SYNOPSIS

AMENDED VERSION NUMBER 2.2 - 14 FEBRUARY 2025

Title	Evaluation of the effect of lomitapide treatment on major adverse cardiovascular events (MACE) in patients with homozygous familial hypercholesterolemia
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Background and rationale	Homozygous familial hypercholesterolemia (HoFH) is a rare, life-threatening condition characterized by a severe elevation of LDL cholesterol (LDL-C) and accelerated atherosclerosis. In these patients, an aggressive therapy to reduce LDL-C is mandatory to control the high risk of CHD associated with this disease. Lomitapide, an inhibitor of microsomal triglycerides transferase protein (MTP) has been demonstrated to be very effective in reducing LDL-C in HoFH in both clinical trial and real-world experience. However, limited information is available on how this drug affects cardiovascular risk. Due to the rarity of the disease, a randomized controlled trial testing the effect of lomitapide on the incidence of major adverse cardiovascular events (MACE) is not feasible. To overcome this, an observational study with the aim of analyzing the occurrence of MACE in HoFH patients exposed to lomitapide will be performed. In the Italian network of lipid centres, information about MACE in HoFH patients exposed to lomitapide are available for more than 30 patients. The duration of follow-up among these patients was not homogenous. In fact there was a group of patients with barely 1 year of treatment and this may not represent a sufficient time to observe any detectable benefit on cardiovascular risk, especially in adult HoFH patients exposed to high levels of LDL-C since birth. Therefore, to provide a better estimation of the effect of lomitapide therapy on MACE we have designed this observational study with a retrospective phase, in which the data available will be collected, followed by a prospective phase where all patients will be followed up to completion of at least 3 years of treatment. As a parallel cohort of untreated HoFH is not available, we have decided to compare the occurrence of MACE during the 3-year period of lomitapide treatment with that which occurred in the same cohort during the 3-year period before initiation of lomitapide.

Study design	Observational multicenter, open-label, retrospective and prospective study. More than 26 centres from Europe and other regions, as needed, will be involved, approximately 14 of which will be in Italy.
Study duration	The maximum duration of the study will be 37 months, that is approximately 3 years. The enrollment period will be 12 months and the prospective observational phase will last a maximum of 24 months followed by 1 month follow-up. The retrospective observational phase involves the collection of at least 4 years retrospective data (3 years prior to lomitapide and 1 year on lomitapide). In patients that have already completed retrospectively the 37 months of treatment with lomitapide, the observation will be extended forward up to 5 years of treatment. The data related to the further 2 years of observation can be collected prospectively or retrospectively. The end of the study will be the date of the last visit of the last subject. For the purposes of analysis the timepoints of data collection will be classified as "visits".
Population and patient selection criteria	Approximately 72 patients with data from both time periods. **INCLUSION CRITERIA** 1. Adult patients (age ≥18 years) 2. Clinical or genetic diagnosis of HoFH patients treated with lomitapide at any dosage 3. On treatment with lomitapide for at least 12 months at the time of enrollment 4. Availability of 3 years medical records prior to the commencement of lomitapide treatment to confirm the occurrence of MACE 5. Giving written informed consent **EXCLUSION CRITERIA** 1. Patients who were prescribed lomitapide outside of the marketing authorization or in contraindicated patients 2. Patients who are receiving lomitapide in clinical trials 3. Patients receiving an investigational agent, defined as any drug or biologic agent other than lomitapide that has not received Market Authorization in the country of participation, at time of enrolment

Objectives	Primary objective: The primary objective of the study is to evaluate the incidence of MACE during the first three years of treatment with lomitapide compared to the incidence of MACE during the three years prior to initiation of lomitapide. Secondary objectives: • To evaluate the changes in LDL-C and plasma lipids at one, two and three years of lomitapide treatment versus baseline • To evaluate changes in the levels of AST, ALT, GGT at one, two and three years of lomitapide treatment versus baseline, as measures of safety • To evaluate the changes of lipid-lowering treatment (including LDL apheresis and evinacumab) at one, two and three years of lomitapide treatment versus baseline • To evaluate LDL-C and plasma lipids values, LFTs and lipid lowering therapies during untreated period and when new lipid-lowering treatments were added pre-lomitapide Exploratory objectives: • To evaluate additional biomarkers of liver (e.g. FIB4) and vascular damage as outlined in section 3.2.3 after three years of lomitapide treatment, where available • To evaluate the presence and extent of liver steatosis as estimated with liver ultrasound or MRI following three years of lomitapide treatment, wherever possible • To evaluate the results of liver elastography as estimated with fibroscan or other relevant imaging methods, after three years of lomitapide treatment, wherever possible • To evaluate dietary records for patients participating in the prospective study

	• To evaluate adherence to lipid lowering medications for patients participating in the prospective study • Only for patients that have already completed retrospectively the first three years of lomitapide treatment, a descriptive evaluation about further data (clinical, biochemical and instrumental) related to the additional 24 months of lomitapide treatment will be performed
Statistical considerations	Sample size This is an exploratory study and the potential sample size is restricted to what is feasible for this rare disease. Nevertheless, existing data can be used to justify the planned sample size. Preliminary results are from an updated analysis of existing MACE data from a pan-European HoFH cohort in the two years before and the two years after lomitapide treatment ('pan-Euro data'), (D'Erasmo et al 2022). The proportions of subjects with a MACE event are 6/26=23.1% (pre) and 3/26=11.5% (post). The observed concordance probability is 23/26= 88%. Assuming marginal rates of 23% (before) and 11.5% (after) and an assumed true concordance probability of 88% and using standard statistical methodology for sample size estimation for McNemar's test (Connor, 1987), a sample size of 72 subjects will provide 80% power for a two-sided test at the alpha=0.05 level of significance. Statistical Analysis The primary analysis will compare the proportions of subjects who have a MACE event between the before and after periods using a two-sided McNemar's test at the alpha=0.05 level of significance. The analysis will include all subjects with pre and post results. All analyses will be detailed in the Statistical Analysis Plan (SAP) which will be finalized as early as possible in advance of the Database freezing. As for the secondary endpoints, i.e. all the laboratory parameters as detailed in Section 3.2.2, descriptive statistics, as detailed in Section 13.2.1 will be presented. In addition, the

	paired t-test (or the relevant non parametric test in case of non-normal distribution) will be used for the analyses of the changes from baseline to final visit. The safety and tolerability data will include (at least) physical examinations, vital signs, laboratory data and adverse events.
Data Management and Monitoring	Patients will be enrolled from >26 centers. The source data, recorded in the appropriate source documentation, will be reported at site by the Investigator and/or delegates in a web-based Database (eCRF). The patient data will be uploaded in the Database following the study initiation and after patient consent has been obtained. The data cleaning will be performed by the Data Manager of the study through a specific function of the eCRF. The validation of the inconsistencies (change or acceptance) will be made by the Investigator and/or delegate. Limited independent monitoring of data will be performed in accordance with a monitoring plan. Before the data freezing, the specialist in charge will code the medical terms using MedDRA dictionary for the Adverse Events and the Pathologies and the WHO-ATC dictionary for the medications. The Data Manager will also oversee the database freezing and provide the statistician in charge of the study with the cleaned Database for the statistical analyses.
Ethical Considerations	The protocol has been written, and the study will be conducted according to the Declaration of Helsinki and the ICH Harmonized Tripartite Guidelines for Good Clinical Practice. Prior to the collection of any study related data, approval of the competent Ethics Committee (EC) is required. Approval for the protocol, informed consent and all patient enrolment materials will be obtained in each country and for each site, as applicable.