SUPPLEMENTAL MATERIAL

Efficacy and safety of alirocumab in children and adolescents with homozygous familial hypercholesterolemia: Phase 3, multinational open-label study

Running title: Alirocumab in pediatric homozygous FH

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Supplementary information I: Detailed patient inclusion/exclusion criteria.

Inclusion criteria

1. Male and female children and adolescents aged 8–17 years genetically diagnosed with homozygous familial hypercholesterolemia (HoFH)\(^1\) inadequately controlled (see threshold mentioned in exclusion criterion 2) despite treatment with optimal dose of statin\(^2\) with or without other lipid-modifying therapies (LMTs), or non-statin LMTs if statin-intolerant,\(^3\) at stable dose(s) for ≥4 weeks.

2. A signed informed consent indicating parental permission with or without patient assent, depending on capacity for understanding based on developmental maturity. In cases involving emancipated or mature minors with adequate decision-making capacity, or when otherwise permitted by law, a signed informed consent directly from patients.

3. Patients on stable low-density lipoprotein (LDL)-apheresis therapy\(^4\) for ≥4 weeks prior to the screening visit (Week -2) and had initiated apheresis treatment ≥6 months before entering the study.

\(^1\)Diagnosis of HoFH must be made either by previous genotyping (true homozygotes, compound heterozygotes, or double heterozygotes) or current genotyping performed after having been suspected by clinical criteria according to European Atherosclerosis Society (EAS) Consensus Panel recommendations for diagnosis of HoFH. Previous genotyping refers to documented results that are available from prior genotyping testing supporting a diagnosis of HoFH.

\(^2\)The optimal dose of statin is defined as the stable daily dose prescribed based on local guidelines or practice or is the dose that is maximally tolerated due to adverse effects of higher doses. For patients not receiving the maximally tolerated dose of statin, statin intensification should be carefully considered prior to inclusion in this study in order to ensure that the addition of a non-statin LDL-C lowering therapy (ie, alirocumab) would be the next appropriate step in the management of the patient’s hypercholesterolemia. The highest dose of statin should not exceed the maximum labeled dose of statin for pediatric patients as per the local prescribing information.

\(^3\)A statin-intolerant patient is defined as a patient with the documented inability to tolerate at least 2 statins: one statin at the lowest daily starting dose, AND another statin at any dose, due to skeletal muscle-related symptoms, other than those due to strain or trauma, such as pain, aches, weakness, or cramping, that began or increased during statin therapy and stopped when statin therapy was discontinued. Patients not receiving a daily approved regimen of a statin (eg, 1–3 times weekly) will also be considered as not able to tolerate a daily dose.
Stable apheresis is defined as 4 apheresis procedures performed during a 4-week period, approximately 1 week apart, or 4 apheresis procedures performed during an 8-week period, approximately 2 weeks apart.

Exclusion criteria

1. Children and adolescents aged <8 years or >17 years at the time of informed consent signature, unless different local regulation applies (eg, for Russia only: patients aged <12 years or >17 years at the time of informed consent signature). Note: Patients aged 8–<10 years who had not had previous attempts to lower LDL-C by other means were excluded

2. Patients with LDL-C (pre-apheresis, if applicable) <130 mg/dL (3.37 mmol/L) obtained during the screening period after the patient has been on stable apheresis procedure or LMT (ie, stable optimal dose of statin ± other stable LMTs, or stable non-statin LMTs in statin-intolerant patients) treatment for ≥4 weeks

3. Patients with null low-density lipoprotein receptor (LDLR) mutations in both alleles

4. Patients with bodyweight <25 kg

5. Patients aged 8–9 years not at Tanner Stage 1 and patients aged 10–17 years not at ≥Tanner Stage 2 in their development. Note: Tanner Stage 1: girls (breast development): pre-adolescent; elevation of papilla only; boys (external genitalia): pre-adolescent. Testes, scrotum, and penis are of about the same size and proportion as in early childhood. Tanner Stage 2: girls (breast development): breast bud stage; elevation of breast and papilla as a small mound, enlargement of areola diameter; boys (external genitalia): The scrotum and testes have enlarged and there is a change in the texture of the scrotal skin. There is also some reddening of the scrotal skin

6. Daily dose of statin that is above the maximum recommended dose for pediatric patients as per the local prescribing label

7. Patients who would receive statin de novo during the run-in period

8. Use of nutraceutical products or over-the-counter therapies that may affect lipids that have not been at a stable dose for ≥4 weeks prior to the screening visit

9. Patients not previously instructed on a cholesterol-lowering diet prior to the screening visit

10. Use of mipomersen in the last 5 months
11. Patients with uncontrolled Type 1 or 2 diabetes mellitus (ie, hemoglobin A1c >9% at the screening visit)

12. Patients with known uncontrolled thyroid disease (ie, thyroid-stimulating hormone levels outside of the laboratory’s reference range within the past 6 months, or with elevated free T3 or T4 and with clinical symptoms of hyperthyroidism)

13. Patients who used systemic corticosteroids. Note: Topical, intra-articular, nasal, inhaled, and ophthalmic steroid therapies were not considered as “systemic” and were allowed

14. Patients with uncontrolled hypertension (ie, systolic blood pressure or diastolic blood pressure above local guidelines or equivalent)

15. Fasting triglycerides >350 mg/dL (3.95 mmol/L) at the screening visit

16. Severe renal impairment (ie, estimated glomerular filtration rate <30 mL/min/1.73m²) at the screening visit

17. Alanine aminotransferase or aspartate aminotransferase >2 × upper limit of normal (1 repeat laboratory allowed per patient) at the screening visit

18. Creatine phosphokinase (also known as creatine kinase) >3 × upper limit of normal (1 repeat laboratory allowed per patient) at the screening visit

19. Patient/parents who withdrew consent during the run-in or screening period (patient who was not willing to continue or fails to return)

20. Conditions/situations or laboratory findings such as:

- Any clinically significant abnormality identified at the time of screening that in the judgment of the investigator or any sub-investigator would preclude safe completion of the study or constrain endpoints assessment such as major systemic diseases

- Patients considered by the investigator or any sub-investigator as inappropriate for this study for any reason, eg:
  - Those deemed unable to meet specific protocol requirements, such as scheduled visits
  - Those deemed unable to administer or tolerate long-term injections as per the patient or the investigator
  - Presence of any other conditions (eg, geographic, social), actual or anticipated, that the investigator felt would restrict or limit the patient’s participation for the duration of the study
• Uncooperative or any condition that could make the patient potentially non-compliant to the study procedures

21. Known or suspected alcohol and/or drug abuse

22. Patients who had previously received evolocumab

23. Treatment with any investigational medicinal product (IMP) within 8 weeks or 5 half-lives prior to the screening period, whichever is longer. Note: If half-life was not known, then 8 weeks was applied for non-biological IMP and 6 months for biological IMP.

24. All contraindications to the background statins or other lipid-modifying therapies (as applicable) or warning/precaution of use (when appropriate) as displayed in the respective National Product Labeling

25. Hypersensitivity to alirocumab or to any of the ingredients of alirocumab injections

26. Female patients who had experienced menarche who were unwilling or unable to be tested for pregnancy. Note: Females who had experienced menarche must have a confirmed negative pregnancy test at screening and other study visits. Pregnancy tests may be performed more frequently in some countries due to local legislation related to women of childbearing potential randomized in clinical trials

27. Positive pregnancy test in females who had experienced menarche

28. Females who were breastfeeding

29. Females of childbearing potential not protected by highly effective method(s) of birth control. Note: Females of childbearing potential had to use an effective contraceptive method throughout the entire duration of study treatment and for ≥10 weeks after the last injection
Supplementary information II: Detailed information on statistical analyses.

Unless otherwise specified, analyses were performed overall and according to the dose received at study start (alirocumab 75 mg every two weeks [Q2W] and alirocumab 150 mg Q2W). Continuous data were summarized using the number of available data, mean, standard deviation, median, and minimum and maximum. Categorical and ordinal data were summarized using the number and percentage of patients. Baseline value was defined as the last available value obtained up to the date and time of the first open-label alirocumab administration.

Analyses of efficacy endpoints

There was no formal statistical test for the efficacy endpoints in this study since there was no control group. All efficacy analyses were descriptive. Unless otherwise specified, efficacy analyses were performed overall only (all doses combined).

Primary and secondary endpoints were analyzed using an intent-to-treat (ITT) approach including lipid values, regardless of whether the patient was continuing therapy or not (ITT analysis), and also using an on-treatment approach only including lipid data collected during the treatment period (on-treatment analysis).

Primary efficacy analysis

The percent change from baseline in low-density lipoprotein-C [LDL-C] at Week 12 was analyzed in the ITT population using a mixed-effect model with repeated measures (MMRM) approach. All post-baseline data available within the Week 4 to Week 48 analysis windows were used and missing data were accounted for by the MMRM model. The model included the fixed categorical effect of timepoint (Weeks
4, 12, 24, and 48) as well as the continuous fixed covariate of baseline LDL-C value.

The model provided baseline adjusted least-squares mean estimates at Week 12 with their corresponding standard errors and 95% confidence intervals (CIs).

However, considering the small number of patients, the authors decided that it was more appropriate to present raw data analysis as it was less sensitive to potential effect related to the inclusion of missing data. Accordingly, LS mean and standard deviation data for the primary endpoint were generated from raw data analysis and are presented in the manuscript.

**Sensitivity analyses**

To assess the robustness of the primary efficacy analysis, additional analyses were performed to check the MMRM assumptions and to evaluate the model’s sensitivity to handling of missing data.

**Analyses of secondary efficacy endpoints**

Continuous efficacy variables anticipated to have a normal distribution (LDL-C, high-density lipoprotein-C [HDL-C], total cholesterol, non–HDL-C, apolipoprotein B, and apolipoprotein A-1) were analyzed using the same MMRM model as for the primary endpoint with fixed planned post-baseline timepoints up to Week 48 as well as the continuous fixed covariate of corresponding baseline value for analysis with ITT estimand.

Continuous efficacy variables anticipated to have a non-normal distribution (ie, triglycerides and lipoprotein a) were analyzed with ITT estimand using multiple imputation approach for handling of missing values. The percent change from
baseline at timepoint of interest was derived from observed and imputed lipid values at this timepoint. Multiple imputations were followed by robust regression model with endpoint of interest as response variable using M-estimation with baseline value as effect. Combined means estimates with their corresponding SEs and 95% CIs (not shown) were provided.

Binary secondary efficacy endpoints (ie, proportion of patients with ≥15% reduction in LDL-C at Weeks 12, 24, 48, ITT estimand) were analyzed using multiple imputation approach for handling of missing values. The binary endpoint at timepoint of interest were derived from observed and imputed lipid values at this timepoint. Combined proportion with their corresponding 95% CIs (not shown) were provided.

**Analyses of safety data**

All safety analyses were descriptive. The primary focus of adverse event reporting was on treatment-emergent adverse events (TEAEs). Summaries of TEAEs were provided on the safety population, comprising enrolled patients who had received ≥1 dose or partial dose of alirocumab. In addition, patients for whom it was unclear whether they received study medication were also to be included in the safety population.
Figure S1: Study Design

*Follow-up call to be performed 10 weeks after the last injection for the patients who complete the study and for the patients who discontinue early

†Primary efficacy endpoint at Week 12

ATPIII = Adult Treatment Panel III; D = Day; E = enrollment; LMT = Lipid-Modifying therapy; NCEP = National Cholesterol Education Panel; TLC = Therapeutic Lifestyle Changes; W = Week
Figure S2: LDL-C over time (A) and percent change from baseline (B), according to apheresis treatment.

Non-apheresis: n was 11 from Week 24 onwards. LDL-C, low-density lipoprotein cholesterol; SD, standard deviation; W, Week.
Figure S3: Total and free serum PCSK9 concentrations over time for the PK population, in patients on LDL apheresis* (A, B, respectively) and patients not on LDL apheresis (C, D, respectively)
Measurements were performed pre-apheresis. PCSK9, proprotein convertase subtilisin/kexin type 9; PK, pharmacokinetic; Q2W, every 2 weeks; SE, standard error; W, Week.
Figure S4: $C_{\text{trough}}$ alirocumab concentrations (µg/mL) over time (PK population), (A) patients not on apheresis, (B) patients on apheresis (pre-apheresis).

$C_{\text{trough}}$, lowest concentration before administration of the next dose; D, day; PK, pharmacokinetic; Q2W, every 2 weeks; SE, standard error, W, Week.
| Country             | Principal Investigator | Institution Name                                           | City, State (if applicable) |
|---------------------|------------------------|-------------------------------------------------------------|-----------------------------|
| Brazil              | Santos Filho, R        | INCOR - Instituto do Coracao                               | Sao Paulo                   |
| Canada              | Bergeron, J            | Clinique des maladies lipidiques de Quebec Inc              | Quebec                      |
| Taiwan, R.O.C.      | Charng, M-J            | Taipei Veterans General Hospital                            | Taipei                      |
| Denmark             | Klausen Ib, C          | Regionshospitalet Viborg Hjertesygdomme                    | Viborg                      |
| Mexico              | Zarate Morales Cesar, A| Oaxaca Site Management Organization S.C.                   | Oaxaca                      |
| Netherlands         | Gerdes, V              | Amsterdam Universitair Medische Centra Academisch Medisch Centrum | Amsterdam                   |
| Russian Federation  | Barbarash, O           | Federal State Budgetary Scientific Institution Scientific Research Institute of Complex problems Of cardiovascular disease | Kemerovo                    |
| Slovenia            | Battelino, T           | University Medical Centre in Ljubljana                      | Ljubljana                   |
| Spain               | Diaz-Diaz, JL          | Hospital Abente Y Lago                                      | A Coruna                    |
| Turkey              | Kalkan-Ucar, S         | Cocuk Sagligi ve Cocuk Hastalikleri BD                     | Izmir                       |
Table S2: Details of patient genotype data and LDLR functional status accessed from literature and databases.

| Patient No | Mutated Gene | Zygosity mutation | Formal HGVS annotation | Functional status |
|------------|--------------|-------------------|------------------------|-------------------|
| 1          | LDLR         | True homozygosity | c.2271del; p.(Leu759Serfs*6) / c.2271del; p.(Leu759Serfs*6) | Null/null (LDLR) |
| 2          | LDLR         | Compound heterozygosity | c.1694G>A; p.(Gly565Arg) / c.1747C>T; p.(His583Tyr) | Defective/defective |
| 3          | LDLR         | Compound heterozygosity | c.(-139_-130)del; p.(?) / c.2271del; p.(Leu759Serfs*6) | Null/null (LDLR) |
| 4          | LDLR         | True homozygosity | c.431dup; p.(His144Glnfs*27) / c.431dup; p.(His144Glnfs*27) | Null/null (LDLRAP1) |
| 5          | LDLR         | True homozygosity | c.313+2dup; p.(?) / c.313+2dup; p.(?) | Null/null (LDLR) |
| 6          | LDLR         | Compound heterozygosity | c.682G>A; p.(Glu228Lys) / c.1747C>T; p.(His583Tyr) | Defective/defective |
| 7          | LDLR         | Compound heterozygosity | c.917C>T; p.(Ser306Leu) / c.314-1G>A; p.(?) | Null/defective |
| 8          | LDLR         | True homozygosity | c.1646G>A; p.(Gly549Asp) / c.1646G>A; p.(Gly549Asp) | Defective/defective |
| 9          | LDLR         | True homozygosity | c.1215C>G; p.(Asn405Lys) / c.1215C>G; p.(Asn405Lys) | Defective/defective |
| 10         | LDLR         | Compound heterozygosity | missense in LDLR exon 4!; splice site mutation in LDLR intron 1! | Null/null (LDLR) |
| 11         | LDLR         | True homozygosity | c.818-1G>A; p.(?) / c.818-1G>A; p.(?) | Null/null (LDLR) |
| 12         | LDLR         | Compound heterozygosity | c.1449G>A; p.(Trp483*) / c.2099A>G; p.(Asp700Gly) | Null/defective |
| 13         | LDLR         | True homozygosity | c.81C>G; p.(Cys27Trp) / c.81C>G; p.(Cys27Trp) | Defective/defective |
|   | Gene   | Status                  | Variant Details                                                                 | Notes              |
|---|--------|-------------------------|--------------------------------------------------------------------------------|--------------------|
|14 | LDLR   | Compound heterozygosity | c.2417_2418insG; p.(Phe807Leufs*10) / c.910G>A; p.(Asp304Asn)                    | Null/defective     |
|15 | LDLR   | True homozygosity       | c.313+2dup; p.(?) / c.313+2dup; p.(?)                                           | Null/null (LDLR)   |
|16 | LDLR   | True homozygosity       | c.1729T>C; p.(Trp577Arg) / c.1729T>C; p.(Trp577Arg)                             | Defective/defective|
|17 | LDLRAP1| True homozygosity       | c.431dup; p.(His144Glnfs*27) / c.431dup; p.(His144Glnfs*27)                      | Null/null (LDLRAP1) |
|18 | LDLR   | Compound heterozygosity | c.2043C>A; p.(Cys681*) / c.761A>C; p.(Gln254Pro)                               | Null/defective     |

1, unofficial notation; LDLR, low-density lipoprotein receptor; LDLRAP1, low-density lipoprotein receptor adapter protein 1.
**Table S3**: Participants reaching ≥15% reduction in LDL-C versus baseline according to assigned functional status and apheresis status at Week 12, Week 24, and Week 48.

| n/N (%) | Week 12 | Week 24 | Week 48 |
|---------|---------|---------|---------|
| Overall | 50.0 (9/18) | 52.9 (9/17) | 41.2 (7/17) |
| Functional status | | | |
| Null/null (LDLR) (n=6*) | 4/6 (66.7) | 4/5 (80.0) | 3/5 (60.0) |
| Null/null (LDLRAP1) (n=2) | 0/2 (0.0) | 1/2 (50.0) | 1/2 (50.0) |
| Null/defective (n=4) | 2/4 (50.0) | 2/4 (50.0) | 1/4 (25.0) |
| Defective/defective (n=6) | 3/6 (50.0) | 2/6 (33.3) | 2/6 (33.3) |
| Apheresis status, | | | |
| On apheresis (n=6) | 2/6 (33.3) | 3/6 (50.0) | 1/6 (16.7) |
| Not on apheresis (n=12†) | 7/12 (58.3) | 6/11 (54.5) | 6/11 (54.5) |

* n=5 from Week 24 onwards; † n=11 from Week 24 onwards.

LDL-C, low density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; LDLRAP1, low-density lipoprotein receptor adapter protein 1.
Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below.

Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (in vivo studies)

| Species | Vendor or Source | Background Strain | Sex | Persistent ID / URL |
|---------|------------------|-------------------|-----|---------------------|
| NA      |                  |                   |     |                     |

Genetically Modified Animals

| Species | Vendor or Source | Background Strain | Other Information | Persistent ID / URL |
|---------|------------------|-------------------|-------------------|---------------------|
| Parent - Male | NA |                   |                   |                     |
| Parent - Female | NA |                   |                   |                     |

Antibodies
| Target antigen | Vendor or Source | Catalog # | Working concentration | Lot # (preferred but not required) | Persistent ID / URL |
|----------------|-----------------|-----------|-----------------------|-----------------------------------|---------------------|
| NA             |                 |           |                       |                                   |                     |

**DNA/cDNA Clones**

| Clone Name | Sequence | Source / Repository | Persistent ID / URL |
|------------|----------|---------------------|---------------------|
| NA         |          |                     |                     |

**Cultured Cells**

| Name       | Vendor or Source | Sex (F, M, or unknown) | Persistent ID / URL |
|------------|------------------|------------------------|---------------------|
| NA         |                  |                        |                     |

**Data & Code Availability**

| Description | Source / Repository | Persistent ID / URL |
|-------------|---------------------|---------------------|
| NA          |                     |                     |
| Description | Source / Repository | Persistent ID / URL |
|-------------|---------------------|---------------------|
| NA          |                     |                     |
The ARRIVE guidelines ([https://arriveguidelines.org/](https://arriveguidelines.org/)) are a checklist of recommendations to improve the reporting of research involving animals. Key elements of the study design should be included below to better enable readers to scrutinize the research adequately, evaluate its methodological rigor, and reproduce the methods or findings.

### Study Design

| Groups     | Sex | Age | Number (prior to experiment) | Number (after termination) | Littermates (Yes/No) | Other description |
|------------|-----|-----|------------------------------|-----------------------------|----------------------|-------------------|
| Group 1 (Control) | NA  |     |                              |                             |                      |                   |
| Group 2    | NA  |     |                              |                             |                      |                   |

**Sample Size:** Please explain how the sample size was decided. Please provide details of any a prior sample size calculation, if done.

**Inclusion Criteria**

**Exclusion Criteria**

**Randomization**

**Blinding**