Supplementary material

Appendix 1

Key inclusion and exclusion criteria for the phase III ORION studies

Inclusion Criteria

ORION 9

1) History of HeFH with a diagnosis of HeFH by genetic testing; and/or a documented history of untreated LDL-C of >4.9 mmol/L, and a family history of FH, elevated cholesterol or early heart disease that may indicate FH (Section 1.1).
2) Serum LDL-C ≥2.6 mmol/L at screening

ORION 10 and 11

1) History of ASCVD (CHD, CVD or PAD; Section 1.2) or in ORION-11 ASCVD-risk equivalents (type 2 diabetes, familial hypercholesterolemia (FH), and including subjects whose 10-year risk of a CV event assessed by Framingham Risk Score for Cardiovascular Disease or equivalent has a target LDL-C of <2.6 mmol/L). FH was defined using standard clinical criteria used in the region. These include Dutch Lipid Clinic Network (DLCN) criteria, Simon Broome criteria and Make Early Diagnosis to Prevent Early Deaths (MEDPED criteria).
2) Serum LDL-C ≥1.8 mmol/L for ASCVD subjects or ≥2.6 mmol/L for ASCVD-risk equivalent subjects at screening

And

3) Male or female subjects ≥18 years of age
4) Fasting triglyceride <4.52 mmol/L at screening
5) Calculated glomerular filtration rate >30 mL/min/ 1.73 m^2 by estimated glomerular filtration rate (eGFR) using standardized local clinical methodology.
6) Subjects on statins should be receiving a maximally tolerated dose. Maximum tolerated dose is defined as the maximum dose of statin that can be taken on a regular basis without intolerable adverse events. Intolerance to any dose of any statin must be documented as historical AEs attributed to the statin in question in the source documentation and on the Medical History page of the electronic case report form (eCRF) (Section 1.3).
7) Subjects not receiving statin must have documented evidence of intolerance to all doses of at least two different statins (Section 1.3).
8) Subjects on lipid-lower therapies (such as a statin and/or ezetimibe) should be on a stable dose for ≥30 days before screening with no planned medication or dose change during study participation.
9) Subjects must be willing and able to give informed consent before initiation of any study related procedures and willing to comply with all required study procedures.

Exclusion Criteria

1) Any uncontrolled or serious disease, or any medical or surgical condition, that may either interfere with participation in the clinical study, and/or put the subject at significant risk (according to investigator's [or delegate] judgment) if he/she participates in the clinical study.
2) An underlying known disease, or surgical, physical, or medical condition that, in the opinion of the investigator (or delegate) might interfere with interpretation of the clinical study results.
3) New York Heart Association (NYHA) class IV heart failure or last known left ventricular ejection fraction <25%.
4) Cardiac arrhythmia within 3 months prior to randomization that is not controlled by medication or via ablation.
5) Major adverse cardiovascular event within 3 months prior to randomization.
6) Uncontrolled severe hypertension: systolic blood pressure >180 mmHg or diastolic blood pressure >110 mmHg prior to randomization despite anti-hypertensive therapy.
7) Active liver disease defined as any known current infectious, neoplastic, or metabolic pathology of the liver or unexplained elevations in ALT, aspartate aminotransferase (AST), >3x the ULN, or total bilirubin >2x ULN at screening confirmed by a repeat abnormal measurement at least 1 week apart.
8) Severe concomitant non-cardiovascular disease that carries the risk of reducing life expectancy to less than 2 years.
9) History of malignancy that required surgery (excluding local and wide-local excision), radiation therapy and/or systemic therapy during the three years prior to randomization.
10) Females who are pregnant or nursing, or who are of childbearing potential and unwilling to use at least two methods of highly effective contraception (failure rate less than 1% per year) (e.g., combined oral contraceptives, barrier methods, approved contraceptive implant, long- term injectable contraception, or intrauterine device) for the entire duration of the study. Exemptions from this criterion:
    a. Women >2 years postmenopausal (defined as 1 year or longer since last menstrual period) AND more than 55 years of age.
    b. Postmenopausal women (as defined above) and less than 55 years of age with a negative pregnancy test within 24 hours of randomization.
    c. Women who are surgically sterilized at least 3 months prior to enrolment.
11) Males who are unwilling to use an acceptable method of birth control during the entire study period (ie, condom with spermicide).
12) Known history of alcohol and/or drug abuse within the last 5 years.
13) Treatment with other investigational products or devices within 30 days or five half-lives of the screening visit, whichever is longer.
14) Planned use of other investigational products or devices during the course of the study.
15) Any condition that according to the investigator could interfere with the conduct of the study, such as but not limited to:
    d. Subjects who are unable to communicate or to cooperate with the investigator.

e. Unable to understand the protocol requirements, instructions and study-related restrictions, the nature, scope, and possible consequences of the study (including subjects whose cooperation is doubtful due to drug abuse or alcohol dependency).
f. Unlikely to comply with the protocol requirements, instructions, and study-related restrictions (e.g., uncooperative attitude, inability to return for follow-up visits, and improbability of completing the study).
g. Have any medical or surgical condition, which in the opinion of the investigator would put the subject at increased risk from participating in the study.
h. Persons directly involved in the conduct of the study.

16) Treatment (within 90 days of screening) with monoclonal antibodies directed towards PCSK9.

Subjects excluded for any of the above reasons may not be re-screened for participation at any time even if the exclusion characteristic has changed.

1.1: Simon Broome diagnostic criteria for familial hypercholesterolaemia

Definite Familial Hypercholesterolemia:
Required laboratory = high cholesterol levels:

• Adult = Total cholesterol levels >7.5 mmol/L or LDL-C >4.9 mmol/L
• Child less than 16 years of age = Total cholesterol levels >6.7 mmol/L or LDL-C >4.0 mmol/L

Plus at least one of the two:
1) Physical finding = tendon xanthomas, or tendon xanthomas in first or second degree relative
2) DNA-based evidence of an LDL-receptor mutation, familial defective apo B-100, or a PCSK9 mutation

Possible Familial Hypercholesterolemia:
Laboratory - high cholesterol levels:

• Adult = Total cholesterol levels >7.5 mmol/L or LDL-C >4.9 mmol/L
• Child less than 16 years of age = Total cholesterol levels >6.7 mmol/L or LDL-C >4.0 mmol/L

Plus at least one of the two:
1) Family history of at least one of the following.
    a. Family history of myocardial infarction at:
        i. Age 60 years or younger in first-degree relative
        ii. Age 50 years or younger in second-degree relative
2) Family history of elevated total cholesterol

a. Greater than 7.5 mmol/L in adult first- or second-degree relative
b. Greater than 6.7 mmol/L in child, brother or sister aged younger than 16 years

Reference: Austin MA, Hunter CM, Zimmern RL, Humphries SE. Genetic causes of monogenic
heterozygous familial hypercholesterolemia: a HuGE prevalence review. American Journal of
Epidemiology 2004; 160:407-420.

1.2: Patients with established ASCVD

Coronary heart disease (CHD):

- Prior myocardial infarction
- Prior coronary revascularization (PCI or CABG)
- Angiographic or CT-imaging (e.g., MDCT/CTA) evidence of coronary atherosclerosis (>70% stenosis in at least one major epicardial coronary artery)

Cerebrovascular disease (CVD):

- Prior ischemic stroke confirmed by a brain imaging study – CT or MRI; thought not to be caused by atrial fibrillation, valvular heart disease or mural thrombus
- Carotid artery stenosis >70% on prior angiography or ultrasound
- History of prior percutaneous or surgical carotid artery revascularization

Peripheral arterial disease (PAD):

- Prior documentation of a resting ankle-brachial index ≤ 0.85
- History of prior percutaneous or surgical revascularization of an iliac, femoral, or popliteal artery
- Prior non-traumatic amputation of a lower extremity due to peripheral artery disease

1.3: Requirements for background lipid-lowering treatment

There should be no plans at the time of screening and randomization to modify the dose of statin or other lipid-lowering medication such as ezetimibe for the duration of the trial. Unless the background lipid-lowering treatment exceptions described below are met, subjects must have been treated with one of the following highly effective statins at the specified daily doses and at a stable dose, preferably for 6 weeks but for at least 30 days, prior to screening for the study:

- atorvastatin, 40 or 80 milligrams (mg) once a day
- rosuvastatin, 20 or 40 mg, once a day
- simvastatin 40 mg, once a day or, if a subject has been on that dose for >1 year, 80 mg once a day

- Combination medications that contain atorvastatin, rosuvastatin, or simvastatin components described at the aforementioned doses will be permitted

Background lipid-lowering treatment exceptions

The following background lipid-lowering treatment exceptions are permitted:

1. Lower doses of statins due to partial statin intolerance: Subjects may be on a lower dose of one of the highly effective statins described above if there is documented intolerance to any one of them (atorvastatin, rosuvastatin, or simvastatin) at the aforementioned doses. Intolerance to any dose of any statin must be documented as historical adverse events attributed to the statin in question, in the source documentation and electronic case report form (eCRF).

2. Regulatory limitations: Subjects may be on a lower dose of one of the highly effective statins described above if the highest locally approved dose for one of the stated statins is lower than those doses shown above (e.g., in some countries, atorvastatin 20 mg, once a day, is the highest locally approved dose).

3. Alternative statins: Subjects may be treated with other statins (pravastatin, fluvastatin, pitavastatin, or lovastatin), different from the highly effective statins listed above, if there is documented intolerance to any two different highly effective statins (atorvastatin, rosuvastatin, simvastatin) at the lowest available daily dose for at least one of those highly effective statins. Intolerance to any statin must be documented as historical adverse events attributed to the statin in question, in the source documentation and eCRF.

4) No background statin therapy: Subjects may be enrolled who are only on non-statin lipid lowering therapy if complete statin intolerance has been documented. Subjects with complete statin intolerance must be unable to tolerate at least two statins: one statin at the lowest available daily dose AND another statin at any dose. Intolerance to any statin must be documented as historical adverse events attributed to the statin in question, in the source documentation and eCRF. The sole exception, for which a subject may participate in the study with documentation of intolerance to only one statin, is a documented history of rhabdomyolysis attributed to that statin.

Appendix 2

Genotypes of South African participants randomized to receive inclisiran or placebo in the ORION-9 study only

Variants	**Inclisiran (n=88)**	**Placebo (n=89)**
***LDLR* Total**	55 (62.5)	57 (64.0)
LDLR Pathogenic	54 (61.4)	55 (61.8)
LDLR Likely Pathogenic	1 (1.1)	2 (2.2)
***LDLR* function**		

Negative	52 (59.1)	52 (58.4)
Defective	1 (1.1)	1 (1.1)
Unknown	2 (2.3)	4 (4.5)
Double variants	6 (6.8)	5 (5.6)
Double heterozygous	5 (5.7)	3 (3.4)
Compound heterozygous	1 (1.1)	2 (2.2)
Other		
ApoB	4 (4.5)	3 (3.4)
PCSK9 GOF	1 (1.1)	0 (0)
No variants identified	15 (17.0)	16 (18.0)
No genotyping conducted	7 (8.0)	8 (9.0)

LDLR = low-density lipoprotein receptor gene; ApoB = apolipoprotein B gene; PCSK9 GOF = proprotein convertase subtilisin/kexin 9 gain of function gene variant

Appendix 3

Background statin dose categories

High-intensity statins	**Medium-intensity statins**	**Low-intensity statins***
Atorvastatin 40 – 80 mg	Atorvastatin 10 – 20 mg	Simvastatin 10 mg
Rosuvastatin 20 – 40 mg	Rosuvastatin 5 – 10 mg	Pravastatin 10 – 20 mg
Simvastatin 80 mg	Simvastatin 20 – 40 mg	Lovastatin 20 mg
	Pravastatin 40 – 80 mg	Fluvastatin 20 -40 mg
	Lovastatin 40 mg	Pitavastatin 1 mg
	Fluvastatin XL 80 mg	
	Fluvastatin 40 mg twice daily	
	Pitavastatin 2 – 4 mg	

*Low-intensity statins also include those patients taking low-dose statins using an alternate regimen (i.e., every other day or for a specified number of times per week).

Appendix 4

Proportion of South African participants attaining LDL-C targets at day 510

Participants	**<1.8 mmol/L**	**<1.4 mmol/L**
All		
Inclisiran (N=130)	71 (54.6)	54 (41.5)
Placebo (N=137)	2 (1.5)	0 (0)
ASCVD		
Inclisiran (N= 79)	51 (64.6)	42 (53.2)
Placebo (N= 92)	2 (2.2)	0 (0)
ASCVD risk equivalent		
Inclisiran (N= 51)	20 (39.2)	12 (23.5)
Placebo (N= 45)	0 (0)	0 (0)
FH		
Inclisiran (N= 84)	35 (41.7)	20 (23.8)
Placebo (N= 94)	1 (1.1)	0 (0)

Count and percentage of South African participants who attained LDL-C levels below specific targets. The percentage of participants attaining goal reflect the population with available data (observed values) at the specified time interval (day 510). Reasons for missing data included subject discontinued study, a sample issue, or a missed visit.