obtained during the DB part were listed and summarized with the use of descriptive statistics (such as mean, geometric mean, median, standard deviation [SD], standard error of the mean [SEM], coefficient of variation [CV], minimum, and maximum). Individual and mean SAR443060 (DNL747) plasma concentration-time profiles were presented graphically using a linear and/or log scale as appropriate. Accumulation ratio of Day 29 (or Day 71) versus Day 1 (on Day 43) on log-transformed parameters C_{max} and $AUC_{0\text{-}tau}$ were estimated using a linear model. The ratio CSF to plasma on Days 29 and 71 was assessed.

For the OLE part, the PK analyses were planned to be performed using the OLE pharmacokinetic population and plasma PK parameters were to be listed and summarized by descriptive statistics.

Pharmacodynamics:

All patients included in the DB safety population who had evaluable (non-missing) PD data at baseline and at least one post-baseline visit within at least one of both treatment periods were included in the DB PD population.

All patients included in the OLE Safety population who had evaluable (non-missing) PD data at baseline and at least one post-baseline visit were included in the OLE PD population.

The PD parameters of pSer166-RIPK1 measurements of PBMC lysates from blood were listed, plotted and summarized using descriptive statistics. The list of potential biomarkers analyzed as PD parameters were analyzed using descriptive statistics. Graphical representations over time of selected biomarkers and statistical analyses were considered.

Summary Results:

Population characteristics:

Fifteen patients were randomized and were exposed to the IMP during each treatment period of the study DB part. There were 12 males and 3 females in the study, with 12 patients (80%) in the age group 18 - 64 years and 3 patients (20%) in the age group 65 - 84 years. All 15 patients were identified as white. Among those only 1 was identified as ethnicity Hispanic or Latino. Regarding rate of progression at entry, 4 (26.7%) patients were fast progressors and 11 (73.3%) were slow progressors, by criteria defined in the statistical analysis plan (SAP). Overall, the demographic and disease characteristics of the randomized and treated ALS patients who entered the study were balanced between the 2 cross-over sequences at entry.

One patient (Sequence: SAR443060 (DNL747)/Placebo) prematurely withdrew from the study during the treatment Period 2 after 28 days of treatment. This patient reported being unable to travel to the clinic anymore due to disease progression. An adverse event of “ALS worsening” was reported on the same day of the study discontinuation.

A second patient prematurely discontinued the treatment in period 1 after 21 days of treatment (with treatment regimen started with the IMP dose of high dose BID and then changed to low dose BID due to study protocol amendment). The patient decided and was allowed to forgo the rest of treatment period 1 without withdrawing from the study, and subsequently the patient completed the treatment period 2 [Sequence: SAR443060(DNL747)/Placebo].

Eight patients who had completed the DB part through the FFU visit were allowed to enter the OLE part and were treated with oral dosing of SAR443060 (DNL747) low dose BID. Among them, 6 patients were treated until the study was terminated by the Sponsor, and 2 patients discontinued from the study treatment due to “withdrawal from treatment by subject”. Overall, 7 patients completed with the FFU visit, and 1 patient withdrew from the study treatment without completing the FFU visit. There were 6 (75%) males and 2 (25%) females in the OLE part, with 6 patients (75%) in the age group from 18 to 64 years and 2 patients (25%) in the age group from 65 to 84 years. All 8 patients were identified as white.

Efficacy/pharmacodynamics results:

Overall, SAR443060 (DNL747) did not reach a desired level of RIPK1 inhibition (>90%), in all patients, as measured by the pSer166-RIPK1 target engagement assay at the dose level used in this study. Measurement of key cytokines and chemokines did not reveal any disease modulating or apparent safety signals with treatment. Transient changes in specific targets were noted for individual patients which may be reflective of individual disease state. A summary heatmap of mean changes for selected biomarkers at each specific sampling timepoint indicated changes in certain biomarkers during the placebo period following the SAR443060 (DNL747) treatment period that exceeded the levels measured during the placebo period of the Placebo-to-SAR443060 (DNL747) treatment sequence.

Analysis of the biomarkers of neurodegeneration and disease progression in ALS patients (neurofilament light chain [Nf-L], Chitinase 3-like-1 [CHI3L1/YKL40], soluble triggering receptor expressed on myeloid cells 2 [sTREM2]), confirmed baseline elevation of these biomarkers in plasma, CSF, and urine (for neurotrophin receptor p75 extracellular domain [$p75^{ECD}$]) samples. This is consistent with published reports of these biomarker as related to neurodegenerative diseases such as ALS, multiple sclerosis and Alzheimer's disease. Absolute levels of these markers varied between patients and did not appear to significantly change with treatment during the DB part. For sTREM2, there was no change in plasma values but an increased percent change from baseline in CSF was noted during SAR443060 (DNL747) treatment periods in the DB part.

Analysis of a limited set of samples collected during the OLE part also did not demonstrate a significant change in these markers even with evaluating the effect of longer treatment.

Safety results:

In the DB part, the mean exposure was comparable between treatment groups. The majority of patients in both groups were treated for the whole treatment period of up to the morning of Day 29.

Overall, in the DB part, 12 out of 15 patients (80.0%) reported TEAEs on SAR443060 (DNL747) and 11 out of 15 patients (73.3%) reported TEAEs on placebo. The safety profile and the nature of the events were comparable between the SAR443060 (DNL747) and placebo.

The most common TEAE by SOC in the DB part was "Nervous system disorders" which were reported in 5 out of 15 patients in both SAR443060 (DNL747) and placebo, including Headache in 3 out of 15 patients (20.0%) in SAR443060 (DNL747) and in 2 out of 15 patients (13.3%) in placebo. Neither severe TEAEs nor any TEAE leading to definitive study/treatment discontinuation were observed during the DB part.

The safety profile and the nature of events were also similar when comparing the results between SAR443060 (DNL747) and placebo within each sequence separately.

There were no death or serious adverse event (SAE) reported during the DB part.

There were two AESIs reported in the DB part (both of mild intensity), 1 in SAR443060 (DNL747) and 1 in placebo. Both AESIs were in the SOC "Skin and subcutaneous tissue disorders" with the PTs "Erythema" in SAR443060 (DNL747) and "Seborrheic Dermatitis" in placebo. Of these two AESIs only the Erythema was deemed, by the site Investigator and Sponsor, to be related to SAR443060 (DNL747). There were no other reported AESIs. Specifically, none of the patients had clinically significant anemia, thrombocytopenia, or elevation in liver enzymes during the double-blind part of the study.

There were very few PCSA events reported among laboratory parameters, vital signs or ECG data in the DB part. When reported they were mostly single events in either SAR443060 (DNL747) or placebo. Heart rate of > 90 beats/min was recorded in 3/15 patients in both SAR443060 (DNL747) and placebo. QRS Interval > 110 msec was recorded in 3/15 and 1/15 patients in SAR443060 (DNL747) and placebo, respectively. PR Interval > 200 msec was recorded in 2/15 and 1/15 patients in SAR443060 (DNL747) and placebo, respectively. None of the patients had a QTcF interval >480 ms or QTc interval change from baseline >60 ms during the double-blind part of the study. Few PCSAs were observed in hematology or chemistry parameters and none in liver function.

Overall, the results in DB part show the patients tolerated SAR443060 (DNL747) well with a safety profile that is comparable to placebo.

In the OLE part, due to the early termination decision by Sponsor, no safety conclusion regarding the long-term safety can be derived from the OLE part.

Pharmacokinetic results:

In the DB part, pharmacokinetic parameters after a first administration at Day 1 (or Day 43) and at Day 29 (or Day 71) after the last administration of oral doses of SAR443060 in patients with ALS are summarized in Table 1 and Table 2, respectively. SAR443060 (DNL747) accumulation ratio for C_{max} and for $AUC_{0\text{-}tau}$ are provided in Table 3. The overall SAR443060 (DNL747) CSF to plasma ratio on Day 29 (or 71) is provided in Table 4.

The Patient was excluded from the analysis of the accumulation ratio (Table 3) and from the CSF to plasma ratio (Table 4) as per SAP, and was kept in the presentations of the descriptive statistics of plasma PK parameters in Table 1 and Table 2.

Table 1 - Descriptive statistics of plasma pharmacokinetic parameters following SAR443060 single oral administration

Mean ± SD (Geometric Mean) [CV%]	PLASMA SAR443060
	low dose BID SAR443060
N	15
C_{max}	0.581 ± 0.405
(µM)	(0.512) [69.7]
t_{max}*a*	1.05
(h)	(0.47 - 4.00)
AUC_{0-12}*b*	2.11 ± 1.89
(µM•hr)	(1.74) [89.8]

a Median
(Min - Max)

b N=14, One patient not included in calculation of summary statistics

One patient who received high dose twice daily for 21 days is included in the descriptive statistics of the PK parameters after the 1st administration.

Source = PKS Study : TDR16536; Scenario: TDR16536-P-D-A-EV-BID, Version 14

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Table 2 - Descriptive statistics of plasma pharmacokinetic parameters following SAR443060 29-days repeated BID oral administration

Mean ± SD (Geometric Mean) [CV%]	PLASMA SAR443060
	low dose BID SAR443060
	Day 29
N	14
C_{max}	0.638 ± 0.267
(µM)	(0.590) [41.9]
t_{max}***Error! Reference source not found.***	1.25
(h)	(0.50 - 4.52)
AUC_{last}	4.10 ± 1.71
(µM•hr)	(3.76) [41.6]
AUC_{0-12}	3.12 ± 1.20
(µM•hr)	(2.89) [38.3]

a Median
(Min - Max)

Source = PKS Study : TDR16536; Scenario: TDR16536-P-D-A-EV-BID, Version 14

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Table 3 - Accumulation ratio (Day 29/Day 1) for SAR443060 C_{max} and $AUC_{0\text{-}tau}$ after 29-days repeated oral administration of low dose BID SAR443060 - DB PK population

Sequence	Parameter	Point estimate	90% CI
Overall	Ratio(C_{max})	1.11	(0.88 to 1.39)
	Ratio($AUC_{0\text{-}tau}$)	1.81	(1.60 to 2.04)
Placebo/SAR443060 (DNL747)	Ratio(C_{max})	1.04	(0.67 to 1.60)
	Ratio($AUC_{0\text{-}tau}$)	1.78	(1.48 to 2.12)
SAR443060 (DNL747)/Placebo	Ratio(C_{max})	1.09	(0.72 to 1.65)
	Ratio($AUC_{0\text{-}tau}$)	1.80	(1.32 to 2.45)

PGM=PRODOPS/SAR443060/DNLI_D_0003/CSR_01/REPORT/PGM/pk_tdr16536_i.sas
OUT=REPORT/OUTPUT/pk_ac_k_t_2_i.rtf (26MAY2020 9:36)

Table 4 - Ratios CSF to unbound plasma on Day 29 (or Day 71) for SAR443060 - DB PK population

Parameter	Statistics	Placebo/SAR443060 (DNL747)	SAR443060 (DNL747)/Placebo	Overall sequence
CSF/Plasma concentration ratio at Day 29 - T4H	N	8	5	13
	Mean	0.916	1.134	1.000
	Geom. Mean	0.884	1.125	0.970
	SD	0.280	0.151	0.256
	CV	31%	13%	26%
	Median	0.879	1.155	0.955
	Minimum	0.63	0.89	0.63
	Maximum	1.50	1.27	1.50

Source = Appendix 16.2.5.4.2.1.1.1.

SAR443060 (DNL747) was rapidly absorbed following oral administration of low dose BID, with median t_{max} value of approximately 1 hour.

After low dose BID dosing, at steady state, the overall accumulation ratios was 1.11 (90% CI: 0.88 ; 1.39) for C_{max} and 1.81 (90% CI: 1.60 ; 2.04) for $AUC_{0\text{-}tau}$.

The mean CSF-to-unbound plasma concentration ratio was about 1.

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