NONINTERVENTIONAL STUDY REPORT SYNOPSIS

Title	Retrospective chart review analysis of pairs of siblings with Mucopolysaccharidosis type II to evaluate the effectiveness of idursulfase started at 12 months of age and younger
Study Short Title	Takeda MPS II Siblings Study
Study Acronym	N/A
Protocol Number	TAK-665-5001
Study Registration Number	This study will not be registered on ClinicalTrials.gov
Active substance & ATC code	Other alimentary tract and metabolism products – enzymes (ATC code: A16AB09)
Medical Product	Idursulfase
Product Reference	Elaprase®
Sponsor/Marketing Authorization Holder (MAH)	Shire Human Genetic Therapies, Inc., part of the Takeda Group 300 Shire Way, Lexington, MA 02421 USA
Dates on which the study was initiated and completed	Start of data collection: 04 March 2022 End of data collection: 08 February 2023
Date of final study report	16 August 2024
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This study was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki, Good Clinical Practice (GCP), the International Society for Pharmacoepidemiology (ISPE) Guidelines for Good Pharmacoepidemiology Practice (GPP), and any applicable local regulations.

Synopsis[11]

Title	Retrospective chart review analysis of pairs of siblings with Mucopolysaccharidosis type II to evaluate the effectiveness of idursulfase started at 12 months of age and younger
Study team and collaborators	● ICON Clinical Research Limited ● Takeda Pharmaceutical Company Limited The study is sponsored by Shire Human Genetic Therapies, Inc., part of the Takeda Group
Rationale and background	Mucopolysaccharidosis type II (MPS II; Hunter syndrome) is an inherited, multisystemic, genetic disorder characterized by a deficiency of the lysosomal enzyme iduronate-2-sulphatase (IDS). Enzyme replacement therapy (ERT) with IV idursulfase (Elaprase®) has demonstrated clinical benefits in patients with MPS II within both clinical trials and post-marketing observational studies. Given the nature of the primary endpoints, patient recruitment in the pivotal Elaprase study (TKT024) was limited to those able to perform pulmonary function tests and the 6-minute walk test. Therefore, children less than 5 years of age were excluded from this study. The results of a subsequent open-label study demonstrated that Elaprase at a weekly dose of 0.5 mg/kg was safe and efficacious in children between 1.4 and 7.5 years of age (median age in the study: 4.0 years). In addition, case reports have described the safe and effective use of Elaprase treatment initiated in children less than 18 months of age. Despite the clinical benefits of Elaprase in patients with MPS II demonstrated by studies conducted to date, limited evidence has so far been generated in very young patients who initiated Elaprase at an age of 12 months or less. This retrospective study, in which data were collected from patients who initiated Elaprase at an age of 12 months or less and their outcomes compared with their older siblings who initiated treatment at an age of 36 months or more, was conducted to address the lack of scientific evidence of the effectiveness of Elaprase in this patient population and the impact of early initiation of Elaprase on clinical outcomes.
Research question(s) and objectives	What impact does early treatment initiation with Elaprase have on treatment effectiveness in patients with MPS II who initiated ERT at 12 months of age and younger compared with their siblings who initiated ERT at 36 months of age and older? The overarching goal of this study was to gain a more complete understanding of the real-world practice of early treatment initiation with Elaprase in very young patients with MPS II and evaluate the impact of Elaprase treatment initiated at an early age. To achieve this goal, the study had the following objectives: I. Primary objective ● To evaluate real-world effectiveness of Elaprase in patients with MPS II who initiated this treatment at 12 months of age and younger.

[11] This Clinical Study Report Synopsis will be reported in British English

	II. Exploratory objectives ● To assess the impact of Elaprase treatment in early age by comparing effectiveness parameters in children who initiated this treatment at 12 months of age and younger and their siblings who initiated this treatment at 36 months of age and older. ● To compare effectiveness parameters in children who initiated Elaprase treatment at up to 20 months of age with their siblings who initiated Elaprase treatment at an older age, on condition that the older sibling initiated Elaprase treatment at an age at least 12 months older than the age of younger sibling at Elaprase treatment initiation.
Study design	This study was a global, multi-centre, non-interventional retrospective chart review study to evaluate the real-world effectiveness of Elaprase in siblings with MPS II. Patient data were collected from existing medical charts at participating sites in Europe, Latin America, North America and Kazakhstan selected based on information gathered through the Medical Insights Questionnaire (MIQ) applied by Takeda Global Medical Affairs. A retrospective chart review was considered the most appropriate design for this study, given the interest in evaluating the effectiveness and outcomes of early ERT initiation with Elaprase. To ensure that the study would address the objective of evaluating long-term outcomes in the study population, it was necessary to limit the study to patients who had been receiving Elaprase for treatment of MPS II for at least 2 years. Utilising a controlled design, such as matching, which is difficult for rare diseases, was an important approach to addressing the study objectives. As siblings are similar with respect to genetic disposition, each sibling acted as a control for each other.
Setting	The study was conducted in 14 hospitals across Europe, Latin America, North America, Turkey, and Kazakhstan. The participating countries were Argentina, Brazil, Colombia, France, Greece, Kazakhstan, Mexico, Russia, Turkey, United Kingdom (UK), and the United States of America (USA).
Ethics	The study was conducted in compliance with local, legal, and regulatory requirements. The study protocol, informed consent form (ICF) (if applicable), and other relevant documents were submitted to Institutional Review Board(s) / Ethic Committee(s) in the countries where the study was conducted for evaluation and approval.
Patients	Sibling pairs with MPS II who initiated ERT with Elaprase at different time in their lives (≤20 months for the younger sibling and the age of the younger sibling at treatment initiation plus 12 months or more for the older sibling) were enrolled in this study. **Inclusion criteria (per protocol):** 1. The patient is male. 2. The patient has diagnosed with MPS II (biochemically and/ or genetically). 3. The patient is a sibling of at least 1 male patient diagnosed with MPS II who received Elaprase treatment.

	4. The patient and his sibling(s) received Elaprase for treatment of MPS II for at least 2 years. 5. The younger sibling must have started Elaprase treatment at a maximum age of 20 months and there is a confirmed minimum of 12 months age difference at Elaprase treatment initiation between the younger and older sibling(s). **Exclusion criteria (per protocol):** 1. Patients or sibling(s) who received treatment for MPS II with an ERT or an investigational product other than idursulfase (intrathecal or intravenous) prior to or within 2 years of initiating Elaprase treatment. 2. Patients or sibling(s) who underwent bone marrow transplantation (BMT) or hematopoietic stem cell transplantation (HSCT) prior to or within 2 years of initiating Elaprase treatment.
Data source and data collection	Data were collected retrospectively from patients' medical charts from the date of diagnosis of MPS II to the last post-Elaprase treatment initiation follow-up data available (ie, minimum follow-up time was 2 years). Data collection started on 04 March 2022, and end of data collection was 08 February 2023. All relevant chart data were abstracted and entered by the site principal investigator or delegated site staff into an online electronic case report form (eCRF) programmed by ICON in an electronic data capture system (Dacima), which is fully compliant with Food and Drug Administration 21 CRF Part 11 requirements. If any relevant data were not available from medical charts, the eCRF offered unknown/not available answer options where needed. Sites completed an eCRF for each eligible patient and his sibling(s) identified.
Variables	**Variables (Exposures, Outcomes and/or Endpoints)** The following variables were abstracted from patients' medical charts, depending on availability. Evaluation of dosing was performed based on available data points for administered dose (mg/kg). **Exposure** ● Patients' age at Elaprase treatment initiation ● Elaprase dosing [labelled dose (yes/no)] and frequency ● Compliance to Elaprase treatment (>80% of scheduled infusions) **Outcomes and/or Endpoints** Even though MPS II is a progressive disease, the course of the disease is highly heterogeneous. In previous chart review studies, there was no pattern or single outcome endpoint that could determine effectiveness in MPS II. Therefore, the following clinical outcomes were assessed: ● Urinary glycosaminoglycan (GAG) levels ● Signs and symptoms by organ system (presence of symptoms, age at onset, frequency of recurrence, and severity) ● Growth parameters (height, weight, and corresponding z-scores) ● Cardiac parameters: o Left ventricular mass index (as calculated by echocardiography) o Onset, type, severity, and progression of cardiac valve disease (assessed by echocardiography)

	● Respiratory parameters: o Hospitalisations for respiratory infections o Frequency and severity of respiratory infections and associated antibiotic use ● Ear, nose, and throat parameters: o Frequency and severity of ear infections, treatment utilisation (including antibiotic use) o Presence/ age at onset of enlarged tonsils and adenoids, its treatment, and outcomes (including reoccurrence) o Presence/ age at onset of obstructive sleep apnoea o Presence and severity of hearing problems, treatment utilisation (including T-tubes insertion and hearing aids) and outcomes ● Abdominal/Gastrointestinal tract parameters: o Liver and spleen size (as estimated by palpation and/ or abdominal ultrasounds, if available) o Presence of chronic diarrhoea ● Musculoskeletal parameters: o Joint stiffness [ie, joint range of motion (wrist, elbow, shoulder, hip, knee, ankle)] o Presence/ age at onset of claw hands o Presence/ age at onset of coarse facial features o Presence/ description of dysostosis multiplex (including radiology findings) o Presence/ age at onset of spinal abnormality (scoliosis, kyphosis) o Presence/ age at onset of immobility o Occurrence and severity of vertebral collapse (including associated nerve damage) ● Neurological parameters: o Presence/ age at onset of carpal tunnel syndrome, its treatment, and outcomes (including reoccurrence) o Presence/ age at onset of peripheral nerve (non-carpal tunnel) involvement, including sensory-motor investigations (e.g. evoked potentials o Presence/ age at onset of neurocognitive decline o Diagnosis of cognitive impairment (CImp) (Yes/ No). If Yes: ▪ Age at CImp diagnosis ▪ Method of CImp diagnosis (formal evaluation or clinical judgement) ▪ Results of formal testing (if available) ● Other parameters: o Frequency and causes of hospitalisations o Frequency and causes of admissions to the emergency room o Frequency and reasons for surgeries (including types and specific surgeries performed)

	Other Study Variables ● Age at MPS II diagnosis ● Enzyme level at treatment baseline[2] ● IDS genetic mutations ● Elaprase treatment discontinuation (if applicable) ● BMT/HSCT treatment (if applicable) ● Death, including age at time of death and cause of death (if applicable)
Sample size	The MIQ feasibility study was able to estimate that ~25 sibling pairs met the criteria for the primary objectives. The inclusion of up to 25 sibling pairs (50 patients) with MPS II was planned, based on the anticipated availability of patient charts at the sites. If enrolling 25 sibling pairs was not achievable, a minimum of 15 sibling pairs (30 patients) with MPS II was expected to be enrolled. All eligible sibling pairs at all sites were anticipated to be included; therefore, no formal sample size testing was conducted. It was anticipated that sibling pairs would be enrolled in this study, however, in the event that more than two siblings from one family met the eligibility criteria, all of them were enrolled in the study and included in the analysis.
Data analysis	Primary and exploratory analyses were performed using subpopulations defined by age of Elaprase treatment initiation and sibling relationship. The subpopulations of interest by objective were: **Primary Objective:** ● Cohort 1 – Younger Sibling - Elaprase Initiation: ≤ 12 months ● Cohort 2 – Matching Older Siblings **Exploratory Objectives:** ● Cohort 1 – Younger Sibling - Elaprase Initiation ≤ 12 months ● Cohort 3 – Matching Older Sibling - Elaprase Initiation ≥ 36 months AND ● Cohort 4 – Younger Sibling - Elaprase Initiation: ≤ 20 months ● Cohort 5 – Matching Older Sibling - who initiated Elaprase treatment at an older age, on condition that the older sibling initiated Elaprase treatment at an age of at least 12 months older than the age of the younger sibling at Elaprase treatment initiation. This was an observational study, and epidemiological methods were employed for the data analyses. The overall analytic strategy for this study was determined dependent on key outcomes and data structure. The analyses were descriptive; the mean, standard deviation (SD), minimum, maximum, median, and interquartile range were presented for continuous variables. Categorical variables were summarized by the number and percentage of each response. The 95% confidence interval (CI) was estimated using standard methods for continuous and dichotomous outcomes. The study was not analysed as a case-control study. The Kaplan-Meier (KM) method was used to describe cumulative incidence of "first events" from birth per cohort (per objective) for key variables. KM curves

[2] This variable was not included in the CRF to avoid extensive site burden

	were presented graphically. For a selected number of binary and continuous variables, and based on the completeness of information, an adjusted analysis with covariates was performed using a generalized linear mixed model (GLMM). This analysis was purely descriptive in the sense that no pre-defined hypothesis was tested. However, for some analyses and statistical procedures, such as Cox proportional hazard models and GLMM, hazard ratios (HR), confidence intervals and p-values were calculated; however, these should not be interpreted in the strict sense of confirmatory testing but rather served as an indicator of precision and uncertainty that is associated with the derived estimates.
Results	Overall, 38 patients (19 sibling pairs) were included for analyses in this chart review study. For the purposes of this study, 5 subpopulations (Cohorts 1 [n=15], 2 [n=15], 3 [n=11], 4 [n=19] and 5 [n=19]) were defined and patients were placed into these subpopulations based on the fulfilment of the specific subpopulation criteria. The mean and median ages at MPS II diagnosis were much lower among Cohort 1 (mean 3.1 months; median 2.0 months, respectively) when compared with the other cohorts. The mean and median times from diagnosis to treatment initiation were slightly lower among Cohort 1 (mean: 2.0 months; median: 1.0 month, respectively) compared with the other cohorts. The mean and median follow-up times were longest in patients in Cohort 3 (mean: 148.3 months [SD:37.4], median: 166.5 months, respectively). Cohort 1 had the highest percentage of patients still alive (93.3%), while Cohort 3 had the highest percentage of deceased patients (45.5%). Across all 5 cohorts the mean age at Elaprase treatment initiation ranged from 5.1 months (Cohort 1, [SD: 4.0]) to 61.8 months (Cohort 3, [SD: 22.8]). The mean Elaprase dose administered to patients at treatment initiation was 0.6 mg/kg [SD: 0.3] in Cohort 1 and 4, and 0.5 mg/kg in Cohort 2 [SD: 0.1], Cohort 3 [SD: 0.0] and Cohort 5 [SD: 0.1]. The frequency of once per week for Elaprase administration was 100% across all 5 cohorts. Majority of patients across all five cohorts did not discontinue Elaprase treatment. The probability of experiencing a first event of hearing problem (HR: 0.84; 95% CI: 0.35, 2.0), sleep apnoea (HR: 0.67; 95% CI: 0.13, 3.60), joint stiffness (HR: 0.69; 95% CI: 0.27, 1.73), coarse facial features (HR: 0.83; 95% CI: 0.34, 2.07), abnormal liver size (HR: 0.32; 95% CI: 0.04, 2.98), chronic diarrhoea (HR: 0.41; 95% CI: 0.08, 2.13), carpal tunnel syndrome (HR: 0.28; 95% CI: 0.03, 2.37), enlarged tonsils and adenoids (HR: 0.36; 95% CI: 0.10, 1.33), and spinal abnormality (HR: 0.74; 95% CI: 0.17, 3.12) was less likely in Cohort 1 compared with Cohort 2. The probability of experiencing a first event of cardiac valve disease (HR: 1.075; 95% CI: 0.407, 2.835), and surgery (HR: 1.35; 95% CI: 0.56, 3.28) was higher in Cohort 1 compared with Cohort 2. Regarding the GLMM analyses, stronger effects (p-values <0.05) were seen in several outcomes: younger siblings (Cohort 1) had an additional 1.1 height-for-age Z-score points (p=0.0022), lower levels (-43.7 g/m2) of left ventricular mass (LVMI) (p=0.0051), lower presence (-1.7) of cardiac valve disease (p=0.0201), lower counts (-2.3) of joint stiffness (p=0.0249), lower presence (-6.7) of coarse facial features (p=0.0230), more measurements (6.8) of “normal liver size” estimated by palpation (p=0.0038); lower counts (-3.8) of carpal tunnel syndrome (p=0.0031), and lower counts (-3.1) of enlarged tonsils and adenoids (p=0.0025) compared with older siblings (Cohort 2). For the other outcomes examined, effects were not strong (p-values >0.05): younger siblings (Cohort 1) had lower measurements (-1.8 cm) for height (p=0.5608), slightly

	higher (0.1 cm) liver size measurements (p=0.9421), higher (236.2 µg/mg) urinary GAG levels (p=0.2171), more measurements (2.8) of "normal liver size" determined by abdominal ultrasound (p=0.3007) and higher "counts of surgeries" (0.3 [p=0.5468]), and lower counts of hearing problems, sleep apnoea, and chronic diarrhoea attributable to MPS II (-1.3 [p=0.1147], -1.0 [p=0.4988], -2.5 [p=0.1169], respectively) compared with older siblings (Cohort 2). Similar findings were observed for the exploratory analyses when Cohort 1 (younger siblings) was compared with Cohort 3 (older siblings), or Cohort 4 (younger siblings) was compared with Cohort 5 (older siblings).
Discussion	This study analysed data from clinical charts from 19 sibling pairs (38 total patients) with MPS II treated with Elaprase and summarized the treatment effectiveness on several clinical outcomes amongst the sibling pairs. Across all patients in this study, the mean age at diagnosis ranged from 3.1 months (<1 year old) to 53.9 months (~ 4.5 years old). This age range is consistent with published literature, which reports mean age at diagnosis of MPS II from 12 months of age to 7 years old. Regarding Elaprase treatment, among the patients in this study, the mean age at Elaprase treatment initiation ranged from 5.1 months (<1 year old) to 61.8 months (~5 years old), which is at least partially in line with the existing literature. The findings from this study regarding Elaprase treatment dose (a mean dose of 0.5 mg/kg in Cohorts 2, 3 and 5) and frequency are similar to previous reported literature and in line with recommendations. The results of this research are consistent with prior publications showing improvement in height measurements, joint mobility, sleep apnoea, liver size, cardiac valve disease, and LVMI measurements post-initiation of idursulfase. Moreover, this study demonstrated effects on clinical outcomes, such as enlarged tonsils and adenoids, coarse facial features, carpal tunnel syndrome, chronic diarrhoea and hearing problems post-initiation of idursulfase, contributing to the body of evidence. One main strength of this study was the use of matched sibling pairs design. Such a design minimizes potential confounding bias, as demographic and baseline clinical characteristics were collected to allow for comparison of outcomes. Another strength of this study was the wide geographic coverage of data that were collected; due to this, the findings from this study may be generalizable to other MPS II populations in various regions. Furthermore, the data collected focused on raw clinical variables rather than classification outcomes, which minimized the impact of potential misclassification or differences in categorization across different sites and regions. Due to the nature of this study as a retrospective chart review, and the rarity of MPS II, this study also had important limitations. One of the limitations of this study was the use of ICFs for some sites (France & UK), which introduced selection bias, as data could not be collected for patients who did not provide consent. Moreover, this study focused primarily on matched sibling pairs, which limits the availability and diversity of the study sample, thus also introducing selection bias. In addition to this, the inclusion criteria for this study included matched sibling pairs that had a 12-month difference in the time of Elaprase treatment initiation between the younger sibling and the older sibling, indicating that other sibling pairs may have been excluded from this analysis. As a result, data from this study may not necessarily be representative of the full spectrum of clinical outcomes amongst Elaprase-treated sibling pairs with MPS II. Secondly, it should be noted that comparisons between the different cohorts reported in the results section and interpretation of the results may need to be viewed and interpreted with caution, due to the small sample size. Furthermore, the events were reported regardless of severity, which may have led to

	capturing new symptoms and events for the younger siblings earlier and in a less severe form compared with the same events reported in the older siblings. A further limitation pertains to the KM curves: the effect of left censoring can't be excluded as information between birth and treatment for the older siblings might be missing. Lastly, some fields in the eCRF were programmed as free text fields, to enable site staff to enter in information pertaining to relevant variable. Such free text fields could have introduced reporting bias as it is possible only a selection of the results or outcomes were captured for the related variable, which may only cover a fraction of the real-world data.
Conclusions	This study provided descriptive evidence of benefit for some of the evaluated outcomes in patients with MPS II who initiate treatment with Elaprase at an earlier age. Furthermore, this study has demonstrated (supported by stronger effects observed in the GLMM analyses) that Elaprase appears effective in improving some clinical outcomes, such as height, joint stiffness, LVMI, cardiac valve disease, liver size, coarse facial features, carpal tunnel syndrome, and enlarged tonsils and adenoids. This study further highlights the importance of early Elaprase treatment initiation within this patient population. Moreover, the information from this study further supports the notion that newborn screening (for MPS II) should be an option included in healthcare facilities or institutions as a means of achieving diagnosis and treatment sufficiently early to optimize therapeutic benefit.