2 SYNOPSIS

Name of Sponsor/Company Dynacure	Name of Finished Product DYN101	Name of Active Ingredient 3-10-3 Ionis constrained ethyl gapmer
INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER:	PAGE:	
(FOR NATIONAL AUTHORITY USE ONLY)		
Title of Study: A Phase 1/2 trial on the safety, tolerability, pharmacokinetics, pharmacodynamics and exploratory efficacy of DYN101 in patients ≥ 16 years of age with centronuclear myopathies caused by mutations in DNM2 or MTM1.		
Protocol Number: DYN101-C101		
Trial Registry Name and Number: 2018-004089-33		
Study Centers: A total of 8 sites in 6 EU countries (Netherlands, Belgium, Germany, France, Denmark, UK).		
Phase of Development: Phase 1/2		
Study Period: Date of first subject enrollment: 04 March 2020 Date of last subject's last visit: 08 July 2022		
Reporting Period: Date of database lock: 05 September 2022 Date of final analysis: 13 October		
Background: Centronuclear myopathies (CNMs) are a group of severe, debilitating, non-dystrophic, congenital myopathies. CNMs are characterized by muscle weakness, fiber atrophy, predominance of type I fibers, and increased centralization of nuclei not secondary to muscle regeneration. Three classical forms of CNM have been characterized: • X-linked CNM (XLCNM), caused by mutations in the myotubularin 1 (MTM1) gene • Autosomal dominant CNM (ADCNM), caused by mutations in the dynamin 2 (DNM2) gene • Autosomal recessive CNM (ARCNM), caused by mutations in the amphiphysin II (BIN1) Currently, there is no cure or disease-modifying therapy for CNM. Antisense oligonucleotide (ASO) technology represents a promising therapeutic approach for diseases caused by gain of function mutations. It either reduces expression of the mutant gene or induces the production of a functional or partially functional protein through a number of mechanisms including splicing modulation for loss-of-function genetic diseases. In neuromuscular diseases, this approach had been mainly applied to dystrophic muscle diseases where uptake may be		

facilitated by disruption of membrane integrity, or to diseases with nuclear accumulation of pathogenic ribonucleic acid (RNA) that favors RNase H1 dependent degradation of the targeted transcript.

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Objectives and Endpoints:

Objective	**Endpoint**
Primary study objective:	
To assess the safety and tolerability of 3 SAD levels and 3 MAD levels of DYN101	Comparison of adverse event (AE, SAE, AESI) frequency and severity by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) and overall using the Safety Analysis Set following 12 weeks of MAD treatment and after 24 weeks of MAD treatment (12 weeks in the MAD part + 12 weeks in the MAD extension part; Week 25 visit) or discontinued earlier
Secondary study objectives:	
To assess the PK of SAD and MAD of DYN101	Comparison of means for PK parameters of DYN101 in plasma (AUCτ, AUClast, AUC∞, Rac(AUC), Rac(Cmax), Cmax, Cav SS, CL, λz, t½, tmax, Vz) by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) and overall using the PK Set Comparison of geometric means for PK parameters of DYN101 in plasma (Cmax and AUCs) by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) and overall using the PK Set
To explore target engagement in muscle of DYN101	Comparison of means on DNM2 mRNA levels and DYN101 concentrations using muscle biopsy data by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) and overall using the PK Set. Comparison of means change from baseline in vital signs, laboratory data, and ECG by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) and overall using the Safety Analysis Set.
Exploratory study objectives:	

To investigate the effect of DYN101 treatment on the clinical assessments in various affected domains (respiratory, muscle strength and function, dysphagia)	Difference in mean change from baseline for clinical assessments in various affected clinical domains (respiratory, muscle strength and function, dysphagia) using the PK Set by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) at each post-baseline visit. The parameters to be analyzed for respiratory function are FVC (L), %FVC predicted, FEV1 (L), %FEV1 predicted, FEV1/FVC, MEP (cm H2O), %MEP predicted, MIP (cm H2O), %MIP predicted, oxygen saturation (%). The MyoGrip Test score will be used for analysing muscle strength. The following parameters will be analysed for muscle function: D1 Standing and transfers (%), D2 Axial and proximal motor function (%), D3 Distal motor function (%), total MFM32 score (%). Dysphagia will be assessed using the EAT-10 total score.
To explore the impact of CNM and its treatment on symptoms, functioning, and health related QoL	Difference in mean change from baseline for PROMIS® Questionnaire T-scores for the following domains (Anxiety, Depression, Fatigue, Pain Interference, Ability to Participate and Physical Function) using the PK Set by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) at each post-baseline visit Comparison of proportion of CGI-I responders (responders defined as responding 1 or 2) using the PK Set by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) at each post-baseline visit Comparison of proportion of PGI-C responders (responders defined as responding 1 or 2) using the PK Set by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) at each post-baseline visit. Comparison of proportion of GAS Questionnaire responders (responders defined as responding “YES – MUCH BETTER,” “YES – A LITTLE BETTER”) using the PK Set by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) at each post-baseline visit
To assess the presence of ADA against DYN101 by collecting blood samples	Comparison of means of ADA against DYN101 using the PK Set by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) at each post-baseline visit
To assess the metabolic profile of DYN101 by collecting blood and urine samples	Comparisons of the metabolic profile of DYN101 in blood and urine using the PK Set by dose (cohort), for single or multiple administration, and by mutated gene (subcohort) at each post-baseline visit

To contribute to the global understanding of CNM and its treatment by collecting blood and muscle samples for further exploratory biomarker/genetic research	Not applicable

Abbreviations: ADA = anti-drug antibodies; AE = adverse event; AESI = adverse event of special interest; AUC = area under the curve; CGI-I = Clinical Global Impression of Improvement; CL = clearance; Cmax = maximum concentration; CNM = centronuclear myopathy; *DNM2* = dynamin 2; EAT-10 = Eating Assessment Tool; ECG = electrocardiogram; FEV = forced expiratory volume; FVC = forced vital capacity; GAS = Goal Attainment Scaling; MAD = multiple ascending dose; MEP = maximum expiratory pressure; MFM = Motor Function Measure; MIP = maximum inspiratory pressure; mRNA = messenger ribonucleic acid; PGI-C = Patient Global Impression of Change; PK = pharmacokinetics; PROMIS = Patient-Reported Outcomes Measurement Information System; QoL = quality of life; SAD = single ascending dose; SAE = serious adverse event; t1/2 = half-life.

Methodology: This was a first-in-human, Phase 1/2, open-label, multicenter trial to evaluate the safety, tolerability, PK, PD, and preliminary efficacy of DYN101 after single ascending dose (SAD) and multiple ascending dose (MAD) in subjects ≥16 years of age with CNMs caused by mutations in DNM2 or MTM1.
There were to be stand-by subjects for replacement of subjects who discontinued the trial early, if needed. Stand-by subjects performed run-in visits until the last MAD subject had completed Week 13, unless needed for replacement.

The trial consisted of a pre-screening consent, a screening period, a run-in period (if applicable), a SAD part with 4 weeks of follow-up after investigational medicinal product (IMP) administration and a washout period of at least 12 weeks (followed by follow-up phone calls until the MAD part starts), a MAD part of 12 weeks, and a MAD extension part of 12 weeks. All subjects were to participate in the SAD, MAD, and MAD extension parts, unless they withdrew. End of Treatment (EoT) assessments were to be performed after 24 weeks of MAD treatment had been completed, i.e., at the Week 25 visit. Subjects were to return to the clinic 3 months after the last IMP administration to follow-up on the subject's status including abnormal laboratory results, adverse events (AEs), and concomitant medications.

In the SAD part, subjects received a single dose of DYN101 at Day 1. In the MAD part, subjects may have received the same or a different dose as in the SAD part, with weekly administrations of IMP for a period of 12 weeks, followed by a MAD extension part with an additional 12 weeks of treatment (weekly administrations). After Week 24, all subjects were to be remained on treatment with weekly IMP administrations and biweekly (every 2 weeks) assessments until the last Cohort MAD3 subject were to be completed the MAD Week 25 visit. In order to avoid any overlaps, a given MAD Cohort may not have started before its corresponding SAD Cohort was finished.

Subjects received DYN101 in a low (1.5 mg/kg), middle (4.5 mg/kg), or high (up to 9 mg/kg) dose level in Cohorts 1, 2, and 3, respectively. In the MAD part, subjects may have received a different dose than what they received in the SAD part. In each cohort, there were up to 6 subjects with a mutation in *DNM2* (subcohort a) and up to 3 subjects with a mutation in *MTM1* (subcohort b).

Cohorts were enrolled in a sequential, staggered approach, with an interval of at least 7 days between dose administration of the first and the next subject in a cohort, irrespective of their subcohort. The first subject (i.e., the sentinel subject) in each cohort was ≥18 years of age. The 48-hour safety data from the first subject in a cohort were reviewed by the medical monitor before the next subject was to be dosed. In addition, any serious adverse event (SAE) data from the first subject received up until the moment of treatment of the second subject was taken into account.

Number of Subjects (planned and analyzed):
Planned: 14 Subjects (randomized);
Analyzed for safety: 13 subjects

Diagnosis and Main Criteria for Inclusion and Exclusion:
Inclusion Criteria:

1. Male or female aged ≥16 years of age on the date of signing the main ICF. The first subject (i.e., the sentinel subject) in each cohort was ≥18 years of age.

2. Had a documented mutation in *DNM2* or *MTM1*.

3. Platelet count >150,000/µL.

4. Had a symptomatic CNM in the opinion of the investigator, at least mild to moderately affected, i.e., showed clinical symptoms in at least 2 of the 4 relevant domains that were investigated in this trial (respiratory function, muscle strength, muscle function, and dysphagia), and was ambulatory, i.e., was able to walk 10 steps, if needed with support/assisted. If a subject was non-ambulatory but highly functioning in the view of the investigator, he/she may have been included following discussion with the sponsor.

5. Had an understanding, ability, and willingness to fully comply with visit frequency, trial procedures and restrictions, including contraceptive requirements.

Exclusion Criteria:

1. Had clinically significant liver disease.

2. Had clinically significant renal disease.

3. Had presence of significant comorbidities or conditions other than CNM or clinically significant findings during screening of medical history, physical examination, laboratory testing, vital signs or ECG recording for which, in the opinion of the investigator and the medical monitor, participation would not have been in the best interest of the subject (e.g., would have compromised the safety or well-being) or that could have prevented, limited, or confounded the protocol-specified assessments (e.g. taking a muscle biopsy).

4. For female subjects of child-bearing potential: were pregnant or breastfeeding or planned to become pregnant during the trial.

5. Existing or past abuse of alcohol or recreational/narcotic drugs (with the exception of caffeine and nicotine), which in the investigator's opinion would have compromised the subject's safety and/or compliance with the trial procedures.

6. Enrolled at that time in any interventional trial or scheduled to participate in such a trial whilst participated in this trial. Subjects were allowed to participate in registry studies.

7. Existing or relevant history of physical or psychiatric illness, any medical disorder that may have required treatment or made the subject unlikely to fully complete the trial, or any condition that presented undue risk from the IMP or procedures.

8. Intake of any disallowed therapies as had been noted in the protocol within 12 weeks before the planned first IMP administration.

9. Had known or suspected intolerance or hypersensitivity to IMP ingredients or closely-related compounds, or history of a significant allergic reaction to IMP ingredients as had been determined by the investigator, such as anaphylaxis requiring hospitalization.

10. Was legally incapacitated or had limited legal capacity. Had lack of mental capacity to fully understand the protocol requirements and complete all trial required procedures.

Test Product, Dose, Mode of Administration, and Batch Number(s):
DYN101 200 mg/ml concentrate for solution for IV infusion
Route of administration: intravenous
Batch number(s): 18177019127, 18177021048

Duration of Treatment: 24 weeks

Statistical Methods:

A hierarchical model was to be fitted to each of the responses using Bayesian methods. Only noninformative priors were to be considered. The data used for the modeling consisted of the NHS data or the run-in for subjects who did not participate in the NHS. This hierarchical model was to contain a random slope and intercept for each subject. The other covariates of the model were time in months and treatment group. The baseline was at time 0. These models were to be performed for each mutation separately.

Handling of Withdrawals, Discontinuations, or Missing Data

For the analysis early withdrawal visits were to be assigned to the next scheduled visit where the relevant variable would have been assessed. For example, subjects who withdrew after MAD Week 5 had their withdrawal visit assigned as follows: vital sign data assigned to MAD Week 6 but laboratory data assigned to MAD Week 9.

It was not expected that there were to be any missing dates; however, in the rare case that an AE start date or time was missing, and it was unclear whether the AE was treatment-emergent

or not then a conservative approach was to be taken and it was to be assumed that the AE occurred after first dosing (earliest date of all doses during the whole study).

Interim Analyses and Data Monitoring

Study was terminated before the interim analysis.
The purpose of the IDMC was to review study data of the Cohorts, before proceeding to the next dose level.
Analysis Populations:
Pre-Screened Set - Subjects who signed pre-screening consent**.**
Screened Set - Subjects who signed main informed consent.
Safety Analysis Set – Screened subjects who received at least 1 IMP administration.
Pharmacokinetic Set – Subjects in the Safety Analysis Set, for whom the primary PK parameters can be calculated.

Summary of Results:
Study Subjects:
A total of 14 subjects were randomized: 6 subjects in Cohort 1 (3 subjects with a mutation in *DNM2* and 3 subjects with a mutation in *MTM1*) received 1.5 mg/kg dose and 8 subjects in Cohort 2 (5 subjects with a mutation in *DNM2* and 3 subjects with a mutation in *MTM1*) received 4.5 mg/kg dose. None of the subjects completed the study. Study terminated by sponsor was the most common reason for early withdrawal (6 [100.0%] in Cohort 1 and 7 [87.5%] subjects in Cohort 2, respectively)Efficacy Results:

The majority of the SAD Cohort (12 subjects [85.7%]) had no change in response on Day 15 and all 14 subjects (100.0%) were considered non-responders. In MAD Cohort, 6 subjects (42.9%) were non-responder. In total, there was no improvement observed. In MAD Cohort Week 13, a total of 3 subjects (21.4%) had minimally improved, 2 subjects (14.3%) had no change and 1 subject (7.1%) had minimally worsen.

Safety Results: Overall, the majority of subjects (9 subjects [64.3%]) had at least one pre-therapy AE in the SAD Cohort. All subjects (14 subjects [100.0%]) experienced at least one TEAE.

- Of the 14 subjects reporting a TEAE, 11 subjects (78.6%; 5 subjects [62.5%] in SAD Cohort and 6 subjects [100.0%] in MAD Cohort) experienced treatment related TEAEs.
- There were no deaths reported in the study and no TEAEs led to withdrawal from study treatment.
- The most common treatment related TEAEs by SOCs occurring in ≥20% overall subjects were blood and lymphatic system disorders (5 subjects [35.7%]), and investigations (8 subjects [57.1%]).

- The most common treatment related TEAEs by PTs occurring in ≥20% overall subjects were thrombocytopenia (5 subjects [35.7%]), hepatic enzyme increased (4 subjects [28.6%]), and liver function test increased (3 subjects [21.4%]).

- The SAD 1.5 mg.kg dose was well tolerated. In the SAD 4.5 mg/kg subgroup, liver elevations were observed in 5 of the 8 patients. Based on these findings, the dosing was changed from actual to ideal body weight. Despite this change in dosing regimen, 6 out of 6 MAD1 patients at low dose (1.5 mg/kg) showed liver enzyme elevations and 5 out of 6 showed low platelet counts while on treatment. In addition, a slight downward trend for red blood cell count as well as white blood cell count was observed.

Pharmacokinetic, Pharmacodynamic, and Other Analysis Results:
The measured DYN101 concentrations in plasma were analyzed using the non-compartmental analysis (WinNonLin Phoenix version 8.3). The estimation of Lambda_z was done using the linear trapezoidal and linear interpolation approach. For the estimation of Lambda z only the Rsq adjusted to equal or greater than 0.8 were accepted.

PK results in Plasma

Plasma concentration of DYN101 following the single administration, based on visual inspection of the graph, declined in multiexponential fashion for the doses 1.5 mg/kg/and 4.5 mg/kg.
Overall, based on the AUC_{last} and C_{max}, the exposure of DYN101 was similar within the same dose level of 1.5 mg/kg, when comparing the first dose from SAD and MAD, and increased with increasing doses. Limited accumulation was observable when comparing AUC_{last} for the first and last dose from the MAD phase. The half-life for DYN-101 ranged from 128 hours (geometric mean for SAD 1.5 mg/kg dose group) to 203 hours (geometric mean for SAD 4.5 mg/kg dose group) and was 237 hours for the subject in the MAD 1.5 mg/kg first dose group. The AUC_{inf} increased almost proportionally with increasing dose within the SAD group, with geometric mean values of 45293 h*ng/mL for the 1.5 mg/kg dose group and 147277 h*ng/mL for the 4.5 mg/kg dose group. The observed clearance of 2853 mL/h (geometric mean for 1.5 mg/kg group) was slightly higher than the geometric mean value of 2321 ml/h (for the 4.5 mg/kg group). Similarly, the steady state volume of distribution (V_{ss}) was slightly higher for the 1.5 mg/kg dose. The slightly higher values for AUCinf, CL and Vss can partly be explained by the change in dosing of DYN-101 for the mid dose in SAD and doses in MAD which are based on ideal body weight.

PK results in Muscle

Muscle biopsy samples were taken at baseline SAD part and after 6 to 12 weeks of MAD treatment in order to determine DYN101 muscle uptake. Overall, 5 baseline and 5 MAD samples were collected. All samples at baseline were BLQ as expected, while mean muscle concentration of 240 .08 ng/mL ranging from 52 ng/mL to 584 ng/mL.

This study was originally designed to test the safety, tolerability and efficacy of DYN101 and to examine the pharmacokinetic behavior of 3 different doses (low: 1.5 mg/kg, mid: 4.5 mg/kg and high: 9 mg/kg). Due to safety concerns the weight-based dosing was changed from actual body weight to ideal body weight dosing and the study was early terminated, leading to reduced information on PK.

Conclusions:

- DYN101 at a single dose of 1.5 mg/kg weekly was sufficiently well tolerated in CNM patients to continue with this dose.
- DYN101 at a single dose of 4.5 mg/kg induced reversible liver enzyme elevations in CNM patients, even after adjustment of dosing to lean body weight.
- DYN101 at multiple doses of 1.5 mg/kg was not well tolerated in CNM patients, with reversible laboratory parameters: liver enzyme elevations, low platelet counts while on treatment and a downward trend for red blood cell count as well as white blood cell count.
- DYN101 at multiple doses of 1.5 mg/kg weekly did not show any clinical efficacy in CNM patients.

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