CLINICAL TRIAL PROTOCOL

Trial Title:	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*.
Trial Design:	This is a first-in-human, Phase 1/2, open-label, multi-center trial, to evaluate single ascending doses (SAD) and multiple ascending doses (MAD) of DYN101.
Short Title:	Research Using an Investigational Treatment for CNM (Unite-CNM)
Protocol Identification:	DYN101-C101
EudraCT No.:	2018-004089-33
Name of Investigational Medicinal Product:	DYN101 Sterile concentrated solution for reconstitution into an infusion solution for intravenous administration.
IND No.:	Not applicable
Indication Studied:	Centronuclear myopathies (CNMs) in subjects ≥ 16 years of age with mutations in dynamin 2 (*DNM2*) or myotubularin 1 (*MTM1*).
Developmental Phase of Trial:	Phase 1/2
Name of the Sponsor/Company:	Dynacure Bioparc 3 850 Boulevard Sébastien Brant 67400 Illkirch-Graffenstaden France
Coordinating Investigator:	[redacted]
Protocol Version and Date:	Version 8.1 (non-substantial administrative correction), EU version 01 December 2021, Final

This trial, including the archiving, will be conducted in compliance with Good Clinical Practice (GCP) according to the International Council for Harmonisation (ICH) Harmonized Tripartite Guideline (CPMP/ICH/135/95).

Confidentiality Statement

Clinical Trial Protocol Signature Page

Sponsor's Approval

Signature:	**Date:**
[redacted]	

Version History

Version	Final Version Date	Countries Concerned	Comment
1.0	06 Dec 2018	All	*Not submitted outside of company*
2.0	01 Feb 2019	All	*Not submitted outside of company*
3.0	25 Feb 2019	All	Submitted to MHRA (rejected)
4.0	11 Apr 2019	All	Substantial Amendment 01
5.0	09 Aug 2019	All	Substantial Amendment 02
6.0	26 May 2020	All	Amendment 03
6.1	24 Aug 2020	US only	US version Amendment 03
6.2	17 May 2021	US only	FDA feedback
7.0	31 Mar 2021	All	Amendment 04
7.1	12 Aug 2021	US only	US version Amendment 04
8.0	15 Sep 2021	All	Substantial Amendment 05
8.1	01 December 2021	All	Non-substantial administrative correction

Investigator's Acknowledgement
I have read this protocol.

Protocol ID: DYN101-C101

Title: 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*.

I have fully discussed the objectives of this trial and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the trial, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a subject in order to obtain his/her consent to participate.

I agree to conduct this trial according to this protocol and any trial specific manuals, to comply with its requirements, ethical and safety considerations and guidelines, and to conduct the trial in accordance with the International Council for Harmonisation guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to my participation as an investigator for this trial to be terminated.

I understand that the sponsor may decide to suspend or prematurely terminate the trial at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the trial, I will communicate my intention immediately in writing to the sponsor.

Investigator Name: Site Number: (please handwrite, print or type) Institution and Address: Phone:	

Signature: _____ Date: _____

If the address or telephone number of the investigator changes during the course of the trial, written notification will be provided by the investigator to the sponsor, and a protocol amendment will not be required.

Emergency Contact Information

In the event of a serious adverse event (SAE), the investigator must complete the electronic case report form (eCRF) and submit within 1 business day to the contract research organization (CRO) responsible for Pharmacovigilance:

Worldwide Clinical Trials
Email: drugsafety@worldwide.com
Fax: +44 (0)115 922 096

In case of **medical questions** during the trial, the investigator must contact the medical monitor:

Worldwide Clinical Trials Medical Monitor
Email: UniteCNM_MM @worldwide.com
Phone: the number is on contact list in the Site File
If after working hours: dyn101@promedim.com or +44 203 356 9696

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

ADA	anti-drug antibodies
ADCNM	autosomal dominant centronuclear myopathy
ADR	adverse drug reaction
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
ARCNM	autosomal recessive centronuclear myopathy
ASO	antisense oligonucleotide
AST	aspartate aminotransferase
BIN1	amphiphysin II
BUN	blood urea nitrogen
C3	complement component 3
C5a	complement component 5a
CBb	complement factor B
CGI-I	Clinical Global Impression of Improvement
CI	confidence interval
CNM	centronuclear myopathy
CRO	contract research organization
CRP	c-reactive protein
DNM2	dynamin 2
EAT-10	Eating Assessment Tool
ECG	electrocardiogram
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EoT	End of Treatment
ESR	erythrocyte sedimentation rate
EU	European Union
FDA	Food and Drug Administration
FEV	forced expiratory volume
FU	follow-up
FVC	forced vital capacity
GCP	Good Clinical Practice
GGT	gamma glutamyltransferase
GLP	Good Laboratory Practice
hCG	human chorionic gonadotropin
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation

IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IgG	immunoglobulin G
IgM	immunoglobulin M
IMP	investigational medicinal product
INR	International normalized ratio
IPF	immature platelet fraction
IRB	Institutional Review Board
IRT	Interactive Response Technology
IV	intravenous(ly)
LLN	lower limit of normal
MAD	multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
MEP	maximum expiratory pressure
MFM	Motor Function Measure
MHRA	Medicines and Healthcare products Regulatory Agency (UK)
MIP	maximum inspiratory pressure
MRSD	minimal recommended starting dose
MTM1	myotubularin 1
NHP	non-human primate(s)
NHS	Natural History Study
NOAEL	no observed adverse effect level
PD	pharmacodynamic(s)
PGI-C	Patient Global Impression of Change
PK	pharmacokinetic(s)
PT	prothrombin time
QoL	quality-of-life
RBC	red blood cell
SAD	single ascending dose
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	subcutaneous
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
ULN	upper limit of normal
WBC	white blood cell
WHO	World Health Organization
XLCNM	X-linked centronuclear myopathy

Definition of Terms

For the definitions of the pharmacokinetic parameters, refer to Section 6.2.3.4.

CLINICAL TRIAL PROTOCOL SYNOPSIS

Protocol Number: DYN101-C101	**Investigational Medicinal Product:** DYN101
Title of the Trial: 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*.	
Planned Number of Subjects: The target is to enroll 18 subjects, with approximately 6 subjects per dose level. In each cohort, the target will be up to 6 subjects with a mutation in dynamin 2 (*DNM2*) (sub-cohort a) and up to 3 subjects with a mutation in myotubularin 1 (*MTM1*) (sub-cohort b).	
Planned Number of Sites: This trial is planned to be conducted at 5 to 10 sites in Europe and the United States of America, with an estimation of 1 to 5 subjects per site.	
Coordinating Investigator: Dr. N. Voermans	
Trial Period (Planned): First Subject Screening: September 2019 Last Subject Last Visit: April 2023	**Clinical Phase:** 1/2
Indication/Trial Population: Centronuclear myopathies (CNMs) in subjects ≥ 16 years of age* with mutations in *DNM2* or *MTM1*. *for Germany, the subject must be ≥ 18 years of age in accordance with local and national regulations.	
Objectives: **Primary:** • To assess the safety and tolerability of 3 single ascending dose (SAD) levels and 3 multiple ascending dose (MAD) levels of DYN101. **Secondary:** • To assess the pharmacokinetics (PK) of SAD and MAD of DYN101. • To explore target engagement in muscle of DYN101. **Exploratory:** • To investigate the effect of DYN101 treatment on the clinical assessments in various affected domains (respiratory function, muscle strength and function, dysphagia). • To explore the impact of CNM and its treatment on symptoms, functioning, and health-related quality-of-life (QoL). • To assess the presence of anti-drug antibodies (ADA) against DYN101 by collecting blood samples. • To assess the metabolic profile of DYN101 by collecting blood and urine samples. • To contribute to the global understanding of CNM and its treatment by collecting blood and muscle samples for further exploratory biomarker/genetic research.	
Rationale: There are currently no available treatments aside from supportive care for subjects with CNM. This trial will assess the safety, tolerability, PK and PD/preliminary efficacy of DYN101 in subjects ≥ 16 years of age* with CNM caused by mutations in *DNM2* or *MTM1* (*for Germany,	

the subject must be ≥ 18 years of age in accordance with local and national regulations). DNM2 protein is ubiquitously present and a complete knock-out of *DNM2* mRNA is lethal in mice. Elevations in DNM2 protein levels or *DNM2* function have been demonstrated in preclinical models and human tissues of X-linked CNM (XLCNM) and autosomal dominant CNM (ADCNM), respectively. Preclinical models of these mutations have shown the potential of reducing *DNM2* mRNA to prevent and reverse the clinical status. [redacted] Moreover, an increased uptake of ASO was seen in muscle of diseased mice over their wild-type counterparts. This first-in-human trial for DYN101 will therefore be conducted in patients and not in healthy volunteers to assess safety, PK and dose selection based on PD/preliminary efficacy.

Investigational Medicinal Product, Dose and Mode of Administration:

[redacted]

Low (1.5 mg/kg), middle (4.5 mg/kg) and high (up to 9 mg/kg) dose levels of DYN101 were selected based on preclinical and toxicology data from ongoing studies. These may be adjusted based on recommendations of the Independent Data Monitoring Committee (IDMC) (see further).

The investigational medicinal product (IMP) will be administered IV as single and multiple (weekly) doses.

Trial Design/Methodology:

This is a first-in-human, Phase 1/2, open-label, multi-center trial to evaluate the safety, tolerability, PK, PD and preliminary efficacy of DYN101 after SAD and MAD in subjects ≥ 16 years of age with CNMs caused by mutations in *DNM2* or *MTM1*.

Sufficient subjects will be screened in order to ascertain that approximately 18 subjects will be dosed and finish the trial. Additionally, there will be stand-by subjects for replacement of subjects who discontinue the trial early, if needed. Stand-by subjects will perform run-in visits until the last MAD subject has completed Week 13, unless needed for replacement.

The trial will consist of a pre-screening consent, a screening period, a run-in period (if applicable), a SAD part with 4 weeks of follow-up after 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 will participate in the SAD, MAD, and MAD extension parts, unless they withdraw. End of Treatment (EoT) assessments will be performed after 24 weeks of MAD treatment have been completed, i.e. at the Week 25 visit. Subjects will 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.

Subjects will give a 'pre-screening' consent to be entered as 'pre-screened' in the Interactive Response Technology (IRT) system. The pre-screening consent process can be managed remotely. Subsequently, subjects will sign the main informed consent form (ICF) and screening will be performed to assess eligibility.

Subjects who are participating in the Natural History Study (NHS) and have at least 2 data time points available from the NHS to assess their pre-trial condition do not require a mandatory run-in visit. For these subjects, screening should be performed within 6 weeks before the baseline visit. There should be a minimum of 3 data time points to assess their pre-trial condition. The screening visit can be the third data time point.

Subjects who have not participated in the NHS or who do not have at least 2 data time points from the NHS will have a mandatory run-in period before continuing to the baseline visit. At least 1 run-in visit needs to be performed in order to obtain 3 data time points to assess their pre-trial condition; screening can be the first data time point, baseline can be the third data time point. There should be a minimum of 28 days between screening and the first run-in visit.

At baseline, subjects will be enrolled into the trial (if all in- and exclusion criteria are still met), until recruitment in the last cohort is reached, including replacement subject(s) who were amongst the stand-by subjects.

In the SAD part, subjects will receive a single dose of DYN101 at Day 1. In the MAD part, subjects may receive the same or a different dose as in the SAD part, with weekly administrations of DYN101 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 will remain on treatment with weekly IMP administrations and assessments every 2 weeks until the last MAD3 subject has completed the MAD Week 25 visit.

Subjects will receive 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 receive a different dose than what they received in the SAD part. In each cohort, the target will be up to 6 subjects with a mutation in *DNM2* (sub-cohort a) and up to 3 subjects with a mutation in *MTM1* (sub-cohort b).

Cohorts will be 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 sub-cohort. The first subject (i.e. the sentinel subject) in each cohort will be ≥ 18 years of age. The 48-hour safety data from the first subject in a cohort will be reviewed by the medical monitor before the next subject can 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 will be taken into account.

Pausing/stopping rules will be applied in this trial.

An IDMC will be established to monitor the safety data on a regular basis to ensure the continuing safety of the enrolled subjects. The IDMC may meet at any time based on emerging safety and tolerability data. At a minimum, the IDMC must meet after all subjects of a cohort have been dosed, to advise to continue each *MTM1* and/or *DNM2* sub-cohort to the next dose level or discontinue the *MTM1* and/or *DNM2* sub-cohort of the trial. Escalation of each *MTM1* and/or *DNM2* sub-cohort to the next increased dose level will not take place until the IDMC has reviewed the data of the previous (sub-)cohort (including subjects who have discontinued the trial). The data reviewed by the IDMC for dose escalation decisions will be as clean as possible prior to their review. The IDMC can recommend adjusting dose levels or stopping the dose escalation scheme for each *MTM1* and/or *DNM2* sub-cohort and/or individual subjects based on the totality of available data (safety and tolerability data and/or level of target engagement). At each IDMC review, different decisions for the sub-cohorts (*DNM2* vs *MTM1*) can be made based on the emerging data. If needed, the IDMC review can be split over separate meetings for the sub-cohorts, although the available data from the other sub-cohort will also be taken into account at each meeting.

The IDMC will convene at least 4 times:

- The trial will start with the enrollment of approximately 6 subjects in Cohort SAD1.
- Cohort SAD2 can start after a favorable IDMC review of the 2-week safety and PK data of SAD1 (IDMC1).

- Cohorts SAD3 and MAD1 can start after a favorable IDMC review of the 2-week safety and PK data of SAD2 (IDMC2).
- Cohort MAD2 can start after a favorable IDMC review of the 2-week safety and PK data of SAD3, if available, as well as the 6-week safety and PK data of MAD1 (IDMC3).
- Cohort MAD3 can start after a favorable review of the 12-week safety and PK data of MAD1 and MAD2 as well as the MAD1 and MAD2 12-week PK muscle biopsy data (IDMC4). In this meeting the IDMC will also recommend a starting dose for the pediatric DYN101-C102 study.

Assessments:

Safety and tolerability will be evaluated throughout the trial from signing of the main ICF onwards until the last trial visit (i.e. EoT follow-up visit 3 months after the last IMP administration). Safety evaluations will include monitoring of SAEs/AEs, clinical laboratory evaluations, electrocardiograms (ECGs), vital signs, and physical examinations at specified time points.

All enrolled subjects will undergo blood sampling for PK evaluations at specified time points. In addition, blood and/or urine samples will be collected for analysis of ADA and metabolic profiling.

PD/exploratory efficacy parameters including respiratory function, muscle strength and function, and dysphagia will be assessed, and a muscle biopsy will be taken at specified time points.

For subjects who provided a separate informed consent, blood samples will be collected for further exploratory research on blood biomarkers (including DNA). Participation in this part of the trial is optional and does not impact the subject's eligibility for participation in the trial. Subjects may continue to participate in the trial if they refuse to provide a sample or if they withdraw their samples.

The impact of CNM and its treatment (safety and efficacy) on QoL and health outcomes will be evaluated at specified time points, using questionnaires.

Inclusion and Exclusion Criteria:

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 must be ≥ 18 years of age.

   *for Germany, the subject must be ≥ 18 years of age in accordance with local and national regulations.
2. Have a documented mutation in *DNM2* or *MTM1*.
3. Platelet count >150,000/µL.
4. Have a symptomatic CNM in the opinion of the investigator, at least mild to moderately affected, i.e. showing clinical symptoms in at least 2 of the 4 relevant domains that will be investigated in this trial (respiratory function, muscle strength, muscle function, and dysphagia), and be ambulatory, i.e. being able to walk 10 steps, if needed with support/assisted. If a subject is non-ambulatory but highly functioning in the view of the investigator, he/she may be included following discussion with the sponsor.
5. Have an understanding, ability and willingness to fully comply with visit frequency, trial procedures and restrictions, including contraceptive requirements.
6. Able to provide written, signed and dated informed consent/assent to participate in the trial. Parental consent (one or both parents) and an assent for subjects < 18 years may be required per local legislation.

Exclusion criteria:

1. Clinically significant liver disease.
2. Clinically significant renal disease.
3. Presence of significant co-morbidities 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 be in the best interest of the subject (e.g. compromise the safety or well-being) or that could prevent, limit, or confound the protocol-specified assessments (e.g. taking a muscle biopsy).
4. For female subjects of child-bearing potential: pregnant or breastfeeding or planning to become pregnant during the trial.
5. Current or past abuse of alcohol or recreational/narcotic drugs (with the exception of caffeine and nicotine), which in the investigator's opinion would compromise the subject's safety and/or compliance with the trial procedures.
6. Currently enrolled in any interventional trial or scheduled to participate in such a trial whilst participating in this trial. Subjects are allowed to participate in registry studies.
7. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to fully complete the trial, or any condition that presents undue risk from the IMP or procedures.
8. Intake of any disallowed therapies as noted in the protocol within 12 weeks before the planned first IMP administration.
9. Known or suspected intolerance or hypersensitivity to IMP ingredients or closely-related compounds, or history of a significant allergic reaction to IMP ingredients as determined by the investigator, such as anaphylaxis requiring hospitalization.
10. Legally incapacitated or have limited legal capacity. Lack of mental capacity to fully understand the protocol requirements and complete all trial required procedures.

Note: Retesting of subjects should always be discussed with the sponsor and/or medical monitor. Retesting of laboratory values that lead to exclusion will be allowed once using an unscheduled visit during the screening period to assess eligibility. This visit should be at least 2 weeks later than the original screening visit.

Duration of Subject Involvement in the Trial:

The duration of participation in the trial for an individual subject in any cohort, from screening (excluding the run-in period) to the last trial visit (i.e. EoT follow-up visit), will be at least 60 weeks.

The end of the trial is defined as the last visit of the last subject.

Endpoints and Statistical Analysis:

Trial Endpoints:

- **Primary Endpoint:**
  - Comparison of adverse event (AE, SAE, AESI) frequency and severity by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) 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 Endpoints**

- Comparison of means for PK parameters of DYN101 in plasma (AUC_{τ}, AUC_{last}, AUC_{∞}, $R_{ac(AUC)}$, $R_{ac(C_{max})}$, C_{max}, C_{av} SS, CL, λ_z, $t_{1/2}$, t_{max}, V_z) by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) and overall using the Pharmacokinetic Set.
- Comparison of geometric means for PK parameters of DYN101 in plasma (C_{max} and AUCs) by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) and overall using the Pharmacokinetic Set.
- 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 (sub-cohort) and overall using the Pharmacokinetic 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 (sub-cohort) and overall.

- **Exploratory Endpoints**
  - Difference in mean change from baseline for clinical assessments in various affected clinical domains (respiratory, muscle strength and function, dysphagia) using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) 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 H_2O), %MEP Predicted, MIP (cm H_2O), %MIP Predicted, Oxygen Saturation (%). The MyoGrip Test score will be used for analyzing muscle strength. The following parameters will be analyzed 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.
  - 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 Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit.
  - Comparison of proportion of CGI-I responders (responders defined as responding 1 or 2) using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit.
  - Comparison of proportion of PGI-C responders (responders defined as responding 1 or 2) using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit.
  - Comparison of proportion of Goal Attainment Scaling (GAS) Questionnaire responders (responders defined as responding 'YES - MUCH BETTER', 'YES - A LITTLE BETTER') using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit.
  - Comparison of means of ADA against DYN101 using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit.
  - Comparisons of the metabolic profile of DYN101 in blood and urine using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit.

Hypothesis:

As this trial is designed to provide descriptive information regarding safety and tolerability, no formal statistical hypothesis testing is planned.

Statistical Analysis:

An interim analysis will be performed when all subjects in Cohorts 1 and 2 have completed 12 weeks of MAD treatment or discontinued earlier, or if the sponsor deems a risk/benefit analysis necessary at any time point.

The primary analysis will be performed when all subjects in all cohorts have completed 12 weeks of MAD treatment or discontinued earlier. The final analysis will be performed when all subjects have completed 24 weeks of MAD treatment (12 weeks in the MAD part + 12 weeks in the MAD extension part; Week 25 visit) or discontinued earlier.

In general, descriptive statistics and/or frequency tabulations will be presented overall and by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort). Listings and figures presenting individual data will be generated as appropriate. Advanced analyses and models for the respiratory function and the muscle strength and function tests data will be used to summarize in a more effective way the potential effect of the treatment and are not aimed for inferential purposes since the trial is not powered for that purpose.

TRIAL SCHEDULES

Subjects will be confined to the clinic for at least 7 hours after SAD dosing, , the first dosing during MAD or a change in dosing during MAD, for at least 4 hours during all other clinic visits during MAD, and may be confined to the clinic for any 24-hour urine collection. Additionally, the subject may be confined to the clinic for their convenience if assessments will be performed on consecutive days.

1) PRE-SCREENING, SCREENING, AND RUN-IN PERIOD

PRE-SCREENING, SCREENING, and RUN-IN PERIOD			
Trial Period	**Pre-screening [a)]**	**Screening Period [b),c)]**	**[Run-in Period] [c),d)]**
Time Point	**Any time before screening**	**Maximum 6 weeks [minimum 28 days in case of run-in period]**	**[Minimum 28 days; can be 1 or more visits]**
Screening/Administrative			
Pre-screening consent (confirmation of NHS, age and mutation)	✔		
Informed consent/(assent) [e)]		✔	
Inclusion/exclusion criteria		✔	
Demographics and medical history		✔	
Concomitant medications		✔	✔
Safety Assessments			
Physical examination [f)]		✔	✔
Triplicate 12-lead ECG		✔	
Vital signs (pulse rate, BP, RR, temperature, pulse oximetry)		✔	✔
Liver ultrasound including hepatic elastography		✔	
Platelet count [g)]		✔	(✔) [h)]
Clinical laboratory assessments [i)]		✔	(✔) [h)]

PRE-SCREENING, SCREENING, and RUN-IN PERIOD			
Trial Period	**Pre-screening** [a]	**Screening Period** [b],[c]	**[Run-in Period]** [c],[d]
Time Point	**Any time before screening**	**Maximum 6 weeks [minimum 28 days in case of run-in period]**	**[Minimum 28 days; can be 1 or more visits]**
Complement assessments		✔	(✔) [h]
Coagulation assessments [j]		✔	(✔) [h]
Urinalysis (dipstick)		✔	
Urine pregnancy test [k]		✔	✔
Collect AEs/SAEs		✔	✔
PD/Exploratory Efficacy Assessments			
PROMIS questionnaires		✔	✔
GAS questionnaire		✔	
EAT-10 Dysphagia questionnaire		✔	✔
Respiratory function [l]		✔	✔
MyoGrip [l]		✔	✔
MFM32 [l]		✔	✔
Muscle ultrasound imaging [l] [m]		✔	

AE: adverse event; BP: blood pressure; EAT-10: Eating Assessment Tool; ECG: electrocardiogram; GAS: Goal Attainment Scaling; MFM: Motor Function Measure; NHS: Natural History Study; PD: pharmacodynamics; PROMIS: Patient Reported Outcomes Measurement Information System; RR: respiratory rate; SAE: serious adverse event.

a) Before entering a potentially eligible subject in the Interactive Response Technology (IRT) system, at least a pre-screening consent needs to be fully signed by the subject (and his/her parent(s), as locally required for subjects < 18 years of age). The pre-screening consent process can be managed remotely (please refer to the Site Operations Manual for more details).
b) Subjects who are participating in the NHS and have at least 2 data time points available from NHS to assess their pre-trial condition do not require a mandatory run-in visit. There should be a minimum of 3 data time points to assess their pre-trial condition. The screening visit can be the third data time point. For subjects who are participating in the NHS, the screening visit should be performed within 6 weeks before the baseline visit. Subjects who have not participated in the NHS or who do not have at least 2 data time points from the NHS will have a mandatory run-in period before continuing to the baseline visit.
c) Assessments of a single visit can be split over 2 consecutive days, if required.
d) Subjects who previously participated in the NHS and have at least 2 data time points available from NHS to assess their pre-trial condition do not require a mandatory run-in visit; screening should be performed within 6 weeks before the baseline visit. There should be a minimum of 3 data time points to assess their pre-trial condition. The screening visit can be the third data time point. The run-in period is mandatory for subjects who did not participate in the NHS or who do not

have at least 2 data time points from the NHS. At least 1 run-in visit needs to be performed in order to obtain 3 data time points to assess their pre-trial condition; screening can be the first data time point, baseline can be the third data time point. The run-in period is optional for stand-by subjects who participated in the NHS and have at least 3 data time points. Run-in visits should be repeated until the sponsor deems there are sufficient data time points for an individual subject, or until a cohort is declared open. During the run-in period, if there was an unscheduled visit for additional laboratory assessments, the initial visit date is taken for the calculation to schedule the next visit. There should be a minimum of 28 days between screening and the first run-in visit. Thereafter, run-in visits may be performed every 12 weeks (±14 days), until the cohort is declared open. If there is only 1 run-in visit, there should be a minimum of 28 days between the run-in visit and the baseline visit. If there is more than 1 run-in visit, there should be 12 weeks (±14 days) in between the run-in visits unless it is known that the baseline visit can be scheduled within 14 days following a run-in visit; in that case, the last run-in visit should be dropped. Stand-by subjects will perform run-in visits until the last MAD subject has completed Week 13, unless needed for replacement.

e) Informed consent (and assent, if applicable) must be obtained before any trial procedures are performed. This applies to the main informed consent form (ICF) and optional ICFs as applicable.
f) A complete physical examination (including height, body weight and body systems) will be performed at screening. At all other time points, a symptom-directed physical examination will be performed.
g) Platelet counts will be assessed by the local as well as the central laboratory. If platelet clumping is observed in a local sample, the test should be repeated locally before IMP administration. In addition, a smear may be performed locally at the investigator's discretion.
h) Only to be performed during the run-in period in case the screening laboratory results became available more than 12 weeks before the baseline visit.
i) All parameters that are not described in a separate assessment in this Trial Schedule are to be performed by the central laboratory, as specified in Section 6.2.2.2.
j) Coagulation parameters will be assessed by the local laboratory only.
k) Only applicable for female subjects of child-bearing potential.
l) Assessments should be performed in the order they are listed after all the questionnaires.
m) To determine which muscle should be taken for muscle biopsies in this trial.

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

2) BASELINE AND SAD

	BASELINE and SAD						
Trial Period	**Single Dose Treatment**						
Time Point: Day (D)	**Day 0 or 1 [a)]**	**Day 1**					
Time Point	**Pre-dose/ Baseline**	**Pre-dose**	**0.5h after start of infusion**	**Immediately after end of infusion**	**1h after end of infusion**	**3h after end of infusion**	**7h after end of infusion**
Time Window			**±10 min**	**±10 min**	**±10 min**	**±10 min**	**±1h**
Administrative							
Enrollment	✔						
Concomitant medications	✔						
Safety Assessments							
Physical examination [b)]	✔						
Vital signs (pulse rate, BP, RR, temperature, pulse oximetry)	✔ c)	✔ d)	✔ d) e) f)	✔ d) e) f)	✔ d)	✔ d)	✔ d)
Liver ultrasound including hepatic elastography	(✔) [g)]						
Platelet count [h)]	✔						
Clinical laboratory tests [i) u)]	✔						✔
Cardiac troponin [j)]	✔						
Complement assessments [i) j)]	✔				✔		✔
Coagulation assessments [k)]	✔						
Erythrocyte sedimentation rate [k)]	✔						
Urinalysis (dipstick)	✔						
Urine pregnancy test [l)]	✔						
Collect AEs/SAEs	✔						

	BASELINE and SAD						
Trial Period	**Single Dose Treatment**						
Time Point: Day (D)	**Day 0 or 1** [a)]	**Day 1**					
Time Point	**Pre-dose/ Baseline**	**Pre-dose**	**0.5h after start of infusion**	**Immediately after end of infusion**	**1h after end of infusion**	**3h after end of infusion**	**7h after end of infusion**
Time Window			**±10 min**	**±10 min**	**±10 min**	**±10 min**	**±1h**
PD/Exploratory Efficacy Assessments							
Blood sample for biomarkers (optional)	✔						
DNA sample (optional)	✔						
PROMIS and GAS questionnaires	✔						
EAT-10 Dysphagia questionnaire	✔						
Respiratory function [m)]	✔						
MyoGrip [m)]	✔						
MFM32 [m)]	✔						
Muscle ultrasound imaging [m)]	(✔) [n)]						
Muscle biopsy [m)]	✔ [o)]						
Single Dose Treatment							
Triplicate 12-lead ECG and ECG monitoring [p)]		→	→	Until end of infusion	→		
Start of IMP administration (IV) [q) r)]		✔					
PK Assessments							
Blood sample for PK [s)]	(✔) [t)]	✔	✔	✔	✔	✔	✔

AE: adverse event; BP: blood pressure; EAT-10: Eating Assessment Tool; ECG: electrocardiogram; EoT: End of Treatment; FU: follow-up; GAS: Goal Attainment Scaling; IMP: investigational medicinal product; IPF: immature platelet fraction; IV: intravenous; MFM: Motor Function Measure; PD: pharmacodynamics; PK: pharmacokinetics; PROMIS: Patient Reported Outcomes Measurement Information System; RR: respiratory rate; SAD: single ascending dose; SAE: serious adverse event.

a) If needed, all assessments that need to be performed pre-dose can be done over 2 consecutive days (i.e. Day 0 or Day 1); any exceptions to doing over 2 consecutive days due to an open biopsy with general anesthetic must be discussed with the medical monitor in advance for approval. There must be at least 24 hours between an open biopsy with general anesthesia and IMP administration. The investigator is to get approval from the medical monitor if additional time is required to perform all needed Pre-dose/Baseline assessments.
b) A symptom-directed physical examination (including a neurologic assessment) and measurement of body weight will be performed. A neurologic assessment is also to be performed.
c) If baseline and IMP administration occur on the same day, vital signs should only be performed once, i.e. immediately before dosing.
d) Vital signs are to be obtained prior to any PK samples.
e) Vital signs are to be monitored continuously during infusion such that vital signs will be taken every 5 minutes for the first 15 minutes of the infusion and every 15 minutes until the infusion is completed. If the infusion is stopped for any reason and restarted, vital signs are to be taken according to this schedule until the infusion is completed. All vital signs will be noted as normal, abnormal not clinically significant, or abnormal clinically significant. Any abnormal clinically significant vital signs results are to be recorded as an AE.
f) Pulse oximetry device should remain in place throughout the IMP administration; any clinical findings during the continuous monitoring are to be reported.
g) Only applicable if the screening liver ultrasound and/or hepatic elastography is older than 12 months.
h) Platelet counts will be assessed by the local as well as the central laboratory. Local results should be available and within acceptable ranges to allow the IMP infusion. If platelet clumping is observed in a local sample, the test should be repeated locally before IMP administration. In addition, a smear may be performed locally at the investigator's discretion. If thrombocytopenia is reported after the first IMP administration, and if an IPF test is available at the local laboratory, an IPF should be obtained in addition to the platelet count. The IPF is the ratio of immature platelets to the total number of platelets, indicating the percentage of young, so-called 'reticulated' platelets.
i) All parameters that are not described in a separate assessment in this Trial Schedule are to be performed by the central laboratory, as specified in Section 6.2.2.2.
j) Will be assessed by the central laboratory.
k) Will be assessed by the local laboratory only.
l) Only applicable for female subjects of child-bearing potential.
m) Assessments should be performed in the order they are listed after all the questionnaires.
n) Only applicable if the screening muscle ultrasound imaging is older than 6 months.
o) The muscle biopsy should be obtained after all muscle assessments have been performed. Platelet counts as determined by the local laboratory need to be within normal ranges before performing the muscle biopsy. In case general anesthesia is used for an open biopsy (see Section 6.2.6), there should be at least 24 hours between the muscle biopsy and the IMP administration. Consequently, platelet counts will need to be verified again before IMP administration (local assessment) only if previous platelet count is more than 3 days old.
p) A triplicate 12-lead ECG will be performed on Day 1, within 30 minutes before the start of IMP administration and within 15 minutes after the end of infusion, and ECG monitoring will be performed during the infusion (accompanied by oximetry and vital signs monitoring).
q) Platelet counts as determined by the local laboratory need to be within normal ranges before IMP administration. If a decreased platelet count is detected (value < LLN), a retest should be performed immediately in the local laboratory. If this retest confirms that the platelet count value is below LLN, the subject may NOT be dosed. For details see Section 7.11.2.
r) Slow infusion over 1 hour; subjects will be monitored on site by qualified site personnel for a minimum of 1 hour after the infusion for safety.
s) Blood samples for PK will be taken pre-dose (i.e. before the start of infusion), at 30 minutes after start (during infusion), immediately after the end of infusion, and at 1, 3, and 7 hours after the end of infusion (on Day 1). See also Section 6.2.3.1.

t) The pre-dose PK sample should be taken on Day 1 immediately before the start of IMP infusion.
u) At timepoints when platelets will be counted, a blood sample will be analyzed centrally for biochemistry and hematology. At timepoints without platelet count, only biochemistry samples will be analyzed centrally.

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

Note: In case of early discontinuation, the subject should be encouraged to perform end-of-treatment assessments as detailed for the MAD Week 25 visit (excluding IMP administration). SAD subject who discontinued prior to MAD part do not need to have a second muscle biopsy. If subjects agree, they will also have an EoT FU visit 3 months after their last IMP administration.

3) SAD FOLLOW-UP AND WASHOUT

	SAD FOLLOW-UP and WASHOUT												
Trial Period	**Single Dose Follow-up and Washout Periods** [a),b)]												
Time Point: Week (W)	**SAD W1**		**SAD W2**		**SAD W3**	**SAD W5**	**SAD W7**	**SAD W9**	**SAD W11**	**SAD W13**	**SAD W15**	**SAD W17**	**Phone call** [c)]
Time Point: Day (D)	**D2**	**D3**	**D8**	**D11**	**D15**	**D29**	**D43**	**D57**	**D71**	**D85**	**D99**	**D113**	**Every 2 weeks after W17**
Time Window			**±1d**	**±1d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**
Timing Relative to Baseline	+24h	+48h	+7d	+10d	+2w	+4w	+6w	+8w	+10w	+12w	+14w	+16w	
Administrative													
Concomitant medications	✔	✔	✔		✔	✔	✔	✔	✔	✔	✔	✔	✔
Safety Assessments													
Physical examination [d)]			✔		✔	✔	✔	✔	✔	✔	✔	✔	
Vital signs (pulse rate, BP, RR, temperature, pulse oximetry)	✔	✔	✔		✔	✔	✔	✔	✔	✔	✔	✔	
Platelet count [e)]			✔		✔	✔	✔	✔	✔	✔	✔	✔	
Clinical laboratory tests [f) k)]	✔	✔	✔	✔	✔	✔		✔		✔		✔	
Cardiac troponin [g)]					✔	✔		✔		✔		✔	
Complement assessments [g)]					✔	✔		✔		✔		✔	
Coagulation assessments [h)]			✔		✔	✔	✔	✔	✔	✔	✔	✔	
Erythrocyte sedimentation rate [h)]			✔		✔	✔	✔	✔	✔	✔	✔	✔	
Urinalysis (dipstick)					✔	✔		✔		✔		✔	
Urine pregnancy test [i)]						✔		✔		✔		✔	
Collect AEs/SAEs	✔	✔	✔		✔	✔	✔	✔	✔	✔	✔	✔	✔

	SAD FOLLOW-UP and WASHOUT												
Trial Period	**Single Dose Follow-up and Washout Periods** [a),b)]												
Time Point: Week (W)	**SAD W1**		**SAD W2**		**SAD W3**	**SAD W5**	**SAD W7**	**SAD W9**	**SAD W11**	**SAD W13**	**SAD W15**	**SAD W17**	**Phone call** [c)]
Time Point: Day (D)	**D2**	**D3**	**D8**	**D11**	**D15**	**D29**	**D43**	**D57**	**D71**	**D85**	**D99**	**D113**	**Every 2 weeks after W17**
Time Window			**±1d**	**±1d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**
Timing Relative to Baseline	+24h	+48h	+7d	+10d	+2w	+4w	+6w	+8w	+10w	+12w	+14w	+16w	
PK Assessments													
Blood sample for PK [j)]	✔	✔	✔		✔	✔		✔		✔		✔	
PD/Exploratory Efficacy Assessments													
CGI-I and PGI-C questionnaires					✔								
Respiratory function					✔								

AE: adverse event; BP: blood pressure; CGI-I: Clinical Global Impression of Improvement; EoT: End of Treatment; FU: follow-up; IPF: immature platelet fraction; IV: intravenous; MFM: Motor Function Measure; PD: pharmacodynamics; PGI-C: Patient Global Impression of Change; PK: pharmacokinetics; RR: respiratory rate; SAD: single ascending dose; SAE: serious adverse event.

Due to restrictions imposed in response to a pandemic that restrict subject visits, please contact the medical monitor to determine strategy for continuing the subject in the trial. All efforts must be employed to maintain subject safety. Assessments, if feasible, must be collected during a phone call with the subject. The site should arrange the collection of the blood and urine samples, if at all possible.

a) Assessments of a single visit can be split over 2 consecutive days, if required.
b) Where possible, the visits as of Day 8 will take place on the same day of the week as the baseline visit (Day 1).
c) Subjects will be contacted by phone by qualified site personnel every 2 weeks following Week 17, to follow-up on concomitant medications and AEs, until their assigned MAD cohort has been opened.
d) Symptom-directed physical examination. A neurologic assessment is also to be performed at Week 17.
e) Platelet counts will be assessed by the local as well as the central laboratory. Local results should be available and within acceptable ranges to allow the IMP infusion. If a decreased platelet count is detected (value < LLN), a retest should be performed immediately in the local laboratory. If this retest confirms that the platelet count value is below LLN, the subject may NOT be dosed. For details see Section 7.11.2. If platelet clumping is observed in a local sample, the test should be repeated locally before IMP administration. In addition, a smear may be performed locally at the investigator's discretion. If thrombocytopenia is reported, and if an IPF test is available at the local laboratory, IPF test results should be obtained in addition to the platelet count. The IPF is the ratio of immature platelets to the total number of platelets, indicating the percentage of young, so-called 'reticulated' platelets.
f) All parameters that are not described in a separate assessment in this Trial Schedule are to be performed by the central laboratory, as specified in Section 6.2.2.2.
g) Will be assessed by the central laboratory.

h) Will be assessed by the local laboratory only.
i) Only applicable for female subjects of child-bearing potential.
j) Blood samples for PK will be collected at 23 (Day 2), 47 (Day 3), and 167 hours (Day 8) after the end of infusion, and at 2 (Day 15) and 4 weeks (Day 29) post-dose, and monthly during the washout period. See also Section 6.2.3.1.
k) At timepoints when platelets will be counted, a blood sample will be analyzed centrally for biochemistry and hematology. At timepoints without platelet count, only biochemistry samples will be analyzed centrally.

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

Note: In case of early discontinuation, the subject should be encouraged to perform end-of-treatment assessments as detailed for the MAD Week 25 visit (excluding IMP administration). SAD subjects who discontinued prior to MAD part do not need to have a second muscle biopsy. If subjects agree, they will also have an EoT FU visit 3 months after their last IMP administration.

4) MAD PART

MAD PART															
Trial Period	**Multiple Dose Treatment Period [a) b)]**														
Time Point: Day (D)/Week (W)	**MAD W1**			**MAD W2**		**MAD W3**	**MAD W4**	**MAD W5**	**MAD W6**	**MAD W7**	**MAD W8**	**MAD W9**	**MAD W10**	**MAD W11**	**MAD W12**
	D1 [c)]	**D2**	**D4**	**D8**	**D11**										
Time Window	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**
Administrative															
Concomitant medications	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
IMP administration (IV) [d)]	✔			✔		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Safety Assessments															
Physical examination [e)]	✔									✔					
Triplicate 12-lead ECG	✔									✔					
Vital signs (pulse rate, BP, RR, temperature, pulse oximetry) [g)]	✔		✔	✔		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Liver ultrasound including hepatic elastography	(✔)[g) h)]														
Platelet count [f) g) i)]	✔		✔ k)	✔		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Clinical laboratory assessments [f) g) j)]	✔ l)	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Cardiac troponin [f) g) k)]	✔					✔		✔				✔			
Complement assessments [f) g) k)]	✔					✔		✔				✔			
Coagulation assessments [f) g) m)]	✔		✔	✔		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Erythrocyte sedimentation rate [f) g) m)]	✔		✔	✔		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Urinalysis (dipstick) [g)]	✔					✔		✔				✔			
Urine pregnancy test [g) n)]	✔							✔				✔			
Collect AEs/SAEs	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔

MAD PART															
Trial Period	**Multiple Dose Treatment Period [a) b)]**														
Time Point: Day (D)/Week (W)	**MAD W1**			**MAD W2**		**MAD W3**	**MAD W4**	**MAD W5**	**MAD W6**	**MAD W7**	**MAD W8**	**MAD W9**	**MAD W10**	**MAD W11**	**MAD W12**
	D1 [c)]	**D2**	**D4**	**D8**	**D11**										
Time Window	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**
PK Assessments															
Blood sample for PK	✔ o)	✔		✔											✔ o)
Blood sample for anti-drug antibodies	✔									✔					
Blood sample for metabolic profiling															✔ p)
24-hour urine collection for metabolic profiling															✔ q)
PD/Exploratory Efficacy Assessments															
Blood sample for biomarkers (optional) [g)]	✔									✔					
PROMIS and GAS questionnaires [g)]	✔									✔					
EAT-10 Dysphagia questionnaire [g)]	✔														
Respiratory function [g) r)]	✔			✔						✔					
MyoGrip [g) r)]	✔									✔					
MFM32 [g) r)]	✔									✔					

AE: adverse event; BP: blood pressure; EAT-10: Eating Assessment Tool; ECG: electrocardiogram; EoT: End of Treatment; FU: follow-up; GAS: Goal Attainment Scaling; IMP: investigational medicinal product; IPF: immature platelet fraction; IV: intravenous; MAD: multiple ascending dose; MFM: Motor Function Measure; PD: pharmacodynamics; PK: pharmacokinetics; PROMIS: Patient Reported Outcomes Measurement Information System; RR: respiratory rate; SAE: serious adverse event.

a) Where possible, the visits in the MAD part will take place on the same day of the week as Day 1 (and of the SAD part, if applicable). A window of ±3 days is allowed for visits as of Week 3, with a minimum of 4 days between 2 subsequent infusions. Assessments of a single visit can be split over 2 consecutive days, if required.

b) Replacement subjects starting in the MAD part should sign an ICF for participation in the MAD part, and their eligibility will be assessed (see Trial Schedule for the Screening Period) before the first IMP administration in the MAD part (MAD Week 1 Day 1). If needed (to be discussed with the sponsor), these subjects will have a run-in period before they can start in the MAD part (see Trial Schedule for Run-in Period). A DNA sample (optional) and a muscle biopsy should be taken before the first IMP administration. If needed, replacement subjects in MAD1 and MAD2 may have the second muscle biopsy taken after 6 to 12 weeks of MAD treatment, after discussion with the sponsor.

c) All assessments need to be done pre-dose.

d) Slow infusion over 1 hour; subjects will be monitored on site by qualified site personnel for a minimum of 1 hour after the infusion for safety. Platelet counts as determined by the local laboratory need to be within normal ranges before IMP administration.

e) A symptom-directed physical examination (and measurement of body weight at MAD Week 1 Day 1) will be performed.
f) During an unscheduled visit which will occur between 6 weeks and 7 days prior to the baseline visit for subjects for whom the last visit in the SAD part will be more than 3 months before the baseline visit in the MAD part.
g) To be performed pre-dose.
h) Only applicable if a liver ultrasound and/or hepatic elastography was not performed during the screening period.
i) Platelet counts will be assessed by the local as well as the central laboratory. Platelet counts as determined by the local laboratory need to be within normal ranges before IMP administration. If a decreased platelet count is detected (value < LLN), a retest should be performed immediately in the local laboratory. If this retest confirms that the platelet count value is below LLN, the patient may NOT be dosed. For details see Section 7.11.2. If platelet clumping is observed in a local sample, the test should be repeated locally before IMP administration. In addition, smear may be performed locally at the investigator's discretion. If thrombocytopenia is reported, and if an IPF test is available at the local laboratory, IPF test results should be obtained in addition to the platelet count. The IPF is the ratio of immature platelets to the total number of platelets, indicating the percentage of young, so-called 'reticulated' platelets.
j) All parameters that are not described in a separate assessment in this Trial Schedule are to be performed by the central laboratory, as specified in Section 6.2.2.2.
k) Will be assessed by the central laboratory.
l) Blood samples for clinical laboratory assessments will be collected at 7, 23 (Day 2), 71 (Day 4), and 167 (Day 8) hours after end of infusion on Day 1.
m) Will be assessed by the local laboratory only.
n) Only applicable for female subjects of child-bearing potential.
o) Blood samples for PK will be collected at MAD Week 1 pre-dose, at 30 minutes after start (during infusion), immediately after the end of infusion, and at 1, 3, 7, 23 (Day 2), 71 (Day 4), and 167 (Day 8) hours post-dose (i.e. before infusion in Week 2) of MAD Week 1 Day 1 infusion, and at MAD Week 12 pre-dose, at 30 minutes after start (during infusion), immediately after the end of infusion, and at 1, 3, 7, 23 (Day 2), 71 (Day 4), and 167 (Day 8) hours after end of infusion (i.e. before infusion in Week 13) of MAD Week 12 Day 1 infusion.
p) A sample will be taken at 1 and 167 hours after the end of infusion (i.e. before infusion in Week 13) in MAD Week 12 Day 1 infusion.
q) Urine for PK (metabolic profiling) will be collected over a 24-hour interval. The window of collection will be based on the start of the infusion.
r) Assessments should be performed in the order they are listed after all the questionnaires.

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

Note: In case of early discontinuation, the subject should be encouraged to perform end-of-treatment assessments as detailed for the MAD Week 25 visit (excluding IMP administration). In addition, subjects will be encouraged to have the second muscle biopsy taken if the subject had at least 6 weeks of treatment during the MAD part of the trial and discontinued prior to having the Week 13 biopsy obtained. If subjects agree, they will also have an EoT FU visit 3 months after their last IMP administration.

5) MAD EXTENSION PART

MAD EXTENSION PART														
Trial Period	**Multiple Dose Treatment Period [a)]**												**EoT/ED**	**EoT FU**
Time Point: Week (W)	**MAD W13**	**MAD W14**	**MAD W15**	**MAD W16**	**MAD W17**	**MAD W18**	**MAD W19**	**MAD W20**	**MAD W21**	**MAD W22**	**MAD W23**	**MAD W24**	**MAD W25**	**MAD Last dose + 3 months [b)]**
Time Window	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**
Administrative														
Concomitant medications	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
IMP administration (IV) [c)]	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔ [d)]	
Safety Assessments														
Physical examination [e)]	✔						✔						✔ [f)]	✔
Triplicate 12-lead ECG	✔						✔						✔	
Vital signs (pulse rate, BP, RR, temperature, pulse oximetry) [f)]	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Platelet count [f) g)]	✔		✔		✔		✔		✔		✔		✔	✔
Clinical laboratory tests [f) h)]	✔		✔		✔		✔		✔		✔		✔	✔
Cardiac troponin [f) i)]	✔				✔				✔				✔	✔
Complement assessments [f) i)]	✔				✔				✔				✔	✔
Coagulation assessments [f) j)]	✔		✔		✔		✔		✔		✔		✔	✔
Erythrocyte sedimentation rate [f) j)]	✔		✔		✔		✔		✔		✔		✔	✔
Urinalysis (dipstick) [f)]	✔				✔				✔				✔	
Urine pregnancy test [f) k)]	✔				✔				✔				✔	
Collect AEs/SAEs	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
PK Assessments														
Blood sample for PK	✔ [l)]												✔ [l)]	
Blood sample for anti-drug antibodies [f)]	✔						✔						✔	

MAD EXTENSION PART														
Trial Period	**Multiple Dose Treatment Period [a)]**												**EoT/ED**	**EoT FU**
Time Point: Week (W)	**MAD W13**	**MAD W14**	**MAD W15**	**MAD W16**	**MAD W17**	**MAD W18**	**MAD W19**	**MAD W20**	**MAD W21**	**MAD W22**	**MAD W23**	**MAD W24**	**MAD W25**	**MAD Last dose + 3 months [b)]**
Time Window	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**	**±3d**
Blood sample for metabolic profiling	✔ [l)]													
PD/Exploratory Efficacy Assessments														
Blood sample for biomarkers (optional) [f)]	✔						✔						✔	
PROMIS and GAS questionnaires [f)]	✔						✔						✔	
CGI-I and PGI-C questionnaires [f)]	✔												✔	
EAT-10 Dysphagia questionnaire [f)]	✔												✔	
Respiratory function [f) m)]	✔						✔						✔	
MyoGrip [f) m)]	✔						✔						✔	
MFM32 [f) m)]	✔						✔						✔	
Muscle biopsy [m)]	✔ [n)]												(✔)[o)]	

AE: adverse event; BP: blood pressure; CGI-I: Clinical Global Impression of Improvement; EAT-10: Eating Assessment Tool; ECG: electrocardiogram; ED: early discontinuation; EoT: End of Treatment; FU: follow-up; GAS: Goal Attainment Scaling; IMP: investigational medicinal product; IPF; immature platelet fraction; IV: intravenous; MAD: multiple ascending dose; MFM: Motor Function Measure; PD: pharmacodynamics; PGI-C: Patient Global Impression of Change; PK: pharmacokinetics; PROMIS: Patient Reported Outcomes Measurement Information System; RR: respiratory rate; SAE: serious adverse event.

a) Where possible, the visits in the MAD extension part will take place on the same day of the week as MAD Week 1 Day 1. A window of ±3 days is allowed, with a minimum of 4 days between 2 subsequent infusions. Assessments of a single visit can be split over 2 consecutive days, if required.
b) If a subject is NOT on treatment after the trial, the subject will return to the clinic 3 months after the last IMP administration, to follow-up on the subject's status including abnormal laboratory test results, AEs, and concomitant medications.
c) Slow infusion over 1 hour; subjects will be monitored on site by qualified site personnel for a minimum of 1 hour after the infusion for safety.
d) The subject may continue to receive IMP weekly with clinic visits every 2 weeks with assessments as defined in the Trial Schedule for Follow-up post MAD Week 25.
e) A symptom-directed physical examination (and measurement of body weight at MAD Week 13) will be performed. A neurologic assessment is also to be performed at the EoT/ED and EoT FU visit.
f) To be performed pre-dose.

g) Platelet counts will be assessed by the local as well as the central laboratory. Platelet counts as determined by the local laboratory need to be within normal ranges before IMP administration. If a decreased platelet count is detected (value < LLN), a retest should be performed immediately in the local laboratory. If this retest confirms that the platelet count value is below LLN, the subject may NOT be dosed. For details see Section 7.11.2. If platelet clumping is observed in a local sample, the test should be repeated locally before IMP administration. In addition, smear may be performed locally at the investigator's discretion. If thrombocytopenia is reported, and if an IPF test is available at the local laboratory, IPF test results should be obtained in addition to the platelet count. The IPF is the ratio of immature platelets to the total number of platelets, indicating the percentage of young, so-called 'reticulated' platelets.
h) All parameters that are not described in a separate assessment in this Trial Schedule are to be performed by the central laboratory, as specified in Section 6.2.2.2.
i) Will be assessed by the central laboratory.
j) Will be assessed by the local laboratory, only.
k) Only applicable for female subjects of child-bearing potential.
l) Blood samples for PK and metabolic profiling at MAD Week 13 must be collected 167 hours after the end of infusion from MAD Week 12 Day 1 visit and before the infusion of MAD Week 13 visit. Blood sample for PK at MAD Week 25 must be collected 167 hours after the end of infusion from MAD Week 24 Day 1 visit and before the infusion of MAD Week 25 visit, where applicable.
m) Assessments should be performed in the order they are listed after all the questionnaires.
n) The muscle biopsy should be obtained after all muscle assessments have been performed. Platelet counts as determined by the local laboratory need to be within normal ranges before performing the muscle biopsy. In case general anesthesia is used for an open biopsy (see Section 6.2.6), there should be at least 24 hours between the muscle biopsy and the IMP administration. Consequently, platelet counts will need to be verified again before IMP administration (local assessment) only if previous platelet count is more than 3 days old.
o) See note about early discontinuation assessments below.

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

Note: In case of early discontinuation, the subject should be encouraged to perform end-of-treatment assessments as detailed for the MAD Week 25 visit (excluding IMP administration). In addition, subjects will be encouraged to have the second muscle biopsy taken if the subject had at least 6 weeks of treatment during the MAD part of the trial and discontinued prior to having the Week 13 biopsy obtained. If subjects agree, they will also have an EoT FU visit 3 months after their last IMP administration.

6) FOLLOW-UP POST MAD WEEK 25

All subjects will remain on treatment with weekly IMP administrations and routine safety assessments performed as noted in the table below until the last MAD3 subject has completed the MAD Week 25 visit. All assessments are to be performed prior to IMP administration.

12-week Follow-up Cycle Repeated After Week 25						
Time Point	**Weeks 1, 3, 5, 7, 9, and 11**	**Weeks 2, 6, and 10**	**Weeks 4 and 8**	**Week 12**	**EoT**	**EoT FU Visit**
Time Window [a)]	**±3d**	**±3d**	**±3d**	**±3d**	**±7d**	**3 months after last IMP administration**
Administrative						
Concomitant medications	✔	✔	✔	✔	✔	✔
IMP administration (IV) [b)]	✔	✔	✔	✔		
Safety Assessments						
Physical examination [c)]				✔	✔	✔
Triplicate 12-lead ECG				✔	✔	
Vital signs (pulse rate, BP, RR, temperature, pulse oximetry) [d)]	✔	✔	✔	✔	✔	✔
Platelet count [d) e)]		✔	✔	✔	✔	✔
Clinical laboratory tests [d) f)]		✔	✔	✔	✔	✔
Cardiac troponin [d) g)]			✔	✔	✔	✔
Complement assessments [d) g)]			✔	✔	✔	✔
Coagulation assessments [d) h)]		✔	✔	✔	✔	✔
Erythrocyte sedimentation rate [d) h)]		✔	✔	✔	✔	✔
Urinalysis (dipstick) [d)]		✔	✔	✔	✔	
Urine pregnancy test [d) i)]		✔	✔	✔	✔	
Collect AEs/SAEs	✔	✔	✔	✔	✔	✔
PK Assessments						
Blood sample for anti-drug antibodies [d)]				✔	✔	
PD/Exploratory Efficacy Assessments						
Blood sample for biomarkers (optional) [d)]				✔	✔	

12-week Follow-up Cycle Repeated After Week 25						
Time Point	**Weeks 1, 3, 5, 7, 9, and 11**	**Weeks 2, 6, and 10**	**Weeks 4 and 8**	**Week 12**	**EoT**	**EoT FU Visit**
Time Window [a)]	**±3d**	**±3d**	**±3d**	**±3d**	**±7d**	**3 months after last IMP administration**
PROMIS and GAS questionnaires [d)]				✔	✔	
CGI-I and PGI-C questionnaires [d)]					✔	
EAT-10 Dysphagia questionnaire [d)]				✔	✔	
Respiratory function [d) j)]				✔	✔	
MyoGrip [d) j)]				✔	✔	
MFM32 [d) j)]				✔	✔	
Muscle biopsy [d) j)]						

AE: adverse event; BP: blood pressure; CGI-I: Clinical Global Impression of Improvement; EAT-10: Eating Assessment Tool; ECG: electrocardiogram; EoT: End of Treatment; FU: follow-up; GAS: Goal Attainment Scaling; IMP: investigational medicinal product; IPF: immature platelet fraction; IV: intravenous; MAD: multiple ascending dose; MFM: Motor Function Measure; PD: pharmacodynamics; PGI-C: Patient Global Impression of Change; PK: pharmacokinetics; PROMIS: Patient Reported Outcomes Measurement Information System; RR: respiratory rate; SAE: serious adverse event.

a) Where possible, the visits in the MAD extension part will take place on the same day of the week as MAD Week 1 Day 1. A window of ±3 days is allowed, with a minimum of 4 days between 2 subsequent infusions. Assessments of a single visit can be split over 2 consecutive days, if required.
b) Slow infusion over 1 hour; subjects will be monitored on site by qualified site personnel for a minimum of 1 hour after the infusion for safety.
c) A symptom-directed physical examination and measurement of body weight. A neurologic assessment is also to be performed at EoT and EoT FU.
d) To be performed pre-dose.
e) Platelet counts will be assessed by the local laboratory only at weeks 2, 6, and 10 and both at the local and central laboratory at weeks 4, 8, and 12. Platelet counts as determined by the local laboratory need to be within normal ranges before IMP administration. If a decreased platelet count is detected (value < LLN), a retest should be performed immediately in the local laboratory. If this retest confirms that the platelet count value is below LLN, the subject may NOT be dosed. For details see Section 7.11.2. If platelet clumping is observed in a local sample, the test should be repeated locally before IMP administration. In addition, a smear may be performed locally at the investigator's discretion. If thrombocytopenia is reported, and if an IPF test is available at the local laboratory, IPF test results should be obtained in addition to the platelet count. The IPF is the ratio of immature platelets to the total number of platelets, indicating the percentage of young, so-called 'reticulated' platelets.
f) All parameters that are not described in a separate assessment in this Trial Schedule, as specified in Section 6.2.2.2, will be evaluated by the central laboratory at weeks 4, 8, and 12. Local results should be available and within acceptable ranges to allow the IMP infusion. If the local test result is abnormal, the test is to be repeated at the local laboratory immediately. If the repeat test is still abnormal, the test is to be repeated at the central laboratory at the next visit.
g) Will be assessed by the central laboratory.
h) Will be assessed by the local laboratory only.
i) Only applicable for female subjects of child-bearing potential.
j) Assessments should be performed in the order they are listed after all the questionnaires.

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

1. INTRODUCTION

Centronuclear myopathies (CNMs) are a group of severe, debilitating, non-dystrophic, congenital myopathies, for which no effective therapy is currently available. CNMs are characterized by muscle weakness, fiber atrophy, predominance of type I fibers, and increased centralization of nuclei not secondary to muscle regeneration (Jungbluth et al., 2008; Romero and Bitoun, 2011). Three main forms of CNM have been characterized:

- X-linked CNM (XLCNM), caused by mutations in the myotubularin 1 gene (*MTM1*) (Laporte et al., 1996);
- Autosomal dominant CNM (ADCNM), caused by mutations in the dynamin 2 gene (*DNM2*) (Bitoun et al., 2005);
- Autosomal recessive CNM (ARCNM), caused by mutations in the amphiphysin II gene (*BIN1*) (Nicot et al., 2007).

The 3 genes with mutations causing CNM (*MTM1*, *DNM2* and *BIN1*) are involved in membrane remodeling and membrane trafficking, and impairment of these processes could be a common CNM pathomechanism.

Diagnosis of CNM is the same for all phenotypes: the presence of centrally placed nuclei on muscle biopsy in addition to clinical features and confirmative genotyping.

Currently, there is no cure or disease-modifying therapy for CNM. Treatment of CNM is supportive.

In this trial, the focus is on XLCNM and ADCNM as there are currently not enough *BIN1* model data to support the inclusion of subjects with ARCNM.

1.1 Condition Background and Current Treatment

1.1.1 X-linked CNM

XLCNM, also referred to as myotubular myopathy, is the best characterized form of CNM. Typically associated with congenital onset, profound muscle weakness, respiratory failure, and high mortality rates, it is considered to be the most severe form. The large majority of patients die within the first months of life, but a relatively small proportion of boys may be less affected in the neonatal period and survive into childhood and even adulthood (McEntagart et al., 2002). Many patients surviving through infancy usually remain severely impaired and require intensive physiotherapy and adapted rehabilitation (permanent ventilation, brace with head support, feeding tube) (Romero and Bitoun, 2011). Female carriers can also be affected to a variable degree (Jungbluth et al., 2003; Kristiansen et al., 2003).

XLCNM is caused by mutations in the *MTM1* gene, located on human chromosome Xq28. Myotubularin belongs to the large family of dual-specificity phosphatases, playing a role in the epigenetic regulation of signaling pathways involved in muscle growth and differentiation (Laporte et al., 1998).

Currently, an incidence of 1 in 50,000 births has been reported for severe male XLCNM (Bertini et al., 2004; Laporte et al., 2001; Jungbluth et al., 2013), and pediatric point prevalence of CNM has been estimated to be < 1 in 100,000 (Biancalana et al., 2012; Fattori et al., 2015), both likely underestimate the current pool (Jungbluth et al., 2013; Verhaart et al., 2017a; Verhaart et al.,

2017b). An integrated model utilizing available literature provides a prevalent patient pool of 776 severe, 334 moderate and 1,542 mild XLCNM cases worldwide (Vandersmissen et al., 2018).

Data from 4 natural history studies (NHSs) evaluating XLCNM are currently available. (Annoussamy et al., 2016; Annoussamy et al., 2017; McEntagart et al., 2002; Amburgey et al., 2017; Beggs et al., 2018). The data illustrate the substantial clinical and economic burden on XLCNM patients and their families. Increasing cumulative lifetime need for ventilation and tracheostomy with age place a substantial quality-of-life (QoL) burden on patients and their families, likely including cost, anxiety about the machines failing, difficulty communicating, and limitations on mobility and social activities.

The overall median survival in XLCNM patients was 29 months (McEntagart et al., 2002).

1.1.2 Autosomal Dominant CNM

The clinical phenotypes of ADCNM range from mild and moderate forms in adult patients to severe forms in infants. In general, adolescent and young adult patients with moderate phenotype have moderate skeletal muscle weakness associated with ptosis and ophthalmoplegia and had delayed motor milestones as children. Pediatric patients usually have generalized weakness, hypotonia, moderate degree of facial weakness with open mouth, ptosis, and ophthalmoplegia (Romero and Bitoun, 2011). Many children with the disease also have stiff joints and sideways curvature of the spine (scoliosis). In childhood, as in the neonatal period, there is a risk of respiratory failure which can become life-threatening in serious situations (e.g. if the patient develops pneumonia). Adults may experience respiratory failure, which in turn may cause cardiac insufficiency. A significant portion of patients become wheelchair-dependent in their fifties (Bitoun et al., 2005; Verhaart et al., 2017a; Verhaart et al., 2017b; Voermans, 2017).

ADCNM is caused by mutations in the *DNM2* gene on chromosome 19p13.2 (Bitoun et al., 2005). Dynamins are structurally complex proteins composed of 5 different domains. CNM-causing mutations identified to date mainly localize to the middle domain involved in protein self-assembly and centrosome localization.

The incidence of ADCNM is currently unknown, although it has been reported to account for approximately 50% of CNM cases (Romero, 2010). The patient pool is estimated to have 553 prevalent cases worldwide (Vandersmissen et al., 2018). No NHS data of ADCNM have been published to date.

The prognosis of ADCNM is currently unknown. However, it appears to be quite heterogeneous, with some patients exhibiting a rapid deterioration and others a slow progressive loss of muscle function.

1.2 RNA-targeted Therapeutic Concept

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 has 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 RNA that favors RNase H1-dependent degradation of the targeted transcript (Wheeler et al., 2012).

As *MTM1* and *DNM2* are involved in membrane remodeling and membrane trafficking, impairment of these processes could be a common CNM pathomechanism. The physiological interplay is complex and not completely understood. *MTM1* and *DNM2* function in a common pathway, where either loss-of-function mutations in *MTM1* or gain-of-function mutations in *DNM2* lead to the CNM phenotype. *MTM1* is a regulator of *DNM2* in skeletal muscle in vivo (Cowling et al., 2014).

Nonclinical data in mouse models of XLCNM and ADCNM have demonstrated a rescue of the CNM phenotype by reducing *Dnm2* mRNA (Cowling et al., 2014;Tasfaout et al., 2017; Buono et al., 2018).

Based on these nonclinical results, the sponsor has decided to develop DYN101, an ASO targeting reduction of human *DNM2* mRNA, for subjects with mutations in *MTM1* (XLCNM) and mutations in *DNM2* (ADCNM).

1.3 Investigational Medicinal Product Background

[redacted]

Mutations in the *DNM2* gene or genes upstream of *DNM2* in the molecular pathway result in higher activity of DNM2 protein (either by activation or higher accumulation of the protein), which in turn plays a critical role in manifestation of CNM of different types in pediatric and adult patients (Cowling et al., 2014; Cowling et al., 2017; Buono et al., 2018).

A summary of the experiments with DYN101 is provided below. For detailed information on DYN101, refer to the latest version of the Investigator's Brochure (IB) (Dynacure).

Compatibility of DYN101 200 mg/mL eq. sterile solution with the selected infusion solution and the selected infusion tubing for clinical study DYN101-C101, was evaluated in a compatibility study. The compatibility study mimicked the worst-case interaction of DYN101 200 mg/mL eq. sterile solution with tubing, infusion solution and infusion bag.

The compatibility study evaluated both the physico-chemical and chemical stability of DYN101 in the infusion solution after adding, after 6 hours and after 24 hours. Two infusion bags were prepared (3.5 mL DYN101 added to 250 mL sodium chloride 0,9%) and 20 mL samples were taken through the tubing at T0. The prepared infusion bags were stored at room temperature and at T6h (bag 1) and T24h (bag 2), a second 20 mL sample per bag is taken though the tubing. Test parameters were appearance, pH, viscosity, assay DYN101, chromatographic purity and osmolality, determined at time points 0, 6, and 24 hours.

No physico-chemical or chemical degradation was observed in any of the samples tested in the compatibility/stability study. Based on the test results obtained in the study, it can be concluded that the proposed study set-up, using 1-mL or 3-mL syringes with luer lock, together with 0.9% sodium chloride infusion bags and the proposed infusion tubing is compatible with the DYN101 solution.

1.3.1 Nonclinical Information

Potency and efficacy of DYN101 were tested in vitro in human cell lines and in vivo in transgenic mice expressing human *DNM2*. Tolerability and safety were assessed in rodents (mice, rats) and in non-human primates (NHP). Single nucleotide polymorphism and off-target analysis was done in silico and in vitro.

Proof-of-concept studies in mouse CNM disease models have demonstrated the efficacy of *DNM2* mRNA targeting, DNM2 protein level reduction and disease improvement/stabilization. Reduction of *DNM2* mRNA was already seen 1 week after dosing. In addition, pharmacology/tolerability and toxicity studies in mice, rats, and Cynomolgus monkeys have demonstrated the safe dosing of DYN101 up to/higher than the targeted human dose levels.

In general, ASOs show a fast distribution (minutes to hours) into tissue, a rapid transmission into cells and long half-lives (2-4 weeks). In addition, pharmacokinetic (PK) properties of ASOs are similar across species (Yu et al., 2013) especially NHP PK data can be used to predict human PK behavior, including steady state in humans. Based on toxicokinetic results of a 13-week NHP study, DYN101 exposures increased in a dose-dependent manner with only moderate accumulation. As typical for ASOs, the subcutaneous (SC) administration of DYN101 exhibited a fast absorption (t_{max}=2 hours) with lower C_{max} in comparison to the intravenous (IV) group and 100% bioavailability based on AUC. After reaching C_{max}, DYN101 concentration decreased in a biphasic manner with a fast-distributional half-life ($t_{1/2}$) and a slower terminal elimination $t_{1/2}$ of 21 days (see Section 3.2.1 of the IB). A population PK modeling and simulation approach showed 90% of steady state concentration will be reached with the planned dosing regimen (weekly administrations in MAD part), after 6 weeks, justifying a biopsy sample taken for early drop outs (see Section 3.1.2).

1.3.2 Clinical Information

No clinical studies have been conducted with DYN101 so far.

1.4 Rationale for the Trial

There are no available treatments aside from supportive care for patients with CNM. This first-in-human trial with DYN101 aims to assess the safety, tolerability, PK and pharmacodynamics (PD)/preliminary efficacy of DYN101 in subjects ≥ 16 years of age with CNM caused by mutations in *DNM2* or *MTM1*. Targeted lowering of *DNM2* is a novel therapeutic approach and is expected to benefit these patients. DNM2 protein is ubiquitously present in human cells and elevations in DNM2 protein levels or *DNM2* function have been demonstrated in muscle tissue in preclinical models and in human tissue from CNM patients. Preclinical models of these mutations have shown the potential of reducing *DNM2* mRNA to prevent and reverse the clinical status. [redacted] A translation of similar changes in efficacy parameters in these knock-out mouse models to patients has been demonstrated by *MTM1* gene therapy in an ongoing clinical trial conducted by Audentes Therapeutics in patients with XLCNM up to 4 years of age (ASPIRO trial, ClinicalTrials.Gov Identifier: NCT03199469). Moreover, an increased uptake of ASO was seen in muscle of diseased mice over their wild-type counterparts. This first-in-human trial for DYN101 will therefore be conducted in patients and not in healthy volunteers to assess safety, PK and dose selection based on PD/preliminary efficacy.

1.5 Risk/Benefit Considerations

As this trial is investigational, there is no defined, expected benefit for subjects who participate in this trial except a better knowledge of their pathology, and the knowledge that they contribute to RNA-targeted therapy for CNM patients carrying *MTM1* and *DNM2* mutations. Should a clear benefit be shown, the sponsor will consider prolonging therapy for those who participated in the trial.

Due to the encouraging results in animal models of the disease, the translation of changes in efficacy parameters from animals to humans seen in the ongoing gene therapy trial conducted by Audentes Therapeutics (see Section 1.4), and a minimum treatment duration of 24 weeks in this trial, it is possible that subjects may exhibit some clinical benefits during the multiple ascending dose (MAD) or MAD extension.

The risks as described in the following paragraphs are considered to be potential risks to trial subjects, based on what is known about ASOs and the mechanism of action of DYN101. The choice of the eligibility criteria and assessments for this trial in relation to risk mitigation is explained in Section 3.2.

Although this will be the first trial with an ASO specifically directed at lowering *DNM2* mRNA, it can be expected that similar ASO-platform toxicities could occur (adverse events [AEs] of special interest, see Section 7.2):

- A decrease in platelets to low levels resulting in bleeding has been observed with other ASOs. Subjects must have normal platelet levels upon entry into the trial, and will be monitored throughout the trial (see also Sections 4.1 and 7.11). In addition to central laboratory testing, platelet counts will be performed locally before investigational medicinal product (IMP) administration at the time points specified in the TRIAL SCHEDULES and before performing a muscle biopsy.

- ASOs are generally directed to the liver, and transient elevation of liver enzymes may be observed as a result. Subjects will be excluded from this trial if they have clinically significant evidence of liver disease. In case any liver abnormalities are observed at baseline, there should be a medically plausible reason for these abnormalities, and a discussion with the medical monitor should occur in this situation before the subject may be dosed for the trial). Liver enzymes will be closely monitored throughout the trial (see also Sections 4.2 and 7.11).

- ASOs are metabolized by cellular endonucleases and the metabolites are then excreted by the kidneys. Estimated glomerular filtration rate (eGFR) must be within normal range upon inclusion into the trial and will be monitored throughout the trial (see also Sections 4.1 and 7.11). Any abnormal results of these tests should have a medically plausible reason and should be discussed with the medical monitor before the subject may be screened and/or dosed for the trial.

Infusion reactions after ASO administration have been known to occur, and are possibly related to complement activation, resulting in local inflammation at the injection site. The DYN101 infusion will be given at a slow rate over 1 hour to minimize the potential of an infusion reaction. Additionally, complement factors will be assessed after each SAD infusion and repetitively during the MAD and extension phase (refer to TRIAL SCHEDULES).

Should there be significant infusion reaction symptoms, the infusion should be paused for 30 minutes and then restarted at half the rate. If this is still followed by symptoms, the subject should be treated per local standard practice (e.g. a steroid bolus), or the infusion should be stopped altogether.

In addition, several safety measures have been proposed to minimize risk to subjects, including:

- Utilization of selection criteria which exclude subjects who may potentially be at higher risk of an AE (refer to Section 4).
- Utilization of withdrawal criteria (refer to Section 4.5). Subjects withdrawn from IMP or from the trial (except when due to withdrawal of consent), will be encouraged to complete the assessments of the early discontinuation visit (refer to Section 6.1.5).
- AEs assessed as "related" to IMP and all serious adverse events (SAEs) and AEs of special interest (regardless of their relationship to IMP) still ongoing after end of trial participation should be followed on a regular basis according to the investigator's clinical judgment until the condition is stable (refer to Section 7.10).
- Close monitoring and careful dose escalation as well as the implementation of an IDMC (see Sections 3.1.1 and 9.12).
- The dosing schedule takes into account the possible side effect of C_{max} exposures (t_{max} expected: 2-4 hours) and the observed high plasma exposures (during distribution phase) (see IB, Dynacure). The distribution phase lasts approximately 24-48 hours. Therefore, the medical monitor will review the 48-hour safety data of the first subject in a cohort before the next subject can be dosed, and there will be an interval of 7 days between dosing of the first and second subject in a cohort (see Section 3.1.1), to see if any delayed toxicities occur. In addition, any SAE data from the first subject received up until the moment of treatment of the second subject will be taken into account.
- Safety evaluations will be done at several time points throughout the trial (refer to the TRIAL SCHEDULES). Blood samples for biochemistry and hematology (including platelet counts), coagulation tests, complement tests, and urine samples for urinalysis will be collected. Vital signs and triplicate 12-lead electrocardiograms (ECGs) will be recorded, as well as ECG monitoring which will be performed during the single dose infusion (see Section 6.2.2.5). Physical examinations will be performed. In addition, liver ultrasound including hepatic elastography will be performed. IMP can be stopped or interrupted at the investigator's discretion (refer to Sections 4.5 and 7.11.2).
- For all subjects, there are pre-specified rules that would result in pausing/stopping of IMP administration if predefined conditions occur, preventing exposure of further subjects to IMP until the IDMC has reviewed all safety data (refer to Section 7.11.1).
- The blood sample collection scheme is designed to perform the safety, tolerability and PK (optional for biomarkers and DNA) assessments with a minimum number of blood samples being collected. The total blood volume to be collected is considered to be within the limits of standard blood donation (refer to Section 6.2.86.2.7). Blood samples will be collected by indwelling catheters to minimize discomfort and distress due to repeated venipunctures for blood sampling.
- Pregnancy and breastfeeding are exclusion criteria. In addition, all subjects are required to use contraceptive methods, as detailed in Section 4.3.

More detailed information about the known and expected benefits and risks of DYN101 can be found in the IB for DYN101 (Dynacure).

2. TRIAL OBJECTIVES AND HYPOTHESIS

2.1 Primary Objective

- To assess the safety and tolerability of 3 single ascending dose (SAD) levels and 3 MAD levels of DYN101.

2.2 Secondary Objectives

- To assess the PK of SAD and MAD of DYN101.
- To explore target engagement in muscle of DYN101.

2.3 Exploratory Objectives

- To investigate the effect of DYN101 treatment on the clinical assessments in various affected domains (respiratory, muscle strength and function, dysphagia).
- To explore the impact of CNM and its treatment on symptoms, functioning, and health-related QoL.
- To assess the presence of anti-drug antibodies (ADA) against DYN101 by collecting blood samples.
- To assess the metabolic profile of DYN101 by collecting blood and urine samples.
- To contribute to the global understanding of CNM and its treatment by collecting blood and muscle samples for further exploratory biomarker/genetic research.

2.4 Hypothesis

As this trial is designed to provide descriptive information regarding safety and tolerability, no formal statistical hypothesis testing is planned.

3. TRIAL DESIGN

3.1 Overall Trial Design

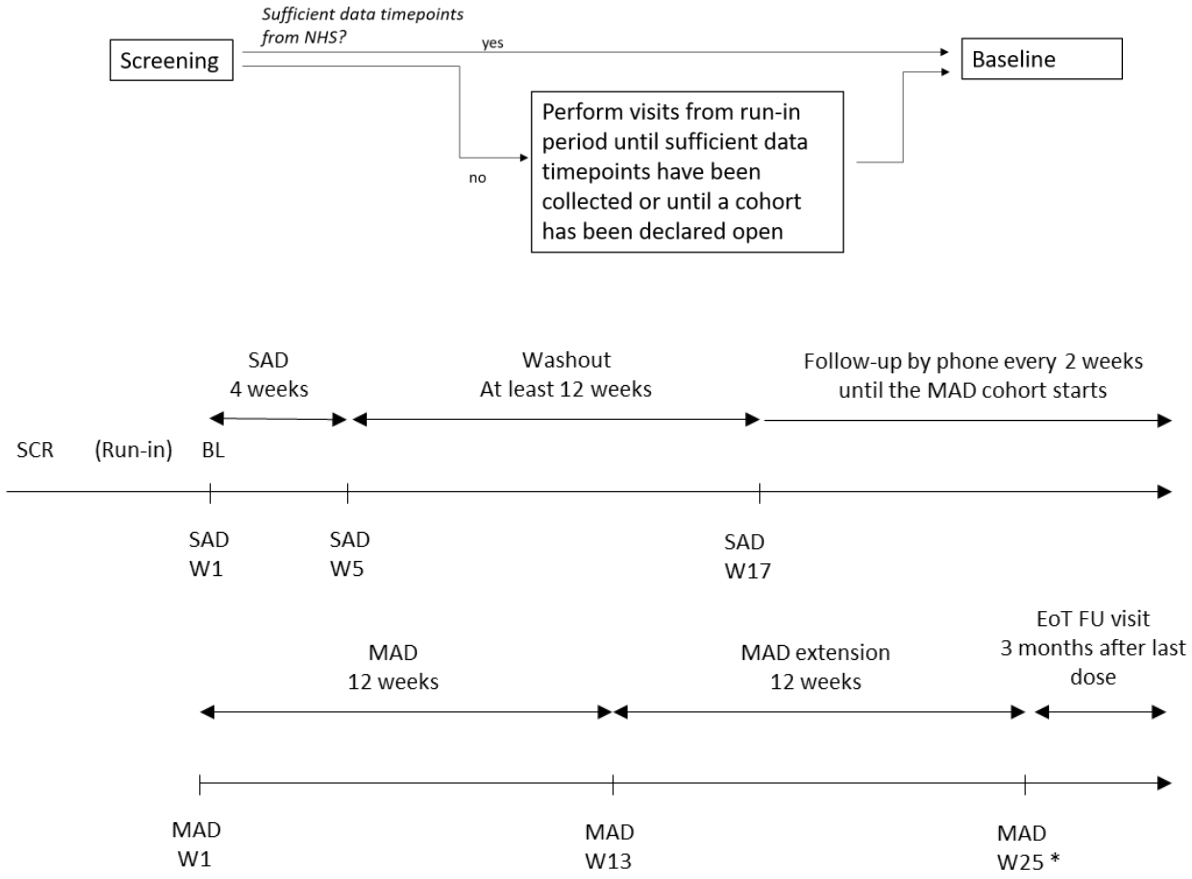
3.1.1 Overview of Trial Periods

This is a first-in-human, Phase 1/2, open-label, multi-center trial to evaluate the safety, tolerability, PK, PD and preliminary efficacy of DYN101 after SAD and MAD in subjects ≥ 16 years of age* with CNMs caused by mutations in *DNM2* or *MTM1* (*for Germany, the subject must be ≥ 18 years of age in accordance with local and national regulations).

Sufficient subjects will be screened in order to ascertain that approximately 18 subjects will be dosed and finish the trial. Subjects may be recruited from an ongoing NHS (Nat-His-CNM Trial Protocol, 2017).

There will be stand-by subjects for replacement of subjects who discontinue the trial early, if needed. Stand-by subjects will perform run-in visits until the last MAD subject has completed Week 13, unless needed for replacement.

The trial will consist of a pre-screening consent, a screening period, a run-in period (if applicable), a SAD part with 4 weeks of follow-up after 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 will participate in the SAD, MAD and MAD extension parts, unless they withdraw. End of Treatment (EoT) assessments will be performed after 24 weeks of MAD treatment have been completed, i.e. at the Week 25 visit. Subjects will return to the clinic 3 months after the last IMP administration, to follow-up on the subject's status including abnormal laboratory results, AEs, and concomitant medications. The trial design is presented in Figure 1.



Abbreviations: BL: baseline, EoT: End of Treatment, FU: follow-up, MAD: multiple ascending dose, SAD: single ascending dose, SCR: screening, W: week

* All subjects will remain on treatment with weekly IMP administrations and bi-weekly (every 2 weeks) assessments until the last MAD3 subject has completed the MAD Week 25 visit. The data generated from these bi-weekly assessments will not be part of the statistical analysis for this trial (see Section 9.1).

Figure 1: Trial Design: Trial Periods

Subjects will give a 'pre-screening' consent to be entered as 'pre-screened' in the Interactive Response Technology (IRT) system. The pre-screening consent process can be managed remotely (please refer to the Site Operations Manual for more details). Subsequently, subjects will sign the main informed consent form (ICF) and screening will be performed to assess eligibility. Subjects who are participating in the NHS and have at least 2 data time points available from the NHS to assess their pre-trial condition do not require a mandatory run-in visit.

Subject screening should be performed within 6 weeks before the baseline visit. There should be a minimum of 3 data time points to assess their pre-trial condition. The screening visit can be the third data time point.

Subjects who have not participated in the NHS or who do not have at least 2 data time points from the NHS will have a mandatory run-in period before continuing to the baseline visit. At least 1 run-in visit needs to be performed for these subjects in order to obtain 3 data time points to assess their pre-trial condition; screening can be the first data time point, baseline can be the third data time point. There should be a minimum of 28 days between screening and the first run-in visit. Run-in visits may be repeated until the assigned cohort can start (see Section 6.1.2). The run-in period is optional for stand-by subjects who participated in the NHS and have at least 3 data time points.

At baseline, subjects will be enrolled into the trial (if all in- and exclusion criteria are still met) until recruitment in the last cohort is reached, including replacement subject(s) who were amongst the stand-by subjects. Stand-by subjects will perform run-in visits until the last MAD subject has completed Week 13, unless needed for replacement.

In the SAD part, subjects will receive a single dose of IMP at Day 1. In the MAD part, subjects may receive 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 will remain on treatment with weekly IMP administrations and bi-weekly (every 2 weeks) assessments until the last MAD3 subject has completed the MAD Week 25 visit. In order to avoid any overlaps, a given MAD cohort may not start before its corresponding SAD cohort is finished.

Subjects will receive 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 receive a different dose than what they received in the SAD part. In each cohort, there will be up to 6 subjects with a mutation in *DNM2* (sub-cohort a) and up to 3 subjects with a mutation in *MTM1* (sub-cohort b).

Cohorts will be 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 sub-cohort. The first subject (i.e. the sentinel subject) in each cohort will be ≥ 18 years of age. The 48-hour safety data from the first subject in a cohort will be reviewed by the medical monitor before the next subject can be dosed. In addition, any SAE data from the first subject received up until the moment of treatment of the second subject will be taken into account.

Pausing/stopping rules will be applied in this trial (see Section 7.11).

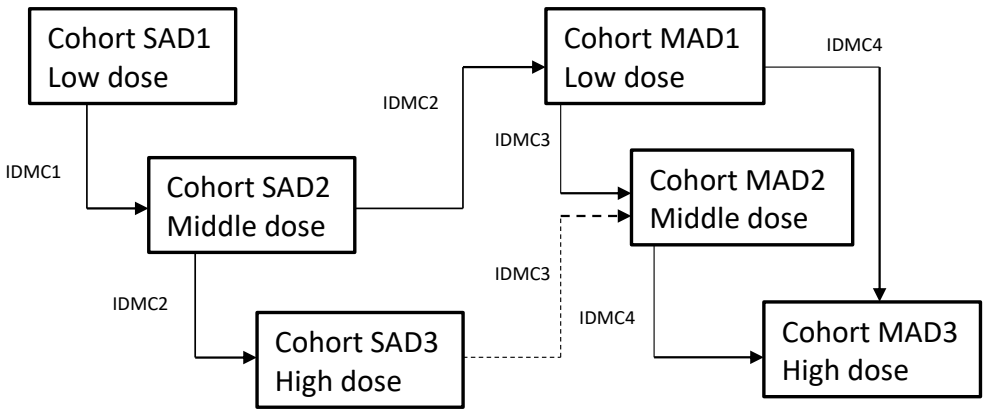
An IDMC will be established to monitor the safety data on a regular basis to ensure the continuing safety of the enrolled subjects. The IDMC may meet at any time based on emerging safety and tolerability data. At a minimum, the IDMC must meet after all subjects of a cohort have been dosed to advise to continue each *MTM1* and/or *DNM2* sub-cohort to the next dose level/or discontinue the *MTM1* and/or *DNM2* sub-cohort of the trial. Escalation of each *MTM1* and/or *DNM2* sub-cohort to the next increased dose level will not take place until the IDMC has reviewed the data of the previous sub-cohort (including subjects who have discontinued the trial). The data reviewed by the IDMC for dose escalation decisions will be as clean as possible prior to their review. The IDMC can recommend adjusting dose levels or stopping the dose escalation scheme for each *MTM1* and/or *DNM2* sub-cohort and/or individual subject based on the totality of available data (safety and tolerability data and/or level of target engagement). At each IDMC review, different decisions for the sub-cohorts (*DNM2* vs *MTM1*) can be made based on the

emerging data. If needed, the IDMC review can be split over separate meetings for the sub-cohorts, although the available data from the other sub-cohort will also be taken into account at each meeting.

The IDMC will convene at least 4 times:

- The trial will start with the enrollment of approximately 6 subjects in Cohort SAD1.
- Cohort SAD2 can start after a favorable IDMC review of the 2-week safety and PK data of SAD1 (IDMC1).
- Cohorts SAD3 and MAD1 can start after a favorable IDMC review of the 2-week safety and PK data of SAD2 (IDMC2).
- Cohort MAD2 can start after a favorable IDMC review of the 2-week safety and PK data of SAD3, if available, as well as the 6-week safety and PK data of MAD1 (IDMC3).
- Cohort MAD3 can start after a favorable review of the 12-week safety and PK data of MAD1 and MAD2 as well as the MAD1 and MAD2 12-week PK and muscle biopsy data (IDMC4). In this meeting the IDMC will also recommend a starting dose for the pediatric DYN101-C102 study.

Details of the IDMC review will be described in the IDMC charter (see Section 9.12).



IDMC1: review of 2-week safety and PK data of SAD1; IDMC2: review of 2-week safety and PK data of SAD2; IDMC3: review of 2-week safety and PK data of SAD3, if available, and 6-week safety and PK data of MAD1; IDMC4: review of 12-week safety and PK data of MAD1 and MAD2, and 12-week PK and muscle biopsy data of MAD1 and MAD2.

Figure 2: Trial Cohorts and IDMC Reviews

Safety and tolerability will be evaluated throughout the trial from signing of the main ICF onwards until the last trial visit (i.e. EoT follow-up visit 3 months after the last IMP

administration). Safety evaluations will include monitoring of SAEs/AEs, clinical laboratory tests, ECGs, vital signs, and physical examinations at specified time points.

All enrolled subjects will undergo blood sampling for PK evaluations at specified time points. In addition, blood and/or urine samples will be collected for analysis of ADA and metabolic profiling.

PD/exploratory efficacy parameters including respiratory function, muscle strength and function, and dysphagia will be assessed, and a muscle biopsy will be taken at specified time points.

For subjects who provided a separate informed consent, blood samples will be collected for further exploratory research on blood biomarkers (including DNA). Participation in this part of the trial is optional and does not impact the subject's eligibility for participation in the trial. Subjects may continue to participate in the trial if they refuse to provide a sample or if they withdraw their samples.

The impact of CNM and its treatment (safety and efficacy) on QoL and health outcomes will be evaluated at specified time points, using questionnaires.

For an overview of the safety, PK and PD/exploratory efficacy assessments, please refer to the TRIAL SCHEDULES.

An interim analysis will be performed when all subjects in Cohorts 1 and 2 have completed 12 weeks of MAD treatment or discontinued earlier, or if the sponsor deems a risk/benefit analysis necessary at any time point. The primary analysis will be performed when all subjects in all cohorts have completed 12 weeks of MAD treatment or discontinued earlier. The final analysis will be performed when all subjects have completed 24 weeks of MAD treatment (12 weeks in the MAD part + 12 weeks in the MAD extension part; Week 25 visit) or discontinued earlier.

The duration of participation in the trial for an individual subject in any cohort, from screening (excluding run-in period) to the last trial visit, will be at least 60 weeks.

The end of the trial is defined as the last visit of the last subject.

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

3.1.2 Replacement of Subjects

Enrolled subjects who are withdrawn (see Section 4.5) can be replaced after discussion with the sponsor, the medical monitor and the investigator in the following circumstances:

- Enrolled subjects who participated in the SAD part and drop out before the start of the MAD part can be replaced (for the MAD part).
- Enrolled subjects who drop out and received less than 6 weeks of MAD treatment can be replaced.
- Enrolled subjects who drop out but received between 6 and 12 weeks of MAD treatment and do not consent to a second muscle biopsy can be replaced. However, subjects will not be replaced if they received between 6 and 12 weeks of MAD treatment and consent to a second muscle biopsy, or if they received more than 12 weeks of MAD treatment.

There will be stand-by subjects for replacement of subjects who discontinue the trial early, if needed. Stand-by subjects will perform run-in visits until the last MAD subject has completed Week 13, unless needed for replacement.

Replacement subjects starting in the MAD part should sign an ICF for participation in the MAD part, and their eligibility will be assessed before the first IMP administration in the MAD part (MAD Week 1 Day 1). Replacement subjects may or may not need a run-in period, in consultation with the sponsor. A muscle biopsy and a DNA sample should be taken before the first IMP administration. If needed, replacement subjects in MAD1 and MAD2 may have the second muscle biopsy taken after 6 to 12 weeks of MAD treatment, after discussion with the sponsor.

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

3.2 Trial Design Rationale

3.2.1 Rationale for Choice of Design

Both XLCNM and ADCNM are ultra-rare diseases, and adult patients who are ambulatory comprise a very small proportion of the total population. A limited number of patients thus meet the criteria for eligibility into this Phase 1/2 trial. In these circumstances, ultra-orphan diseases often do not include a placebo arm in their first in human studies.

The primary and secondary endpoints to this trial are safety/tolerability and PK/PD, with efficacy being a tertiary endpoint. An NHS that has been ongoing for several years has had safety parameters captured and it is intended to compare the AEs and SAEs in the Phase 1/2 trial to those recorded in the NHS. The PK/PD results do not need any comparison to placebo.

The NHS is demonstrating that patients are very stable for all the assessment measures that are included in this Phase 1/2 trial (except for dysphagia which is a new parameter). These assessments were repeated 3 times and were shown to be reliable with little variability. In this Phase 1/2 trial, assessments will be repeated 3 times with an accepted variability of 10% for the recording of the best value, which is difficult to assign to a placebo effect. These tertiary results will only be used to strengthen the choice of a dose for pivotal trials that will be primarily based on the safety, tolerability, PK, ASO concentration and *DNM2* mRNA reduction in muscle biopsies.

3.2.2 Rationale for Choice of Eligibility Criteria

As explained in Section 1.4, changes in efficacy parameters observed in mouse models can be translated to patients. This first-in-human trial for DYN101 will therefore be conducted in patients and not in healthy volunteers to assess safety, PK and dose selection based on PD and possibly efficacy.

Subjects ≥ 16 years will be included in this Phase 1/2 trial. The metabolism of ASOs occurs via nucleases which are ubiquitously expressed at similar levels in children and adults (Wójcik et al., 2007). Thus, it could be expected that the dose for children will be the same as for adults on a mg per kg body weight basis. Inclusion of children at an early stage of clinical development (i.e. during dose finding) could be seen as subjecting them to unnecessary interventions

(e.g. muscle biopsies). Therefore, it is preferable to only dose children once an appropriate dose for lowering of *DNM2* mRNA is achieved in adults and then used for children in subsequent trials.

It is intended to include mild to moderately affected subjects in this trial, with clinical symptoms in at least 2 of the domains that will be investigated (respiratory function, muscle strength and function, and dysphagia). Due to the frequency of trial assessments at a site and associated travel, inclusion of ambulatory subjects will facilitate recruitment and retention. Furthermore, the possibility of seeing an efficacy signal in mild to moderately affected subjects will be greater due to the amount of recoverable muscle mass remaining. Ambulatory subjects can have more efficacy assessments carried out (upper and lower limbs) and are less prone to be affected by contractures that are very often seen in more severe subjects.

ASO platform side effects include liver toxicity where a significant portion of administered drug accumulates. Furthermore, rare instances of peliosis hepatis have been reported in untreated XLCNM patients (Motoki et al., 2013; Wang et al., 2001). Moreover, some degree of liver dysfunction has been previously reported in long-term survivors (Herman et al., 1999). Recently, investigators for an ongoing clinical trial in children with XLCNM mention that >50% of subjects enrolled in the trial displayed some evidence of pre-existing hepatobiliary disease (Shieh et al., 2020). Therefore, liver function tests, liver ultrasound, and hepatic elastography will be carried out to ensure that these are normal prior to entry. In case of slight liver function abnormalities, there should be a medically plausible reason for the elevation of these parameters outside of the normal range, and a discussion with the medical monitor should occur in this situation before the subject may be screened and/or dosed for the trial.

ASOs are slowly metabolized to chain-shortened metabolites and excreted by the kidneys (Geary et al., 2009). Therefore, only subjects with normal kidney function will be included.

Symptomatic decreases in platelet count have been reported for some other ASOs, and these were managed by implementation of monitoring of platelet count and cessation of treatment once they fell to a predetermined level. This mostly led to a normalization of platelet count with no further reports of untoward effects. As a result, only subjects with a platelet count > 150,000/µL will be included in this trial.

3.2.3 Rationale for Choice of Safety Assessments

Safety assessments are based on preclinical toxicology results, prior experience with platform safety of ASOs (see also Section 3.2.2), as well as the evaluation of potential on-target effects of decreasing *DNM2* levels with the DYN101 ASO.

Liver function tests, kidney function tests, platelet counts, coagulation tests, and complement testing will be monitored regularly in this trial. In addition to central laboratory testing, platelet counts will be assessed by the local laboratory and need to be within normal ranges before IMP administration and before performing a muscle biopsy.

In a 9-month preclinical study with DYN101 in NHP, vasculitis which appeared to be associated with chronic complement activation with subsequent depletion and inflammation was observed. Therefore, subjects with any suspicion for vasculitis (vasculitis of any origin, including the complement pathway) will trigger a consult with an appropriate specialist for further investigation.

As there are no validated specific diagnostic markers to detect vasculitis, monitoring and assessment should be based on a high level of clinical awareness/suspicion and on physical examination and objective studies specific to the end organs that could be involved in drug-induced small or medium vessel vasculitis. These include, but are not limited to, the heart, kidney, lungs, liver, peripheral nervous system, and skin.

3.2.4 Rationale for Choice of PD/Exploratory Efficacy Assessments

An NHS is currently ongoing in subjects of all ages with CNM resulting from a mutation in the *MTM1* and *DNM2* genes. This study is assessing the natural history and functional status of these subjects by assessing, amongst others, respiratory function, motor function, muscle strength, cardiac function, liver function, kidney function, QoL and health outcomes. The aim is to include subjects mostly from this ongoing NHS into the current trial, although other subjects can also participate. Several of the aforementioned assessments have been performed at baseline and at several other time points in the NHS, which will allow to assess the validity and variability of the results at baseline as well as the individual subject's course and inter-occasion variability for the various measurements prior to entry into the current trial for eventual comparative analyses. Subjects who are participating in the NHS and have at least 2 data time points available from the NHS to assess their pre-trial condition do not require a mandatory run-in visit. (see Section 6.1.2).

Due to the extreme rarity, severity, and heterogeneity of CNM as well as the rapid benefit seen in mouse models and human studies, the set of exploratory efficacy assessments planned in this trial covers a wide range of areas affected by the disease and should allow for a decision on the most reliable and important endpoints for pivotal trials as well as potential efficacy signals for dose selection.

The PD/exploratory efficacy assessments that will be performed in this trial will include QoL and health outcomes (by means of questionnaires), respiratory function tests, MyoGrip, Motor Function Measure (MFM), muscle biopsy, and dysphagia assessments. Details on these assessments are provided in Section 6.2.4.

The sponsor intends to apply biomarker/genetic research across the DYN101 development program to explore how biomarker/genetic variations may affect the clinical parameters associated with and response to DYN101 (and any background products, comparators and concomitant medications), and potentially the basis of the indications under study in the protocol, in this case CNM. Collection of appropriate samples from populations with well described clinical characteristics may lead to improvements in the design and interpretation of clinical studies, biomarker-guided treatment strategies and a better understanding of disease etiology which may lead to new therapeutic approaches.

Candidate biomarkers/genes which may be studied include those potentially related to the mechanism of action of DYN101 and comparators and concomitant medications as well as those potentially responsible for absorption, disposition, metabolism and excretion of DYN101 and comparators and concomitant medications. Future research may suggest other genes, gene categories, proteins etc. as candidates for influencing not only response to DYN101 and comparators and concomitant medications but also susceptibility to CNM for which DYN101 may be evaluated. Thus, this additional biomarker/genetic research may involve the future study of additional unnamed genes or gene categories, but only as they relate to CNM and drug action.

3.2.5 Rationale for Choice of Doses, Route of Administration and Dosing Regimen

Dose selection was based on the following data/assumptions:

1. Safety profile of DYN101 derived from Cynomolgus monkey studies.
2. Target knock-down: 30 to 50% knock-down of *DNM2* mRNA resulted in a significant reduction of myopathic features in mice. It is assumed that a similar target knock-down level in humans would translate into clinically meaningful effects.
3. Translatability of effective concentrations: the required effective concentration in human tissue (muscle) is expected to be the same as the observed effective concentration in transgenic mouse tissue expressing human *DNM2*.
4. Translation of effective concentrations in humans into doses can be extrapolated from non-human primate data.

See the latest version of the IB for the supportive information on dose selection (Dynacure).

The minimal recommended starting dose (MRSD) is 1.5 mg/kg (i.e. low dose level in this trial). This MRSD is selected based on the no observed adverse effect level (NOAEL) of 10 mg/kg from a Good Laboratory Practice (GLP), 13-week toxicity study with an 8-week recovery period in Cynomolgus monkeys. The most noteworthy finding during the 13-week treatment was a marked platelet reduction in two female monkeys at 30 mg/kg. This type of platelet change has occasionally been observed in monkeys treated with ASOs of similar chemistry, where a small proportion of individual animals appear to be susceptible to marked effects on platelet counts following ASO treatment. Platelet counts will be closely monitored in this trial to ensure subjects' safety and mitigate the risk. In addition, recovery of platelet counts occurs after drug withdrawal and/or treatment with steroids. Moreover, sudden decreases in platelets have not been observed in humans so far with other constrained ethyl ASOs at dose levels of 1.5 mg/kg.

In addition, a human dose of 1.5 mg/kg has the possibility to provide a beneficial effect (on-target) to patients with this life-threatening, rare disease. Since this first-in-human study will not be conducted in healthy volunteers, but in patients with a severely debilitating disease, a possible benefit also at the lowest dose level is desired if patients' safety is ensured. Preclinical data in mouse models of XLCNM and ADCNM suggest that therapeutic efficacy might be seen at 15% target knock-down. Based on quadriceps tissue concentration data derived in NHP, this level of knock-down could be achieved after administration of 1.5 mg/kg in humans.

The suggested middle dose level will be 4.5 mg/kg and the high dose level will be up to 9 mg/kg. These dose levels should be sufficient to achieve the target knock-down *DNM2* mRNA levels of 30% and 50%, respectively (based on animal data from quadriceps muscle tissue) (Table 1). The predicted *DNM2* mRNA knock-down of 30% to 50% is anticipated to be efficacious in humans. Moreover, these target knock-down levels have not raised any safety concerns in nonclinical studies, as reduction of *DNM2* mRNA and DNM2 protein by more than 80% in key tissues such as liver and skeletal muscle was safe and well tolerated in mice. In addition, 9 mg/kg is below the NOAEL of 10 mg/kg, as derived from the GLP, 13-week toxicity study with an 8-week recovery period in Cynomolgus monkeys.

Table 1: Calculated Weekly Human Dose*

Level of mRNA Knock-down		**15%**	**30%**	**50%**
Muscle	Concentration in NHP Muscle (µg/g) [a)]	Human Dose (mg/kg) [b)]		
Quadriceps at 25 mg/kg SC	9.3±0.8	1.9	4.2	9.7
Quadriceps at 30 mg/kg IV	9.8±2.9	2.1	4.7	11.1

* Based on Kim, 2018.

a) Data from a 12-week NHP study at 25 mg/kg SC (non-GLP; Kim, 2018) and a 13-week toxicity study with an 8-week recovery period in NHP at 30 mg/kg IV ().

b) In a humanized, transgenic *DNM2* mouse model, effective DYN101 concentrations in quadriceps muscle were 3.62, 1.55, and 0.7 µg/g, respectively at EC_{50}, EC_{30}, and EC_{15} on mRNA knock-down. These concentrations were used to calculate the human doses required to reach a level of KD of 50%, 30%, and 15% respectively.

Escalation to the next dose level will be done carefully and the decision will be made by an IDMC. To decide on dose escalation to the highest dose level, the IDMC will be allowed to adjust the dose level to a lower level, driven by the safety, tolerability and expected target engagement in quadriceps or biceps muscle.

DYN101 will be administered via IV infusion over 1 hour. The most widely used route of administration for ASOs is SC injection; however, there are some limitations as follows. For SC administration, it is recommended not to exceed an injection volume of 2 mL due to the tolerability at the injection site. For instance, IV infusion accommodates for dosing up to 9 mg/kg, whereas the limit of 2 mL will be exceeded in the targeted population if SC administration is conducted. Moreover, injection site reactions are known to occur after SC injection of ASOs: this was reported by 79% of patients treated with weekly SC injections of 300 mg/1.5 mL volanesorsen in a pivotal clinical trial (FDA, 2018).

The doses will be administered weekly in the MAD part of the trial. This dosing regimen was used to establish proof-of-concept in mice (see IB, Dynacure) and is an established regimen for other ASOs. Steady state will be reached after 12 weeks of weekly dosing, when the second muscle biopsy will be taken. This muscle biopsy will be used to determine DYN101 uptake in muscle and target engagement (see Section 6.2.6).

3.3 Trial Endpoints

3.3.1 Primary Endpoint

- Comparison of adverse event (AE, SAE, AESI) frequency and severity by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) 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.

3.3.2 Secondary Endpoints

- Comparison of means for PK parameters of DYN101 in plasma (AUC_{τ}, AUC_{last}, AUC_{∞}, $R_{ac}(AUC)$, $R_{ac}(C_{max})$, C_{max}, C_{av} SS, CL, λ_z, $t_{1/2}$, t_{max}, V_z) by dose (cohort), for

single or multiple administration, and by mutated gene (sub-cohort) and overall using the Pharmacokinetic Set.

- Comparison of geometric means for PK parameters of DYN101 in plasma (C_{max} and AUCs) by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) and overall using the Pharmacokinetic Set.
- 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 (sub-cohort) and overall using the Pharmacokinetic 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 (sub-cohort) and overall.

3.3.3 Exploratory Endpoints

- Difference in mean change from baseline for clinical assessments in various affected clinical domains (respiratory, muscle strength and function, dysphagia) using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) 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 H_2O), %MEP Predicted, MIP (cm H_2O), %MIP Predicted, Oxygen Saturation (%). The MyoGrip Test score will be used for analyzing muscle strength. The following parameters will be analyzed 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.
- 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 Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit.
- Comparison of proportion of CGI-I responders (responders defined as responding 1 or 2) using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit.
- Comparison of proportion of PGI-C responders (responders defined as responding 1 or 2) using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit.
- Comparison of proportion of Goal Attainment Scaling (GAS) Questionnaire responders (responders defined as responding 'YES – MUCH BETTER', 'YES – A LITTLE BETTER') using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit
- Comparison of means of ADA against DYN101 using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit.

- Comparisons of the metabolic profile of DYN101 in blood and urine using the Pharmacokinetic Set by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort) at each post-baseline visit.

3.4 Sample Size

Sufficient subjects will be screened to ascertain approximately 18 subjects who will be dosed and finish the trial. The target is approximately 6 subjects per dose level. In each dose level, the target will be up to 6 subjects with a *DNM2* mutation and up to 3 subjects with an *MTM1* mutation. This sample size is deemed to be appropriate for safety and tolerability purposes and are not based on any power analysis (see Section 9.11).

3.5 Sites and Regions

This trial is planned to be conducted at 5 to 10 sites in Europe and the United States of America, with an estimation of 1 to 5 subjects per site.

4. TRIAL POPULATION

4.1 Inclusion Criteria

The subject cannot be enrolled in the trial before all of the following inclusion criteria (including test results) are met:

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 will be ≥ 18 years of age.

   *for Germany, the subject must be ≥ 18 years of age in accordance with local and national regulations.

2. Have a documented mutation in *DNM2* or *MTM1*.
3. Platelet count > 150,000/µL
4. Have a symptomatic CNM in the opinion of the investigator, at least mild to moderately affected, i.e. showing clinical symptoms in at least 2 of the 4 relevant domains that will be investigated in this trial (respiratory function, muscle strength, muscle function, and dysphagia), and be ambulatory, i.e. being able to walk 10 steps, if needed with support/assisted. If a subject is non-ambulatory but highly functioning in the view of the investigator, he/she may be included following discussion with the sponsor.
5. Have an understanding, ability, and willingness to fully comply with visit frequency, trial procedures and restrictions, including contraceptive requirements.
6. Able to provide written, signed and dated informed consent/assent to participate in the trial. Parental consent (one or both parents) and an assent for subjects < 18 years may be required per local legislation.

4.2 Exclusion Criteria

Subjects are excluded from the trial if any of the following exclusion criteria are met:

1. Clinically significant liver disease.
2. Clinically significant renal disease.
3. Presence of significant co-morbidities 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 be in the best interest of the subject (e.g. compromise the safety or well-being) or that could prevent, limit, or confound the protocol-specified assessments (e.g. taking a muscle biopsy).
4. For female subjects of child-bearing potential: pregnant or breastfeeding or planning to become pregnant during the trial.
5. Current or past abuse of alcohol or recreational/narcotic drugs (with the exception of caffeine and nicotine), which in the investigator's opinion would compromise the subject's safety and/or compliance with the trial procedures.
6. Currently enrolled in any interventional trial or scheduled to participate in such a trial whilst participating in this trial. Subjects are allowed to participate in registry studies.
7. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to fully complete the trial, or any condition that presents undue risk from the IMP or procedures.
8. Intake of any disallowed therapies as noted in Section 5.5 within 12 weeks before the planned first IMP administration.
9. Known or suspected intolerance or hypersensitivity to IMP ingredients or closely-related compounds, or history of a significant allergic reaction to IMP ingredients as determined by the investigator, such as anaphylaxis requiring hospitalization.
10. Legally incapacitated or have limited legal capacity. Lack of mental capacity to fully understand the protocol requirements and complete all trial required procedures.

Note: Retesting of subjects should always be discussed with the sponsor and/or medical monitor. Retesting of laboratory values that lead to exclusion will be allowed once using an unscheduled visit during the screening period to assess eligibility. This visit should be at least 2 weeks later than the original screening visit.

4.3 Reproductive Potential

At screening, female subjects should be either:

- Post-menopausal (12 consecutive months of spontaneous amenorrhea and ≥ 51 years of age), or
- Surgically sterile (i.e. hysterectomy, bilateral salpingectomy, or bilateral oophorectomy, or otherwise be incapable of pregnancy) and at least 6 weeks post-sterilization, or

- Of child-bearing potential with a negative pregnancy test at screening AND prior to the first IMP administration and
  - not heterosexually active but agree to use acceptable contraception as defined below if they become sexually active during the trial and until at least 90 days after the last IMP administration in this trial.
  - heterosexually active and have a vasectomized partner (confirmed sterile)
  - heterosexually active and using or agree to use acceptable contraception as defined below throughout the trial and until at least 90 days after the last IMP administration.

The following forms of contraception are considered highly effective and acceptable:

- Use of a hormone-based contraceptive (oral, depot, patch, injectable, or vaginal ring) or an intrauterine device in combination with condoms. Hormonal contraceptives should be administered according to the package insert.
- Intrauterine device or intrauterine hormone-releasing system used/administered in accordance with the package insert.

Male subjects who did not have a vasectomy performed more than 1 year prior to screening and who are sexually active with a female partner of child-bearing potential must use condoms for sexual intercourse throughout the trial and until at least 90 days after the last IMP administration in this trial. Their female partner should be advised to use an acceptable form of contraception as stated above.

4.4 Prohibitions and Restrictions

Subjects must be willing and able to adhere to the following prohibitions and restrictions during the course of the trial to be eligible for participation:

1. Female subjects of child-bearing potential must remain on a highly effective method of birth control consistent with local regulations (see Section 4.3) until at least 90 days following the last IMP administration in this trial.
2. Female subjects are not allowed to breastfeed their child during this trial.
3. Male subjects with a female partner of child-bearing potential must agree to use effective contraceptive methods (see Section 4.3) during the trial until at least 90 days following the last IMP administration in this trial.
4. Strenuous activities should be avoided during the whole trial (from screening to the last visit in the extension period, or early discontinuation visit).
5. Subjects are not allowed to take alcohol/recreational or narcotic drugs 24 hours before each IMP administration and should abstain from alcohol/recreational or narcotic drugs for 24 hours after dosing. During the treatment period, the subject must be willing to limit alcohol consumption to < 5 drinks per day for men and < 4 drinks per day for women. One drink is defined as 1 bottle (360 mL) of beer, 1 glass (120 mL) of wine, or 30 mL of liquor. The abstinence of the use of alcohol/recreational or narcotic drugs will be confirmed verbally by site personnel before infusion.

6. Subjects are asked to refrain from using tobacco or nicotine-containing products during the treatment period.
7. Subjects are not allowed to take any new medication (including over-the-counter medication and herbal supplements) to treat CNM or that are known to affect *DNM2* levels (see Section 5.5).
8. Subjects should follow restrictions imposed in response to a pandemic closely (e.g. social distancing).
9. Subjects should refrain from sharing information about the trial within a patient community, on social media, etc. until the last subject in the trial has completed treatment.

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

4.5 Subject Withdrawal and Trial Termination

It is the investigators responsibility to stop dosing a subject(s) at any stage if they deem it in the subjects' best interest to do so and take any urgent safety measures required to safeguard the subject. Such measures should be reported to the sponsor immediately and to the regulatory authorities or the Independent Ethics Committee/Institutional Review Board (IEC/IRB). Pausing/stopping rules (Section 7.11) should be taken into account.

A subject may be withdrawn from the trial at any time, for any reason, without prejudice to their future medical care by his/her physician or at the institution. Subjects withdrawing from the trial will be encouraged to complete the assessments of the early discontinuation visit, see Section 6.1.5), particularly safety evaluations in the subject's interest and to ensure that data can be recorded in the same way as for subjects who completed the trial. If subjects agree, they will also have the follow-up visit 3 months after their last IMP administration (see Section 6.1.4). Comments (spontaneous or elicited) or complaints made by the subject must be recorded in the electronic case report form (eCRF).

Reasons for withdrawal from the trial may include, but are not limited to:

- For subject's best interest
- SAE
- Any pausing/stopping rule for the trial (as described in Section 7.11)
- Pregnancy
- Protocol violation (except if related to a restriction imposed in response to a pandemic)
- Withdrawal of consent by subject
- Lost to follow-up

In case of temporary IMP discontinuation, it should be discussed with the medical monitor whether continued participation is possible and whether any (pre-) treatment/therapy before resuming IMP administration can be considered useful in order to ensure subject safety. The withdrawal of a subject from IMP by the investigator should be discussed, where possible, with the medical monitor before the subject stops with the IMP. The reason for IMP discontinuation and date of stopping IMP must be recorded on the eCRF and source documents. The total amount of IMP received is registered on the drug accountability form.

Replacement of withdrawn subjects can occur under the circumstances described in Section 3.1.2.

At least 3 documented attempts must be made to contact any subject lost to follow-up at any time point prior to the last scheduled contact (e.g. office visit or telephone contact). One of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that they return to the site for final safety evaluations.

The sponsor has the right to terminate the trial at any time in case of safety concerns or if special circumstances concerning the IMP or the company itself occur, making further treatment of subjects impossible. In this event, the investigator(s) and relevant authorities will be informed of the reason for trial termination.

If a subject withdraws his/her consent to continue with the trial, the analysis and reporting of samples collected to that point, cannot be withdrawn.

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

Withdrawal from the Use of Research Samples

Subjects may withdraw their consent for storage of samples for additional research at any time (see Section 10.4.4), in which case the samples will be destroyed, and no further testing will take place.

5. TRIAL TREATMENT

5.1 Investigational Medicinal Product

[redacted] he dose levels will be 1.5 mg/kg (Cohort 1), 4.5 mg/kg (Cohort 2), and up to 9 mg/kg (Cohort 3), unless the IDMC advises otherwise. Additional information is provided in the IB (Dynacure).

5.2 Dosage and Administration

The IMP will be prepared by the pharmacist or delegated qualified site personnel. The ideal body weight determined at SAD Day 1, MAD Week 1 Day 1, and MAD Week 13 will be used to calculate the applicable dose. In case the ideal body weight is higher than real body weight, real body weight will be used to calculate the applicable dose. Refer to the Pharmacy Manual for the exact calculations of the volume to be administered. The corresponding volume of IMP will be taken from the vial and diluted into a 0.9% sodium chloride solution (in an infusion bag), resulting in an IV solution with the respective concentration of the dose level. A substantial change (as judged by the investigator) in body weight will be reported as an AE, and it will be discussed with the sponsor whether the dose calculation should be adjusted.

Detailed instructions for aseptic preparation of the applicable doses and all items related to IMP administration will be described in the Pharmacy Manual.

The IMP will be dispensed IV using a validated infusion pump. Subjects will be dosed by qualified site personnel. A physician will be available on-site during infusions.

In each cohort, there will be an interval of at least 7 days between dose administration of the first and second subject in a cohort, irrespective of their sub-cohort. The 48-hour safety data from the first subject in a cohort will be reviewed by the medical monitor before the next subject can be dosed. In addition, any SAE data from the first subject received up until the moment of treatment of the second subject will be taken into account.

In the SAD part, subjects will receive a single IV administration of IMP over approximately 1 hour at a low (1.5 mg/kg), middle (4.5 mg/kg), or high (up to 9 mg/kg) dose level in Cohorts SAD1, SAD2, or SAD3, respectively (unless the IDMC advises otherwise).

In the MAD part, subjects will receive weekly IV administrations of IMP over approximately 1 hour for a period of 12 weeks, at the same or different dose level (low, middle, or high) as in their SAD cohort in Cohorts MAD1, MAD2, or MAD3, respectively (unless the IDMC advises otherwise).

After MAD, subjects will continue on the IMP dose level of their assigned MAD cohort in the extension part of this trial for an additional 12 weeks (weekly IV administrations), unless the IDMC decides to change the dose for a (sub)-cohort. After Week 24, all subjects will remain on treatment with weekly IMP administrations until the last MAD3 subject has completed the MAD Week 25 visit.

All subjects will be monitored on site by qualified site personnel for a minimum of 1 hour after the infusion for safety. The infusion time including pauses may not exceed 3 hours (unless the pauses are due to technical issues).

The planned dose levels may be adjusted upon IDMC recommendation, based on emerging data (see Section 3.1), taking into account pre-specified pausing/stopping rules (refer to Section 7.11 for details).

5.3 Treatment Allocation

Subjects will be assigned to a dose level (low, middle, or high), just before baseline, as detailed in the Cohort Management Plan.

The target will be up to 6 subjects with *DNM2* and up to 3 subjects with *MTM1* mutation in each cohort.

The first subject in each cohort will be a 'sentinel' subject. The sentinel subject in each cohort will be ≥ 18 years of age. There will be an interval of at least 7 days between dose administration of the first and second subject in a cohort, irrespective of their sub-cohort.

The aim is to allocate the NHS subjects to the cohorts as evenly and as balanced as possible, given the logistical constraints of the trial.

Family members, including direct family and siblings, are allowed to participate. Family members will be allocated to different cohorts as often as possible, taking into account the logistical constraints of the trial.

A temporary unique number will identify all subjects during their pre-screening. Re-screened subjects will retain the same temporary unique number for all assessments. A cohort-subject number which is the final unique identifier will be assigned once eligibility has been determined. Once a unique identifier has been assigned, that number must not be used again if, for example,

a subject is withdrawn from the trial. If a temporary or final number is allocated incorrectly, the monitor must be notified as soon as the error is discovered.

5.4 Labeling, Packaging, Storage, and Handling

5.4.1 Labeling and Packaging

Labels containing trial information and packaging identification are applied to the IMP vial.

The IMP will be labelled according to local requirements. All manufacturing, packaging, and labeling operations will be performed according to Good Manufacturing Practice for Medicinal Products and the relevant regulatory requirements. Further details will be provided in the Pharmacy Manual.

5.4.2 Storage

The investigator has overall responsibility for ensuring that IMP is stored in a secure, limited-access location. Responsibility will be delegated to the pharmacy; this delegation must be documented.

IMP must be stored in accordance with labeled storage conditions (temperature range of +2°C to +8°C in the carton and protected from light). Temperature monitoring is required at the storage location to ensure that the IMP is maintained within an established temperature range (as defined in the Pharmacy Manual). The pharmacist is responsible for ensuring that the temperature is monitored throughout the duration of the trial and that records are maintained.

5.4.3 Handling

The pharmacist must keep an accurate account of the number of IMP vials delivered to the site used to prepare infusion bags for administration to trial subjects and stored at the site, during the trial. IMP must be administered to subjects only by an appropriately qualified person. Investigators will maintain records that document adequately that the subjects were administered the IMP dose specified by the protocol. The pharmacist will reconcile all IMP vials received at the site before final disposition.

At the completion of the trial after the last monitoring, any empty vials and unused IMP will be returned to the sponsor or its designee or destroyed with the sponsor's written permission. For the latter, a certificate of destruction will be provided.

Details will be provided in the Pharmacy Manual.

5.5 Prior and Concomitant Medication/Therapy

5.5.1 Prior Medication/Therapy

Prior medication/therapy includes medication/therapy (including herbal treatments, vitamins, non-pharmacological treatment such as psychotherapy or physiotherapy as appropriate) received within 5 times the half-live of the drug in question and discontinued prior to the date of first IMP administration. Prohibited medication/therapy is provided in Table 2.

Prior medication/therapy information must be recorded on the appropriate eCRF page. Disease-specific therapy should be recorded regardless of date of therapy.

Table 2: Prohibited Medication and Washout Period

Medication/Therapy	Washout Period	Drug
Drugs affecting *DNM2* levels	8 weeks	Tamoxifen

5.5.2 Concomitant Medication/Therapy

Concomitant medication/therapy is defined as any medication/therapy being continued by the subject at the date of signing of the main ICF, and any new medication/therapy received during the trial. Subjects may get breathing technique advice during the trial as long as it is started at or before screening.

Concomitant medication/therapy taken must be recorded on the appropriate eCRF page.

Treatments not listed in Table 2 above are considered allowable.

New medications (including herbal treatment) for the treatment of CNM are not allowed to be started during the trial, unless for the management of concurrent illness or AEs. Medications already used for the treatment of CNM (e.g. Mestinon) may be continued if subjects are on a stable treatment regimen for at least 3 months and the dose will not be increased during the trial.

Administered vaccines, including COVID-19 vaccines, should be reported as a concomitant medication. In case of a (planned) vaccination, the investigator should contact the medical monitor to discuss the optimal timing of the vaccination in relation to dosing of the IMP.

5.6 Treatment Compliance and Drug Accountability

Sites will be provided with sufficient amounts of the IMP and related materials for infusion (described in the Pharmacy Manual) to carry out this protocol for the agreed number of subjects. The investigator or designee will acknowledge receipt of the IMP by documenting shipment content and condition. Accurate records of all IMP dispensed, used, returned, and/or destroyed must be maintained.

The investigator has the overall responsibility for administering the IMP. Where permissible, tasks may be delegated to a qualified designee (e.g. a pharmacist) who is adequately trained in the protocol and who works under the direct supervision of the investigator. This delegation must be documented in the applicable trial delegation of authority form.

The investigator or designee (as documented by the investigator in the applicable trial delegation of authority form) will administer the IMP only to subjects included in this trial following the procedures set out in the trial protocol. Each subject will be given only the dose of IMP carrying his/her treatment assignment. All dispensing of IMP will be documented on the drug accountability form and infusion information will be entered in the eCRF. Refer to Section 7.4 for details on overdose.

The sponsor or its representatives must be permitted access to review the supplies storage and distribution procedures and records.

Based on entries in the IRT and site drug accountability forms, it must be possible to reconcile IMP delivered with those used, unused and returned to sponsor or designee. All IMP must be accounted for, and all discrepancies must be investigated and documented to the sponsor's satisfaction.

6. TRIAL SCHEDULE AND ASSESSMENTS

6.1 Trial Schedule

Refer to the TRIAL SCHEDULES for an overview of the frequency and timing and sequence of the trial measurements and procedures. Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual).

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

For subjects suffering from an intercurrent illness (e.g. viral or bacterial infection) that could interfere with the PD/exploratory efficacy assessments (see Section 6.2.4), these assessments should be postponed for at least a week until the subject has recovered.

Subjects will be confined to the clinic for at least 7 hours after SAD dosing, the first dosing or a change of dosing) during MAD, for at least 4 hours during all other clinic visits during MAD, and may be confined to the clinic for any 24-hour urine collection. Additionally, the subject may be confined to the clinic for their convenience if assessments will be performed on consecutive days.

6.1.1 Pre-screening and Screening Period

Subjects will give a 'pre-screening' consent to be entered as 'pre-screened' in the IRT system. During this pre-screening, NHS participation and data, age and type of mutation will be confirmed. The pre-screening consent process can be managed remotely (please refer to the Site Operations Manual for more details).

Subsequently, screening will be performed to assess eligibility. Subjects who are participating in the NHS and have at least 2 data time points available from NHS to assess their pre-trial condition do not require a mandatory run-in visit. For subjects not performing run-in visits, the screening visit should be performed within 6 weeks before the baseline visit. There should be a minimum of 3 data time points to assess their pre-trial condition. The screening visit can be the third data time point. Subjects who have not participated in the NHS or who do not have at least 2 data time points from the NHS will have a mandatory run-in period before continuing to the baseline visit (see Section 6.1.2). The run-in period is optional for stand-by subjects who participated in the NHS and have at least 3 data time points. Informed consent (and assent, if applicable) must be obtained before any trial procedures are performed. This applies to the main ICF and optional ICFs as applicable.

Eligibility of subjects will be checked according to the in- and exclusion criteria (see Sections 4.1 and 4.2). The screening assessments can be split over 2 consecutive days, if required; if more than 2 days are needed, approval is required from the medical monitor in advance.

Retesting of subjects should always be discussed with the sponsor and/or the medical monitor. Retesting of laboratory values that lead to exclusion will be allowed once using an unscheduled visit during the screening period to assess eligibility. This visit should be at least 2 weeks later than the original screening visit.

Screen failed subjects can be re-screened up to 2 times after discussion with the medical monitor. The screening procedures to be repeated in case of re-screening will depend on the reason for re-screening and the time passed between the initial screening and the re-screening. Therefore,

the screening procedures to be repeated will be determined following discussion with the medical monitor. Re-screened subjects will be prioritized in the cohort assignment queue. A re-screening can also be only due to timing issues (e.g. government/hospital restrictions or a pause in IMP administration). In all cases, there should be no more than 6 weeks between the acceptable re-screening and Pre-dose/Baseline assessments. Re-screened subjects will retain the same temporary unique number for all assessments.

6.1.2 Run-in Period (if Applicable)

Subjects who are participating in the NHS and have at least 2 data time points available from the NHS to assess their pre-trial condition do not require a mandatory run-in visit. For these subjects, screening should be performed within 6 weeks before the baseline visit. There should be a minimum of 3 data time points to assess their pre-trial condition. The screening visit can be the third data time point. Subjects who have not participated in the NHS or who do not have at least 2 data time points from the NHS will have a mandatory run-in period before continuing to the baseline visit. Subjects will be asked to visit the site for run-in visits to assess their pre-trial condition. At least 1 run-in visit needs to be performed in order to obtain 3 data time points to assess their pre-trial condition; screening can be the first data time point, baseline can be the third data time point. Run-in visits should be repeated until the sponsor deems there are sufficient data time points for an individual subject, or until a cohort is declared open. Stand-by subjects will perform run-in visits until the last MAD subject has completed Week 13, unless needed for replacement. There should be a minimum of 28 days between screening and the first run-in visit. Thereafter, run-in visits may be performed every 12 weeks (±14 days), until the cohort is declared open. If there is only 1 run-in visit, there should be a minimum of 28 days between the run-in visit and the baseline visit. If there is more than 1 run-in visit, there should be 12 weeks (±14 days) in between the run-in visits unless it is known that the baseline visit can be scheduled within 14 days following a run-in visit; in that case, the last run-in visit should be dropped.

In case the screening laboratory results (clinical laboratory assessments, including platelet count) became available more than 12 weeks before the baseline visit (due to a long run-in period), these laboratory tests should be repeated during the run-in period.

Assessments of a single visit can be split over up to 2 consecutive days, if required. If more than 2 days are needed, approval is required from the medical monitor in advance.

The run-in period is optional for stand-by subjects who participated in the NHS and have at least 3 data time points.

6.1.3 Treatment Periods

Assessments of a single visit can be split over up to 2 consecutive days, if required. If more than 2 days are needed, approval is required from the medical monitor in advance.

6.1.3.1 Baseline Visit

If needed, all assessments that need to be performed pre-dose can be done over 2 consecutive days (i.e. Day 0 or Day 1); any exceptions to doing over 2 consecutive days due to an open biopsy with general anesthetic must be discussed with the medical monitor in advance for approval. There must be at least 24 hours between the biopsy and IMP administration. The investigator is

to get approval from the medical monitor if additional time is required to perform all needed Pre-dose/Baseline assessments.

6.1.3.2 Single Ascending Dose Part

Subjects will receive a single IV administration of DYN101 on Day 1, after which they are followed for 4 weeks up to Day 29.

There will be a subsequent washout period of at least 12 weeks: during Weeks 5-17, subjects will be asked to visit the site every 2 weeks for safety follow-up, even if they do not continue to the MAD part. Where possible, the visits will take place on the same day of the week as the visit on Day 1.

Due to restrictions imposed in response to a pandemic that restrict subject visits, please contact the medical monitor to determine the strategy for continuing the subject in the trial. All efforts must be employed to maintain subject safety. Assessments, if feasible, must be collected during a phone call with the subject. The site should arrange the collection of the blood and urine samples, if at all possible.

Every 2 weeks following the Week 17 visit, subjects will be contacted by phone by qualified site personnel to follow-up on concomitant medications and AEs, until their assigned MAD cohort has been opened.

Subjects who participated in the SAD part will subsequently participate in the MAD part (provided the IDMC has given a favorable advice to proceed, see Section 3.1.1).

6.1.3.3 Multiple Ascending Dose Part

For subjects for whom the last visit in the SAD part was more than 3 months before the baseline visit in the MAD part, there will be an unscheduled visit (physical examination and clinical laboratory parameters) which will occur between 6 weeks and 7 days prior to the MAD baseline visit. Liver ultrasound and hepatic elastography have to be performed before the baseline visit in the MAD part. The first IMP administration in the MAD part cannot be started before the liver ultrasound and hepatic elastography results are available.

Subjects will receive weekly IV administrations of DYN101 for a period of 12 weeks, i.e. from MAD Week 1 to Week 12.

Replacement of withdrawn subjects can occur under the circumstances described in Section 3.1.2. New subjects who did not participate in the SAD part should sign an ICF for participation in the MAD part, and the eligibility will be assessed (see Section 6.1.1) before the first IMP administration in the MAD part (MAD Week 1 Day 1). Replacement subjects may or may not need a run-in period, in consultation with the sponsor (see Section 6.1.2). A DNA sample (optional) and a muscle biopsy should be taken before the first IMP administration.

6.1.3.4 MAD Extension Part

After completion of the MAD part, subjects will continue on the IMP dose level of their assigned MAD cohort for an additional 12 weeks with weekly IV administrations (MAD Weeks 13-24) (unless the IDMC advises otherwise).

End-of-treatment assessments will be performed after 24 weeks of MAD treatment have been completed, i.e. at the Week 25 visit.

After Week 24, all subjects will remain on treatment with weekly IMP administrations and assessments every 2 weeks until the last MAD3 subject has completed the MAD Week 25 visit. Data collected during this period will be listed and tabulated in the clinical trial report but will not be included in the statistical modeling.

6.1.4 End of Treatment Follow-up Visit

If a subject is NOT continuing on IMP treatment after the trial, the subject will return to the clinic for an EoT follow-up visit 3 months after the last IMP administration, to follow-up on the subject's status including abnormal laboratory results, AEs, and concomitant medications. The follow-up information will be recorded in the eCRF.

6.1.5 Early Discontinuation

In case subjects are withdrawn from IMP or from the trial, there will be a follow-up visit to query for AEs/SAEs and concomitant medications. In addition, safety, PK and PD/exploratory efficacy assessments may be performed at this early discontinuation visit. The assessments to be performed at this visit correspond to the assessments of the MAD Week 25 visit (excluding IMP administration) (refer to the TRIAL SCHEDULES). In addition, subjects participating in MAD are encouraged to have a second muscle biopsy taken if the subject had at least 6 weeks of treatment during the MAD part of the trial and discontinued prior to having the Week 13 biopsy obtained; SAD subjects do not need a second muscle biopsy.

If subjects agree, they will also have an EoT follow-up visit 3 months after their last IMP administration.

6.1.6 Additional Care of Subjects After the Trial

The sponsor will consider providing IMP to subjects after the last visit in this trial, including stand-by subjects.

6.2 Trial Evaluations

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

6.2.1 Demographic and Other Baseline Characteristics

Demographic data such as age, gender, race and ethnicity, and other baseline characteristics including medical history will be recorded.

6.2.2 Safety Evaluations

The investigators, together with the medical monitor, will be responsible for the safety monitoring of the trial, and will pause IMP administration of further subjects in case any of the pre-specified pausing/stopping rules described in Section 7.11.1 occur.

An IDMC will be appointed by the sponsor before the start of the trial to perform regular reviews of the emerging safety data during the trial. Details regarding the IDMC are provided in Section 9.12.

Abnormal results for any safety evaluations, which are unexpected or not explained by the subject's clinical condition may be, at the discretion of the investigator or sponsor, repeated until confirmed, explained, or resolved as soon as possible.

6.2.2.1 Adverse Events

At each trial visit, subjects will be questioned in a general way to ascertain if AEs have occurred since the previous visit (e.g. "Have you had any health problems since your last visit?"). AEs are collected from signing of the main ICF until the last trial visit.

For definitions of AEs and SAEs, and procedures regarding reporting of SAEs refer to Section 7. Adverse events of special interest are defined in Section 7.2, and will be reported as AEs.

6.2.2.2 Clinical Laboratory Tests

Clinical laboratory parameters should be monitored at the time points specified in the TRIAL SCHEDULES.

In addition to testing of all parameters (except coagulation and erythrocyte sedimentation rate [ESR]) at the central laboratory, platelet counts will also be assessed locally prior to IMP administration and muscle biopsy. Platelet counts as determined by the local laboratory need to be within normal laboratory references ranges before IMP administration (at the time points specified in the TRIAL SCHEDULES), or before performing a muscle biopsy. The samples for platelet count verification cannot be older than 3 days. Any discrepancy between the results of the local and central laboratory should be discussed with the medical monitor before any further IMP administration. The coagulation panel and ESR will only be tested locally; they will NOT be repeated at the central laboratory. If thrombocytopenia is reported during the trial after the first IMP administration, and if an immature platelet fraction (IPF) test is available at the local laboratory, IPF test results should be obtained in addition to the platelet count. The IPF is the ratio of immature platelets to the total number of platelets, indicating the percentage of young, so-called 'reticulated' platelets.

All clinical laboratory assays will be performed according to the local or central laboratory's normal procedures. Normal reference ranges which are age- and gender-specific (as applicable) are supplied by the respective laboratories and used to assess the clinical laboratory data for clinical significance and out-of-range pathological changes. The investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant.

The following clinical laboratory assessments will be performed:

Biochemistry:

General	Liver function tests	Kidney function tests	Other
- sodium	- AST	- BUN	*Cardiac Safety* - creatine kinase myocardial band (CK-MB)
- potassium	- ALT	- creatinine	- cardiac troponin
- chloride	- ALP	- cystatin C	
- calcium	- GGT	- eGFR [a)]	
- magnesium	- bilirubin: direct, indirect, and total		
- phosphorus	- albumin		*Muscle injury*
- bicarbonate	- total protein		- creatine kinase
- glucose	- bile acids		
- CRP			
- IgG and IgM			
- LDH			

a) Calculation of eGFR will be done by the central laboratory. The same method of calculation must be maintained for an individual subject.

Complement:

- C3	- C5a
- CBb	

Hematology:

- hemoglobin	- WBC differential [b)]
- hematocrit	- neutrophils
- red blood cell (RBC) count [a)]	- lymphocytes
- RBC parameters [a)]:	- monocytes
- mean corpuscular hemoglobin	- eosinophils
- mean corpuscular hemoglobin concentration	- basophils
- mean corpuscular volume	- platelet count [c)]
- white blood cell (WBC) count [b)]	- ESR

a) RBC evaluation may include abnormalities in RBC count and/or RBC parameters and/or RBC morphology, which will then be reported by the laboratory.
b) WBC evaluation may include any abnormal cells, which will then be reported by the laboratory. In addition, any other abnormal cells in a blood smear will also be reported
c) If platelet clumping is observed in a local sample, the test should be repeated locally before IMP administration. In addition, a smear may be performed locally at the investigator's discretion.

Coagulation:

- PT [a)]	- INR
- aPTT	

a) Depending on local laboratory's routine practice Coagulation panel test result may or may not include PT.

Urinalysis (dipstick):

- proteinuria	- hematuria

6.2.2.3 Physical Examination

A complete physical examination (including height, body weight and body systems) will be performed at screening. At all other time points, a symptom-directed physical examination will be performed. At baseline and after 24 weeks of MAD treatment (Week 25 visit), the symptom-directed physical examination will include a neurologic assessment. Physical examination results will be reported as normal/abnormal. Abnormal results will include underlying disease abnormalities. A neurologic assessment will be performed at Screening, Day 1 for each SAD and MAD cohort, Week 17 for each SAD cohort, and Week 25/EoT for each MAD cohort and at EoT and EoT follow-up for any subject in the MAD extension. Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

Body weight will be measured at the indicated time points. A substantial change (as judged by the investigator) in body weight will be reported as an AE, and it will be discussed with the sponsor whether the dose calculation should be adjusted (see Section 5.2).

Treatment of any abnormality observed during this examination has to be performed according to local medical practice outside this trial or by referral to an appropriate health care provider. Abnormalities identified at screening (compared to a healthy person) will be documented in the subject's source documents and on the medical history eCRF. Worsening after screening will be captured as AEs on the AE eCRF page, when deemed clinically significant in the opinion of the investigator.

6.2.2.4 Vital Signs

Vital signs (i.e. pulse rate, systolic and diastolic blood pressure, respiratory rate, pulse oximetry, and body temperature) will be assessed in a standardized manner. Pulse rate and blood pressure will be measured after 5 minutes in supine position. Blood pressure should be determined by cuff (using the same method, the same arm, and in the same position throughout the trial). Any deviations from baseline vital signs, which are deemed clinically significant in the opinion of the investigator, will be recorded as an AE. The vital signs monitoring during the infusion should be done every 5 minutes for the first 15 minutes and every 15 minutes until the infusion is completed. If the infusion is stopped for any reason and restarted, vital signs are to be taken according to this schedule until the infusion is completed. Pulse oximetry will measure the oxygen saturation level using a small device attached to the subject's finger. The pulse oximetry device should remain in place throughout the IMP administration; any clinical findings during the continuous monitoring are to be reported. All vital signs will be noted as normal, abnormal not clinically significant, or abnormal clinically significant. Any abnormal clinically significant vital signs results are to be recorded as an AE.

6.2.2.5 Triplicate 12-Lead ECG and ECG Monitoring

Triplicate 12-lead ECGs will be recorded and interpreted locally at the specified time points. On Day 1 (SAD), a triplicate 12-lead ECG will be performed, within 30 minutes before the start of the IMP administration and within 15 minutes after the end of the infusion, and ECG monitoring will be performed during the infusion (accompanied by oximetry and vital signs monitoring). Additional ECG monitoring may be done during treatment if, in the opinion of the investigator, this is clinically indicated.

The subject will be instructed to rest in the supine position for 5 minutes before having an ECG assessment performed. If blood sampling or vital sign measurements are scheduled at the same

time point as the ECG recording, the procedures should preferably be initiated in the following order: ECG, vital signs and blood draw.

Any deviations from baseline values, which are deemed clinically significant in the opinion of the investigator, will be recorded as an AE.

6.2.2.6 Liver Ultrasound and Hepatic Elastography

A liver ultrasound including hepatic elastography to assess hepatic peliosis and other structural abnormalities will be performed during the screening period, at the baseline visit of SAD (if the screening liver ultrasound and/or hepatic elastography is older than 12 months), or before the baseline visit in the MAD part (if not performed during the screening period). The liver ultrasound and hepatic elastography results should be available before IMP administration.

Abnormalities identified at baseline will be documented in the subject's source documents and on the medical history eCRF page.

6.2.2.7 Pregnancy Test

A urine beta human chorionic gonadotropin (hCG) pregnancy test with a minimal sensitivity of 25 mL/IU will be performed on all female subjects of child-bearing potential at the specified time points, in case pregnancy is suspected, and/or upon withdrawal of the subject from the trial.

6.2.3 Pharmacokinetic Evaluations

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual, Pharmacy Manual).

6.2.3.1 Collection of Blood Samples for PK Evaluations

Blood samples for the determination of DYN101 levels in plasma will be collected by an indwelling catheter in the arm into vacuum tubes at the time points indicated in Table 3. A sample should be taken immediately after the end of the infusion. The following post-dose samples should be taken relative to the end of the infusion.

Table 3: Pharmacokinetic Sampling Schedule

Time Point	Time Relative to Dosing on Day 1 (SAD/MAD)	Sampling Window
SAD and Washout		
Week 1, Day 1	Pre-dose (0h)	
	30 min after start of infusion (i.e. during infusion)	±10 min
	Immediately after the end of infusion	±10 min
	1h after the end of infusion	±10 min
	3h after the end of infusion	±10 min
	7h after the end of infusion	±1h
Week 1, Day 2	23h after the end of infusion	±3h
Week 1, Day 3	47h after the end of infusion	±3h
Week 2, Day 8	167h after the end of infusion	±1d
Week 3, Day 15	2 weeks post-dose	±3d
Week 5, Day 29	4 weeks post-dose	±3d
Week 9	8 weeks post-dose	±3d
Week 13	12 weeks post-dose	±3d
Week 17	16 weeks post-dose	±3d

Time Point	Time Relative to Dosing on Day 1 (SAD/MAD)	Sampling Window
MAD		
MAD Week 1, Day 1	Pre-dose (0h)	
	30 min post start of infusion (i.e. during infusion)	±10 min
	Immediately after the end of infusion	±10 min
	1h after the end of infusion	±10 min
	3h after the end of infusion	±10 min
	7h after the end of infusion	±1h
MAD Week 1, Day 2	23h after the end of infusion	±3h
MAD Week 1, Day 4	71h after the end of infusion	±3h
MAD Week 2	Week 1, Day 1* 167h after the end of infusion	±1d
MAD Week 12, Day 1	Pre-dose (0h)	
	30 min after start of infusion (i.e. during infusion)	±10 min
	Immediately after the end of infusion	±10 min
	1h after the end of infusion	±10 min
	3h after the end of infusion	±10 min
	7h after the end of infusion	±1h
MAD Week 12, Day 2	23h after the end of infusion	±3h
MAD Week 12, Day 4	71h after the end of infusion	±3h
MAD Week 13	Week 12, Day 1* (167h after the end of infusion)	±1d
MAD Week 25	Week 24* (167h after the end of infusion)	±1d

* Sample to be taken before the next infusion (if applicable).

6.2.3.2 Collection of Samples for Exploratory Pharmacokinetics

Collection of Blood Samples for Anti-Drug Antibodies

Blood samples for determination of ADA will be taken in the MAD part and the MAD extension part at the time points specified in the TRIAL SCHEDULES.

Collection of Blood Samples for Metabolic Profiling

Blood samples for metabolic profiling of DYN101 will be taken in MAD Week 12 at 1 and 167 hours post-dose (see Table 3).

Collection of Urine for Metabolic Profiling

Urine for metabolic profiling will be collected in MAD Week 12 in the following manner:

- Subject should empty their bladder prior to, but as close as feasibly possible to, dose administration.
- Urine will be collected over a 24-hour interval. The window of collection will be based on the start of the infusion (e.g. if infusion start is recorded as 13:00, then the collection window will end the following day at 12:59).

Complete instructions on the collection of urine will be provided to the subject prior to the start of the collection. The subject may be confined to the clinic to collect this 24-hour urine sample.

Further details will be described in the site manuals (e.g. Laboratory Manual, Site Operations Manual).

6.2.3.3 Analytical Methods

Plasma samples will be analyzed to determine concentrations of DYN101 using a validated, specific and sensitive method.

DYN101 metabolites in plasma and urine will be identified and profiled in an exploratory manner.

A validated ELISA method will be used to determine the presence and level of anti-DYN101 antibodies in plasma.

6.2.3.4 PK Parameters

The PK parameters of DYN101 will be derived using non-compartmental analysis methods with the plasma concentration-time data from all evaluable subjects. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times. The following PK parameters will be calculated for plasma concentration values of DYN101.

Parameter	Definition
AUC_{τ}	Area under the plasma concentration-time curve during a dosing interval
AUC_{last}	Area under the plasma concentration-time curve from time 0 to time of the last quantifiable concentration
AUC_{∞}	Area under the plasma concentration-time curve from time 0 to infinity
$R_{ac(AUC)}$	Accumulation ratio based on AUC_{τ}
$R_{ac(Cmax)}$	Accumulation ratio based on C_{max}
C_{max}	Maximum plasma concentration
$C_{av, SS}$	Average plasma concentration during a dosing interval at steady state
CL	Clearance after IV administration
λ_z	Terminal disposition phase rate constant
$t_{1/2}$	Terminal disposition phase half-life
t_{max}	Time of first occurrence of C_{max}
V_z	Apparent volume of distribution during the terminal disposition phase

The effects of ADA on the PK parameters will be assessed.

6.2.4 Pharmacodynamic/Exploratory Efficacy Evaluations

The following PD/exploratory efficacy evaluations will be performed at specified time points as indicated in the TRIAL SCHEDULES. The questionnaires should be completed first, then the other assessments will be performed in the order listed:

1) QoL and health outcome questionnaires
2) EAT-10 Dysphagia questionnaire
3) Respiratory function tests
4) Muscle Strength and Function Tests:
    a. MyoGrip
    b. MFM32

All assessments, including their collection, management, and reporting, are described in detail in a separate Site Operations Manual.

6.2.4.1 Quality of Life and Health Outcome Questionnaires

The following questionnaires will be assessed:

- Patient Reported Outcomes Measurement Information System (PROMIS®) is a National Institutes of Health Initiative, created to assess patient–reported health status for physical, mental, and social well–being to reliably and validly measure patient-reported outcomes in chronic diseases for clinical research and practice. The PROMIS domains tested in this trial are Anxiety, Depression, Fatigue, Pain Interference, Ability to Participate, and Physical Function.
- Clinical Global Impression of Improvement (CGI-I) is a 7-point scale ranging from (1) very much improved to (7) very much worse and is used to assess global changes in the subject's condition compared to the condition before treatment. Wherever possible, the same clinician must complete the CGI-scale during the trial for the same subject. Results will be documented in the source documents.
- Patient Global Impression of Change (PGI-C) is a scale similar to the CGI-I. It is a 7-point scale ranging from (1) very much improved to (7) very much worse and needs to be completed independent of the investigator who completes the CGI-I, so neither party knows how the other has rated the scale. The subject (assisted by site personnel if needed) will complete PGI-C scale in the subject's source documents.
- Goal Attainment Scaling is an instrument that is intended for individual evaluation of an intervention. It allows subjects to set individual goals, together with their physician. The number of goals and the content of these goals may differ per subject, but the attainment of the goals is measured in a standardized way. This makes a standardized evaluation of an intervention possible, even when the subjects are all in a different stage of their disease. The physician selects objectives for each affected domain (respiratory, muscle strength and function, dysphagia) together with the subject, and records this in the eCRF. At the final assessment, the goal is evaluated on a 3-point scale by the physician and the subject.

6.2.4.2 Dysphagia Questionnaire

Dysphagia is a relatively frequent problem in patients with CNM, particularly in those with XLCNM, and increases the risk of aspiration pneumonia, requiring the use of gastrostomy feedings. The Eating Assessment Tool (EAT-10) questionnaire will be used to assess swallowing difficulties and will be completed by the subject.

6.2.4.3 Respiratory Function Tests

Assessment of the respiratory function will be performed with a handheld device, by qualified site personnel. To prevent air leaks, the nose will be blocked by a nose clip for every respiratory assessment.

For each parameter, the attempt should be repeated at least 3 times making sure that the subject has recovered between attempts. If, at each attempt, there is an improvement in the results, further tests can be conducted until the subject has achieved his/her best result.

The following measurements will be performed:

- **Forced Vital Capacity** (FVC): the maximum amount of air that a person can exhale as hard and as long as possible from the lungs after a maximum inspiration.
- **Forced Expiratory Volume** (FEV1) in the first second of exhalation: FEV1 is the most frequently used index for assessing airway obstruction, bronchoconstriction or bronchodilatation. The ratio of FEV1 and FVC is the standard index (also called Tiffeneau index) for assessing and quantifying airflow limitation.
- **Maximum Expiratory and Inspiratory Pressures** (MEP and MIP): these measurements assess the overall respiratory muscle strength. MIP reflects the strength of the inspiratory muscles, while MEP reflects the strength of the abdominal muscles and other expiratory muscles. This examination can detect early respiratory muscle weakness before the onset of a decrease in vital capacity.

Measurements will be expressed as percent of predictive value.

In addition, ventilation requirements will be recorded in the eCRF.

6.2.4.4 Muscle Strength and Function Tests

All muscular testing will be done by a certified physiotherapist.

The measurements will take place in a quiet and acclimatized room. All tests will be performed with the subject sitting in a regular chair or in their wheelchair facing a table, preferably adjustable in height with the forearm placed on the table or on the wheelchair shelf. Before each test, subjects will be given a description of the task, a demonstration of the expected movement and advice on the correct practice. If necessary, the correct body position will be manually guided. If standard upper limb position described in the assessment protocol (which will be provided to the sites) cannot be maintained because of contractures, an alternative position will be allowed.

The subjects will perform the strength tests first (MyoGrip), followed by the functional tests (MFM32). To limit fatigue, only the subject's dominant side will be tested, i.e. the hand preferred for fine motor skills such as writing. Each strength test must be repeated at least 3 times or repeated until the last attempt is less successful than the previous one. The retained value will be the maximal reproducible strength and should be within 10% of another attempt. Subjects will be given a 3-minute rest between each test.

Functional tests will be video-recorded after signed consent to constitute a visual record of the subject's ability. The subject or his/her representative have the right to refuse the video recording or request filming of only a specific frame (e.g. only the hand), a specific time period, or anonymization of the recording. In case the video will be used outside of the study team involved in the trial as a visual representation of the effect, the subject will be asked for a separate consent.

- **MyoGrip** is an extremely sensitive tool to measure muscle strength in severely disabled subjects where current tools are unable to measure changes or benefit (Servais et al., 2013). For disorders such as CNM, the ability to use the joystick of their electric wheelchair or other fine motor functions is important to the subject. These sensitive measures can also assist in detecting early signals of efficacy of an administered drug (Seferian et al., 2015).
- **Motor Function Measure** (MFM) is classically used as a measure of functional capacities in subjects with neuromuscular disorders (Bérard et al., 2005). The MFM 32-item list (MFM32) is a scale to enable an objective assessment of the motor abilities of subjects with neuromuscular diseases whatever the motor function deficiency. Three dimensions are taken into account: D1: standing position and transfers, D2: axial and proximal motor function, and D3: distal motor function.

6.2.5 Collection of Optional Samples for Exploratory Research (Blood Biomarkers)

Samples will be taken and stored from subjects who signed a separate ICF for further exploratory research (refer to Section 10.4.4). Blood-based biomarkers allow to explore how biomarker/genetic variations may affect the clinical parameters associated with the response to DYN101 and potentially the basis of CNM.

If the subject is very light weight, anemic, or presenting with symptoms of fatigue, main study samples are to be taken first, exploratory samples may to be skipped due to the subject's status.

A blood sample for DNA will be taken only once at SAD baseline (or at MAD baseline for replacement subjects who did not participate in SAD) and blood samples for biomarkers will be taken at the time points defined in the TRIAL SCHEDULES.

6.2.6 Muscle Biopsy

Muscle biopsies will be performed at the time points defined in the TRIAL SCHEDULES. Details will be described in the Site Operations Manual.

Muscle ultrasound imaging of both the vastus lateralis and biceps brachii will be performed at screening to determine which muscle should be biopsied. This imaging should not be older than 6 months at baseline, otherwise it should be repeated. Muscle biopsies should be obtained from the vastus lateralis according to the site's standard practice (open or needle biopsy) if sufficient muscle mass is identified after ultrasound imaging and muscle depth and location allow. In the event inadequate muscle tissue is identified in the vastus lateralis after ultrasound imaging, or it is determined that the location of a suitable vastus lateralis muscle is not easily accessible, a biopsy should be obtained from the biceps brachii (open or needle biopsy). For subjects undergoing an open muscle biopsy, general anesthesia can be applied. There should be 24 hours between the muscle biopsy and the IMP administration.

Platelet counts need to be verified before a muscle biopsy is performed (the samples for verification of the platelet counts cannot be older than 3 days). The muscle biopsy should be the last assessment at the visit. Platelet counts will need to be verified again before IMP administration (local assessment) only if the previous platelet count is more than 3 days old.

The level of *DNM2* mRNA as well as the concentration of DYN101 will be measured. This evaluation will allow for a measure of target engagement (i.e. the amount of decrease of *DNM2*) and will support the start of the MAD3 cohort and a dose selection for further clinical trials based on the PK/PD modelling, in addition to preclinical data, and results of some of the exploratory efficacy assessments.

In addition, a section of the muscle will be prepared for histology.

In case sufficient muscle biopsy material is left, the material will be stored for further exploratory research (see Section 10.4.4).

6.2.7 Volume of Blood to be Drawn from Each Subject

All blood samples will be collected via an indwelling catheter. The volume of blood to be drawn from each subject is presented in Table 4.

Table 4: Volume of Blood to be Drawn from Each Subject

Assessment	Sample Volume (mL)	Number of Samples [a)]	Total Volume (mL)
Safety samples – clinical laboratory tests			
Biochemistry (8.5 mL)	8.5	16	136.0
Biochemistry (5.0 mL)	5.0	19	95.0
Bile Acids	3.5	35	122.5
Complement	5.0	1	5.0
Hematology	2.0	31	62.0
Coagulation - local	4.5	31	139.5
Platelet count – local	1.0	31	31.0
Cardiac troponin	2.5	14	35.0
Erythrocyte sedimentation rate - local	2.0	30	60.0
PK samples [b)]			
PK evaluations	4.0	33	132.0
Anti-drug antibodies	4.0	5	20.0
Metabolic profiling	4.0	2	8.0
Blood biomarkers samples (optional)	9.0	6	54.0
DNA sample (optional)	2.5	1	2.5
Total Volume of Blood (mL)	**57.5**		**902.5**

a) Not including any additional samples taken after MAD Week 25.
b) If a catheter is used, the first 1 mL is to be discarded and 3 mL is taken into an appropriate tube for PK samples. Therefore, a total of 4 mL is indicated as the sample volume.

Note: The above volume of blood to be taken for each assessment is an estimate. The volume of blood to be taken may vary according to the instructions provided by the manufacturer or laboratory for an individual assessment. The estimated total blood volume does not include blood draws during unscheduled visits. When more than 1 blood assessment is to be done at the same time point/period, if they require the same type of tube, the assessments may be combined.

The total blood volume to be collected is considered to be within the limits of standard blood donation.

6.2.8 Sample Management

Samples will be labeled with the protocol number, the subject's trial identification number and information related to the sample. No personal identifiers (e.g. date of birth, subject initials) will be recorded on the sample labels. Leftover samples may be used in the future for additional exploratory research.

Sample collection and processing will be performed by qualified site personnel according to current versions of approved standard operating procedures. The Laboratory Manual contains further details regarding the collection, handling, labeling, and shipment of biological samples to the respective laboratories.

7. SAFETY DEFINITIONS, REPORTING AND FOLLOW-UP

7.1 Adverse Event Definitions

An AE, an adverse drug reaction (ADR), and an SAE are defined according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline E2A.

An AE is any untoward medical occurrence in a subject administered the IMP and which does not necessarily have a causal relationship with this IMP or the IMP administration procedure. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the IMP including the IMP administration procedure. The definition also covers medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

An ADR is an untoward and unintended response to the IMP related to any dose administered. A causal relationship between the IMP and the AE is at least a reasonable possibility.

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it was more severe)
- Requires in-subject hospitalization or prolongation of existing hospitalization
    - The hospitalization of a subject who decides to stay in the hospital overnight in advance or following IMP administration, for convenience based on personal choice or at the recommendation of the investigator without the presence of an AE warranting such hospitalization, is not considered an SAE.
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

- Is judged medically important by the investigator (this refers to an event, not resulting in any of the outcomes listed above, but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed)

A suspected unexpected serious adverse reaction (SUSAR) is any suspected adverse reaction that is both serious and unexpected.

The IB should be referenced for events that are considered expected.

In the following situations events are not defined as an AE:

- Medical or surgical procedure (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the trial that do not worsen.
- Condition(s) for which pre-planned procedure(s) have been recorded at screening, including hospitalization(s), unless the condition(s) for which the procedure and/or hospitalization was planned has worsened from the first trial-related activity after the subject has signed the main ICF.
- Concomitant illness identified during the screening procedures will be recorded as medical history. However, whenever symptoms for these condition(s) worsen and/or become serious, then these events must be reported as an AE or SAE, as applicable.

7.2 Adverse Events of Special Interest

The events described in this section will be reported as AEs, will be marked as AEs of special interest in the eCRF, and reported within 24 hours of the investigator becoming aware of the AE of special interest.

Thrombocytopenia, impaired hepatic or kidney function are AEs of special interest, as they have been described with the use of ASOs previously, both in the preclinical and the clinical setting. Therefore, subjects experiencing reduced platelet counts or showing signs of impaired hepatic or kidney function, as evident by laboratory testing (see Section 7.11.2) should be examined by a physician as soon as possible and retested.

Vasculitis is an AE of special interest as vasculitis which appeared to be associated with chronic complement activation with subsequent depletion and inflammation was observed in a 9-month preclinical study with DYN101 in NHP. Therefore, subjects with any suspicion for vasculitis (vasculitis of any origin, including the complement pathway) will trigger a consult with an appropriate specialist for further investigation.

Although there is no clinical experience with DYN101 yet, some ASOs have been associated with an increased risk for thrombocytopenia. All subjects enrolled in clinical studies with DYN101 must be informed of the potential risk of severe thrombocytopenia.

The investigator will diligently monitor each subject's platelet count and must be in receipt of an interpretable platelet result before a scheduled IMP administration on the time points indicated in the TRIAL SCHEDULES. Investigators should ensure that subjects are queried about the possibility of spontaneous bleeding at routine study visits.

Subjects must also be instructed to urgently notify their doctor if they develop any symptoms consistent with thrombocytopenia or abnormal bleeding. It is recommended that subjects should be provided with an information leaflet describing these alert symptoms. These symptoms are as follows:

- Nosebleed, bleeding from gums (either spontaneously or on brushing teeth), dark or bloody stools or blood in urine, vomiting blood, unusually heavy menstrual bleeding (period), or other unusual bleeding (for example prolonged bleeding after a cut or abrasion);
- More frequent or easy bruising or bruising without injury;
- Pinpoint-size red or purple spots on the skin, in the lining of the mouth, or the white of the eye;
- Neck stiffness, unusual drowsiness, worsening headache, blurred or double vision;
- Worsening abdominal pain.

As there are no validated specific diagnostic markers to detect vasculitis, monitoring and assessment should be based on a high level of clinical awareness/suspicion and on physical examination and objective studies specific to the end organs that could be involved in drug-induced small or medium vessel vasculitis. These include, but are not limited to, the heart, kidney, lungs, liver, peripheral nervous system, and skin. In order to monitor for potential cases of vasculitis the following aspects need to be taken into account:

- The physical examination performed by the investigator should include head, eyes, ears, nose, throat, heart, lungs, abdomen, extremities, examination of the skin for purpura, as well as neurological assessments for any new onset of paresthesia or dysesthesia.
- Laboratory parameters, including cardiac troponin, complete blood count, clinical chemistry, liver and kidney function tests, urinalysis, and acute phase reactants like CRP and ESR have to be reviewed with the awareness of potential signals of end organs and non-specific markers of inflammation.

In case of suspicion of vasculitis, refer to the Site Operations Manual for guidance on further work-up and consults with appropriate specialists.

The investigator should also consider whether alternative explanations for the finding may be likely (e.g. recent antibiotic use when the subject presents with a rash) and how characteristic the finding is of vasculitis (e.g. a non-specific skin rash should be further investigated, but a purpuric rash should raise a higher level of suspicion, as it is more suggestive of vasculitis).

7.3 Clinical Laboratory Abnormalities and Other Abnormal Assessments as Adverse Events or Serious Adverse Events

Laboratory abnormalities without clinical significance based on the investigator's judgment, are not considered AEs or SAEs. However, laboratory abnormalities (e.g. clinical chemistry, hematology) or other abnormal (clinical trial-specific) assessments (e.g. ECG, vital signs) that require medical or surgical intervention, are associated with signs and/or symptoms, lead to IMP

interruption, modification, or discontinuation must be reported as an AE or SAE if they meet the definitions as described in Section 7.1. If the laboratory abnormality is part of a syndrome, the syndrome or diagnosis is to be reported (e.g. anemia instead of decreased hemoglobin).

If a laboratory abnormality meets the criteria of a pausing stopping rule, appropriate action should be taken, as explained in Section 7.11.

7.4 Overdose

An overdose is defined as a known deliberate or accidental administration of IMP to or by a subject, at a dose greater than 110% of the dosage assigned to that individual subject according to the trial protocol.

All cases of overdose (with or without associated AEs) will be documented in the eCRF. Cases of overdose without manifested signs or symptoms are not considered AEs. AEs associated with an overdose will be documented in the eCRF.

SAEs associated with overdose should be reported according to the procedure outlined in Section 7.7.

In the event of overdose, the subject should be treated symptomatically.

7.5 Adverse Event Assessment Definitions

7.5.1 Severity

The investigator should assess the severity of all AEs according to the following definitions:

- **Mild**: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- **Moderate**: A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.
- **Severe**: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

For reporting of (S)AE related laboratory abnormalities, the severity (intensity) needs to be evaluated by the investigator in the accordance with the defined criteria for assessment of laboratory value abnormalities, following the CTCAE criteria (version 5, November 2017) (the investigator will be requested to provide the values for severity grades,).

Note the distinction between seriousness and severity: the term severe is used to describe the intensity of the event and a severe event is not necessarily serious (e.g. a severe headache would probably not constitute an SAE; however, a mild myocardial infarction could constitute an SAE). The seriousness criteria serve as a guide for defining regulatory reporting obligations.

If an AE changes severity over time, a new AE has to be reported with the new severity.

7.5.2 Relationship to Investigational Medicinal Product

The investigator must assess the causal relationship of the IMP for each (S)AE. The investigator should decide whether, in his or her medical judgment, a possibility exists that the event may have been caused by the IMP. If there is a valid reason, even if undetermined or untested, for suspecting a cause-and-effect relationship between the IMP and the occurrence of the AE, then the AE should be considered 'related'. If there is no valid reason for suggesting a relationship, i.e. when a relationship to IMP is excluded, then the AE should be classified as 'not related'. The causality assessment must be documented in the source document.

The following additional guidance may be helpful:

Term	Relationship	Definition
Related	Yes	As a therapeutic dose has not yet been established for the IMP, at this time, all noxious and unintended responses to the IMP are considered to be related to the IMP unless they are reasonably attributed to another cause. There is a temporal relationship between the event and the administration of the IMP.
Not Related	No	The event can be readily explained by other factors such as the concomitant therapy or accident and no plausible temporal or biologic relationship exists between the IMP and the event. In addition, this assessment can be used in cases where the subject did not receive any treatment with IMP.

7.6 Reporting of Adverse Events

All events meeting the definition of an AE must be reported from signing of the main ICF onwards until the last trial visit. Only medically qualified personnel (investigators) must assess AEs.

AEs must be reported by the investigator in the source data and the eCRF. The diagnosis will be recorded, if available and applicable. If no diagnosis is available, each sign and symptom will be recorded as individual AEs in the eCRF.

Recurring AEs with fluctuations in severity should be reported separately, i.e. with separate start date and time and stop date and time. Recurring AEs for which the severity is stable, could be reported as intermittent AEs, with the start date being the start of the first episode of the AE. If the severity changes, the intermittent AE should get a stop date, and a new AE with the new severity should be reported.

Investigators will receive copies of initial and follow-up expedited safety reports (unexpected SAEs that are determined to be associated with the use of IMP) from the sponsor or designee. The investigator is responsible for fulfilling applicable local reporting requirements to their IEC/IRB. Investigators must forward copies of the IEC/IRB notification to the sponsor or designee. In the European Union (EU), the sponsor or designee is responsible for notifying the European Medicines Agency (EMA), IEC/IRB, and competent authorities. In regions/countries other than the EU, reporting of events to IEC/IRB or local authorities will be performed by the investigator/sponsor or designee and in accordance with local procedures/regulations.

7.7 Prompt Reporting of Serious Adverse Events

All events meeting the definition of an SAE must be reported from signing of the main ICF onwards until the last trial visit. SAEs must be reported immediately to the sponsor as soon as the investigator determines that the event meets the protocol definition for that event. Further details will be described in the site manuals (e.g. Site Cohort Management Manual, Site Operations Manual).

The information will be reported on the SAE eCRF and will include assessment of seriousness, severity, causal relationship to the IMP or trial procedures, outcome, and a narrative description of the course of the event. Additional information may be subsequently provided.

All SAEs, regardless of causality, must be reported to the sponsor or designee (the contract research organization [CRO] responsible for Pharmacovigilance) within 24 hours of the first awareness of the event in the eCRF. In the event the eCRF system is unavailable, a paper back-up SAE form should be completed and submitted (see Emergency Contact Information).

All relevant documents including but not limited to diagnostic procedures, hospital records, autopsy reports after redaction of all subject identification details must be faxed or e-mailed (see Emergency Contact Information).

SAEs occurring in a subject after the subject has completed the clinical trial and for which a reasonable possibility of a causal relationship is assessed by the investigator should be reported by the investigator to the sponsor, if the investigator becomes aware of them regardless of the time that has elapsed (post-trial events).

7.8 Regulatory Reporting Requirements for Serious Adverse Events and Other Events

Prompt notification of SAEs by the investigator to the sponsor is essential, so that legal obligations and ethical responsibilities towards the safety of subjects are met. This is particularly true for this trial as this involves the first use of DYN101 in humans; safety concerns drive the Pausing/Stopping rules for the trial (Section 7.11.1). Further details will be described in the site manuals (e.g. Site Cohort Management Manual, Site Operations Manual).

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. The sponsor will comply with the ICH/EMA and country-specific regulatory requirements relating to safety reporting to the regulatory authority, IEC/IRB, and investigators.

Expedited reporting of SUSARs will be taking place within 7 or 15 days depending on fatal/life threatening status, seriousness, expectedness, and causality from all clinical trials globally (SUSAR reporting in EU). Any suspected adverse reaction that is both serious and unexpected according to the IB will be reported provided there is evidence to suggest a causal relationship between the IMP and the AE.

Relevant follow-up information to a SUSAR will be submitted as soon as the information is available.

Information about SUSARs is also forwarded to the investigators and IEC/IRB. Additionally, safety information will be forwarded to Ionis Pharmaceuticals, Inc, owner of DYN101.

An investigator who receives a SUSAR describing (an) SAE(s) or other specific safety information (e.g. summary or listing of SAEs) from the sponsor will file it with the IB, and will notify the IEC/IRB, if appropriate according to local requirements.

In addition to submission of SAEs, an annual development safety update report will be prepared and submitted to the EMA (and locally, if applicable), according to the development international birth date.

7.9 Reporting of Pregnancies

Should a pregnancy occur in a female subject or female partner of a male subject, it will be recorded separately from AEs, but will be reported by the investigator in a manner identical to the reporting of SAEs (see Section 7.7), by completing the pregnancy eCRF.

All attempts will be made to follow the pregnancy until the outcome of the pregnancy has been determined, and to capture information on the development of the infant in the period up till and including the age of 1 year. This information will only be collected if separate informed consent is given by the subject and the subject's sexual partner.

Any report of a congenital abnormality/birth defect is an SAE and should be reported as such. Any complication of a pregnancy occurring during this trial, including elective termination for medical reasons, must be reported in the pregnancy eCRF.

In the event the eCRF system is unavailable, a paper back-up SAE form should be completed and submitted (see Emergency Contact Information).

7.10 Follow-up on Adverse Events

All AEs and SAEs should be followed until stable, resolved or they have reached a "final outcome" or the subject's participation in the trial ends, whichever comes first.

Adverse events assessed as "related" to IMP and all SAEs and AEs of special interest (regardless of their relationship to IMP) still ongoing after the end of trial participation (i.e. after the follow-up call) should be followed on a regular basis according to the investigator's clinical judgment until the condition is stable.

The outcome "recovering" can be used as the "final outcome" for events that are stabilized (i.e. no further worsening is expected) and expected by the investigator to resolve over time.

The outcome "not recovered" can be used as the "final outcome" for events that are not expected to resolve over time (e.g. cancer).

The outcome "unknown" can be used as the "final outcome" for events for which the outcome is not known.

If a subject dies during the reporting period, attempts should be made to obtain muscle biopsy material to assess muscle histology, ASO concentration, and *DNM2* mRNA.

Follow-up information received after the end of the trial should be sent to the sponsor.

7.11 Pausing/Stopping Rules

7.11.1 Pausing/Stopping Rules for the Trial

The investigators, together with the medical monitor, will be responsible for the safety monitoring of the trial, and will pause IMP administration in case any of the pre-specified pausing/stopping rules have been met. The sponsor's medical lead will be involved in all discussions and decisions.

If any of the following events occur in any subject in any cohort who received at least one IMP administration in the trial (at any site), the investigator will pause the IMP administration of further subjects in all cohorts and will notify the medical monitor immediately. The medical monitor will inform all the other investigators to pause further IMP administration as well and will notify the IDMC immediately.

- Death in any subject, considered related to IMP; OR
- An anaphylactic reaction within 24 hours of the start of the infusion or the presence of generalized urticaria within 72 hours of the start of the infusion in any subject, considered related to IMP; OR
- An SAE in any subject, considered related to IMP.

  Events which would not meet the hospitalization criterion to be reported as an SAE for this trial are:

  - Overnight stay for convenience based on the subject/caregiver personal choice
  - Overnight stay offered by the investigator based on non-clinical grounds
  - For observation of minor reactions which would not on their own warrant admission to hospital, but is preferred in this vulnerable population; OR
- ALT or AST > 8 × ULN, considered related to IMP and confirmed by repeat testing.

Subjects experiencing any of the events defined above will be permanently withdrawn from IMP administration and will discontinue the trial (see Section 4.5).

Within 3 business days, the IDMC will convene to review the available safety data as outlined in the charter and to discuss further actions (e.g. continue either as planned or with modifications, or to stop the trial). In the event a stopping rule is met, the national authorities will be informed through a substantial amendment. The sites will be allowed to resume activities after the national authorities have received, reviewed, and approved the available safety data and any protocol amendment, if any, and upon receipt of a written notification from the sponsor to allow continued treatment and enrollment.

In case the pause is lifted, withdrawn subjects can be replaced to maintain full enrollment within the sub-cohort under the circumstances described in Section 3.1.2.

Subjects who were awaiting the start of their cohort and whose screening period is longer than the protocol-defined 6 weeks as a result of a pause in IMP administrations will be allowed to be re-screened in line with the re-screening procedures outlined in Section 6.1.1.

7.11.2 Individual Treatment Interruptions and Dose Adjustments

As thrombocytopenia is a known side effect of ASOs, it is important to always check that the subject has a normal platelet count before dosing. If a decreased platelet count is detected (i.e. value < lower limit of normal [LLN]), a retest should be performed immediately in the local laboratory. If the retest confirms a platelet count value < LLN, the subject may NOT be dosed.

Treatment interruptions will be considered in the event of any suspicion of vasculitis or in the event of any of the following situations that may occur, considered related to IMP:

- Platelet count is between 75,000 and 150,000/µL; in that case the subject can be dosed as soon as the platelet count has returned to a value > LLN and dosing can resume at the same dose.
- Platelet count is < 75,000/µL; in that case the subject can be dosed as soon as the platelet has returned to a value > LLN, however dosing should be resumed at 50% of the previous dose.
- Platelet count is < LLN and a previous dose interruption had taken place because of decreased platelet values; in this case, the medical monitor should be contacted to discuss possible additional treatment interruptions and/or dose adjustments.

Furthermore, treatment interruptions and/or dose adjustments for an individual subject should be considered in the event of any of the following events:

- Elevation of AST and/or ALT > 3 × ULN
- Elevation of bilirubin > 2 × ULN
- Elevation of ALP > 1.5 × ULN
- Elevation of ALT and/or AST in temporal association with nausea, vomiting, anorexia, abdominal pain, or fatigue
- Clinically significant deteriorating kidney function during treatment
- Any other clinically significant deterioration during treatment considered related to IMP.

In these instances, the medical monitor should be contacted to discuss potential treatment interruptions and/or dose adjustments.

In case a treatment interruption is needed for any safety reason for more than 4 consecutive weeks, or if there is a second treatment interruption on the initial or reduced dose level, the site should always contact the medical monitor to determine the best strategy for the subject, considering the individual risk-benefit ratio.

Note: If treatment is interrupted for circumstances other than safety reasons, the site should also contact the medical monitor to determine whether it is allowed or possible to restart treatment, and if so, at what dose.

8. DATA MANAGEMENT

8.1 Data Collection

The investigators' authorized site personnel must enter the information from the source data (see Section 10.2.3.2), as required by the protocol, in the eCRF (see Section 10.2.3.1). A monitor will visit each site in accordance with the monitoring plan and review the eCRF data against the source data for completeness and accuracy. Discrepancies between source data and data entered in the eCRF will be addressed by qualified site personnel. When a data discrepancy warrants correction, the correction will be made by authorized site personnel. Data collection procedures will be discussed with the site personnel at the site initiation visit and/or at the investigator's meeting.

8.2 Clinical Data Management

Data are to be entered into a clinical database as specified in the Data Management Plan. Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections have to be documented in an auditable manner.

9. STATISTICAL METHODS

9.1 Statistical Analysis

Statistical analysis will be done by the sponsor or under the authority of the sponsor. Statistical analyses will be performed using Version 9.2 or higher of SAS® (SAS Institute, Cary, NC 27513). Details regarding the statistical methods and definitions will be provided in the Statistical Analysis Plan (SAP), and will include templates for the tables, figures and listings to be provided. The SAP will be finalized before the database lock; output for the IDMC will be finalized before the first subject receives the first dose of IMP. Any deviations from the SAP are to be justified in the clinical trial report. A general description of the statistical methods to be used to analyze the safety, PK, PD and preliminary efficacy data is outlined below.

In general, descriptive statistics and/or frequency tabulations will be presented overall and by dose (cohort), for single or multiple administration, and by mutated gene (sub-cohort). Listings and figures presenting individual data will be generated as appropriate.

Endpoints are described in Section 3.3.

An interim analysis will be performed when all subjects in cohorts 1 and 2 have completed 12 weeks of MAD treatment or discontinued earlier, or if the sponsor deems a risk/benefit analysis necessary at any time point.

The primary analysis will be performed when all subjects in all cohorts have completed 12 weeks of MAD treatment or discontinued earlier.

The final analysis will be performed when all subjects have completed 24 weeks of MAD treatment (12 weeks in the MAD part + 12 weeks in the MAD extension part; Week 25 visit) or discontinued earlier.

The interim, primary and final analyses will be performed on the Safety Analysis Set (see Section 9.3) for the primary objective. Secondary and exploratory objectives will be conducted on the Pharmacokinetic Set. Safety analyses (AE, clinical laboratory tests, physical examination, vital signs, and ECG) will be performed on the Safety Analysis Set. Results from the interim, primary, and final analysis will be described in the clinical trial report.

Note that after Week 24, all subjects will remain on treatment with weekly IMP administrations and bi-weekly (every 2 weeks) assessments until the last MAD3 subject has completed the MAD Week 25 visit. The data from this period for these subjects will be tabulated and listed in the clinical trial report but will not be taken into account in the statistical modeling.

9.2 Planned Interim Analysis and Adaptive Design

To allow robust data, an interim analysis will be performed when all subjects in Cohorts 1 and 2 have completed 12 weeks of MAD treatment or discontinued earlier, or if the sponsor deems a risk/benefit analysis necessary at any time point.

9.3 Selection of Subjects to be Included in the Analyses

Pre-Screened Set – Subjects who have a signed pre-screening consent.

Screened Set – Subjects who have signed main informed consent.

Safety Analysis Set – Screened subjects who have received at least 1 IMP administration.

Pharmacokinetic Set – Subjects in the Safety Analysis Set for whom the primary PK data are considered sufficient and interpretable.

9.4 Subject Disposition

Subjects in each analysis set as well as subjects who complete the trial or who discontinue from the trial will be summarized by sub-cohort using frequency tabulations. In addition, for subjects who discontinue from the trial, the reasons for discontinuation will be summarized by cohort.

9.5 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be presented for the Safety Analysis Set. Continuous variables such as subject age, body weight, height and body mass index will be summarized using the number of observations, mean, standard deviation, median, minimum and maximum values. Categorical variables such as subject gender and race/ethnicity will be summarized using the number of observations and percentages for each category.

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized using the number of observations and percentages of subjects reporting each category.

9.6 Investigational Medicinal Product Exposure

Descriptive statistics for the duration of exposure to IMP will be calculated. The number of subjects receiving each dose and compliance rates will be summarized at each scheduled time point.

9.7 Prior and Concomitant Medication

Prior and concomitant medications will be coded using the World Health Organization (WHO) drug dictionary and summarized using the number of observations and percentages of subjects reporting for each preferred drug name.

9.8 Safety Analyses

Safety analyses will be presented for the Safety Analysis Set.

Baseline for all clinical laboratory tests, physical examination results, vital signs, liver ultrasound and hepatic elastography, and ECG will be defined as the last assessment prior to IMP administration.

Safety data will be analyzed using descriptive statistics (arithmetic mean, geometric mean, SD, %CV, median, minimum and maximum values, including 95% confidence intervals (CIs), if applicable).

Adverse Events

AEs will be coded using the MedDRA version available at the start of the trial. The number of events as well as the incidence and percentage of subjects with treatment-emergent adverse events (TEAEs) will be calculated overall, by system organ class, and by preferred term. TEAEs will be further summarized by severity and relationship to IMP. AEs related to IMP, AEs of special interest, AEs leading to withdrawal, SAEs and deaths will be summarized or listed.

Clinical Laboratory Tests

Descriptive statistics of observed values and changes from baseline will be calculated for each laboratory analyte at each scheduled time point. The frequency counts and percentage of subjects with laboratory abnormalities will be tabulated at each scheduled time point. The laboratory abnormalities will be determined according to the CTCAE criteria (version 5, November 2017) and in accordance with the normal ranges of the clinical laboratory if no gradings are available. Potentially clinically important findings will also be summarized or listed.

Physical Examination

Physical examination abnormalities will be listed at each scheduled time point.

Vital Signs and ECG

Descriptive statistics of observed values and changes from baseline will be calculated for each vital signs and ECG parameter at each scheduled time point. The frequency counts and percentage of subjects with abnormalities for each parameter will be tabulated at each scheduled time point.

Liver Ultrasound and Hepatic Elastography

The observed data for liver ultrasound and hepatic elastography assessments will be listed.

9.9 Pharmacokinetics Analyses

Plasma concentrations over each scheduled collection time and PK parameters of DYN101 will be summarized for each dose using descriptive statistics (arithmetic mean, SD, %CV, median, minimum and maximum). In addition, geometric mean and CV will be computed for C_{max} and AUCs.

To assess dose proportionality (as appropriate) following single or multiple doses in all cohorts, regression analysis of natural logarithm (log) transformed C_{max}, AUC_{last} and AUC_{∞} will be performed on log(dose). The power fit will be assumed as described by the following equation:

$$\log(\text{PK parameter}) = \beta_0 + \beta_1 \log(\text{Dose}) + \varepsilon$$

Where β_0 is the intercept and β_1 is the slope. Dose will be the weight adjusted dose.

The 90% CI of the slope estimate will be presented. Dose proportionality will be declared when the 90% CI for β1 lies entirely within the critical region:

$$(1+\log(0.8)/\log(r)) < \beta_1 < (1+\log(1.25)/\log(r))$$

Where r is the ratio of the highest and the lowest dose in this trial. This criterion implies that the 90% CI for the ratio of the central values of PK parameter of interest from the highest dose to the lowest dose is contained completely within the bioequivalence range of (0.80, 1.25).

One-way analysis of variance will be performed for λ_z, with dose as a fixed effect to evaluate the effect of dose on λ_z.

In addition, the impact of immunogenicity (ADA) on the PK of DYN101 will be explored. Furthermore, the metabolic profile of DYN101 will be described.

Any additional analysis that is deemed necessary will be presented in the SAP.

An exploratory population PK model might be developed, of which the results will be reported in a separate report.

9.10 Pharmacodynamics/Exploratory Efficacy Analyses

Descriptive statistics (arithmetic mean, geometric mean, SD, %CV, median, minimum and maximum) will be used to summarize the PD/exploratory efficacy data. Changes from baseline (where applicable) will also be tabulated.

Advanced analyses and models will be used to summarize in a more effective way the potential effect of the treatment and are not aimed for inferential purposes since the trial is not powered for that purpose.

For the respiratory function and the muscle strength and function tests data available from the NHS or the run-in period, an appropriate mixed effect model for repeated measurements will be applied to all the data to evaluate the evolution over time on a subject basis and to compute the

probability to observe the data under treatment assuming a normal disease progression. The model will include necessary covariates such as dose, demographic data, mutated gene, and biomarker values. Depending on the test, transformations may be applied to the results to ensure use of the appropriate model. For tests providing bounded scores, the normalized data between 0 and 100% will be evaluated using a Beta sampling distribution. For unbounded scores, logarithmic transformations will be applied by default since these are strictly positive values. The selection of covariates such as demographic data and biomarker value will be made using quality of fit criteria such as Aikaike Information Criteria.

The analysis of the blood biomarker data will be explorative and will not be performed as part of this trial.

9.11 Sample Size Justification

The sample size of 18 subjects treated with DYN101 is deemed to be appropriate to evaluate the safety and tolerability in a first-in-human trial. No power analysis was conducted to support the sample size.

9.12 Independent Data Monitoring Committee

An IDMC will be involved in the management of this trial. The purpose of the IDMC is to review the safety and tolerability and muscle biopsy data (where applicable) of the cohorts, before proceeding to the next dose level, as specified in Section 3.1.1.

The committee will meet periodically (see Section 3.1 and Figure 2 for the 4 planned IDMC meetings) to monitor the safety data on a regular basis to ensure the continuing safety of the enrolled subjects. The IDMC may meet at any time based on emerging safety and tolerability data. At a minimum the IDMC must meet after all subjects of a cohort have been dosed to advise to continue each *MTM1* and/or *DNM2* sub-cohort to the next dose level/or discontinue the *MTM1* and/or *DNM2* sub-cohort of the trial. Escalation of each *MTM1* and/or *DNM2* sub-cohort to the next increased dose level will not take place until the IDMC has reviewed the data of the previous (sub-)cohort (including subjects who have discontinued the trial). The data reviewed by the IDMC for dose escalation decisions will be as clean as possible prior to their review. The IDMC can recommend adjusting dose levels or stopping the dose escalation scheme for each *MTM1* and/or *DNM2* sub-cohort and/or individual subject based on the totality of available data (safety and tolerability, level of target engagement and/or efficacy). At each IDMC review, different decisions for the sub-cohorts (*DNM2* vs *MTM1*) can be made based on the emerging data. If needed, the IDMC review can be split over separate meetings for the sub-cohorts, although the available data from the other sub-cohort will also be taken into account at each meeting.

The IDMC will review all available safety data on a rolling basis to allow emerging data to contribute to all decisions. An ad hoc IDMC meeting may be requested via the sponsor for any single event or combination of multiple events which are considered to jeopardize the safety of the subjects.

Pausing/stopping rules will be applied in this trial (see Section 7.11).

The IDMC will be appointed by the sponsor before the start of the trial. The IDMC will consist of at least 1 independent medical expert in the relevant therapeutic area and at least 1 independent statistician. The IDMC responsibilities, authorities, and procedures will be documented in its charter. Any safety reports/interim data from the cohorts in this trial reviewed by the IDMC and the recommendations of the IDMC will be shared with the local health authorities and the IEC/IRB.

Further details regarding the IDMC can be found in the IDMC charter, which will be available prior to the administration of DYN101.

9.13 Missing Data

Since efficacy concerns are only exploratory, no missing data imputations methods will be used.

10. SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

This trial will be conducted in accordance with the current applicable regulations, ICH GCP E6 (R2) (https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4_2016_1109.pdf), EU Directive 2001/20/EC and its updates (https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/dir_2001_20/dir_2001_20_en.pdf), the Guideline on Strategies to Identify and Mitigate Risks for First-in-Human and Early Clinical Trials with Investigational Medicinal Products (20July2017EMEA/CHMP/SWP/28367/07 Rev), the guidelines of the Declaration of Helsinki, and local ethical and legal requirements. In the absence of data demonstrating developmental delay in subjects with CNM ≥ 16 years of age, apart from disease associated effect on muscles, these subjects will be treated as adults in this trial from a metabolic and PK perspective. And given that this disease has rare disease status, while the ICH 11 Guideline, Clinical Investigation of Medical Products in the Pediatric Population (http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E11/ICH_E11_R1_Step_2_25Aug2016_Final.pdf), was reviewed, full compliance is not warranted/claimed.

Good Clinical Practice (GCP) is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of clinical trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible.

The name and address of each third-party vendor (e.g. CRO) used in this trial and the sponsor's trial team members will be maintained in the investigator's and sponsor's files as appropriate.

10.1 Sponsor's Responsibilities

10.1.1 Good Clinical Practice Compliance

The sponsor and any third party to whom aspects of the trial management or monitoring have been delegated will undertake their assigned roles for this trial in compliance with the ICH GCP Guideline E6 (R2) as well as with applicable regulatory requirements in the countries where the trial will take place.

The sponsor will provide the investigator with any new safety findings as soon as they are available.

Representatives of the sponsor, and/or the company organizing/managing the research on behalf of the sponsor, conduct visits to sites to inspect trial data, subjects' medical records, and eCRFs in accordance with current GCP and the respective local and (inter)national government

regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities. Any changes from standard agreed upon practices due to restrictions imposed in response to a pandemic will be communicated and agreed to by the sponsor.

The sponsor ensures that local regulatory authority requirements are met before the start of the trial. The sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of IMP for shipment to the site.

A 'serious breach' is defined as a breach likely to effect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial; or
- the scientific value of the trial.

Making a judgment on whether a potential breach or non-compliance (or persistent non-compliance) is 'serious' and requires reporting is the responsibility of the sponsor. The sponsor will notify the licensing authority in writing of any serious breach of:

- the conditions and principles of GCP in connection with the trial; or
- the protocol, as amended from time to time in accordance with local regulations, within 7 days of becoming aware of that breach.

10.1.2 Indemnity/Liability and Insurance

The sponsor ensures that suitable clinical trial insurance coverage is in place prior to the start of the trial. An insurance certificate is supplied to the investigator and the IEC/IRB as necessary.

The sponsor has obtained travel insurance to cover the subject's and their caregiver's travel to and from clinic visits.

10.1.3 Public Posting of Trial Information

The sponsor will assure that key design elements of this protocol will be posted in a publicly accessible database such as EudraCT and clinicaltrials.gov. In addition, upon trial completion and finalization of the trial report the results of this trial will be either submitted for publication and/or posted in a publicly accessible database of clinical trial results. Information included in clinical trial registries may include participating investigators' names and contact information.

10.1.4 Submission of Summary of Clinical Trial Report to Competent Authorities of Member States Concerned and Independent Ethics Committees/Independent Review Boards

The sponsor will provide a summary of the clinical trial report within 1 year of the end of the trial completion date to the competent authority of the member state(s) concerned as required by regulatory requirement(s) and to comply with the community guideline on GCP. The sponsor will provide the IECs/IRBs with a copy of the same summary.

10.1.5 Trial Suspension, Termination, and Completion

The sponsor may suspend or terminate the trial or part of the trial at any time for any reason. If the trial is suspended or early terminated, the sponsor will ensure that applicable regulatory agencies and the IECs/IRBs are notified as appropriate. Additionally, the discontinuation of a registered clinical trial, which has been posted to a designated public website, will be updated accordingly.

Upon completion of the trial, the sponsor will make an end of trial declaration to the relevant competent authority as required by Article 10 (c) of Directive 2001/20/EC.

10.2 Investigator's Responsibilities

10.2.1 Good Clinical Practice Compliance

The investigator must agree to conduct the trial in accordance with ICH GCP Guideline E6 (R2), and applicable regulatory requirements and guidelines. Per the Guideline on Strategies to Identify and Mitigate Risks for First-in-Human and Early Clinical Trials with Investigational Medicinal Products (20July2017EMEA/CHMP/SWP/28367/07 Rev), the investigator should have immediate access to equipment and appropriately qualified staff for resuscitating and stabilizing individuals in an acute emergency including ready availability of intensive care unit and other hospital facilities. Procedures should be established between the clinical research unit and its nearby intensive care unit regarding the responsibilities and undertakings of each in the transfer and care of subjects. Further details will be described in the site manuals (e.g. Site Cohort Management Manual, Site Operations Manual, Pharmacy Manual).

It is the investigator's responsibility to ensure that adequate time and appropriate trained resources are available at the site prior to commitment to participate in this trial. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant trial-related tasks. Curriculum vitae for investigators and sub-investigators are provided to the sponsor (or designee) before starting the trial.

If a potential research subject has a primary care physician, the investigator should, with the subject's consent, inform him or her of the subject's participation in the trial.

A coordinating investigator is appointed to review the final clinical trial report for multi-center trials. Agreement with the final clinical trial report is documented by the signed and dated signature of the coordinating investigator, in compliance with Directive 2001/83/EC as amended by Directive 2003/63/EC and ICH Guidance E3 (1995).

10.2.2 Protocol Adherence and Investigator Agreement

The investigator and any sub-investigators must adhere to the trial protocol. The investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. The investigator agrees to promptly (i.e. immediately but in less than 24 hours) report any SAE in both sentinel and other subjects. Further details will be described in the site manuals (e.g. Site

Operations Manual, Site Cohort Management Manual). Investigators are required to sign an Investigator Agreement to confirm acceptance and willingness to comply with the trial protocol. The investigator is responsible for reporting protocol deviations.

If the investigator terminates the trial at his or her site, the investigator will promptly inform the sponsor and the IEC/IRB and provide them with a detailed written explanation. The investigator will also return all IMP, containers, and other trial materials to the sponsor. Upon trial completion, the investigator will provide the sponsor, IEC/IRB, and regulatory authority with final reports and summaries as required by (inter)national regulations.

Communication with the local IEC/IRB, to ensure accurate and timely information is provided at all phases during the trial, may be done by the sponsor, applicable CRO, investigator, or for multi-center trials, the coordinating investigator according to national provisions. Responsibilities for this communication will be documented in the Investigator Agreement.

10.2.3 Documentation and Retention of Records

10.2.3.1 Electronic Case Report Forms

Electronic CRFs are supplied by the CRO and should be handled in accordance with instructions from the sponsor.

The investigator is responsible for maintaining adequate and accurate medical records (see Section 10.2.3.2) from which accurate information is recorded onto eCRFs, which have been designed to record all observations and other data pertinent to the clinical investigation. The investigator will ensure the accuracy, completeness, legibility, and timelines of the data recorded in the eCRF, and of the provision of answers to data queries. All eCRF entries, corrections, and alterations must be made by the investigator or other authorized site personnel. The investigator will sign the completed eCRF. A copy of the completed eCRF will be sent to the sites for archiving.

The monitor will verify the contents against the source data per the monitoring plan. If the data are unclear or contradictory, queries will be sent for corrections or verification of data.

10.2.3.2 Recording, Access, and Retention of Source Data and Trial Documents

Original source data to be reviewed during this trial will include, but are not limited to the subject's medical file, original clinical laboratory reports, and histology and pathology reports, QoL and health outcome questionnaires, ECG, ICFs, and videos.

All key data must be recorded in the subject's medical records.

The investigator must permit authorized representatives of the sponsor, the respective national, local, or foreign regulatory authorities, the IEC/IRB, and auditors to inspect facilities and to have direct access to original source records relevant to this trial, regardless of media. Any access to original source records not possible due to restrictions imposed in response to a pandemic must be communicated to the sponsor and/or the company organizing/managing the research on behalf of the sponsor as soon as possible. All source records to be used in this trial will be explained to the subject in the ICF.

The monitor (and auditors, IEC/IRB or regulatory inspectors) may check the eCRF entries against the source documents. The ICF includes a statement by which the subject agrees to the monitor/auditor from the sponsor or its representatives, national or local regulatory authorities, or the IEC/IRB having access to source data (e.g. subject's medical file, appointment books, original laboratory reports, X-rays etc.).

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory authority (e.g. the US Food and Drug Administration [FDA], EMA, UK Medicines and Healthcare products Regulatory Agency [MHRA]) or an auditor) (in compliance with the General Data Protection Regulation (EU)2016/679 [27 April 2016]).

The investigator should take all necessary measures to prevent accidental destruction of the essential documents. They must be maintained according to ICH GCP requirements, for at least 15 years or longer if required by local regulatory requirements, and may not be destroyed without written permission from the sponsor.

10.2.3.3 Audit/Inspection

To ensure compliance with relevant regulations, data generated by this trial must be available for inspection upon request by representatives of, for example, the US FDA (as well as other US national and local regulatory authorities), the EMA, the MHRA, other regulatory authorities, the sponsor or its representatives, and the IEC/IRB for each site.

10.3 Trial Documentation

Before initiation of the trial, the following documents must be made available to the sponsor for each participating site:

1) Curriculum vitae of the investigator and any sub-investigators (listed on the 1572 form if applicable);
2) A signed copy of the IEC/IRB approval notice for protocol and ICFs;
3) A copy of the IEC/IRB-approved ICFs;
4) Laboratory certification with a list of normal values for laboratory tests that will be conducted at local laboratories;
5) Completed financial disclosure form for the Investigator and any sub-investigators (listed on the 1572 form if applicable);
6) Authorization to conduct a Phase 1 trial as required per local regulation.

Further details will be described in the site manuals (e.g. Site Cohort Management Manual, Site Operations Manual, Pharmacy Manual).

10.4 Ethical Considerations

10.4.1 Informed Consent

It is the responsibility of the investigator, or designee, to obtain voluntary written informed consent and assent, where applicable from all subjects prior to any trial-related procedures

including screening assessments. All consent and assent documentation must be in accordance with the applicable regulations and GCP. The pre-screening consent process can be managed remotely (please refer to the Site Operations Manual for more details). Each subject or the subject's legally-authorized representative as applicable is requested to sign ICFs after the subject has received and read the written subject information and received an explanation of what the trial involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. A copy of the informed consent and assent documentation (i.e. a complete set of subject information sheets and fully executed signature pages) must be given to the subject or the subject's legally-authorized representative as applicable. Signed consent forms including assent of under-age subjects must remain in each subject's trial file and must be available for verification at any time. A subject will be required to be reconsent when a subject reaches the age of consent based on specific country requirements. If any new information is found during the trial that may affect whether a subject wants to continue to take part in the trial, the subject is to be given this information orally and in writing. The subject may be asked to sign a new consent form if this occurs.

The subject will be provided with 24-hour contact information which can be used to report any safety concerns.

Whenever the IEC/IRB is directly managed by the national coordinating investigator, he/she will provide the sponsor with a copy of the consent form which was reviewed by his/her IEC/IRB, and which received their favorable opinion/approval. A copy of the IEC/IRB written favorable opinion/approval of these documents must also be provided to the sponsor, prior to the start of the trial. Additionally, if the IEC/IRB requires modification of the subject information and consent document provided by the sponsor, the documentation supporting this requirement must be provided to the sponsor.

Whenever the IEC/IRB is not managed by the investigator, the sponsor or its designee will provide the investigators with a copy of the consent form which was reviewed by the IEC/IRB, and which received their favorable opinion/approval. A copy of the IEC/IRB written favorable opinion/approval of these documents must also be provided to the investigator, prior to the start of the trial.

10.4.2 Independent Ethics Committee/Independent Review Board

The investigator (or authorized person) must submit the protocol and all the necessary documents (informed consent document and any materials provided to the subject) to the appropriate Ethics Committee (IEC/IRB), for review and approval, prior to any subject enrollment. Approval by IECs/IRBs is required for each participating site. Any changes from standard agreed upon submission to the IECs/IRBs due to restrictions imposed in response to a pandemic will be communicated and agreed to by the sponsor.

The investigator (or authorized person) should notify the sponsor of the IEC/IRB's approval date. The sponsor should receive a copy of the written and dated approval/favorable opinion signed by the Chairman with Ethics Committee (IEC/IRB) composition, prior to any investigational product drug shipment to the site.

Any substantial amendment to the protocol or any amendment to the ICFs should be submitted for approval to the Ethics Committee (IEC/IRB) prior to its implementation, unless there are overriding safety reasons.

10.4.3 Protocol Amendments

Neither the investigator nor the sponsor will modify this protocol or informed consent forms without a formal amendment by the sponsor. All protocol amendments must be issued by the sponsor and signed and dated by the investigator. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the subjects, in which case the amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the investigator and IEC/IRB must be provided to the sponsor. When the change(s) involve(s) only logistic or administrative aspects of the trial, the IEC/IRB (where required) only needs to be notified.

Substantial amendments have to be submitted to the IEC/IRB and competent authorities. Changes that are not substantial, which have no significant impact on the medical or scientific validity of the trial will be notified to the IEC/IRB and the competent authority, when required.

During the course of the trial, in situations where a departure from the protocol is unavoidable, the investigator or other physician in attendance will contact the appropriate sponsor representative. Except in emergency situations, this contact should be made before implementing any departure from the protocol. In all cases, contact with the sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the CRF and source documents will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it.

10.4.4 Long-term Retention of Samples for Additional Research (Optional)

Samples collected in this trial may be stored for up to 15 years (or according to local regulations) for additional research. Samples will only be used to understand CNM. The research may begin at any time during the trial or the post-trial storage period. All samples will be pseudo anonymized to prevent unnecessarily associating the sample with a specific subject.

Subjects will be asked to consent voluntarily for their samples to be stored for other research studies that may be done after this trial is completed. Participants for whom such consent is not given can participate in the trial without having their samples stored for future testing. In such case, their samples will be destroyed after all the trial assessments have been concluded (as agreed by the sponsor). Any results already generated on the samples will not be removed from any analyses that have already been performed.

Samples for genetic (DNA) research and biomarkers may be taken from subjects who provide separate informed consent for these samples to be collected and for their samples to be stored for other research studies.

Subjects may withdraw their consent for storage of samples for additional research at any time. In such case, their samples will be destroyed, and no further testing will take place.

The sponsor, sponsor's representatives, bio-repositories, and any specialty laboratories will be blinded to the subject's identity.

Results of the genetic analyses may contribute to the global understanding of CNM and its treatment and may be used internally to help support the design of additional studies, form part of scientific publication, or be made known to the regulatory authorities as part of a new drug application. Any results generated will be for exploratory research purposes only and will not be made available unless required by law (i.e. to regulatory authorities). Additionally, as any potential analysis does not form part of pre-defined analysis within the protocol, any results will be reported separately to the main clinical trial report. Subjects can request results of any analysis on their samples.

10.5 Privacy and Confidentiality

All EU-based sites, sponsors and laboratories or entities providing support for this trial must, where applicable, comply with the General Data Protection Regulation (EU)2016/679 (27 April 2016) and EU data protection regulations No. 45/2001 (18 December 2001).

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the trial, the sponsor and/or its representative reviews their medical records and data collected during the trial. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the sponsor; third parties with whom the sponsor may develop, register, or market DYN101; national or local regulatory authorities; and the IECs/IRBs, which gave approval for the trial to proceed. Additionally, the sponsor and/or its representatives may share the data with others confidentially relative to the further development of DYN101. The sponsor and/or its representatives, and Ionis Pharmaceuticals, Inc., owner of DYN101, accessing the records and data will ensure precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number. However, age and birth year will be collected and used to assist the sponsor to verify the accuracy of the data, for example, to confirm that laboratory results have been assigned to the correct subject.

The results of the trials – containing subjects' unique cohort-subject identifying number, relevant medical records, and possibly age and birth year – will be recorded. They may be transferred to, and used in, other countries that may not afford the same level of protection that applies within the countries where this trial is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the trial results, or to answer questions asked by regulatory or health authorities.

10.6 Publication Policy

All manuscripts, abstracts, or other modes of presentation arising from the results of the trial must be reviewed and approved in writing by the sponsor, in advance of submission. The review is aimed at protecting the sponsor's proprietary information either existing at the date of the commencement of the trial or generated during the trial. Authorship will follow the guidelines

established by the International Committee of Medical Journal Editors. The publication policy with respect to the investigator and site will be further detailed in a separate document.

11. ADMINISTRATIVE ASPECTS

Please refer to the trial contact list in the investigator site file.

12. REFERENCE LIST

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