22 December 2025
EMA/CHMP/113960/2025
Committee for Medicinal Products for Human Use (CHMP)

CHMP assessment report

Aqneursa

International non-proprietary name: levacetylleucine

Procedure No. EMEA/H/C/006327/0000

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List of abbreviations

8MWT	8-meter walk test
9HPT(D/ND)	9-hole peg test (dominant hand/non-dominant hand)
ADR	Adverse drug reaction
ADL	Acetyl-D-leucine
ADLL	Acetyl-DL-leucine
ALL	Acetyl-L-leucine
AE	Adverse event
ALP	alkaline phosphatase
ALT	alanine transaminase
API	Active pharmaceutical ingredient
ASIS	Annual severity increment scores
A-T	Ataxia-telangiectasia
ATP	Adenosine triphosphate
BCRP	Breast cancer resistance protein
BCS	Biopharmaceutics Classification System
BSEP	Bile salt export pump
CGI-C	Clinical global impression of change
CGI-I	Clinical global impression of improvement
CGI-S	Clinical global impression of severity
CHO	Chinese hamster ovary
CI	Confidence interval
CI-CS	Clinical impression of change in severity
C_{max}	Maximum concentration
CNS	Central nervous system
CPP	Critical Process Parameters
CSR	Clinical study report
CTD	Common technical document
CV	Cardiovascular
DDI	Drug-drug interaction
DSMB	Data safety monitoring board
ECG	Electrocardiogram
EFD	Embryofoetal developmental
EP	Extension phase
EQ-5D-5L	European Quality of Life 5 Dimensions - 5 Levels
EQ-5D-Y	European Quality of Life, 5-Dimensions, Youth
EU	European Union
GI	Gastrointestinal
GLP	Good laboratory practice
GOF	goodness-of-fit
GSL	Glycosphingolipid
HDPE	High Density Polyethylene
HED	Human Equivalent Dose
HPLC	High Performance Liquid Chromatography
ICE	Intercurrent event
IMP	Investigational medicinal product
INN	International non-proprietary name
i.p.	Intraperitoneal
ISR	Incurred sample reanalysis

ISS	Integrated summary of safety
ITT	Intention-to-treat
JAS	juvenile animal study
LCL	Lower confidence limit
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LDPE	Low Density Polyethylene
LOCF	Last observation carried forward
LS	Least squares
LSD	Lysosomal storage disorder
MAA	Marketing authorisation application
Max	Maximum
MCT	Monocarboxylate transporter
MDR1	Multidrug-resistance-protein 1
mDRS	Modified disability rating scale
Min	Minimum
mITT	Modified intention-to-treat
mITTe	Modified intention-to-treat population of the Extension Phase
MoCA	Montreal cognitive assessment
mSARA	Modified scale for the assessment and rating of ataxia
NADL	N-acetyl-D-leucine
NADLL	N-acetyl-DL-leucine
NALL	N-acetyl-L-leucine
NAS	New active substance
NDA	New Drug Application
NOAEL	No observed adverse effect level
NPC	Niemann-Pick type C disease
NPC-CSS NPC	Niemann-Pick type C disease clinical severity scale
PD	Pharmacodynamic(s)
PedsQL	Paediatric quality of life questionnaire
PEG	Percutaneous endoscopic gastrostomy
PK	Pharmacokinetic(s)
p.o.	Per os (oral)
popPK	Population pharmacokinetic(s)
PRO	Patient-reported outcome
PT	Preferred term
QP	Qualified Person
QoL	Quality of life
RCT	Randomised-controlled trial
RDT	Repeat-dose toxicity
REC	Recommendation
rCGM	regional cerebral glucose metabolic rate
RH	Relative Humidity
RMP	Risk management plan
RRT	Relative retention time
SAE	Serious adverse event
SAF	Safety analysis set
SARA	Scale for the assessment and rating of ataxia
SCAFI	Spinocerebellar ataxia functional index
SD	Standard deviation
SE	Standard error

SmPC	Summary of product characteristics
TDI	time-dependent inhibition
TEAE	Treatment-emergent adverse event
TK	Toxicokinetic
TFEB	Transcription factor EB
TLC	Thin Layer Chromatography
UCL	Upper confidence limit
UV	Ultraviolet
VAS	Visual analogue scale
WOE	Weight of Evidence

1. Background information on the procedure

1.1. Submission of the dossier

The Applicant IntraBio Ireland Limited submitted on 3 June 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Aqneursa, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 30 March 2023.

Aqneursa, was designated as an orphan medicinal product EU/3/17/1848 on 20 March 2017 in the following condition: Treatment of Niemann-Pick disease.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Aqneursa as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/Aqneursa.

The Applicant applied for the following indication:

Aqneursa is indicated in adults and children from birth for chronic treatment of Niemann-Pick Type C (NPC).

The final indication granted by CHMP is the following:

Aqneursa is indicated in combination with miglustat, or as a monotherapy in patients where miglustat is not tolerated, for the treatment of neurological manifestations of Niemann-Pick type C (NPC) disease in adults and children aged 6 years and older and weighing at least 20 kg.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on Applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0043/2024 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No

847/2000, the Applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

1.5. Applicant's request(s) for consideration

1.5.1. New active Substance status

The Applicant requested the active substance levacetylleucine contained in the above medicinal product to be considered as a new active substance in comparison to N-Acetyl-DL-Leucine previously authorised in the European Union as Tanganil, as the Applicant claimed that levacetylleucine differs significantly in properties with regard to safety and/or efficacy from the already authorised active substance.

1.6. Protocol assistance

The Applicant did not seek Protocol assistance from the CHMP.

1.7. Steps taken for the assessment of the product

The application was received by the EMA on	3 June 2024
The procedure started on	20 June 2024
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	9 September 2024
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	n/a
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	23 September 2024
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	03 October 2024
The CHMP agreed on the consolidated List of Questions to be sent to the Applicant during the meeting on	17 October 2024
The Applicant submitted the responses to the CHMP consolidated List of Questions on	29 December 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	03 February 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	13 February 2025
The CHMP agreed on a list of outstanding issues to be sent to the Applicant on	27 February 2025

The Applicant submitted the responses to the CHMP List of Outstanding Issues on	26 March 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	09 April 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	10 April 2025
The CHMP agreed on a list of outstanding issues to be sent to the Applicant on	25 April 2025
The Applicant submitted the responses to the CHMP List of Outstanding Issues on	20 May 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	04 June 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	05 June 2025
Oral explanation	17 June 2025
The CHMP agreed on a list of outstanding issues in an oral explanation to be sent to the Applicant on	19 June 2025
The Applicant submitted the responses to the CHMP List of Outstanding Issues on	26 June 2025
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Aqneursa on	24 July 2025
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	24 July 2025

1.8. Steps taken for the re-examination procedure

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Fátima Ventura Co-Rapporteur: Antonio Gomez-Outes

The Rapporteur appointed by the PRAC was: n/a

The Applicant submitted written notice to the EMA, to request a re-examination of Aqneursa CHMP opinion of 24 July 2025, on	31 July 2025
The CHMP appointed Fátima Ventura (PT) as Rapporteur and Antonio Gomez-Outes (ES) as Co-Rapporteur	18 September 2025
The Applicant submitted the detailed grounds for the re-examination (Appendix 1 of Final Opinion) on	22 September 2025
The re-examination procedure started on	23 September 2025

The CHMP Rapporteur's re-examination assessment report was circulated to all CHMP members on	13 October 2025
The CHMP Co-Rapporteur's assessment report was circulated to all CHMP members on	13 October 2025
The CHMP Rapporteurs circulated the Rapporteurs Joint Assessment Report on the detailed grounds for re-examination to all CHMP members on	05 November 2025
The detailed grounds for re-examination were presented by the applicant during an oral explanation before the CHMP on	10 November 2025
The CHMP, in the light of the scientific data available and the scientific discussion within the Committee, re-examined its initial opinion and in its final opinion concluded that the application satisfied the criteria for authorisation and recommended the granting of the marketing authorisation on The CHMP considered that levacetylleucine is not to be qualified as a new active substance, as insufficient evidence has been provided to demonstrate that it differs significantly in properties with regard to safety and/or efficacy from the previously authorised substance.	13 November 2025
An opinion was adopted by the CHMP on 13 November 2025. A revised opinion was adopted by the CHMP on 22 December 2025 to improve clarity and consistency in the assessment.	

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The Applicant applied for the following indication:

"Aqneursa is indicated in adults and children from birth for chronic treatment of Niemann-Pick Type C (NPC)."

2.1.2. Epidemiology

NPC is a rare, progressive, and fatal neurodegenerative disorder with an estimated incidence of ~1:100,000 live births (Geberhiwot et al. 2018).

NPC is inherited in an autosomal-recessive manner caused by mutation in either NPC1 or NPC2 Both genes encode lysosomal proteins that are essential in intracellular transport and metabolism of lipids. As a result of the dysfunction of either of these NPC proteins, lysosomal function is impaired causing an accumulation of lipids in the lysosomes, which in turn leads to cell stress and toxicity. Over time, this leads to neurodegeneration as well as peripheral organ dysfunction

2.1.3. Aetiology and pathogenesis

NPC is caused by mutations in the NPC1 (~95% of cases) or NPC2 (~5% of cases) genes that encode the NPC1 and NPC2 proteins important for intracellular trafficking and transport of cholesterol and sphingolipids from the late endosome/lysosome (organelles that are involved in the breakdown and recycling of macromolecules in most mammalian cells) [Stern, 2014; Alavi et al. 2013; Fusco et al. 2012; Naureckiene et al. 2000; Park et al. 2003; Steinberg et al. 1994]. NPC1 or NPC2 dysfunction leads to the storage of lipids in the late endocytic. An expanded lysosomal volume (representing lysosomal dysfunction) and the accumulation of cholesterol, sphingosines, and glycosphingolipids (GSLs) in neuronal and non-neuronal tissues are the hallmark phenotypes of NPC and key drivers of the disease [Lloyd-Evans and Platt, 2010; te Vruchte et al. 2014].

Another important contributory factor to the pathophysiology of several LSDs (including NPC and GM2 gangliosidosis) is mitochondrial dysfunction. When the lysosome cannot form contact sites with the endoplasmic reticulum (ER) via NPC1 or NPC2, the lysosome forms excessive contact sites with mitochondria and as a result, lipids stored in the lysosome are transferred to mitochondria. This impairs mitochondrial function, compromising cellular energy status and metabolic flux, and leads to the depletion of cellular energy – adenosine triphosphate (ATP) [Stepien et al. 2020].

2.1.4. Clinical presentation, diagnosis and stage/prognosis

The clinical presentations of NPC disease are characterized by broad heterogeneity in systemic, psychiatric, and neurological symptoms. The age of first symptoms of the disease is highly variable, as are often the first symptoms themselves, rendering diagnoses difficult and the diagnosis odyssey highly variable for each patient.

The course of the disease also varies highly from patient to patient depending on the age of onset of neurological symptoms, from a rapidly progressing neonatal form to an adult-onset chronic neurodegenerative condition. Infantile and juvenile patients often experience the most severe symptoms, including epileptic seizures and cataplexy, while adult patients experience severe cognitive impairment, dementia, and psychosis.

The symptoms of NPC patients who survive infancy are dominated by progressive neurodegeneration of the brain [Patterson et al. 2013].

In NPC, all aspects of neurological function are affected, including:

- Cerebellar dysfunction (ataxia, dysarthrophonia, dysmetria, dysphagia, cognition/language, disdiadochokinesia, dysmetria)
- Cortical dysfunction (cognition, seizures, language [speech], behaviour)
- Dysfunction of the basal ganglia (movement disorders, dystonia, tremor, dyskinesias)
- Dysfunction of the hypothalamus (sleep disorders, cataplexy)
- Dysfunction of pyramidal tract (spasticity)
- Dysfunction of the brain stem (ocular motor disturbances, swallowing, respiratory control)

The neurological symptoms in NPC include a delay in developmental motor milestones (early-infantile period) and problems at school, including difficulties in writing and impaired attention (late-infantile and juvenile period). A cardinal symptom is cerebellar ataxia (present in 70% of patients) where patients have problems with stance and gait ataxia and consequent falls, dizziness, clumsiness, dysmetria, and disdiadochokinesia. Other neurological signs are vertical supranuclear gaze palsy, dysphagia, gait

disorders, and dementia, which can lead to a premature death. Cataplexy, seizures, and dystonia are also common symptoms. Broadly, NPC is characterized for the wide variety of its clinical features: some patients may have no walking issues, but severely impaired upper limb mobility; some patients may be able to talk and use their arms normally but be wheelchair-bound; some patients may have significant cognitive behavioural impairment, but mild motor symptoms. The majority of NPC patients are children and die before the age of 20. Life expectancy varies from death in infancy to >60 years (dependent on age of onset) with a median age of death of around 13 years [Garver et al. 2007; Bianconi et al. 2019].

2.1.5. Management

There are no curative therapies for NPC. Miglustat is the only medicinal product in the EU authorised for NPC. Miglustat is a compound first licensed for type 1 Gaucher disease that has received market authorisation for use in NPC patients in the European Union (EU), Japan, and other countries (but not in the United States [US]). Evidence in support of miglustat in NPC comes from a randomised clinical trial, long-term extension studies, and two retrospective surveys, demonstrating a reduction of the progression of clinically relevant neurological symptoms in patients with NPC. In NPC patients, miglustat is indicated for the treatment of progressive neurological manifestations and has been shown to slow the general progression of neurological symptoms in patients with NPC.

2.2. About the product

The Applicant is seeking marketing approval for levacetylleucine (N-acetyl-L-leucine (NALL); company code: IB1001) for the treatment of adult and paediatric patients with Niemann-Pick type C (NPC) disease.

Levacetylleucine is an amino acid derivative resulting from N-acetylation of the essential amino acid L-leucine. It is the L-enantiomer of N-acetyl-DL- leucine that has been marketed in France since 1957, under the trade name Tanganil for the treatment of acute vertigo in adult.

Levacetylleucine targets the pathology mitochondria and lysosomal function, neuroinflammation, defects in cellular signalling, and neurodegeneration seen in lysosomal storage disorders (LSDs) such as NPC and in other neurological disease models.

2.3. Type of Application and aspects on development

The CHMP did not agree to the Applicant's request for an accelerated assessment as the product was not considered to be of major public health interest. This was based on uncertainty on the strength of the clinical data to be submitted in supporting the intended broad indication covering all NPC sign/symptoms in adults and paediatric patients of all ages.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as granules for oral suspension containing 1000 mg of levacetylleucine as active substance.

Other ingredients are: isomalt (E953), Hypromellose and strawberry flavour (contains propylene glycol (E1520)).

The product is available in single-dose paper-backed aluminium/polyethylene sachets as described in section 6.5 of the SmPC.

2.4.2. Active Substance

2.4.2.1. General information

The chemical name of levacetylleucine is 2(*S*)-(acetylamino)-4-methylpentanoic acid corresponding to the molecular formula $C_8H_{15}NO_3$. The active substance is also known as N-acetylleucine, N-acetyl-L-leucine.

It has a relative molecular mass of 173.21 g/mol and the following structure:

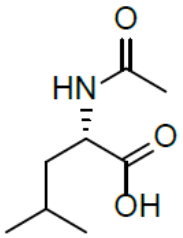


Figure 1: active substance structure

The chemical structure of the active substance was elucidated by elemental analysis, mass spectrometry, FTIR spectroscopy, ultraviolet/visible (UV/Vis) spectroscopy, ^{1}H and ^{13}C NMR spectroscopy, X-ray crystallography and optical rotation. Other physical properties were characterised by differential scanning calorimetry (DSC), dynamic vapor sorption (DVS) analysis, thermogravimetric analysis (TGA), and X-ray powder diffraction (XRPD).

The active substance is slightly hygroscopic, white to off-white crystalline powder. The active substance is slightly soluble in water, soluble in 2-propanol, freely soluble in ethanol and practically insoluble in heptane.

The active substance exhibits stereoisomerism due to the presence of one chiral centre. The absolute configuration at the single stereocenter is (S). Enantiomeric purity is controlled routinely with the optical rotation test in the starting material and with the specification limit for the enantiomeric impurity in the active substance specification.
There is only one crystalline form known and observed. It has been demonstrated that manufacturing process constantly produces this form and consistency of the polymorphic form has been demonstrated by XRPD spectra of aged active substance batches (40 months stored at 25 ºC/60% RH).

The applicant claimed new active substance status (NAS) for levacetylleucine. The racemate, N-acetyl-DL-leucine has been granted a national marketing Authorisation (MA) in France (number 310 337-3, 1992 to present). From the quality side, both the racemate and levacetylleucine expose the patient to the same therapeutic moiety and therefore L-acetyl leucine does not qualify as a NAS on quality grounds.

2.4.2.2. Manufacture, characterisation and process controls

The active substance is manufactured at one site. The QP Declaration provided by the MIAH site is accepted.

The manufacturing process of the active substance is a simple, one step chemical transformation. The starting material is a commercially available natural amino acid with acceptable specifications.

Although there is only one chemical transformation step in the manufacturing process, the selection of starting material is in line with the general principles outlined in the ICH guidelines; this is acceptable. The proposed starting material is a commercially available amino acid used as dietary supplement and a commodity in a pre-existing non-pharmaceutical market. It is a substance with defined chemical properties and structure. It is incorporated as a significant structural fragment. It contains most of the structural elements present in the active substance and has one chiral centre. Its optical purity is confirmed by the optical rotation test, and the chiral integrity is also part of the final active substance specification.

The process description provides sufficient detail and covers reaction conditions, as well as amounts and specifications of materials, reagents and solvents used. Reprocessing may be performed in accordance with the ICH Q7 guideline, and the process is performed under change control which is described in ICH Q7.

Critical process parameters (CPPs) are clearly defined. Key in process control (IPC) tests necessary to control the manufacturing step are included. They are not identified as critical IPCs for the active substance manufacturing process. The methods used for in-process testing are sufficiently described.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

The known and potential impurities have been presented and discussed regarding the origin, fate and control strategy.

Genotoxic impurities have been suitably discussed, and no specific control actions are necessary from the quality perspective. Information on nitrosamines is provided, and potential presence of any N-nitrosamines has not been identified. Nitrosating agents are not present in the API manufacturing process and in materials used in the manufacturing process including API starting materials steps. The risk of contaminating the synthetic process with nitrosating agents originating from purified water and subsequent formation of N-nitroso derivatives is discussed and considered as very low.

The active substance is packed into two low-density polyethylene (LDPE) bags and tied. The LDPE bags containing active substance are placed into a high-density polyethylene (HDPE) drum. The LDPE bags comply with Ph. Eur. 3.1.3. polyolefins and Commission Regulation (EU) 10/2011, as amended.

2.4.2.3. Specification

The active substance specification from the active manufacturer includes tests for appearance (visual), appearance of solution (Ph. Eur.), identification (IR, HPLC), water content (KF), residue on ignition (Ph. Eur.), elemental impurities, assay (anhydrous basis) (HPLC), related substances (HPLC), starting material (HPLC), enantiomeric purity (HPLC) and microbial quality (TAMC, TYMC) (Ph. Eur.).

The active substance specification from the finished product manufacturer is identical to that of the active substance supplier, except that the control of elemental impurities is not included; this is acceptable.

The choice of the parameters to be controlled in the active substance specification are agreed. The maximal daily dose for levacetylleucine is 4 g. Therefore, according to ICH Q3A the reporting level for impurities is 0.03% and identification & qualification threshold is 0.05%.

Stereoisomeric purity is controlled with the optical rotation test in the starting material and with the specification limit for the enantiomeric impurity in the active substance specification.

As requested by CHMP, the applicant tightened the limit for total impurities and each unspecified impurity based on batch results, but the limit for one impurity was not tighten as per the CHMP request. The CHMP recommends the applicant to re-evaluate the specification acceptance criteria of two impurities after 15 batches of active substance batches have been manufactured (REC1).

Acceptable justification for omission of residual solvents control has been provided. The risk of genotoxic impurities and potential nitrosamines is considered negligible, and no furthers controls are needed. Elemental impurities are controlled with the limits according to ICHQ3D (Option 1) by the active substance manufacturer which is found acceptable.

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for identification, assay, related substances and enantiomeric purity testing has been presented.

Forced degradation report performed with the analytical HPLC method for assay, purity and identity of an impurity has been provided. The stability indicating nature of the used procedure is considered confirmed.

The batch analysis results obtained for registration (pilot scale) and validation (commercial scale) batches from the active substance and the finished product manufacturers remain within the proposed specification limits and batch-to-batch consistency is confirmed.

2.4.2.4. Stability

Stability data from three pilot scale (registration) and three commercial scale (validation) batches and one commercial scale post-validation batch of active substance from the proposed manufacturer stored in double LDPE bags representative of the commercial packaging for up to 36 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided.

The following parameters were tested: appearance, water content, assay/purity (anhydrous basis), related substances, starting material, enantiomeric purity and microbial quality (TAMC, TYMC). The analytical methods used were the same as for release and were stability indicating.

All the results of the tested parameters at 25 °C ± 2 °C and 40 °C ± 2 °C conditions comply with proposed specifications, and no trends or significant changes were observed.

As mentioned above, a forced degradation study has been conducted as part of the stability study by the active substance manufacturer to prove that the current analytical method HPLC-1 for assay and related substances is stability indicating. The active substance was exposed to acidic, basic, oxidative, elevated temperature and light exposure (ICH Q1B using the Option 2 light source). The active substance was stable to most tested stress conditions except oxidative and thermal stress conditions.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 48 months for the active substance stored at or below 25 °C in the proposed container.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Aqneursa 1000 mg granules for oral suspension are white to off-white, strawberry flavoured granules.

In the submitted dossier the applicant described the product as granules for oral solution. However, following a Major Objection (MO1) from the CHMP the term 'granules for oral solution' was questioned given that from the solubility characteristics of the active substance complete dissolution would not be expected after reconstitution in aqueous solution as proposed in the SmPC, and the applicant described the appearance after reconstitution as 'homogeneous white cloudy suspension', the standard term was changed to 'granules for oral suspension'. The term is in accordance with the active substance solubility data and is listed in the EDQM standard term database.

There are no overages in the formulation.

As part of development two sources of active substance have been used. Active substance from one source was used from early development to upscale and Phase 3 clinical trial batches. Active substance from the second source was used to manufacture the finished product registration batches and will be the proposed primary commercial source. The two active substance sources have been compared with regards to their physical-chemical properties and impurity profiles and the potential impact on the finished product formulation and the manufacturing process. The results confirmed the differences in physical properties are negligible and do not have a significant impact on finished product quality.

Isomalt 801, hypromellose and purified water are well known pharmaceutical ingredients which comply with Ph. Eur. requirements. The strawberry flavour contains compendial excipients and additional natural flavouring substances, and in house specification is followed. A declaration on the compliance of the flavour with the regulation 1334/2008 has been provided.

There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC.

Taste trials were also successfully completed in which the most appropriate type and percentage of strawberry flavour was chosen for masking the bitterness of the active substance for paediatric purposes.

Compatibility of the active substance with the excipients was not demonstrated through binary mixture studies of the active substance with each excipient in different mixing ratios. This is accepted given the manufacturing experience, the results of the stability and the stress studies presented.

As requested by CHMP (MO2) a comparative dissolution study was conducted to compare the initial powder for oral suspension with the developed granules for oral suspension (clinical batches and batches for commercial use) in multiple media over the physiological pH range. The differences in composition and manufacturing process of all batches were discussed. The clinical product batches are declared as representative of the proposed commercial formulation and commercial manufacturing process - identical composition of granules and manufacturing principle and process steps as proposed for commercial use. Only minor changes were implemented in the production to improve the finished product quality and scalability. The results of this study confirm the similarity between the formulation of granules used in clinical trials and the commercial finished product batches.

The medicinal product is indicated for children weighing at least 15 kg. Following a request from CHMP (MO3), the applicant provided a justification regarding the development of medicinal product in accordance with the "Guideline on pharmaceutical development of medicines for paediatric use"

(dosage form, route of administration, acceptability and palatability, excipient selection, container closure system).

The applicant conducted an in-use stability study to evaluate the reconstitution of the granules with different vehicles (40 mL of water, orange juice or almond milk) and the stability assessment in connection with feeding tubes (gastrostomy tubes).

During the review the CHMP asked the applicant to further justify the selected volume and the need to use a medical device to measure the volume. In response, the applicant provided results for an in-use study and justified the appropriateness of the volume of vehicle needed in relation to the intended posology and the target population. This was acceptable. In addition, following the clinical comments (i.e. lack of pharmacokinetic data), orange juice and almond milk were eliminated as options for dispersing the granules. Water remains as the only vehicle to be used to suspend the product.

In addition, the applicant justified that, in line with the results from clinical studies, there is no need to rinse the cups after administration of the suspension. The SmPC has been updated to clarify this.

The potential use of additional amount of vehicle to withdraw the potential remaining suspension from the administration cup has been discussed.

With regards to the consideration to co-package a medical device for measuring the reconstitution volume, the applicant argued that given the chronic use of the product, the co-packaging of a device would significantly contribute to plastic waste, which is not environmentally sustainable. Instead, patients or caregivers could use a reusable graduated measuring cup which are widely available in pharmacies. Additionally, during clinical trials, an accurate dosage was achieved without the need for a measuring device. This is acceptable.

During the in-use this study it was noted that the appearance of the suspension changes over time as particles settle to the bottom. To address this the SmPC was updated during the procedure to indicate that 'the entire suspension should be drunk immediately after preparation (within 30 minutes). If the suspension is not drunk immediately, it should be stirred again before intake'.

Although the SmPC clearly states that the content of one sachet should be poured into approximately 40 mL of liquid and fully dispersed and consumed (drunk) immediately after preparation, and that all steps must be repeated if a second sachet is required, the applicant is recommended to conduct an experiment of dispersion of two sachets in 80 mL of liquid at the same time by September 2025. This will ensure that a single dose (2 g) can be prepared in one go which is expected to be general practice (REC2).

The feasibility to administer the product through a gastrostomy tube when following the instructions in the SmPC has been sufficiently demonstrated.

A process risk assessment has been performed to identify potential CPP (critical process parameters). Critical process parameters have been identified. Additional risk assessment has been performed to assess impact of these parameters on finished product critical quality attributes. Based on implemented control strategies (defined process limits and detection of the failures) the risks have been mitigated to low.

The proposed dissolution method (MO2) and dissolution media employs paddle apparatus. Discriminatory power of the dissolution medium has not been demonstrated due to rapid dissolution behaviour of the finished product. The full validation report is provided.

The primary packaging of the finished product is single-dose paper-backed aluminium/polyethylene sachets. The material complies with EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

2.4.3.2. Manufacture of the product and process controls

As indicated above, for the production of the granules a wet granulation process is used. The manufacturing process consists of six main steps. The process is considered to be a standard manufacturing process.

The description of manufacturing process is in accordance with the manufacturing process development provided in development section. The description of the procedure is adequately detailed. Process parameters and in-process controls are adequately set to control the process leading to consistent quality. Analytical methods of in-process controls are provided in the documentation.

Stability data for bulk product packed in bulk container has been provided as requested by CHMP and shelf-life for bulk product proposed is acceptable.

The manufacturing process is considered standard. No process validation data has been provided. Before commercial distribution manufacturing process validation will be performed on the first three full-scale commercial batches. The proposed validation protocol is acceptable.

2.4.3.3. Product specification

The finished product release specifications include appropriate tests for this kind of dosage form. It comprises appearance (visual), appearance after reconstitution (visual), identification (HPLC/UV), water content (Ph. Eur.), uniformity of dosage units by content uniformity (Ph. Eur.), assay (HPLC), related substances (HPLC), dissolution (in-house), microbial quality (TAMC, TYMC, *E. coli*) (Ph. Eur.).

As requested by CHMP (MO2), the applicant included a test and acceptance criteria for dissolution in the finished product release and shelf-life specification.

During the review the CHMP requested the applicant to tighten the limit for one specified impurity, total impurities and assay in line with release and stability data. In response the applicant provided justification regarding the qualified limit for the impurity when used in children weighting at least 15 kg. According to toxicological studies in rats and dogs the qualified limit for this impurity is acceptable. Nonetheless, the applicant is recommended to further tighten this limit post approval based on batch analysis result. The applicant tightened the specification limit for total impurities. This is acceptable but the applicant is recommended to re-assess all these limits after 15 finished product batches are manufactured. This is acceptable (REC3).

The release and shelf-life specification limit for assay was tightened.

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Batch analysis data on three commercial scale finished product validation batches using a validated ICP-MS method was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective PDE. Based on the risk assessment and the presented batch data it can be concluded that it is not necessary to include any elemental impurity controls. The information on the control of elemental impurities is satisfactory.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for

marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "European Medicines Regulatory Network approach for the implementation of the CHMP Opinion pursuant to Article 5(3) of Regulation (EC) Nº 726/2004 for nitrosamine impurities in human medicines (EMA/425645/2020). Based on the information provided it is accepted that no risk was identified on the possible presence of nitrosamine impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

The analytical methods used have been adequately described. Full validation data for analytical procedures in accordance with ICH Q2(R2) "Guideline on validation of analytical procedures" has been provided following a request from CHMP. A forced degradation study to evaluate stability indicating nature of the HPLC method has been provided. Satisfactory information regarding the active substance reference standard used for identification, related substances, uniformity of dosage units and assay has been provided.

Batch analysis results are provided for three pilot scale representative batches and three pilot scale registration batches confirming the consistency of the manufacturing process and its ability to manufacture the intended product specification.

2.4.3.4. Stability of the product

Stability data from three pilot scale registration, three pilot scale representative batches and three commercial scale validation batches of finished product stored for up to 48 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The registration and the validation batches were also stored for up to 12 months under intermediate storage conditions (30±2 °C / 65±5% RH). The batches are representative of those proposed for marketing and were packed in the primary packaging proposed for marketing.

All batches were manufactured at the proposed commercial manufacturing site, using the same manufacturing process and the same class of manufacturing equipment. However, the active substance used for manufacture of the representative batches was from a different supplier than the one proposed for registration. Nonetheless, as described above, these were compared and concluded that the quality of the active substance from both sites was comparable.

Samples were tested for appearance, appearance after reconstitution, assay, dissolution, related substances, water content, and microbiological contamination. The analytical procedures used are stability indicating.

Overall, the data presented show that the finished product is stable under all storage conditions with no significant changes or trends observed in the parameters tested. Although some change in assay occurred at 6 months at accelerated storage condition, the investigation concluded that segregation occurred during sachet filling and were not related to a stability issue.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. The results confirm that the finished product is photostable.

Based on available stability data, the proposed shelf-life of 2 years as stated in the SmPC (section 6.3) is acceptable. The applicant confirmed that the start of shelf-life of the finished product is set in accordance with the guidance CPMP/QWP/072/96.

A bulk holding time study was performed on a GMP technical batch. The manufacturing process of the technical batch is similar to the manufacturing process for the proposed commercial batch size. The

results confirm that the physical and chemical integrity of the product remains intact throughout the holding period.

2.4.3.5. Adventitious agents

No excipients derived from animal or human origin have been used.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Regarding the active substance, the relevant aspects have been sufficiently addressed. Tightening of the impurities limits has been performed for one specified impurity, for each unknown impurity and for total impurities. The specification limits for two impurities are accepted as both are considered qualified, including for the use in children weighing at least 15 kg. However, it is recommended that the applicant re-evaluate their specification acceptance criteria after 15 batches of active substance have been manufacture (REC 1).

Regarding the finished product, the three major objections raised during the evaluation (namely MO1 requesting clarification of the characteristics of the product after reconstitution and revision of the proposed standard term for the dosage form; MO2 asking for further information on the finished product batches used in clinical trials and additional in vitro dissolution data; and MO3 on the confirmation of the development of the product in line with the guideline on pharmaceutical development of medicines for paediatric use) have been resolved by provision of additional data and justifications. As a result, the proposed name of the dosage form has been changed to ″granules for oral suspension″, the clinical formulation and registration formulation were shown to be comparable based on provided dissolution data and a justification regarding the development of medicinal product in accordance with the "Guideline on pharmaceutical development of medicines for paediatric use" has been provided.

Although the instructions for administration included in the SmPC have been reviewed during the procedure and are acceptable, given that the posology also includes the possibility to administer 2 sachets and the applicant has not performed an experiment of dispersion of two sachets in 80 mL of liquid at the same time, it is recommended to perform it post-approval (REC2).

Tightening of the limit for total impurities has been performed. The limit for one specified impurity is below the qualified limit. However, it is recommended that the applicant re-evaluate the specification acceptance criteria for said impurity and total impurities after 15 batches of finished product have been manufactured (REC 3).

Overall, information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product, which pertain to re-evaluation of the acceptance criteria of two impurities in the active substance specification, the execution of dispersion experiments of two sachets of finished product in 80 mL of liquid at the same time and the re-evaluation of the limits for one specified impurity and total impurities in the finished product specification. These points are put forward and agreed on as recommendations for future quality development.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.4.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. The CHMP recommends the applicant to re-evaluate the active substance specification acceptance criteria for two specified impurities after 15 batches of active substance have been manufactured (REC 1).

2. The applicant is recommended to conduct an experiment of dispersion of two sachets in 80 mL of liquid at the same time by September 2025 (REC2).

3. The applicant is recommended to re-evaluate the finished product specification acceptance criteria for one specified impurity and total impurities after 15 batches of finished product have been manufactured (REC 3).

2.5. Non-clinical aspects

2.5.1. Introduction

IntraBio Ireland Limited is seeking approval for levacetylleucine (equivalent to "N-acetyl-L-leucine" ("NALL", company code: IB1001, proposed brand name: Aqneursa; proposed international non-proprietary name (INN): levacetylleucine)), a novel drug for the treatment of Niemann-Pick disease Type C (NPC).

Aqneursa is proposed to be indicated in adults and children from birth for chronic treatment of Niemann-Pick Type C (NPC). Niemann-Pick disease type C (NPC) is an autosomal-recessive lysosomal storage disorder caused by mutations in two independent genes, NPC1 or NPC2. It is an ultra-rare, serious indication characterized by rapid neurodegeneration and is prematurely fatal.

Aqneursa contains levacetylleucine, a non-natural amino acid, derivative resulting from N-acetylation of the essential amino acid L-leucine, targeting the pathology mitochondria and lysosomal function, neuroinflammation, defects in cellular signalling, and neurodegeneration seen in lysosomal storage disorders (LSDs) such as NPC.

Levacetylleucine is the L-enantiomer of N-acetyl-DL-leucine, a modified amino acid that has been available in France as a solution for injection and in tablet form since 1957, under the trade name Tanganil (Pierre Fabre Laboratories) as a treatment for acute vertigo. Use of levacetylleucine in the treatment of NPC was first documented in published case series documenting compassionate use of Tanganil in the treatment of NPC; the L-enantiomer was prioritized for development when it was identified to be the active enantiomer, and that the D-enantiomer may inhibit the effects.

A non-clinical testing program was conducted to assess the pharmacologic, pharmacokinetic, and toxicological properties of the molecule. However, the toxicology program is not totally completed: the carcinogenic potential of levacetylleucine will be investigated in a 26-week carcinogenicity study in rasH2

mice, however a carcinogenic risk assessment was prepared for levacetylleucine to waive the 2-year carcinogenicity study in rats which might be conducted after drug approval if considered necessary.

A complete program of reproductive and developmental toxicity studies was conducted with levacetylleucine in rats and rabbits.

Moreover, the validity and the GLP compliance of some toxicity studies, like the repeat-doses toxicity studies in dogs, the Ames test and the chromosome aberrations test, cannot be supported, which will be considered in the risk assessment.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Niemann-Pick disease type C (NPC) is an autosomal-recessive LSD caused by mutations in one of 2 genes, NPC1 which encodes a membrane glycoprotein or NPC2 which encodes a soluble lysosomal protein that binds cholesterol.

NPC is biochemically characterized, by the storage of multiple lipids, including low density lipoprotein (LDL)-derived cholesterol, sphingoid bases (e.g., sphingosine and sphinganine) and all sphingolipids including GSLs and sphingomyelin. At the cellular level, fusion between late endosomes and lysosomes is impaired and LDL-derived cholesterol fails to efficiently reach the endoplasmic reticulum (ER).

In addition, mitochondrial function, responsible for the generation of cellular energy, is also significantly impaired in NPC. The combined effects of the biochemical and cellular biological defects in NPC lead to neurodegeneration and neuroinflammation, leading to the clinical signs of NPC. Most of the in vitro and in vivo primary pharmacological data came from publications by different authors.

In vitro primary pharmacology studies (Churchill et al., 2021; Churchill et al., 2020; Mann, 2018; te Vruchte et al., 2021; Kaya et al., 2021), were performed with acetyl-DL-Leucine and levacetylleucine in non-neuronal NPC1 cells. These in vitro primary pharmacology studies models were performed in different test system: non-neuronal NPC1 cells i.e. NPC1-/- Chinese Hamster Ovary (CHO) cells and fibroblasts from patients with NPC (skin).

No studies researched the mechanism of action on the target. The Applicant affirms that levacetylleucine has been demonstrated to reduce lysosomal volume and glycosphingolipids levels (GSL) and normalize mitochondria numbers and mitochondrial superoxide levels.

The mode of action of levacetylleucine in NPC disease is unclear.

In vivo, the Npc1-/- mice model was selected to study the effects of levacetylleucine in the context of NPC.

In vivo studies performed in Npc1-/- mice, as well as publications on animal models of vestibular impairments, demonstrate that the L-enantiomer, levacetylleucine, mediates the pharmaceutical effect of the racemate, N-acetyl-DL-leucine, and has potential clinical benefits over the racemic mixture when administered independently.

The results of the in vivo study from Kaya *et al*., 2021(only one dose and 5 animals per group) were mainly significant when the treated Npc1-/- mice were compared to untreated Npc1-/- mice but no results with the comparison to the wild type mice Npc1+/+ were reported.

However, untreated 9-week-old Npc1-/- mice exhibit statistically significant ataxia relative to wild type (Npc1+/+) mice.

In addition, levacetylleucine significantly reduced the magnitude of ataxia compared to the untreated Npc1-/- mice. Treatment with levacetylleucine was found to selectively reduce certain GSLs in the forebrain, including GM1a (20.1%) and GM2 (19.6%), and stored lipids in the cerebellum, including GA2 (23.8%) and GA1 (23.5%).

In addition, levacetylleucine causes decreased expression of superoxide dismutase 1 (SOD1) in the cerebellum. However, Kaya et al., 2021 were unable to detect a significant difference in SOD1 levels in the cerebella of Npc-/- and wt animals.

Moreover, it is unclear why the reduction of this important regulator of oxygen metabolism should be of advantage.

In experiments investigating synergistic effects of NADLL plus miglustat on lifespan motor function, and gait [Kaya et al. 2021], miglustat reduces levels of stored lipids and slows disease progression. Miglustat is known to significantly extend the life span of Npc1-/- mice.

When NADLL was used in combination with miglustat to treat Npc1-/- mice: the 2 drugs significantly extended the lifespan of the mice and increased Rotarod performance beyond either individual treatment. Also, the results showed that miglustat monotherapy extended the lifespan and increased Rotarod performance, higher than the levacetylleucine monotherapy.

2.5.2.2. Secondary pharmacodynamic studies

No data on secondary pharmacodynamics were performed.

2.5.2.3. Safety pharmacology programme

Levacetylleucine was tested in the core battery of GLP safety pharmacology studies.

With regards to hERG assay, it is understood that a single concentration (i.e., 525 µM) was tested and as this concentration did not inhibit 30% of the hERG tail current amplitude no dose-response curve was generated and the IC50 value was not estimated. It is emphasized that a 10-fold concentration safety margin is not high and recent literature even proposes a safety margin ≥30 (Vargas HM et al., 2021; (Redfern et al., 2003; Pollard et al., 2017.). However, the actual upper limit of solubility (525 µM) of levacetylleucine in aqueous solution is still considered implausible. Of note, solubility of levacetylleucine as well as hERG testing itself was conducted in accordance with GLP and hence at the highest standard of reliability. A safety margin of ~10 is insisted to be low but can be considered acceptable if otherwise experimentally infeasible – this appears to be this case.

In vivo telemetry study in Beagle dogs' study (Study No. 8440125 (2020), levacetylleucine induced no relevant modifications of cardiac haemodynamics or electrophysiology in the conscious, telemetered dog, after a single oral dose of up to 600 mg/kg. C_{max} in male dogs dosed at 600 mg/kg provides a 10-fold safety margin when compared to the respective steady-state levels in humans for the oral daily total dose of 4000 mg.

In female Sprague-Dawley rats, respiratory function was not modified by a single dose of levacetylleucine administered orally up to 2500 mg/kg (maximum feasible dose level). Similarly, the study in female Sprague-Dawley rats showed that levacetylleucine of up to 2500 mg/kg had no impact on CNS function after single oral administration. C_{max} and exposure values in female rats dosed at 2500 mg/kg provide more than 8-fold safety margins when compared to the respective steady-state levels in humans.

2.5.2.4. Pharmacodynamic drug interactions

No data on pharmacology drug interaction with levacetylleucine were provided. However, during Phase II and III clinical trials, levacetylleucine was combined with a broad spectrum of standard-of-care and supportive-care medication and no potential for respective pharmacodynamic drug-drug interactions was identified.

2.5.3. Pharmacokinetics

A full validation of a bioanalytical (biological samples) or analytical method (formulation) is generally performed for the quantification of the analyte (and its metabolites) in nonclinical studies. The bioanalytical methods using high-performance liquid chromatography (HPLC/MS/MS) for the determination of levacetylleucine used in pharmacokinetic and toxicokinetic studies in dog, rat, rabbit and mouse were validated.
The absorption parameters were based on the single dose (Day1) or repeat-dose (intermediate or final day of the study) administrations from animal toxicity studies. The animals were not fasting.

For this oral formulation, no experimental absorption model was performed.

Regarding the pharmacokinetic in rats, the calculated plasma half-lives (t1/2) of levacetylleucine ranged from 0.80 to 4.22 hours in males and from 0.62 to 6.71 hours in females. The C_{max}-levels and AUC-areas of the high dose level group were slightly lower on test day 91 (males and females) and on test day 178 (males only) compared to the values of test day 1.

In dogs, the dose levels were limited due to the emesis observed for the male and female animals treated with 750 mg /kg/day and above: this is clearly reflected by the AUC/dose values, which were decreased with increasing dose levels. In the 39-week study in male and female dogs, T_{max} was reached at about 1 hour after dosing. The increases in levacetylleucine mean C_{max} and AUC0-24 values were generally dose proportional from 100 to 200 mg/kg/day and less than dose proportional from 200 to 600 mg/kg/day.

Overall, plasma concentrations showed both rapid absorption and clearance of levacetylleucine with half-lives of about 1 to 4 hours. No accumulation nor significant gender difference were noted.

No studies of distribution, of excretion in milk and placental transfer with levacetylleucine have been carried out, as levacetylleucine is metabolized by deacetylation (a robust and ubiquitous mechanism), giving rise to a single metabolite, L-leucine, which is endogenously present in humans. *In vitro* plasma protein binding of levacetylleucine suggested very low binding to plasma proteins in animal species and humans.

In humans, the large volumes of distribution (ca. 200 L) suggest that levacetylleucine distributes extensively beyond the blood volume. Moreover, a publication of Benard et al. (2001) showed that Acetyl-DL-leucine (Tanganil IV) crossed Blood Brain Barrier in two monkeys, since an important uptake of radioactivity is localized in the cortical structures and in several tissues such as nuclei of the vestibular system, including the inner ear.

Concerning the metabolism, the result of an *in vitro* hepatic enzyme (mouse, rat, rabbit, dog, and human liver S9 fraction) study with N-acetyl-DL-leucine-D10 showed that formation of the corresponding metabolite was D10-L-leucine, via deacetylation. The studied enzymes were limited to CYP-, UGT or SULT enzymes. Based upon its chemical nature, it is very likely prone to ubiquitous cleavage into L-leucine and acetate. These 2 metabolites are likely re-used in endogenous, catabolic pathways. Only minor amounts of levacetylleucine are excreted via the urine.

2.5.4. Toxicology

The Applicant has submitted a non-clinical package according to the recommendations mentioned in the ICH guideline M3. Given the long-life-expectancy of the patient and therefore the treatment with levacetylleucine for a long-term duration, non-clinical program should allow an assessment of safety profile after long-term administration. Therefore, three GLP-compliant repeat-dose toxicity (RDT) studies were conducted by the Sponsor with oral administration of levacetylleucine in rats for 26 weeks and in dogs up to the duration of 39 weeks.

2.5.4.1. Single dose toxicity

No single-dose toxicity studies were conducted with levacetylleucine. General acute toxicity information can be obtained from the GLP repeat-dose toxicity studies in rats as well as dogs.

2.5.4.2. Repeat dose toxicity

Repeat-dose toxicity (RDT) studies were conducted in Wistar Han rats for 26 weeks and Beagle Dogs treated with levacetylleucine by oral gavage for up to 39 weeks. The oral route was selected for all studies as is the intended route of administration in humans. Levacetylleucine dosing suspensions were formulated with NaCl 0.9%, w/v, pH 8.0. The reversibility, persistence, or delayed occurrence of levacetylleucine-related effect was evaluated for 4 weeks in the 26-weeks and the 39 weeks toxicity studies respectively, in both rodent and non-rodent species.

<u>RATS – Pivotal study (26-weeks chronic toxicity, GPL study)</u>
Toxicity profile of levacetylleucine was first determined in a 26-week GLP study in rats (with 4-weeks recovery period).
Levacetylleucine was administrated once a day in rats at doses of 250, 800 and 2500 mg/kg/day.
The histopathological examination of the male and female rats revealed dose-related morphological organ changes in the kidneys of the male animals treated with 800 or 2500 mg /kg/day by oral administration for 26 weeks in the form of an increased incidence and severity of tubular basophilia and/or hyaline tubular casts (statistically significantly in left kidneys of the high dose group only).
Indeed, in rats adverse test item-related effects were noted only at the 2500 mg/kg/day dose level.
Adverse effects consisted of decreased body weight (males only), changes of clinical chemistry parameters (males and females): decrease of minerals (chloride, potassium and sodium plasma levels), changes of urine parameters (females only): increase urine pH, increase urea, decrease of albumin, increase of both kidney weights (males and females), histo-pathological changes (increased incidence and severity of tubular basophilia and/or hyaline tubular casts in the kidney; males only).
At the dose level of 800 mg/kg, the kidney histological effects are considered not to be adverse, while adversity is given for the findings at 2500 mg/kg.
These kidney findings were reported in at the end of the 4-week treatment free recovery period at 2500 mg mg/kg/day.
Also, non-adverse, reversible test item-related effects were noted at 2500 mg/kg: soft faeces, hyper-salivation.

No test item-related deaths or premature sacrifices occurred.

No test item-related influence was noted on the haematological and coagulation parameters, the eyes or optic region, the auditory acuity, at necropsy as well as for the myeloid.

Under consideration of the afore mentioned findings, the dose of 800 mg /kg/day was identified as the NOAEL (no-observed-adverse-effect-level) for male and female rats following repeated oral administration.

To conclude, the target organs of levacetylleucine toxicity identified in the 26-weeks rat study were GI tract (soft faeces, hypersalivation), Kidneys (increase in weight of both kidneys, severity of tubular basophilia and/or hyaline tubular casts, urine increased, pH increased, urea increased, albumin decreased, mineral decreased).

The kidney findings in male rats did not reverse at the 4 weeks recovery period despite achieving 5.5 (M)/ 8 (F) the human systemic exposure. However, no kidney toxicity was reported at the clinical dose level. This toxicity was found to be a known male rats-specific toxicity. Thus, it is not relevant for humans.

In the absence of any changes observed in the male or female reproductive organs in the chronic RDT study in rats (Provivo Report No. 36242), a combined male and female dosing study with a 2-week pre-pairing dosing period was considered to evaluate the effect of the test article on fertility and early embryonic development. The total dosing period for the males (approximately 5 weeks), ensured that retained testis were evaluated microscopically for pathological changes, whenever this was considered necessary.

6-week repeat toxicity study in Beagle dogs

The aim of the 6-week subchronic toxicity study of levacetylleucine by repeated oral administration to Beagle dogs (Study No. 36243), was to obtain information on the toxicity of levacetylleucine administered daily over 6 weeks and to assess the reversibility of any effect after a recovery period of 2 weeks.

Therefore, a 6-week preliminary study was conducted in Beagle dogs to assess the toxicity of levacetylleucine following daily oral administration at doses of 225, 750, and 2500 mg/kg/day.
The highest doses (750 and 2500 mg/kg/day) resulted in gastrointestinal (GI) effects, including emesis, increased salivation, and diarrhoea (sometimes greenish), observed shortly after administration.

All these gastrointestinal changes observed for the previously high dosed male and female animals treated once daily with 2500 mg /kg/day in form of emesis, salivation and defaecation (diarrhoea) had completely subsided after the cessation of treatment. Neither the macroscopic inspection at necropsy nor the histopathological examination of the recovery animals at the end of the treatment-free 2-week recovery period did reveal any changes related to the previous treatment with the test item.

These effects were dose-related, non-adverse and appeared to be considered non-specific and attributed to the high dose levels of levacetylleucine.
None of the animals died during the study, and no treatment-related effects were observed on body weight, food consumption, ECG parameters, blood pressure, or clinical laboratory values (haematological, biochemical, and urinary).
Moreover, no abnormalities were detected in ophthalmological or auditory tests, organ weights, or bone marrow myeloid/erythroid ratios. Macroscopic and histopathological examinations also revealed no test item-related morphological changes.
During a 2-week recovery period, gastrointestinal effects in the high-dose group (2500 mg/kg/day) were fully reversible. Based on these findings, the No Observed Adverse Effect Level (NOAEL) was determined to be 2500 mg/kg/day.

DOGS – Pivotal 39-Week Oral (Gavage) Study Followed by a 4-Week Recovery Period (GLP Study)

The main objective of this study was to assess the toxicity profile of levacetylleucine following 39 weeks of daily oral administration in Beagle dogs. A 4-week recovery period was included in the study in order to evaluate the reversibility or persistence of any observed findings. Additionally, toxicokinetic (TK) data were collected in female and male dogs at all doses tested.
The dose regimen in dogs was as follow: dogs were administered levacetylleucine at doses of 100, 200, and 600 mg/kg/day via daily oral gavage for 39 weeks.
This dose level corresponded to mean maximum observed concentration (C_{max}) and area under the concentration-time curve (AUC0-24) values of 61.1 µg/mL and 193 h*µg/mL, respectively, for males and 83.9 µg/mL and 272 h*µg/mL, respectively, for females in Week 39 of the dosing phase.

Indeed, the toxicokinetic profile demonstrated that the mean plasma concentration of levacetylleucine increased proportionally between 100 and 200 mg/kg/day. However, this increase was less than dose-proportional between 200 and 600 mg/kg/day. No significant accumulation of levacetylleucine was observed after multiple doses.
Based on the low severity and lack of any adverse impact on the health of animals administered up to 600 mg/kg/day, the NOAEL for this study was determined by the Applicant to be 600 mg/kg/day as no specific target organs of toxicity were identified except the GI tract (reversible and non-adverse effects). Indeed, dogs showed mild GI symptoms, such as liquid faeces and diarrhoea at the highest dose (600 mg/kg/day), but these effects were considered non-adverse. Other GI symptoms like vomiting, dermatitis, were noted but deemed incidental and not related to levacetylleucine.
At histopathology, no significant macroscopic or microscopic abnormalities were linked to levacetylleucine treatment, even at the highest dose.
In conclusion, in this pivotal 39-week GLP study, there is no concern to raise regarding specific organ toxicity. The gastrointestinal effects observed at the highest dose were non-adverse and reversible, supporting the safety of levacetylleucine in long-term exposure (39 weeks) in Beagle dogs.

Target organs identified in repeated-dose toxicity studies

Gastrointestinal (GI) tract

The GI toxicity in rats after 26 weeks and in dogs after 6 weeks and 39 weeks are similar (in term of reversibility of the symptoms, and type of symptoms). Indeed, toxic reversible findings were observed in the GI system in 26 weeks from the highest dose of 2500 mg/kg/day (5.5 (M) and 8.4 (F) fold the human exposure). The GI toxicity in rat at this dose was salivation (last 60 minutes after administration), and presence of soft faeces (consistency returned to normal). In the 6-week toxicology study in dogs, levacetylleucine related GI intolerance in form of emesis, increased salivation and defecation (diarrhoea) were noted for the intermediate and also the highest dose, respectively 750 and the NOAEL, 2500 mg/kg/day (17.8 (M) and 21 (F) fold the human exposure). The GI effects in the 6-week study at these doses were also completely reversible after a recovery period of 2 weeks. In the 39-week pivotal toxicity study in dogs, GI effect in term of liquid faeces was observed at the NOAEL, also highest dose tested: 600 mg/kg/day (6 (M) and 8 (F) fold the human exposure). The levacetylleucine-related GI findings were also considered as non-adverse. The GI tract intolerance is mentioned as a side effect in clinic. No further concerns are therefore raised in relation to this.

Kidney

In rats treated for 26 weeks, an increase in relative kidney weight was observed, with microscopic and/or biochemistry disorders and clinical correlate.
After a 26-week treatment, increased incidence and severity of tubular basophilia (15/20, M) and/or hyaline tubular (2/18 for 800 and 7/20 for 2500) casts (statistically significantly in left kidneys of the high dose group only) were observed in the kidney in all male groups at 800 and 2500 mg/kg/day.

An increase in urea, increase in urine pH, decrease in electrolytes (Calcium, sodium, potassium) and decrease of albumin were other clinical chemistry disorders and urinalysis disorders associated to the histopathological kidney's findings for the dose of 2500 mg/kg/day.
At the end of the 4-week recovery period, all changes noted for animals treated with 2500 mg/kg/day at the end of the 26-week treatment period had completely subsided with similar incidence and/or severity, thus not considered reversible. However, these findings occurred only in male rats and thus are not relevant as it is a species-specific toxicity.

2.5.4.3. Genotoxicity

A standard battery of genotoxicity tests was conducted with levacetylleucine following ICH guideline S2 and OECD 474, including both in vitro and in vivo studies.

In vitro:

An Ames test was provided: levacetylleucine showed no mutagenic activity up to the maximum dose of 5000 µg/plate.
A Chromosome aberration test was realized in cultured Human peripheral lymphocytes: levacetylleucine did not increase the percentage of cells with chromosomal aberrations compared to controls.

In vivo:

The two in vitro tests concluded that levacetylleucine is non genotoxic.
An additionally in vivo micronucleus test in CD rats was realized with TK based on the 26 weeks rats study (No. 36242). Indeed, levacetylleucine was tested in male CD rats and did not induce micronucleus in bone marrow cells, indicating no mutagenic effects (the in vivo negative results confirmed the two in vitro results). Although specific toxicokinetic (TK) data were not included in the presented in vivo micronucleus study according to ICH S2 and OECD, it is presented in the study No. 36242 that rats were exposed to the maximum ethically acceptable dose (2000 mg/kg/day) in toxicology studies. Additionally, in the 26-week repeated-dose toxicity study (No. 36242), TK data provided, demonstrated that rats were exposed to doses of up to 2500 mg/kg/day, supporting adequate systemic exposure. Then, the absence of toxicokinetic data in the micronucleus study is considered endorsed due to the high exposure levels in other studies. In conclusion, levacetylleucine did not demonstrate any genotoxic potential in either in vitro or in vivo tests. However, there is a concern regarding the GLP validity of the in vitro genotoxicity studies (studies No. 38188 and 38189). The formulation analysis has not been conducted and reported as GLP deviation, in addition the impact on the validity of the study has not been assessed, however genotoxic endpoints are covered by in vivo genotoxic tests and the conclusion on the genotoxic potential remains valid.

2.5.4.4. Carcinogenicity

The carcinogenic risk assessment was conducted for levacetylleucine taking all available information into consideration to support the weight of evidence evaluation and to show that the drug product does not possess a risk for patients.

From a regulatory perspective, carcinogenicity studies for levacetylleucine, an amino acid, can be questionable in accordance with ICH S1 guidelines.
The pivotal repeated-dose toxicology studies in rats (26 weeks) and dogs (39 weeks) did not reveal any findings that suggest a potential carcinogenic effect for levacetylleucine, such as hyperplasia or hypertrophy. Furthermore, there is no genotoxic mechanism specific to levacetylleucine.
Therefore, no elements seem to suggest any carcinogenic risk for levacetylleucine.

In fact, levacetylleucine is an orally administered, acetylated derivate of a ubiquitously present amino acid which is endogenous in humans. Its metabolite (L-leucine) is a naturally occurring protein (α amino acid), which is also endogenously present in humans, as well as in human food, and is considered to be a "Generally Recognized As Safe (GRAS)" as food additive by the United States Food and Drug Administration (FDA). In addition, N-acetyl-DL leucine the racemic form of levacetylleucine has been available in France since 1957 under the trade name Tanganil (Pierre Fabre Laboratories) and clinical experience exists for a long time, without evidence of a genotoxic or carcinogenic potential in treated patients.
Another point is that the metabolism of levacetylleucine (cleavage of the acetic bond releasing acetate and L-leucine) does not result in toxic metabolites, and there is no long-term tissue retention of levacetylleucine or its endogenous metabolites resulting in local tissue reactions or other pathophysiological responses. L-leucine is present in significant amounts in human body fluids – e. g. human blood plasma - as well as in human cells.
It is a basic metabolite and building block of all living organisms and therefore a genotoxic/mutagenic potential could be excluded.
Finally, no carcinogenic potential has been observed in the product class (N-acetylated amino acids) that is considered relevant to humans, and the chemical structure (leucine with an acetate residue) does not indicate a genotoxic/carcinogenic risk. A substantial clinical safety database for levacetylleucine exists. There have been no serious adverse reactions with levacetylleucine administration in clinical studies conducted with the drug.

However, despite all these elements, it can be considered that due to the long-term administration of levacetylleucine in paediatric populations, the Applicant has taken a precautionary approach by assessing the carcinogenic risk for levacetylleucine.

A set of non-clinical and clinical studies using levacetylleucine have been conducted or is still ongoing and demonstrated that levacetylleucine is not genotoxic in vitro (Ames and mouse lymphoma assay) and in vivo (rat micronucleus study). Results from nonclinical repeated-dose safety studies conducted in rats and dogs, including high systemic exposure levels after oral administration, do not suggest tumorigenic safety concerns so far. There have been no histopathological findings indicative of a carcinogenic potential (e.g. hyperplasia) and no evidence of preneoplastic findings has been observed in the 6- and 9-month repeated-dose toxicity studies in rats and dogs.
Results of embryo-foetal developmental (EFD) studies in rats indicate that pregnant rats tolerated treatment very well without relevant findings on embryo-foetal development. Treatment of pregnant rabbits was tolerated by the mothers. However, external/skeletal malformations have been observed. An additional study in rabbits is ongoing at the time point of initial MAA submission to evaluate the effects.

In accordance with ICH S1 guideline, the Applicant has provided a strategy for carcinogenicity assessment based on various criteria to justify the "very low" carcinogenic risk of levacetylleucine. Therefore, based on this rationale, the Applicant presented a justification for conducting the 26-week carcinogenicity study in rasH2 mice (scheduled to begin in mid-2025) post-approval rather than prior to marketing authorization and submitting the results when available. However, although the Weight of Evidence approach (WoE) provided by the Applicant is consistent mainly based on the structure of Tanganil (N-acetyl-DL-leucine), it does not completely exclude the possibility of a carcinogenic risk, based on the planned 26-week carcinogenicity study in rasH2 mice. Furthermore, some factors that should be considered for the WoE assessment are not sufficiently and clearly presented. For example, the pharmacology of levacetylleucine is not well characterized. In addition, the carcinogenicity of Tanganil (N-acetyl-DL-leucine) does not appear to have been specifically studied. The comparison with

Tanganil, which is approved for short term used in adults (or used in medical practice intermittently according to the Applicant), at a different posology, is not directly applicable to the intended conditions of use for Aqneursa in NPC disease.
The CHMP agreed that the Applicant provides the report of the transgenic mouse study post-approval rather than prior to marketing authorization. However, as the potential carcinogenic risk cannot be completely excluded until the final reports of the 26-week carcinogenicity study in rasH2 mice are submitted, to be more cautious, the CHMP recommended that the SmPC section 5.3 on carcinogenesis is amended to reflect an "unknown" (instead of "low") carcinogenic risk until the study results are submitted. The risk was also added as an important identified risk in the risk management plan (RMP). Consequently, the Applicant committed to realize the 26-week carcinogenicity study in rasH2 mice and submit the results when available. The submission of the final report for this study is due by 30 June 2027 (MEA). Moreover, the SmPC (section 5.3) has been updated as requested to reflect that the carcinogenic risk is "unknown" at this stage of the procedure. Indeed, the Applicant mentioned: "No carcinogenicity studies have been conducted. The carcinogenic risk is unknown." The CHMP took note of this commitment and accepted the revised SmPC and RMP.

2.5.4.5. Reproductive and developmental toxicity

A complete program of reproductive and developmental toxicity studies was conducted with levacetylleucine in rats and rabbits.

Levacetylleucine was shown to not affect the fertility of male and female rats at doses of 1000 mg/kg/day corresponding to 1.5- to 2.3-fold human exposure levels

EFD studies were conducted in rats and rabbits dosed once daily by gavage during organogenesis. In both species, 20 mated females per group were used, and dosing was staggered whereby the first 6 animals/group were dosed first (phase 1) and dosing of the remaining 14 animals/group (phase 2) was initiated after necropsy of phase 1 animals. In rats, levacetylleucine did not induce either maternal or developmental toxicity at doses up to 1000 mg/kg/day. In rabbits, high dose level was decreased from 2500 mg/kg/day in phase 1 to 1925 mg/kg/day in phase 2 due to developmental toxicity, but treatment was considered as generally well-tolerated by the dams. A treatment-related decrease in food consumption was observed at all dose levels, but this was considered as non-adverse due to a lack of impact on maternal body weights. No treatment-related effect on intra-uterine survival was observed at C-section but increased incidence of malformations affecting the eye, limb and skull was reported at ≥1250 mg/kg/day. In addition, foetal weights were decreased with subsequent delayed ossification at these dose levels. Therefore, the developmental NOAEL is 675 mg/kg/day in rabbits. Toxicokinetic data indicate that malformations occurred in rabbits at 7.1-fold human exposure whereas at the no observed adverse effect level on embryofoetal development of 1000 mg/kg/day in rats and 675 mg/kg/day in rabbits corresponded to 2.0- and 4.9-fold, respectively, the human exposure levels reached at the recommended dose.

In the pre- and postnatal development study conducted in rats at doses up to 1000 mg/kg/day, non-adverse reductions in body weight gain were noted for female offspring during the postweaning period. No effects on behavioural assessments, maturation, mating or fertility were noted. Increases in the mean number of early resorptions and post-implantation loss (%) were noted for the offspring of females administered ≥600 mg/kg/day. They were considered as not adverse because these changes were not dose-dependent, mean group values for these parameters fell within the historical control range, and in the absence of an effect on the mean number of live embryos. Therefore, the NOAEL for the F1 generation was set at 1000 mg/kg/day.

A dose range-finding toxicity study in juvenile rats was already completed, and a final study report is not yet available for the definitive study. Age of animals at initiation of treatment covers human patients

from birth. Of note, neuro-behavioural examinations and mating procedures were conducted in addition to core study endpoints, without any clear justification. From an EU perspective, it had been concluded during the original PIP application that a juvenile animal study (JAS) was not needed to support development. Considering also the orphan drug status of levacetylleucine and the serious, debilitating nature of the disease, post-approval submission of the definitive juvenile rat study is considered as acceptable.

However, as the potential developmental toxicity risk cannot be completely excluded until the reports of the Intravenous (Slow Bolus) Preliminary Study of Embryo-Foetal Development in the Rabbit and of the 8 Week Once Daily Oral (Gavage) Administration Dose Juvenile Toxicity Study with IB1001 in the Rat are submitted, and in view of the potential deleterious clinical consequences linked to this risk 'developmental toxicity' was added as an important identified risk in the risk management plan (RMP). The submission of the reports for these studies is due by 31 December 2025 (MEA).

2.5.4.6. Toxicokinetic data

The TK evaluations in a series of toxicology studies in rats, and dogs showed that levacetylleucine is rapidly absorbed and cleared from plasma following dosing.

Totality of the provided TK data from rats and dogs indicate that levacetylleucine was rapidly cleared from plasma following p.o. administration with half-lives of approximately 1 hour.

Over the dose range of 250 mg/kg to 2500 mg/kg, maximal plasma concentrations and exposure are dose proportional. Over study periods up to 39 weeks in dogs, no accumulation was shown and no obvious gender differences was observed. Sex differences in levacetylleucine mean C_{max} and AUC0-24 values were less than 2-fold in the 39-week Toxicity Study in the Beagle dogs. After oral gavage administration, levacetylleucine was absorbed, with median Tmax values of 1.00 hour on Day 1, and 1.00- and 1.12-hours during Week 39. After reaching C_{max}, levacetylleucine concentrations declined, with mean half-life (t1/2) values ranging from 1.11 to 1.53 hours on Day 1 and from 1.14 to 1.28 hours during Week 39. Mean concentration values for levacetylleucine were measurable through 6 hours post-dose on Day 1 and during Week 39.
In summary, animals' expositions were discussed in the repeated dose toxicities studies parts. Regarding interspecies comparison and exposure margins to clinical exposure, margins of exposure based on the exposure levels (AUC) of levacetylleucine relative to the reference human exposure at daily dose of 4000 mg of levacetylleucine and at the NOAEL in rats and dogs' studies ranged from 2 to 18. No concerns are raised.

2.5.4.7. Local Tolerance

The intended route of administration for levacetylleucine is oral. The gastrointestinal tract was evaluated in all repeat-dose toxicology studies in rats and dogs. No dedicated local tolerance testing was conducted but the local tolerance after oral administrations was investigated in the RDT studies in rats and dogs and showed a good tolerability.

A non-adverse and reversible GI tract toxicity was observed in both species study. No concerns have been raised in relation to this.

2.5.4.8. Other toxicity studies

<u>Studies on metabolites</u>

The metabolism of levacetylleucine (cleavage of the acetic bond releasing acetate and L-leucine) does not result in toxic metabolites, and there is no long-term tissue retention of levacetylleucine or its endogenous metabolites resulting in local tissue reactions or other pathophysiological responses. L-leucine is present in significant amounts in human body fluids – e. g. human blood plasma - as well as in human cells. Therefore, its chemical nature, is likely to prone to ubiquitous cleavage into L-leucine and acetate. These 2 metabolites are likely re-used in endogenous, catabolic pathway. There are no objections to raise.

Studies on impurities

3 specified impurities were identified in the drug substance, one of which being a food supplement. its additional qualification is therefore not necessary. The 2 other impurities were present in pivotal toxicity studies in rats and dogs. The actual impurity intakes in rats and dogs treated with these doses cover the proposed specifications. Therefore, these 3 specified impurities are considered to be qualified at levels equivalent in a non-clinical standpoint only. NOAELs were determined in rats and dogs and were respectively 800 and 600 mg/kg. The 39-week study in dogs is the critical study under consideration, as dogs have been identified as the most sensitive species with a NOAEL of 600 mg/kg/day.

Moreover, the sponsor has employed a Human Equivalent Dose (HED) approach using data from the 39-week study in Beagle dogs, considering an allometric scaling factor of 1.8 (conversion of animal dose in mg/kg to HED in mg/kg). As the Applicant has excluded newborns and patients weighing less than 20 kg, the HED calculation using the weight of 20 kg is accepted.

Finally, the two impurities according to in silico have been tested negative for mutagenicity alert through two different (Q) SAR models (Derek Nexus and Sarah) and thus classified as 'Class 5' according to ICH M7 guidance because no structural alerts were identified, and no genotoxic risk is to be expected.

Phototoxicity studies

In regard of the phototoxicity potential of levacetylleucine, the UV/Vis light absorption spectrum of levacetylleucine suggests that there is no need to evaluate the phototoxic potential of levacetylleucine since levacetylleucine does not absorb light between 240 to 700 nm. Consequently, levacetylleucine has no phototoxic potential. Therefore, there are no objections to raise.

Excipients studies

The excipients in a 1000 mg sachet of the drug levacetylleucine are: Isomalt , Hypromellose 4000, Strawberry Flavour, and Purified Water (q.s). Isomalt is included in the FDA's Inactive Ingredients Database (IID). As the Applicant has limited the therapeutic indication to children weighing at least 20 kg (and no longer includes newborns), and in accordance with the ICH S11 guideline (ICH guideline S11 on nonclinical safety testing in support of the development of paediatric pharmaceuticals), which states that the gastrointestinal system is mature around 20 kg (approximately 3-4 years of age), we consider the use of Isomalt acceptable.

The Strawberry Flavour, manufactured as "Strawberry flavouring powder", is not referenced in the FDA IID. It contains propylene glycol, maltodextrin (maize), gum arabic, acetic acid (E260), various natural flavouring substances, and natural flavouring preparations. While several strawberry flavours for oral use are listed in the FDA IID, there is no entry for the specific material used as an excipient in the drug levacetylleucine.

According to the Applicant, the indication no longer takes into account neonates but children weighing at least 20 kg. Since metabolic enzymes mature with the development of the gastrointestinal system in

children around the age of 3 years, the safety of chronic exposure to dimethyl hydroxy furanone (DMHF) and methyl cinnamate, is acceptable in the paediatric population from 20 kg.- DMHF is a substrate of UDP-glucuronosyltransferases (UGT), and the majority of UDPGT activities develop to adult levels within 10-20 weeks post-natally. Therefore, at 20 kg, the use of DMHF, a component of the strawberry flavour, is considered acceptable for the intended indication. Regarding methyl cinnamate, another component of the strawberry flavour, it is hydrolysed in vivo with esterases, which are present in most tissues but mostly in hepatocytes. Moreover, the EFSA (European Food Safety Authority) scientific opinion on Flavouring Group Evaluation 68 (FGE.68) concludes that "there is no safety concern at the estimated level of intake as a flavouring substance based on the MSDI (Maximized Survey-derived Daily Intake) approach." Therefore, at 20 kg, as metabolism occurs, the use of methyl cinnamate in the strawberry flavour composition is acceptable.

2.5.5. Ecotoxicity/environmental risk assessment

According to ERA guideline EMEA/CHMP/SWP/4447/00 Rev. 1- Corr. The Log Kow of NALL should be determined using the shake-flask method (OECD 107): the Log P determined for levacetylleucine was 0.62. For substances for which the logarithmic octanol/water partitioning coefficient (log Kow) < 4.5, an assessment of PBT/vPvB properties may not be required. However, since the study to determine the log P of levacetylleucine was not conducted under Good Laboratory Practice (GLP), the Applicant committed to conduct an GLP OECD 107 study to determine the Log Kow concerning the active substance levacetylleucine, and to submit the results by Q1 2026 (REC).

The active substance levacetylleucine is considered as a non-natural amino acid, however the metabolism of levacetylleucine in mammalian species including humans, showed that it is very likely prone to ubiquitous cleavage into L-leucine and acetate. These two metabolites are likely re-used in endogenous, catabolic pathways.

In addition, L-leucine as well as levacetylleucine are widely occurring in the environment being synthesized by microorganisms.

Furthermore, in order to enhance protection of environment, precautionary and safety measures were included in:

- SmPC section 6.6: "Any unused medicinal product or waste material should be disposed of in accordance with local requirements.)",
- PL section 5: "Do not throw away any medicines via wastewater or household waste. Ask your pharmacist how to throw away medicines you no longer use. These measures will help protect the environment".

2.5.6. Discussion on non-clinical aspects

The present pharmacological package almost entirely consists of data derived from published literature. In pertinent publications, levacetylleucine was shown to exert positive effects in in vitro and in vivo models of Niemann-Pick disease Type C. Hence, from a non-clinical pharmacological perspective, levacetylleucine has potential to alleviate symptoms of NPC disease in patients. However, the underlying mode of action remains unclear.
Of note, it should be kept in mind that insufficient evidence was provided in non-clinical studies that levacetylleucine is superior to the racemic NADLL, which is approved as Tanganil in France.

Repeat dose toxicity studies (RDT)

Toxicity profile of levacetylleucine was determined in GLP studies in rats and dogs by repeated oral administration. TK analyses were conducted for all the repeat-dose studies at all dose levels.

Two pivotal repeated-dose toxicity studies were conducted in rats (26 weeks) and dogs (39 weeks) with levacetylleucine (GLP studies). The renal toxicity found in male rats is specific to the species and is not relevant for the clinical aspect.

GI tract disorders/intolerance were observed in both studies in dogs. No renal findings were observed in any dog studies.

To conclude, the toxicity observed though the toxic studies performed in repeated dose pivotal studies in rats and dogs was coherent. GI was the primary target organ identified for levacetylleucine.

Genotoxicity

A standard test battery was performed with levacetylleucine according to ICH guideline S2. levacetylleucine was tested in vitro in gene mutation in bacteria (Ames test) and in vitro chromosome aberration test in human peripheral lymphocytes. In vivo, levacetylleucine was tested in CD rats with a mammalian erythrocytes micronucleus test in line with ICH S2 guideline and OECD 474. In vivo, no evidence of toxicokinetic (TK) evaluation was included in the Micronucleus study (No.38193, 2020). Although there is a deviation from OECD guideline 474 and ICH guideline S2 on genotoxicity study due to the absence of specific toxicokinetic in vivo data in the micronucleus assay, it is known that the rats were exposed to the maximum ethically acceptable dose toxicology studies (2000 mg/kg/day). In the 26-week repeated dose toxicity study in rats (No. 36242), animals were exposed up to 2500 mg/kg/day which corresponds to twice the human systemic exposure.

Standard in vivo and in vitro tests with levacetylleucine did not show any evidence for a relevant genotoxic potential.

Carcinogenicity

Given the product levacetylleucine, and as in the repeat dose toxicity studies, no findings (hyperplasia for instance) suggest any potential carcinogenic risk, at this stage of the submission, the lack of carcinogenicity do not constitute a major issue (as data can be submitted post-approval.)

In accordance with ICH S1 guideline, the Applicant has provided a strategy for carcinogenicity assessment based on various criteria to justify the "very low" carcinogenic risk of levacetylleucine. Therefore, based on this rationale, the Applicant presented a justification for conducting the 26-week carcinogenicity study in rasH2 mice (scheduled to begin in mid-2025) post-approval rather than prior to marketing authorization and submitting the results when available. However, although the Weight of Evidence approach (WoE) provided by the Applicant is consistent mainly based on the structure of Tanganil (N-acetyl-DL-leucine), it does not completely exclude the possibility of a carcinogenic risk, based on the planned 26-week carcinogenicity study in rasH2 mice. Furthermore, some factors that should be considered for the WoE assessment are not sufficiently and clearly presented. For example, the pharmacology of levacetylleucine is not well characterized. In addition, the carcinogenicity of Tanganil (N-acetyl-DL-leucine) does not appear to have been specifically studied. The comparison with Tanganil, which is approved for short term used in adults (or used in medical practice intermittently according to the Applicant), at a different posology, is not directly applicable to the intended conditions of use for Aqneursa in NPC disease.
The CHMP agreed that the Applicant provides the report of the transgenic mouse study post-approval rather than prior to marketing authorization. However, as the potential carcinogenic risk cannot be completely excluded until the final reports of the 26-week carcinogenicity study in rasH2 mice are submitted, and in view of the potential deleterious clinical consequences linked to this risk 'carcinogenicity', the CHMP recommended that section 5.3 of the SmPC is amended to reflect an

"unknown" (instead of "low") carcinogenic risk until the study results are submitted. The risk was also added as an important identified risk in the risk management plan (RMP). Consequently, the Applicant committed to realize the 26-week carcinogenicity study in rasH2 mice and submit the results when available. The submission of the final report for this study is due by 30 June 2027 (MEA). Moreover, the SmPC (section 5.3) has been updated as requested to reflect that the carcinogenic risk is "unknown" at this stage of the procedure. Indeed, the Applicant mentioned: "No carcinogenicity studies have been conducted. The carcinogenic risk is unknown." The CHMP took note of this commitment and accepted the revised SmPC and RMP.

Developmental and reproductive toxicology (DART)

DART studies were conducted in rats and rabbits. In rats, no effect on fertility, embryo-foetal or postnatal development was observed at doses up to 1000 mg/kg/day corresponding to safety margins ranging from 1.5 to 2.3 based on exposure levels measured in the fertility and embryofoetal development studies. In rabbits, developmental toxicity (decreased foetal weight and malformations affecting the eye, limb and skull) was reported at 7.1-fold human exposure in absence of significant maternal toxicity, and with a safety margin of 4.9-fold at the developmental NOAEL of 675 mg/kg/day.

However, as the potential developmental toxicity risk cannot be completely excluded until the reports of the Intravenous (Slow Bolus) Preliminary Study of Embryo-Foetal Development in the Rabbit and of the 8 Week Once Daily Oral (Gavage) Administration Dose Juvenile Toxicity Study with IB1001 in the Rat are submitted, and in view of the potential deleterious clinical consequences linked to this risk 'developmental toxicity' was added as an important identified risk in the risk management plan (RMP). The submission of the reports for these studies is due by 31 December 2025 (MEA).

Impurities

An assessment was performed for the impurities present in the drug product of levacetylleucine because of the difference in the specifications set for these impurities in the drug product compared to the amount present in previously used non-clinical batches. An HED approach was proposed by the Applicant. This assessment was conducted by calculating the amount of each impurity taking the maximum total daily dose of 4 mg/patient and day into consideration and comparing these to the amounts present at the human equivalent dose (HED) levels at the NOAELs obtained (respectively 600 mg/kg/day and 800 mg/kg/day in dogs and rats) in the non-clinical safety studies in rats and dogs. As the Applicant has excluded newborns and patients weighting less than 20 kg, the HED calculation using the weight of 20 kg is accepted. Finally, the two impurities according to in silico have been tested negative for mutagenicity alert through two different (Q) SAR models (Derek Nexus and Sarah) and thus classified as 'Class 5' according to ICH M7 guidance because no structural alerts were identified, and no genotoxic risk is to be expected.

Excipients

As the Applicant has limited the therapeutic indication to children weighing 20 kg (and no longer includes newborns), and in accordance with the ICH S11 guideline (ICH guideline S11 on nonclinical safety testing in support of the development of paediatric pharmaceuticals), which states that the gastrointestinal system is mature around 15 kg (approximately 3-4 years of age), we consider the use of Isomalt and Strawberry flavour acceptable.

Phototoxicity

Levacetylleucine does not absorb light at 240 to 700 nm. Consequently, levacetylleucine has no phototoxic potential.

ERA

The ERA guideline EMEA/CHMP/SWP/4447/00 Rev. 1- Corr. states that "data generated by or on behalf of the Applicant in order to meet the ERA data requirements specified in this guideline should be compliant with Good Laboratory Practice (GLP) where applicable and preferably follow the most recent test guidelines issued by the Organisation for Economic Co-operation and Development (OECD) or comparable international validated test guidelines. The results of a shake-flask method (OECD 107) study were provided on levacetylleucine, however the study to determinate the log P of levacetylleucine was not conducted under Good Laboratory Practice (GLP).

As a result of the above considerations, the available data do not allow to conclude definitively on the potential risk of levacetylleucine to the environment.

The Applicant committed to perform the following studies as follow-up measures: GLP OECD 107 study to determine the Log Kow for levacetylleucine, and to submit the results by Q1 2026 (REC).

Assessment of paediatric data on non-clinical aspects

A dose range-finding toxicity study in juvenile rats was already completed, and a final study report is not yet available for the definitive study. From an EU perspective, it had been concluded during the original PIP application that a JAS was not needed to support development. Considering also the orphan drug status of levacetylleucine and the serious, debilitating nature of the disease, post-approval submission of the definitive juvenile rat study is considered as acceptable (MEA).

2.5.7. Conclusion on the non-clinical aspects

The data assessing the non-clinical aspects are insufficient to rule out the potential risks of Aqneursa, indeed the non-clinical program is incomplete, notably for carcinogenicity, the developmental and reproductive toxicity and environmental risk assessment GLP compliance (log Kow).

Regarding the carcinogenicity, the Applicant committed to realize the 26-week carcinogenicity study in rasH2 mice. This study is due to start in mid-2025. Post-approval submission of the definitive report of this study is acceptable. In the meantime, the Applicant revised Section 5.3 of the SmPC on carcinogenesis to indicate that, the carcinogenic risk is "unknown".

The study to determine the Log P will be carried out under Good Laboratory Practice (GLP) and the results of this new study will be submitted by Q1 2026.

Overall, the non-clinical package is considered acceptable by the CHMP, with the agreed recommendations.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the Applicant.

The Applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

Study ID	Enrolment status Start date	Design Control type	Study & control drugs	Population

	Total enrolment/ enrolment goal		Dose, route of administration and duration Regimen	Main inclusion/ exclusion criteria
IB1001-301 (pivotal)	Parent Study: FPFV: 30 Jun 2022 LPLV: 12 Jun 2023 Extension Phase: ongoing	Multinational, randomized, placebo-controlled, double-blinded, crossover study evaluating 12 weeks of treatment with IB1001.	IB1001, placebo Patients aged ≥13 years or aged <13 years weighing ≥35 kg received 4 g/day of orally administered NALL. Patients aged <13 years weighing <35 kg received weight-tiered doses 2 or 3 times per day	60 (SAF) IB1001: 60 Placebo: 59 Patients with a confirmed diagnosis of NPC aged 4 years or above SARA score of 5≤ X ≤34 points (out of 40) AND Either: Within the 2-7 range (0-8 range) of the Gait subtest of the SARA scale OR able to perform the 9HPT-D (SCAFI subtest) in 20≤ X ≤150 second
IB1001-201, Phase II study in NPC (Parent Study and Extension Phase completed	Parent Study: FPFV: 04 Sep 2019 LPLV: 05 Aug 2020 Extension Phase: FPFV: 11 Dec 2019 LPLV: 07 Nov 2022	Multinational, multi-center, open-label, rater-blinded single-arm study in paediatric and adult patients aged ≥6 years evaluating 6 weeks' treatment with IB1001.	Patients aged ≥13 years or aged <13 years weighing ≥35 kg received 4 g/day of orally administered NALL. Patients aged <13 years weighing <35 kg received weight-tiered doses 2 or 3 times per day	33 (SAF) Patients with a confirmed diagnosis of NPC aged 6 years or above SARA score of 5≤ X ≤33 points (out of 40) AND Either: Within the 2-7 range (0-8 range) of the Gait subtest of the SARA scale OR able to perform the 9HPT-D (SCAFI subtest) in 20≤ X ≤150 seconds
GM2 gangliosidosis (Tay-Sachs and Sandhoff diseases)				
IB1001-202, Phase II study in GM2 gangliosidosis	Parent Study: FPFV: 07-Jun 2019 LPLV: 07-Jan-2021 Extension	Multinational, multi-center, open-label, rater-blinded single-arm study in	Patients aged ≥13 years or aged <13 years weighing ≥35 kg received 4 g/day of	30 (SAF) Patients with a confirmed diagnosis

(Parent Study and Extension Phase completed)	Phase: FPFV: 09-Apr-2020 LPLV: 09-Jan-2023	paediatric and adult patients aged ≥6 years evaluating 6 weeks' treatment with IB1001	orally administered NALL. Patients aged <13 years weighing <35 kg received weight-tiered doses 2 or 3 times per day	of GM2 aged 6 years or above SARA score of 5≤ X ≤33 points (out of 40) AND Either: Within the 2-7 range (0-8 range) of the Gait subtest of the SARA scale OR able to perform the 9HPT-D (SCAFI subtest) in 20≤ X ≤150 seconds

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Levacetylleucine, a modified amino-acid, is the L-enantiomeric form of N-Acetyl-DL-Leucine (racemate), the active substance of Tanganil (Pierre Fabre Laboratories), an anti-vertiginous medication marketed in France for over 60 years.

In the current submission, the Applicant seeks marketing approval for levacetylleucine, a novel agent for the treatment of chronic Niemann-Peak disease Type C (NPC) in both adults and children from birth.

In the final proposed indication, only adults and children aged 6 years and older and weighing more than 20 kg are included.

The proposed recommended oral dose should be taken two to three times daily depending on the patient's weight as below:

Table 5: Recommended dose

Patient's body weight	Morning dose	Afternoon dose	Evening dose
20 to 24 kg	1 g (1 sachet)	No dose	1 g (1 sachet)
25 to 34 kg	1 g (1 sachet)	1 g (1 sachet)	1 g (1 sachet)
35 kg or more	2 g (2 sachets)	1 g (1 sachet)	1 g (1 sachet)

The proposed commercial formulation is a sachet of granules for oral solution supplied at one strength of 1000 mg.

levacetylleucine has a molecular weight of 173.21 Da. The molecular formula is $C_8H_{15}NO_3$ and the chemical structure is presented in Figure 3.

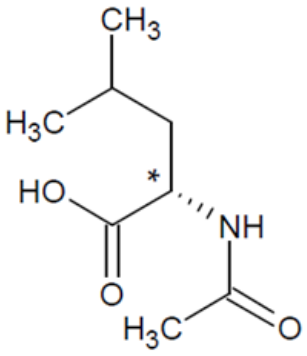


Figure 3: Structure of IB1001 drug substance

Overall, 8 non-clinical pharmacokinetics studies (i.e. protein binding, hepatic metabolism, CYP inhibition/induction, transporter substrate…) as listed in Table 6 were conducted.

Table 6: Studies using human biomaterials

Study type / Test method	GLP	Test system	Route of administration	Dose or concentration	Reference
Pharmacokinetic studies with N-acetyl-L-leucine					
Plasma protein binding	No	CD1-mouse, SD rat, NZW rabbit, beagle dog, and human plasma	*In vitro*	5.77, 57.7 and 577 µM (1, 10 µg/mL and 100 µg/mL)	Study No. ADM-20-2970b
Hepatic metabolism	No	S9 fractions from several species	*In vitro*	40 µM and 2 mM (6.928 µg/ml and 0.346 mg/ml) as well as 1, 10, and 100 µM (0.1732, 1.732, 17.321 µg/ml)	ADM-20-2970a, 2020
Direct and time-dependent Cytochrome P450 (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4) inhibition	No	Human liver microsomes	*In vitro*	37.62 – 11540 µM (6 to 2000 µg/mL)	Study No. ADM-18-2066b, 2018
Cytochrome P450 (1A2, 2B6, and 3A4) induction potency	No	Cryopreserved hepatocytes from 3 human donors	*In vitro*	37.62 – 11540 µM (6 to 2000 µg/mL)	Study No. ADM-18-2066a, 2018
Efflux transporter inhibition (MDR1, BCRP, and BSEP)	No	Inside-out membrane vesicles	*In vitro*	37.62 – 11540 µM (6 to 2000 µg/mL)	Study No. ADM-18-2106a, 2018

Human solute carrier (SLC) transporter inhibition (OATP1B1*1, OATP1B3, OAT1, OAT3, or OCT2)	No	Human embryonic kidney (HEK293) cells	*In vitro*	173.1 to 5193 µM (30 to 900 µg/mL)	Study No. ADM-18-2106c, 2018
Transporter substrate (MDR1 and BCRP)	No	Madin-Darby canine kidney (MDCK)-WT, MDCK-BCRP and MDCK-MDR1 cell monolayers	*In vitro*	57.7 µM (10 µg/mL)	Study No. ADM-18-2106d, 2018
Cellular uptake and inhibition (OAT1, OAT3, and OCT2)	No	Human transporter transfected cells	*In vitro*	57.7 and 577 µM (10 and 100 µg/mL)	Study No. ADM-18-2106e, 2018

The clinical pharmacology program for levacetylleucine presented in Table 7, encompasses 2 completed Phase II studies (IB1001-201 in NPC and IB1001-202 in GM) and 1 ongoing Phase III study (IB1001-301). Another Phase II study IB1001-203 is ongoing but was not included as part of this application. No Phase I studies were performed.

Table 7: Clinical pharmacology studies

	Study IB1001-201	Study IB1001-202	Study IB1001-301
Description	Study IB1001-201 was a multinational, multicenter, open-label, rater-blinded Phase II study investigating the efficacy and safety of N-Acetyl-L-Leucine for the treatment of NPC.	Study IB1001-202 was a multinational, multicenter, open-label, rater-blinded Phase II study investigating the efficacy and safety of N-Acetyl-L-Leucine for the treatment of GM2 gangliosidosis.	Study IB1001-301 is a multinational, randomized, placebo-controlled, double-blinded, cross-over Phase III study investigating the efficacy and safety of N-Acetyl-L-Leucine for the treatment of NPC.
Design	All patients received IB1001, per oral administration, in this single-arm study, during a 6-weeks (42 days + 7 days) treatment period, followed by a 6-weeks washout (Parent study). During Extension Phase I, IB1001 was taken for a 365-day (+/- 14 days) treatment period. The study drug was formulated as a powder for oral suspension in the Parent Phase and as granules for oral solution in the Extension Phase. More details on the formulation of the study drug in the studies can be found in [Module 2.7.1.1.1].		Patients were randomly assigned (1:1) to two randomization sequences of 12-weeks each (Parent study): • Sequence 1: patients received IB1001 during Period I, and immediately crossed over to receive placebo during Period II. • Sequence 2: patients received placebo during Period I, and immediately crossed over to receive IB1001 during Period II. The study drug was formulated as granules for oral solution. More details on the formulation of the study drug in the studies can be found in [Module 2.7.1.1.1]. At the time of preparation of this document, the IB1001-301 Extension Phase was ongoing and thus the rich PK samples were unavailable for PK analysis.
Dose	The patient's dose was determined based on their age and weight: • Patients aged ≥13 years in Europe and aged ≥18 years in the United States will take 4 g per day: 2 g in the morning, 1 g in the afternoon, and 1 g in the evening. • Patients aged 6-12 years weighing 15 to <25 kg will take 2 g per day: 1 g in the morning and 1 g in the evening. • Patients aged 6-12 years weighing 25 to <35 kg will take 3 g per day: 1 g in the morning, 1 g in the afternoon, and 1 g in the evening. • Patients aged 6-12 years weighing ≥35 kg will take 4 g per day: 2 g in the morning, 1g in the afternoon and 1 g in the evening (as per patients aged ≥13).		The patient's dose was determined based on their age and weight: • Patients aged ≥13 years will take 4 g per day: 2 g in the morning, 1 g in the afternoon, and 1 g in the evening. • Patients aged 4-12 years weighing 15 to <25 kg will take 2 g per day: 1 g in the morning and 1 g in the evening. • Patients aged 4-12 years weighing 25 to <35 kg will take 3 g per day: 1 g in the morning, 1 g in the afternoon, and 1 g in the evening. • Patients aged 4-12 years weighing ≥35 kg will take 4 g per day: 2 g in the morning, 1g in the afternoon and 1 g in the evening (as per patients aged ≥13).

Blood sampling	For the Parent study, sparse PK samplings were collected at Visits 1, 2, 3, 4, 5 and 6. • Visit 1, 2: Baseline (no study drug) • Visit 3, 4: IB1001 Treatment Period • Visit 5, 6: Washout from IB1001 (no study drug) For the Extension phase, rich PK profiles were collected at baseline (Visit 7B) and after 1-year treatment (Visit 9B). Visit 7B occurred after a minimum 42-day washout from Parent phase. At Visit 7B, the first sample was taken before the first dosing of N-Acetyl-L-Leucine in the Extension Phase (0 h (pre-dose)); subsequent samples were taken at 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 4 h and 6 h after the first extension phase investigational medicinal product (IMP) dose is taken. At Visit 9B, the first sample was taken directly before last IMP intake of the treatment period (0 h (pre-dose)); subsequent samples were taken at 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 4 h and 6 h after the last extension phase IMP dose is taken.	For the Parent phase of studies IB1001-301 sparse PK samplings were collected at Visits 2, 4 and 6. • Visit 2: Baseline • Visit 4: IB1001 or Placebo Treatment Period • Visit 6: IB1001 or Placebo Treatment Period For the ongoing Extension phase, rich PK profiles will be collected at baseline (Visit 7B) and after 1-year treatment (Visit 9B).

A Population PK analysis (PPK) was developed from which predicted exposure metrics were used as input for two exposure-response (ER) analysis.

Pharmacokinetics

Methods

Bioanalysis

The developed methods for the quantification of levacetylleucine and its D enantiomer in several matrix are adequate and comply with the acceptance criteria of the bioanalytical method validation EMA guideline. Description and validation reports were provided with satisfactory results regarding specificity, sensitivity, precision, accuracy, dilution factor linearity, matrix effect. Short and long-term stability of the analytes in the biological matrix were tested and shown to be satisfactory.

Pharmacokinetic analysis

Standard non-compartmental (model-independent) pharmacokinetic methods were used to calculate PK parameters using Phoenix WinNonlinTM (version 8.3.5.340). Population PK analysis was performed using the non-linear mixed effect modelling implemented in Phoenix WinNonlinTM NLME with the FOCE ELS algorithm.

For single or multiple-dose studies, PK parameters evaluated in plasma include C_{max}, T_{max}, AUCs (AUC0-t, AUC0-24, AUC0-∞), CL/F and Vz/F, T1/2.

Absorption

Following single dose of levacetylleucine (Commercial formulation, Visit 7B, n=14) in subjects from Study IB1001-201, absorption was reasonably rapid with a C_{max} approximately achieved at a median (min-max) T_{max} of 1h (0.5-2 h). Geometric mean dose-normalized (per gram of IB1001) C_{max} and AUC0-24 were 3.3 µg/mL and 7.2 µg.h/mL.

Following multiple doses of levacetylleucine (Commercial formulation, Visit 9B, n=19) in subjects from Study IB1001-201, absorption was reasonably rapid with a C_{max} approximately achieved at a median (min-max) T_{max} of 1h (0.75-1.3 h). Geometric mean dose-normalized (per gram of levacetylleucine) C_{max} and AUC0-24 were 4 µg/mL and 8 µg.h/mL.

Absolute bioavailability

No information was provided with regards to levacetylleucine absolute bioavailability

BCS

No information was provided with regards to levacetylleucine BCS

Relative Bioavailability/Bioequivalence

Two oral formulations for levacetylleucine of equal strength at 1000 mg were developed and had been used in the clinical trials, a powder for oral suspension with Ora-Blend and granules for oral solution (commercial formulation).

No formal rBA study was performed between the two formulations. PK comparability between the two formulations has been performed in 5 subjects. Off note, PK parameters were estimated following a single dose of levacetylleucine (Visit 7B) and multiple dose (Visit 9B) in study IB1001-201. And even a low accumulation after multiple doses is observed, the observed PK metrics were similar with geomean dose normalized C_{max} and AUC0-24 for the powder vs granules of 3.8 vs 4.3 µg/mL and 8.3 vs 8.1 µg.h/mL, respectively.

Influence of food

Although, no formal PK study was performed to investigate the effect of food on levacetylleucine PK, in both studies IB1001-201 and IB1001-301, levacetylleucine was advised to be taken 0.5 h before or 2 h after a meal.

Distribution

Based on *in vitro* investigation (Study ADM-20-2970b), levacetylleucine tested at a concentration of 1, 10 and 100 µg/mL has a protein binding near 0%.

Based on study **IB1001-201**, after multiple doses, geometric mean Vss/F was 227 L.

Elimination

Elimination of levacetylleucine from plasma was rapid with an estimated half-life of 1h. Based on study IB1001-201, after multiple doses, geometric mean CLss/F was 128 L/h.

Metabolism

Levacetylleucine underwent rapid metabolism in liver S9 mix. However, its metabolism was not cofactor-dependent in any of the investigated species (including humans) and thus not mediated via CYP-, UGT-, or SULT enzymes. Based upon its chemical nature, it is very likely prone to ubiquitous cleavage into L-leucine and acetate. These 2 metabolites are likely re-used in endogenous, catabolic pathways.

Interconversion

Interconversion between the L to the D enantiomer is unlikely.

Dose proportionality and time dependencies

Only a single dose level was evaluated in humans and consequently the range of linear PK could not be determined.

Due to the very short half-life (approximately 1 hour), no differences between single and repeat dose administration were expected. Slight differences could be observed in exposure PK parameters between single (Visit 7B) and repeated administration (Visit 9B) in studies IB1001- 201.

Intra-and inter-individual variability

The inter-subject variability in exposure of levacetylleucine in patients with NPC was moderate to high (from single to multiple dose) with range from 30% to 47 % for C_{max} and 36% to 39 %for AUC0-24.

Based on the PPK analysis IIV CL/F and Vd/F were 38.7% and 83%.

Pharmacokinetics in the target population

Two clinical studies have investigated levacetylleucine PK in patients with NPC (IB1001-201 and IB1001-301).

Study IB1001-201 was a multinational, multi-centre, open-label, rater-blinded Phase II study performed in adults and children patients aged 6 to <18 years and weighing at least 15 kg. Two study phases were considered (please refer to section 3):

- A Parent Phase where levacetylleucine dosing was performed during 6 weeks followed by a wash-out period of 6 weeks (Visit 1 to 6)

- An Extension Phase where levacetylleucine dosing was performed during one year (Period 1, Visit 7A/B, Visit 8 180 days after Visit 7A/B and Visit 9A/B, 365 days after Visit 7A/B) followed by a 6 weeks wash-out and followed for another 1 year treatment (Period 2, Visit 10 to 12).

PK parameter estimates are presented in Table 8 and Table 9 for Visit 7b and 9B respectively.

Table 8: PK parameter estimates of IB1001 (Visit 7B)

	C_{max} (µg/mL)	C_{max}/D (µg/mL/g)	$t_{1/2}$ (h)	t_{max} (h)	AUC_{inf} (h*µg/mL)	AUC_{inf}/D (h*µg/mL/g)
N	19	19	19	19	19	19
Mean	**6.695**	**3.693**	**0.992**		**14.639**	**7.956**
SD	1.852	1.463	0.328		4.721	2.864
Min	3.36	1.68	0.63	0.50	5.56	2.78
Median	6.61	3.38	0.91	**1.00**	13.50	7.21
Max	10.04	8.32	1.94	2.50	22.57	14.99
Geom Mean	6.433	3.460	0.948		13.869	7.459
Geom SD	1.349	1.444	1.355		1.419	1.463
Geom CV%	30.62	38.00	31.06		36.11	39.47

	AUC_{0-24} (h*µg/mL)	AUC_{0-24}/D (h*µg/mL/g)	AUC_{0-t} (h*µg/mL)	AUC_{0-t}/D (h*µg/mL/g)	Vz/F (L)	Cl/F (L/h)
N	19	19	19	19	19	19
Mean	**14.636**	**7.955**	**14.078**	**7.647**	**200.502**	**144.311**
SD	4.720	2.863	4.638	2.805	82.518	63.914
Min	5.56	2.78	5.28	2.64	64.75	66.71
Median	13.50	7.21	13.15	7.16	185.05	138.71
Max	22.57	14.99	22.10	14.64	353.49	359.91
Geom Mean	13.866	7.458	13.303	7.155	183.305	134.060
Geom SD	1.419	1.463	1.431	1.471	1.573	1.463
Geom CV%	36.11	39.47	37.06	40.07	47.71	39.47

Table 9: PK parameter estimates of IB1001 (Visit 9B)

	C_{max} (µg/mL)	C_{max}/D (µg/mL/g)	$t_{1/2}$ (h)	t_{max} (h)	AUC_{0-24} (h*µg/mL)	AUC_{0-24}/D (h*µg/mL/g)
N	17	17	17	17	17	17
Mean	**8.324**	**4.470**	**0.958**		**16.614**	**8.789**
SD	3.309	2.250	0.183		6.252	3.686
Min	2.57	1.28	0.61	0.50	8.69	4.34
Median	8.87	4.43	0.93	**1.00**	16.76	8.70
Max	15.84	10.48	1.28	1.50	32.71	16.37
Geom Mean	7.647	3.983	0.941		15.549	8.098
Geom SD	1.563	1.657	1.217		1.460	1.521
Geom CV%	46.99	53.91	19.82		39.24	43.86

	AUC_{0-t} (h*µg/mL)	AUC_{0-t}/D (h*µg/mL/g)	Vss/F (L)	Clss/F (L/h)	MRT0-t (h)
N	17	17	17	17	17
Mean	**16.074**	**8.510**	**252.917**	**138.857**	**1.705**
SD	6.053	3.600	125.193	58.683	0.285
Min	8.05	4.02	87.48	62.22	1.35
Median	16.56	8.39	211.83	119.18	1.67
Max	31.52	16.07	573.82	248.48	2.42
Geom Mean	15.034	7.830	227.227	127.720	1.685
Geom SD	1.464	1.528	1.610	1.528	1.173
Geom CV%	39.58	44.35	50.49	44.35	16.07

Study IB1001-301 (pivotal study) was a multinational, randomized, placebo-controlled, double-blinded, crossover Phase III study of levacetylleucine in patients aged over 4 years and weighing more than 15 kg. Adults and children received levacetylleucine following the dosing schedule presented in Table 10 below. Only the commercial formulation as 1000 mg granules for oral suspension was administered.

Table 10: Dosing regimen in study IB1001-301

Patient Population	Total Daily Dose	Morning Dose	Afternoon Dose	Evening Dose
Patients aged ≥13 years	4 g/day	2 g	1 g	1 g
Patients aged 4 to 12 years weighing ≥35 kg	4 g/day	2 g	1 g	1 g
Patients aged 4 to 12 years weighing 25 to <35 kg	3 g/day	1 g	1 g	1 g
Patients aged 4 to 12 years weighing 15 to <25 kg	2 g/day	1 g	No dose	1 g

No PK analysis was conducted on the sparse PK sampling. PK data from this phase were used as part of the PPK analysis. Overall sparse PK data from 38 subjects are available from which 9 children aged 6 to 12 years were included.

Population pharmacokinetic (PPK) analysis

One PPK analysis aiming to characterize the PK of levacetylleucine in the target population and identifying/quantifying source of variability was developed. From this analysis, predicted exposure metrics were used as input of subsequent ER.

The PPK of levacetylleucine was based on PK results pooling 3 clinical studies from patients aged 4 years and over (IB1001-201/202/301). The concentration-time data of levacetylleucine was modelled using a compartmental approach.

Covariates of interest in levacetylleucine trials were baseline demographic covariates (age, body size, gender, race/ethnicity), disease type (NPC vs GM2 patients), dose, co-medication or meal consumption.

PPK was built using nonlinear mixed effects model with the first order conditional with estimation-extended least squares (FOCE ELS) for parameter estimation implemented in Phoenix WinNonlinTM NLME. Covariate effects were first explored graphically (ETA vs covariates), then testing of the covariate effects was performed using a SCM building strategy (single addition, forward inclusion and backward elimination) with $p<0.01$ for inclusion and $p<0.001$ for exclusion. The PopPK model was evaluated using standard diagnostic plots, visual predictive check and bootstrap.

Overall, 185 subjects with 567 PK observations were included. PK samples exclusion consisted of unplausible values (n=9), no influence on the PPK analysis (n=99), unexpected quantifiable pre-dose concentration (n=4), absence of covariates (n=3).

The final PPK model consisted of a one compartment PK model parameterized with first order absorption and lag time and linear elimination, in terms of Ka, Tlag, CL/F and Vd/F. IIV was considered on, Vd/F and IOV on CL/F. Body weight allometric scaling with fixed exponents to theoretical values was considered on both CL/F and Vd/F.

Overall, all PK parameters were estimated with a good precision (RSE <30% for the fixed and unknown for the random effects).

Final PK parameter estimates, and goodness-of-fit (GOF) plots are presented below.

Table 11: Final PK parameter estimates

Theta	**Estimate**	**Units**	**Stderr**	**RSE%**	**2.5% CI**	**97.5% CI**
tvKa	0.85	1/h	0.04	4.13	0.78	0.92
tvV	64.84	L	11.19	17.26	42.87	86.82
tvCl	148.22	L/h	7.98	5.39	132.53	163.90
tvTlag	0.23	h	0.04	16.96	0.15	0.30
dVdWEIGHT	1.00					
dCldWEIGHT	0.75					
stdev0 (RUV)	0.51		0.03	6.54	0.44	0.57
Omega	**Estimate**	**Shrinkage**	**Stderr**	**RSE%**		
nV	0.49	0.39	0.11	22.45		
IOV on CL	0.18	0.23	0.03	16.67		
EPS Shrinkage	16%					
Condition number	482					

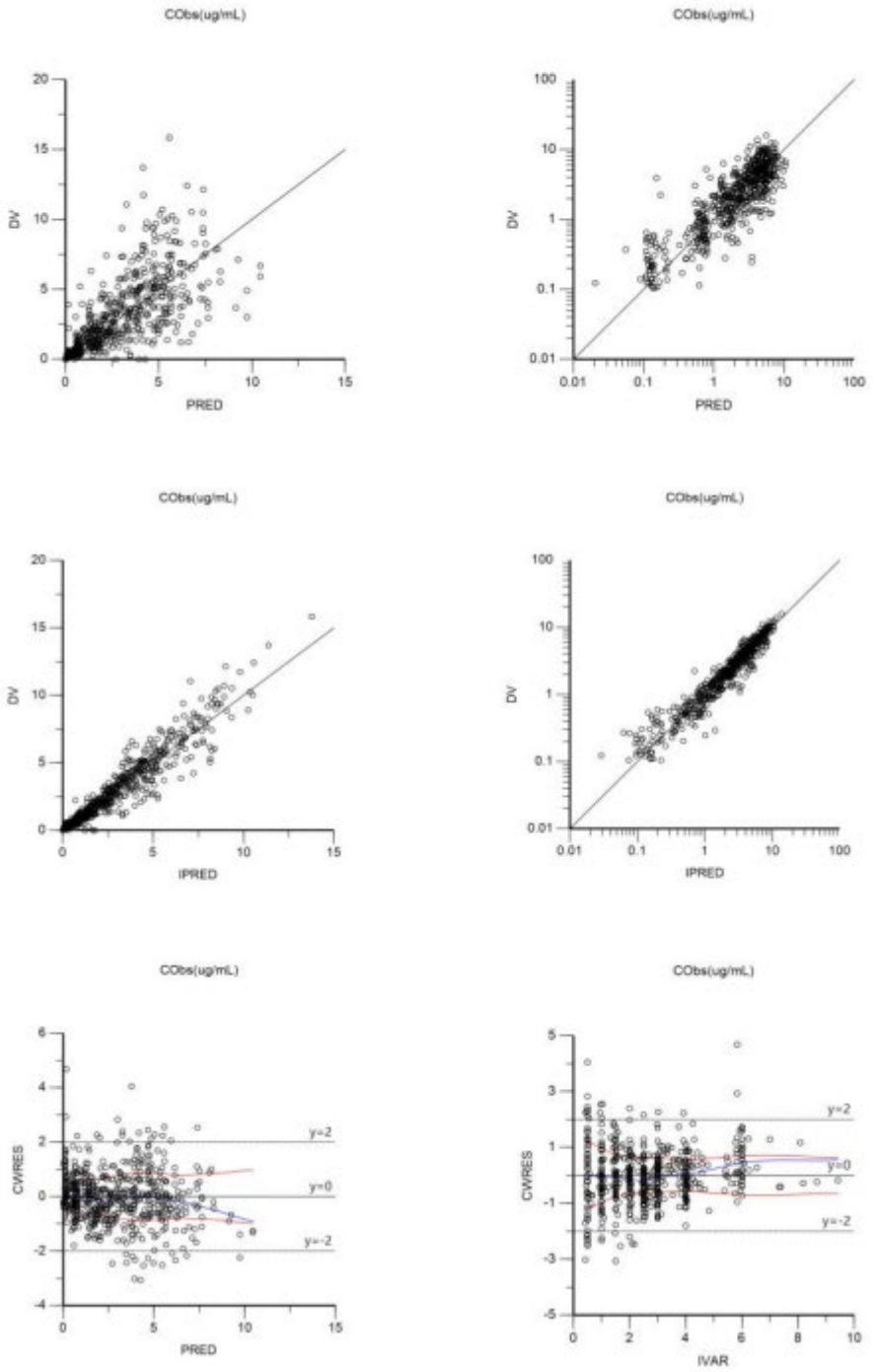


Figure 4: GOF of the final PPK model

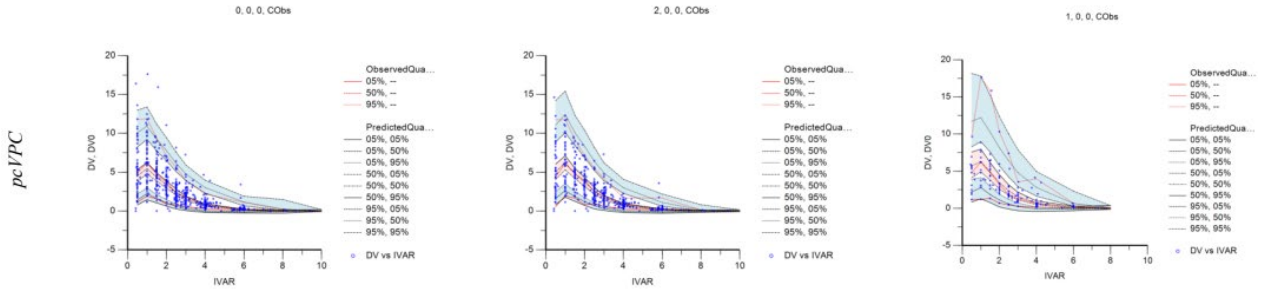


Figure 5: VPC of the final PPK model split by dose

Special populations

Renal impairment

No formal PK study investigating the effect of renal impairment on levacetylleucine PK was performed, since the product is rapidly metabolized into L-leucine and acetate and both are used in further catabolic pathways. Based on non-clinical investigations levacetylleucine, only minor amounts of levacetylleucine are excreted via urine. No impact of renal impairment is expected.

Hepatic impairment

No formal PK study investigating the effect of hepatic impairment on levacetylleucine PK was performed, since the product is rapidly metabolized into L-leucine and acetate and both are used in further catabolic pathways. No impact of hepatic impairment is expected.

Ethnicity

Race was investigated as part of the PPK analysis and was not found to have a significant effect on levacetylleucine PK.

Age

Age was investigated as part of the PPK analysis and was not found to have a significant effect on levacetylleucine CL/F. From the PPK analysis median (min-max) age within the used PK dataset is 26 years (6-67).

Only two subjects from study IB1001-301 had an age over 65 years (66 and 67 years) and none were above 68 years.

Weight

Weight was investigated as part of the PPK analysis and was found to have a significant effect on IB1001 PK. Allometric scaling was applied with fixed coefficient. From the PPK analysis median weight (min–max) was 59.2 kg (20.5-98.43 kg).

Gender

As part of study IB1001-201 the gender effect on levacetylleucine PK was investigated during visit 7B and Visit 9B (rich PK sampling) and no effect was found.

Based on the PPK analysis, gender was not found to have a significant effect on levacetylleucine PK.

Paediatric population

PK data from 14 children aged 6 to 12 years and weighing 20 to 25 kg (n=6), 25 to <35 kg (n=4) and over 35 kg (n=4) were available. Overall levacetylleucine was investigated across studies IB1001-201 and -301 in children aged 6 years and weighing at least 20 kg.

Exposure-response

The paucity of both the PK and PD data from study IB1001-301 cannot allow reliable conclusions from these analyses.

Pharmacokinetic interaction studies

Pharmacokinetics using human biomaterials

Inhibition of CYP enzymes

In the ADM-18-2066b study, direct and time-dependent inhibition (TDI) towards major drug metabolising cytochrome P450 (CYP) enzymes by levacetylleucine at six concentrations ranging from 6 to 2000 µg/mL was investigated. Inhibition was monitored using a cocktail incubation with CYP specific substrates for seven major drug metabolising CYP enzymes (CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4) in incubations with a pool of human liver microsomes. The microsomes were preincubated with the study compound in the presence and absence of NADPH for 30 min to elucidate the possible time-dependency of inhibition. In addition, control compounds for both reversible and time dependent inhibition towards each CYP enzyme were incubated at one concentration.

Direct inhibition (in the absence of NADPH during pre-incubation) towards phenacetin de-ethylation (CYP1A2 probe) with IC50 extrapolated as 3600 µg/mL mg/mL and towards bupropion hydroxylation (CYP2B6 probe) with IC50 estimated as 2000 µg/mL was observed in incubations with levacetylleucine concentration ranging from 6 to 2000 µg/mL. The samples pre-incubated in the presence of NADPH suggested slightly (1.3-fold) lower IC50 towards both CYP1A2 and CYP2B6.

Induction of CYP enzymes

The induction potency of levacetylleucine towards human CYP enzymes 1A2, 2B6, and 3A4 at the mRNA level was studied in cryopreserved hepatocytes from 3 human donors at 6 concentrations ranging from 6 to 2000 µg/mL [Study No. ADM-18-2066a, 2018]. Total exposure time was 48 hours and cell culture media containing the test compound was refreshed after 24 hours incubation. Cytotoxicity was monitored visually and by measurement of lactate dehydrogenase leakage after 24 hours incubation.

CYP1A2, CYP2B6, and CYP3A4 mRNA levels after incubation with of 6 – 2000 µg/mL levacetylleucine remained within 0.5- and 1.7-fold of the solvent control and below 3% of the corresponding positive control inducer. Thus, no induction of these genes was detected by 6 – 2000 µg/mL levacetylleucine. No signs of cytotoxicity or morphological changes caused by the test compound were observed.

Transporter substrate

Study No. ADM-18-2106d (MDR1 and BCRP):

The apparent permeability (Papp) of levacetylleucine was measured bidirectionally (pH 7.4; apical to basolateral (A>B) and basolateral to apical (B>A)) at 10 µg/mL concentration through Madin-Darby canine kidney (MDCK)-WT, MDCK-BCRP and MDCK-MDR1 cell monolayers. Incubations with MDCK-BCRP and MDCK-MDR1 cells were conducted in the presence and absence of chemical BCRP and MDR1 inhibitors, respectively. Respective positive and negative control incubation were included. Transporter inhibition was observed as a reduced level of vesicular uptake of the probe substrate in the presence of ATP. Efflux ratio for levacetylleucine was 1.6 with MDCK-WT cells (similar result on both plates), 0.7 with MDCK-BCRP without inhibitor and 1.3 with inhibitor (2 µM Ko143), and 1.6 with MDCK-MDR1 without inhibitor and 2.6 with inhibitor (50 µM verapamil). Thus, BCRP or MDR1 mediated levacetylleucine active transport was not detected in this study.

Study No. ADM-18-2106e (OAT1, OAT3, and OCT2):

Cellular uptake of levacetylleucine into single human transporter transfected cells was measured at 2 concentrations (10 and 100 µg/mL) in the absence and presence of chemical transporter inhibitors, and into control cells without transfected transporter in the absence of chemical inhibitors. Transporter mediated cellular uptake of the study compounds is observed as difference in the cellular uptake to transporter over- expressing cells and vector transfected control cells and as inhibition of cellular uptake in transporter over-expressing cells by the control inhibitors. Positive control substrates at a single concentration were assayed in parallel. Levacetylleucine was measured to be a substrate of OAT1 and OAT3, but not OCT2 *in vitro*. Uptake of levacetylleucine into OAT1 expressing cells was approximately 5-fold higher and into OAT3 approximately 9-fold higher after 3 minutes of incubation when compared to control cells. Uptake could be abolished by addition of the inhibitor diclofenac. The clinical relevance for this finding is unknown. However, due to the overall safety profile of levacetylleucine, no drug interactions with inhibitors of OAT1 or OAT3 transporters are expected.

Transporter inhibition

Study No. ADM-18-2106a, 2018:

Efflux transporter inhibition was evaluated by measuring the vesicular uptake of a probe substrate into inside-out membrane vesicles expressing a single human ABC transporter (MDR1, BCRP and BSEP) in the absence and presence of levacetylleucine (Mw = 173.21 g/mol) at six concentrations (6 µg/mL – 2000 µg/mL) with and without ATP. Transporter inhibition was observed as reduced level of vesicular uptake of the probe substrate in the presence of ATP. Inhibition towards efflux transporters MDR1, BCRP and BSEP by levacetylleucine was observed and IC50 values were 227 (MDR1), 491 (BCRP) and 408 (BSEP) µg/mL equal to 1.31 (MDR1), 2.83 (BCRP) and 2.36 (BSEP) mM.

Study No. ADM-18-2106c, 2018:

Human solute carrier (SLC) transporter inhibition was evaluated by measuring the cellular uptake of probe substrates into HEK (human embryonic kidney)-293 cells (Corning® TransportoCells™) over-expressing a single human SLC transporter (OATP1B1, OATP1B3, OAT1, OAT3, or OCT2) in the absence and presence of levacetylleucine (Mw = 173.21 g/mol) at concentrations ranging from 30 to 900 µg/mL.

Inhibition towards OATP1B1 and OATP1B3 was evaluated both with and without 30 minutes pre-incubation of the cells with the test compound. Inhibition of active transport was observed as decreased cellular uptake of the probe substrate in the presence of inhibitor. Positive control inhibitors for each transporter were incubated in parallel to confirm active cellular uptake of the probe substrate. Inhibition of OAT1 and OAT3 by levacetylleucine was observed with IC50 values of 1030 (OAT1) and 103 (OAT3) µg/mL equal to 5.9 (OAT1) and 0.59 (OAT3) mM. Additionally, minor inhibition of OCT2 was observed with 61% activity remaining at 900 µg/mL levacetylleucine (extrapolated IC50 1700 µg/mL). Up to 900 mcg/mL (5193 µM) levacetylleucine was not an inhibitor of OATP1B1 or OATP1B3.

Pharmacokinetic interaction studies

Pharmacokinetics using human biomaterials

Inhibition of CYP enzymes

In the ADM-18-2066b study, direct and time-dependent inhibition (TDI) towards major drug metabolising cytochrome P450 (CYP) enzymes by levacetylleucine at six concentrations ranging from 6 to 2000 µg/mL was investigated. Inhibition was monitored using a cocktail incubation with CYP specific substrates for seven major drug metabolising CYP enzymes (CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4) in

incubations with a pool of human liver microsomes. The microsomes were preincubated with the study compound in the presence and absence of NADPH for 30 min to elucidate the possible time-dependency of inhibition. In addition, control compounds for both reversible and time dependent inhibition towards each CYP enzyme were incubated at one concentration.

Direct inhibition (in the absence of NADPH during pre-incubation) towards phenacetin de-ethylation (CYP1A2 probe) with IC50 extrapolated as 3600 µg/mL mg/mL and towards bupropion hydroxylation (CYP2B6 probe) with IC50 estimated as 2000 µg/mL was observed in incubations with levacetylleucine concentration ranging from 6 to 2000 µg/mL. The samples pre-incubated in the presence of NADPH suggested slightly (1.3-fold) lower IC50 towards both CYP1A2 and CYP2B6.

Induction of CYP enzymes

The induction potency of levacetylleucine towards human CYP enzymes 1A2, 2B6, and 3A4 at the mRNA level was studied in cryopreserved hepatocytes from 3 human donors at 6 concentrations ranging from 6 to 2000 µg/mL [Study No. ADM-18-2066a, 2018]. Total exposure time was 48 hours and cell culture media containing the test compound was refreshed after 24 hours incubation. Cytotoxicity was monitored visually and by measurement of lactate dehydrogenase leakage after 24 hours incubation.

CYP1A2, CYP2B6, and CYP3A4 mRNA levels after incubation with of 6 – 2000 µg/mL N- acetyl-L-leucine remained within 0.5- and 1.7-fold of the solvent control and below 3% of the corresponding positive control inducer. Thus, no induction of these genes was detected by 6 – 2000 µg/mL levacetylleucine. No signs of cytotoxicity or morphological changes caused by the test compound were observed.

Transporter substrate

Study No. ADM-18-2106d (MDR1 and BCRP):

The apparent permeability (Papp) of levacetylleucine was measured bidirectionally (pH 7.4; apical to basolateral (A>B) and basolateral to apical (B>A)) at 10 µg/mL concentration through Madin-Darby canine kidney (MDCK)-WT, MDCK-BCRP and MDCK-MDR1 cell monolayers. Incubations with MDCK-BCRP and MDCK-MDR1 cells were conducted in the presence and absence of chemical BCRP and MDR1 inhibitors, respectively. Respective positive and negative control incubation were included. Transporter inhibition was observed as a reduced level of vesicular uptake of the probe substrate in the presence of ATP. Efflux ratio for levacetylleucine was 1.6 with MDCK-WT cells (similar result on both plates), 0.7 with MDCK-BCRP without inhibitor and 1.3 with inhibitor (2 µM Ko143), and 1.6 with MDCK-MDR1 without inhibitor and 2.6 with inhibitor (50 µM verapamil). Thus, BCRP or MDR1 mediated levacetylleucine active transport was not detected in this study.

Study No. ADM-18-2106e (OAT1, OAT3, and OCT2):

Cellular uptake of levacetylleucine into single human transporter transfected cells was measured at 2 concentrations (10 and 100 µg/mL) in the absence and presence of chemical transporter inhibitors, and into control cells without transfected transporter in the absence of chemical inhibitors. Transporter mediated cellular uptake of the study compounds is observed as difference in the cellular uptake to transporter over- expressing cells and vector transfected control cells and as inhibition of cellular uptake in transporter over-expressing cells by the control inhibitors. Positive control substrates at a single concentration were assayed in parallel. Levacetylleucine was measured to be a substrate of OAT1 and OAT3, but not OCT2 *in vitro*. Uptake of levacetylleucine into OAT1 expressing cells was approximately 5-fold higher and into OAT3 approximately 9-fold higher after 3 minutes of incubation when compared to control cells. Uptake could be abolished by addition of the inhibitor diclofenac. The clinical relevance for this finding is unknown. However, due to the overall safety profile of levacetylleucine, no drug interactions with inhibitors of OAT1 or OAT3 transporters are expected.

Transporter inhibition

Study No. ADM-18-2106a, 2018:

Efflux transporter inhibition was evaluated by measuring the vesicular uptake of a probe substrate into inside-out membrane vesicles expressing a single human ABC transporter (MDR1, BCRP and BSEP) in the absence and presence of levacetylleucine (Mw = 173.21 g/mol) at six concentrations (6 µg/mL – 2000 µg/mL) with and without ATP. Transporter inhibition was observed as reduced level of vesicular uptake of the probe substrate in the presence of ATP. Inhibition towards efflux transporters MDR1, BCRP and BSEP by levacetylleucine was observed and IC50 values were 227 (MDR1), 491 (BCRP) and 408 (BSEP) µg/mL equal to 1.31 (MDR1), 2.83 (BCRP) and 2.36 (BSEP) mM.

Study No. ADM-18-2106c, 2018:

Human solute carrier (SLC) transporter inhibition was evaluated by measuring the cellular uptake of probe substrates into HEK (human embryonic kidney)-293 cells (Corning® TransportoCells™) over-expressing a single human SLC transporter (OATP1B1, OATP1B3, OAT1, OAT3, or OCT2) in the absence and presence of levacetylleucine (Mw = 173.21 g/mol) at concentrations ranging from 30 to 900 µg/mL.

Inhibition towards OATP1B1 and OATP1B3 was evaluated both with and without 30 minutes pre-incubation of the cells with the test compound. Inhibition of active transport was observed as decreased cellular uptake of the probe substrate in the presence of inhibitor. Positive control inhibitors for each transporter were incubated in parallel to confirm active cellular uptake of the probe substrate. Inhibition of OAT1 and OAT3 by levacetylleucine was observed with IC50 values of 1030 (OAT1) and 103 (OAT3) µg/mL equal to 5.9 (OAT1) and 0.59 (OAT3) mM. Additionally, minor inhibition of OCT2 was observed with 61% activity remaining at 900 µg/mL N-acetyl- L-leucine (extrapolated IC50 1700 µg/mL). Up to 900 mcg/mL (5193 µM) levacetylleucine was not an inhibitor of OATP1B1 or OATP1B3.

2.6.2.2. Pharmacodynamics

Mechanism of action

Levacetylleucine shows a pharmacological activity *in vivo* in a well-accepted mouse model of NPC but only one dose was tested. *In vitro* experiments demonstrated that levacetylleucine alter mechanisms in relation with NPC (lysosomal storage, mitochondria…) using relevant models. However, a scientific rationale based on a possible mechanism of action is not suggested.

Primary and Secondary pharmacology

The Applicant did not conduct clinical primary or secondary pharmacology studies.

2.6.3. Discussion on clinical pharmacology

Bioanalytical

Bioanalytical report for study IB1001-201 and for parent study IB1001-301 has been provided. The Applicant committed to submit the PK results (rich and sparse PK sampling) and the full bioanalysis report of study 301 (REC) however an approximate date of submission has not been provided.

Regarding the method validation, plasma samples from NPC patients have not been used in validation, only samples from healthy donors have been evaluated.

The concomitant medication has not been tested during bioanalytical method validation. To consider the effect of concomitant medication on the accuracy and precision of the analyte during processing and storage, the incurred sample reanalysis (ISR) should be evaluated in separate runs in different days. For study IB1001-201 – full sampling, 316 samples were delivered to the bioanalytical site.

Three samples were reanalysed with reasoning of quantifiable analyte levels in pre-dose samples. The pre-dose concentrations were in those samples above the LLOQ. 96% of ISR fulfilled acceptance criteria.

Raw data for sample analysis of patient's samples including the samples for the full PK characterisation of levacetylleucine for study IB1001-201 (Visit 7B and 9B) were provided. As has been discussed previously, three pre-dose samples were reconfirmed by reanalysis to be positive for plasma concentration of levacetylleucine (above LLOQ) at the end of the analysis. Carry over (the confirmed carry over in the validation has been detected), cross contamination and/or impact of endogenous levels cannot be fully excluded. Regarding the confirmed carry over effect in the validation, the two blank samples should have been injected after higher concentration samples, and the clinical sample should not be randomized/injected. The Applicant is reminded that the method for PK samples collected for the future food effect study/PK sampling in the paediatric population should be appropriately validated and the potential of the carry over should be excluded in the sample analysis in line with validated method. In addition, the presence of endogenous levels should be appropriately taken into consideration.

Certificates of analysis for standards levacetylleucine, N-acetyl-D-leucin and N-acetyl-deca-deutero-DL-leucine used in bioanalysis and validation of the method are provided.

It was stated by the Applicant that levacetylleucine is present also as endogenously generated metabolite (by endogenous catabolic pathways from L-leucine and acetate) and is acetylated derivate of a ubiquitously present amino acid occurring in human food. The Applicant claims that potential interference from endogenously formed/derived levacetylleucine with administered levacetylleucine has been investigated via results from drug-naïve healthy volunteers and patients' samples and no significant systemic levels of endogenous levacetylleucine interfering with a proper analysis of orally administered levacetylleucine has been detected.

The observed pre-dose concentrations in the PK samples above the LLOQ (observed in study IB1001-201 and study IB1001-301) have been justified by the Applicant by cross-contamination. No thorough discussion of this phenomenon has been provided. The cross-contamination, presence of endogenous levels of levacetylleucine and/or incorrect procedures during the sampling process (critical findings raised during the GCP inspection) cannot be fully excluded.

Considering the findings related to findings for the PK sampling/bioanalysis observed during the GCP inspection for study IB1001-301, the reliability of the PK samples measurement could be questioned and the potential impact of endogenous PK levels cannot be fully excluded considering the measured pre-dose levels (similarly, the pre-dose concentrations above the LLOQ in PK samples for rich sampling in study IB1001-201 have been detected).

The Applicant committed to conduct and submit the results (when available) of a food effect PK study (planned in 2026) (REC) and drug-drug interaction study (REC). In the new planned study, the potential impact of endogenous levacetylleucine and appropriate corrective/preventive action should be considered for the process of collecting, processing and analysing of PK samples to avoid cross-contamination, cross-over of the levacetylleucine from samples in the bioanalysis and misinterpretation of the results. The bioanalysis should be conducted in line with current ICH guideline on bioanalytical method validation and study sample analysis (EMA/CHMP/ICH/172948/2019). All new PK data should be submitted to update the pharmacokinetic sections of SmPC.

Food effect

No data regarding the food effect is currently available and the Applicant plans to perform PK study regarding the food effect. The Applicant commits to provide the results from the food effect study planned in 2025 (REC).

Paediatric population

Based on the available data for studies IB1001-201 and IB1001-301, PK data from 14 children aged 6 to 12 years and weighing 20 to 25 kg (n=6), 25 to <35 kg (n=4) and over 35 kg (n=4) were available. Overall levacetylleucine was investigated in children aged 6 years and weighing at least 20 kg.

Based on the proposed simulation exercise for children aged 6-12 years and weighing 20-25 kg or 25 to 35 kg, the total daily dose of 2g and 3 g can be considered acceptable based on matching exposure (AUC24h). For children weighing 15-20 kg, it is predicted a 24% higher exposure however no PK data are yet available. Therefore, the levacetylleucine dosing should be restricted at this stage to patients aged 6 years and weighing 20 kg at least, since additional PK data are planned to be collected as part of Study 301 in subjects aged from birth to < 4 years and would not be available in due time during this procedure.

The Applicant accepted to restrict the indication to children weighing at least 20 kg but initially did not accept the age restriction of 6 years arguing that age was not found to be a significant covariate influencing the PK in the PopPK model.

However, it should be reminded to the Applicant that body weight allometric scaling with fixed exponent to the theoretical values was the only (significant) covariate retained in the final PopPK model. According to itb-867-poppk-report-31 Appendix 4.5 (Scatterplot matrix and correlation between continuous covariate), one can note that body weight correlate with age (Pearson Corr Coeff of 0.68), BSA (0.98), BMI (0.88). Although, it is not a strong correlation like BSA, it is expected that age has also an effect on levacetylleucine PK.

No PK data from children aged below 6 years have been submitted as part of this application, whereas children of at least 4 years were planned to be enrolled. Consequently, from a clinical PK perspective the restriction of children aged 6 years and weighing at least 20 kg is justified, levacetylleucine PK is considered unknown below these age/weight limits.

Dose rationale

No dose-finding study has been performed by the Applicant. The selection of the dose was purely based on non-clinical data and experience gained from off-label use of racemate N-acetyl-DL-leucine. Based on *in vivo* data studied by Günther et al. 2015, the doses of 3.75 mg/kg (equivalent to human dose 0.25 g/day) and dose 15 mg/kg (equivalent to human dose 1 g/day) in Sprague-Dawley rats had no significant PD effect and i.v dose of 60 mg/kg (equivalent to human dose 4 g/day) was the minimum dose to trigger a biological effect. It was postulated that the dose of 4 g/day is probably in the range of dose response curve where the facilitatory effect is saturated.

Further the daily dose of 4 g should be administered according to the SmPC (Section 4.2) as 3 single doses: 2 g (morning dose)- 1 g (afternoon dose)- 1 g (evening dose) for adults and children weighing over 35 kg. The Applicant was requested to discuss this dosing as no rationale was provided for the administration of the higher first single dose (2 g) administered in the morning. The Applicant justified the different morning dose of 2 g by the need for a loading dose after no dose overnight. No additional pharmacokinetic or pharmacodynamic justification was provided, suggesting rather arbitrary decision to dose the daily dose of 4 g in adult patients. In addition, levacetylleucine was administered with miglustat in most of the NPC patients, thus the true effect of levacetylleucine alone is unknown in the target population.

The CHMP agreed that the dose of 4 g/day for adults and children weighing at least 35 kg has been demonstrated in both non-clinical and clinical studies to be safe and well-tolerated. Non-clinical studies indicate that the dose of 4 g/day is effective, and the clinical trial outcome is reflective of the efficacy of this dose.

Based on a simulation exercise for children aged 6-12 years and weighing 20-25 kg or 25 to 35 kg, the total daily dose of 2 g and 3 g respectively can be considered acceptable based on matching exposure (AUC_{24h}). For children weighing 15-20 kg, a 24% higher exposure is predicted. However, no PK data are yet available in this population at the time of this submission, and it is expected that PK data will be available as part of Study 301. Therefore, the IB1001 dosing should be restricted at this stage to patients weighing at least 20 kg.

Overall, despite some uncertainties, the dose proposed by the Applicant was considered acceptable by the CHMP.

PK interactions:

As victim:

It was shown in vitro that levacetylleucine is metabolized without the involvement of the body's primary detoxification enzymes (CYP, UGT, SULT). It is readily broken down into L-leucine and acetate, which are subsequently reused in the body's natural metabolic pathways to produce proteins and energy. This indicates that levacetylleucine is metabolized efficiently, without the risk of overloading the detoxification enzyme systems, the DDI risk with these enzyme modulators could be ruled out.

The findings from in vitro studies suggest that levacetylleucine is not a substrate of P-gp, BCRP, OATP1B1 and OATP1B3. Therefore, the likelihood of drug-drug interactions between P-gp, BCRP, OATP1B1, OATP1B3 modulators, and N-acetyl-L-leucine as a substrate is not expected.

It was shown in vitro that levacetylleucine is a substrate for OAT1/3. However, since the renal pathway is not the primary route of elimination, the clinical risk of drug-drug interactions with modulators of these transporters is likely minimal.

As precipitant:

Inhibition of CYP enzymes:

The ability of levacetylleucine to be direct or time-dependent inhibitor (TDI) of CYP1A2, CYP2B6, CYP2C8, CYP2C9, 2C19, 2D6 and 3A4 was assessed as part of study ADM-18-2066b study.

The study setups were adequate with an appropriate system, human liver microsomes (HLM), ranges of concentrations, from 6 to 2000 µg/mL (corresponding to 34.62 µM and 11540 µM) covering the worst case expected at both systemic level (i.e. 2165 µM) and intestinal level (0,1× dose/250 ml, i.e. 4618.7 µM), control substrates and inhibitors. Results from this study show that, at the highest tested concentrations of 11540 µM vorasidenib did not exert any or little direct or TDI inhibition towards the tested CYPs (IC50>11540 µM). These finding suggest that the DDI potential between levacetylleucine and the tested CYPs substrates could be ruled out at both systemic and intestinal level.

Induction of CYP450s:

Levacetylleucine induction on CYP1A2, 2B6, 3A4 effect was assessed part of an intro study (ADM-18-2066a) using cryopreserved human primary hepatocytes (3 donors) at 6 concentrations ranging from 6 to 2000 µg/mL (corresponding to 34.62 µM and 11540 µM) covering the worst expected at both systemic level (i.e. 2165 µM) and intestinal level (0,1× dose/250 ml, i.e. 4618.7 µM). The control substrates and control inducers for these enzymes were appropriate. The viability of each hepatocyte preparation was between 89 and 100%.

The results indicate that levacetylleucine does not exhibit an in vitro induction effect on CYP1A2, CYP2B6, and CYP3A4 enzymes. This conclusion is based on the observation that the fold-change in CYP mRNA expression is not greater than or equal to 2-fold at concentrations below 50 times the $C_{max,u}$ of the drug

(i.e. 2165 µM). Furthermore, there was no induction effect observed that reached or exceeded 20% of the induction seen with the positive control inducer.

From a clinical perspective, these findings suggest that there is no risk of CYP enzyme induction-related DDI.

Inhibition of Transporters:

The ability of levacetylleucine to inhibit several transporters was assessed through two assays: Study ADM-18-2106a for P-gp, BCRP, and BSEP transporters, and Study ADM-18-2106c for OATP1B1, OATP1B3, OAT1, OAT3, and OCT2.

The experimental design of Study ADM-18-2106a appears acceptable, utilizing an appropriate system with suitable control substrates and inhibitors. The concentration range tested (34.62 µM to 11540 µM) adequately covers the highest anticipated concentration at the intestinal level (4618.7 µM). The data suggests that levacetylleucine is an inhibitor of P-gp and BCRP, with IC50 values of 1310 µM and 2830 µM, respectively. Since these IC50 are below the concentration expect at worst case a clinical inhibitory effect could not be ruled out. Therefore, a clinical DDI study involving P-gp and BCRP substrates is warranted to evaluate the extent of levacetylleucine's inhibitory effect on these transporters. The Applicant committed to conduct a DDI study to evaluate P-gp and BCRP inhibition. This study is scheduled for 2025, and the results will be submitted when available (REC) and incorporated into a future SmPC update.

Regarding the BSEP transporter, the study demonstrated an inhibitory effect with an IC50 of 2355 µM, suggesting that a clinical inhibitory effect could not be ruled out. Thus, the Applicant has added a warning when levacetylleucine is co-administered with BSEP substrates in section 4.5 of the SmPC The experimental design of Study ADM-18-2106c is well-constructed, incorporating an appropriate system with suitable control substrates and inhibitors. The concentration range tested (173.1 µM to 5194 µM) adequately covers the highest anticipated concentrations at both the renal level (10 × $C_{max,ss,u}$ i.e., 433 µM) and systemic level (50 × $C_{max,ss,u}$ i.e. 2165 µM). The data suggests that levacetylleucine is an inhibitor of OCT2, OAT1, and OAT3, with IC50 values of 981 µM, 594 µM, and 594 µM, respectively. However, since these IC50 values are above the threshold of the expected worst-case concentrations, the inhibitory effect is not considered clinically significant.

Regarding the OATP1B1/3 transporters, the concentration range tested does not cover the worst-case scenario expected at the hepatic level (10 × Cu,inlet i.e., 7649.6 µM). The Applicant has justified the concentration used by the fact that at very high concentrations of levacetylleucine (3000 and 9000 µg/mL), the study encountered issues such as cell detachment and unreliable uptake data, likely due to non-specific or cytotoxic effects. Therefore, the analysis of OATP1B1/3 inhibition focused on concentrations where the cells were stable, and the results were consistent (30 to 900 µg/mL). Since, at these concentrations no OATP1B1/3 inhibition was observed the study results and interpretation could be accepted.

The assessment of the inhibitory effect of levacetylleucine on MATEs transporters was not considered. The Applicant committed to conduct an in vitro study to investigate levacetylleucine’s inhibitory effect on MATEs transporters and to submit the results post-MAA as part of a post-marketing commitment by Q2 2026 (REC).

A warning sentence has been added to the SmPC as follow:

“The potential inhibitory effect of levacetylleucine on MATE transporters is unknown; therefore, caution is advised when co-administering levacetylleucine with substrates of MATE transporters”

Pharmacodynamics

Primary pharmacology

No phase I study in healthy volunteers has been conducted, and the primary pharmacology is primarily based on the non-clinical studies. The Applicant has referred to published literature which concerns the off-label use of the racemate or levacetylleucine as active compound in the treatment of NPC patients and in different rare diseases (e.g. GM2 Gangliosidosis).

A phase II Study IB1001-201 was conducted as a proof-of-concept study showing a significant effect on neurological endpoints CI-CS score on either the 9-Hole Peg Test of the Dominant Hand (9HPT-D) or the 8-Meter Walk Test (8MWT) in NPC patients. The CI-CS score was not used in the pivotal study as the primary endpoint. The Applicant claims that the mechanism of action is rather non-specific and levacetylleucine targets several cell structures.

Mechanism of action

Mechanism of action is described very generally. Symptomatic, neuroprotective and disease-modifying effect is claimed. Based on results from period of 12 weeks of treatment, improvements have been observed, which may indicate symptomatic treatment rather than a disease modifying effect.

Secondary pharmacology

No clinical studies in healthy volunteers have been conducted with regards to the secondary pharmacology.

2.6.4. Conclusions on clinical pharmacology

The clinical pharmacology is based on non-clinical data; no phase I clinical studies were carried out. The mechanism of action of levacetylleucine is presented as rather non-specific and the active substance (L-enantiomer) revealed no main pathway which would account for the mechanism of action at specific cell-target.

Overall, the PK of levacetylleucine has been investigated and characterized. However, from a PK perspective, levacetylleucine dosing should be restricted to children aged 6 years of age and weighing at least 20 kg.

PK DDI:

The Applicant has conducted a series of *in vitro* DDI studies to assess the effects of levacetylleucine on relevant CYP enzymes and transporters, except for the assessment of its inhibitory effect on MATEs transporters which was not considered. The Applicant committed to conduct an in-vitro study to investigate the levacetylleucine's inhibitory effect on MATEs transporters. The study results could be submitted post-MAA as part of a post-marketing commitment (REC).

2.6.5. Clinical efficacy

Study ID	Enrolment status Start date Total enrolment/ enrolment goal	Design Control type	Study & control drugs Dose, route of administration and duration Regimen	Population Main inclusion/ exclusion criteria

IB1001-301 (pivotal)	Parent Study: FPFV: 30 Jun 2022 LPLV: 12 Jun 2023 Extension Phase: ongoing	Multinational, randomized, placebo-controlled, double-blinded, crossover study evaluating 12 weeks of treatment with IB1001.	IB1001, placebo Patients aged ≥13 years or aged <13 years weighing ≥35 kg received 4 g/day of orally administered NALL. Patients aged <13 years weighing <35 kg received weight-tiered doses 2 or 3 times per day	60 (SAF) IB1001: 60 Placebo: 59 Patients with a confirmed diagnosis of NPC aged 4 years or above SARA score of 5≤ X ≤34 points (out of 40) AND Either: Within the 2-7 range (0-8 range) of the Gait subtest of the SARA scale OR able to perform the 9HPT-D (SCAFI subtest) in 20≤ X ≤150 second
IB1001-201, Phase II study in NPC (Parent Study and Extension Phase completed	Parent Study: FPFV: 04 Sep 2019 LPLV: 05 Aug 2020 Extension Phase: FPFV: 11 Dec 2019 LPLV: 07 Nov 2022	Multinational, multi-center, open-label, rater-blinded single-arm study in paediatric and adult patients aged ≥6 years evaluating 6 weeks' treatment with IB1001.	Patients aged ≥13 years or aged <13 years weighing ≥35 kg received 4 g/day of orally administered NALL. Patients aged <13 years weighing <35 kg received weight-tiered doses 2 or 3 times per day	33 (SAF) Patients with a confirmed diagnosis of NPC aged 6 years or above SARA score of 5≤ X ≤33 points (out of 40) AND Either: Within the 2-7 range (0-8 range) of the Gait subtest of the SARA scale OR able to perform the 9HPT-D (SCAFI subtest) in 20≤ X ≤150 seconds
GM2 gangliosidosis (Tay-Sachs and Sandhoff diseases)				
IB1001-202, Phase II study in GM2 gangliosidosis (Parent Study and Extension Phase completed)	Parent Study: FPFV: 07-Jun 2019 LPLV: 07-Jan-2021 Extension Phase: FPFV: 09-Apr-2020 LPLV: 09-Jan-2023	Multinational, multi-center, open-label, rater-blinded single-arm study in paediatric and adult patients aged ≥6 years	Patients aged ≥13 years or aged <13 years weighing ≥35 kg received 4 g/day of orally administered NALL. Patients aged <13 years weighing <35 kg received weight-	30 (SAF) Patients with a confirmed diagnosis of GM2 aged 6 years or above

	evaluating 6 weeks' treatment with IB1001	tiered doses 2 or 3 times per day	SARA score of 5≤ X ≤33 points (out of 40) AND Either: Within the 2-7 range (0-8 range) of the Gait subtest of the SARA scale OR able to perform the 9HPT-D (SCAFI subtest) in 20≤ X ≤150 seconds

Abbreviations: FPFV: first patient first visit; LPLV: last patient last visit; SAF: safety analysis set; SARA: Scale for the assessment and rating of ataxia; SCAFI: spinocerebellar ataxia functional index; 9HPT-D: 9-hole peg test (dominant hand/non-dominant hand)

2.6.5.1. Dose response study(ies)

The Applicant did not conduct dose response studies and the dose of levacetylleucine - approximately 0.1 g/kg/day - used in the clinical studies was selected based on extrapolation of clinical experience with the racemate and in vivo studies in non-clinical models.

2.6.5.2. Main study IB 1001-301

Effects of N-Acetyl-L-Leucine on Niemann-Pick disease type C (NPC): A Phase III, randomized, placebo-controlled, double-blind, crossover study

Methods

This pivotal study is an ongoing a multinational, multicentre, double-blind, placebo-controlled, randomized, crossover Phase III study that enrolled male or female patients aged ≥4 years with a confirmed diagnosis of NPC. Patients were assessed during 3 study periods: a baseline period (with or without a study run-in), followed by 2 treatment periods of equal duration (12 weeks). At the end of Treatment Period I (Visit 4), patients immediately switched to receive the opposite treatment (levacetylleucine or placebo) during Treatment Period II.

At the end of period II, patients were offered the opportunity to participate in an Extension Phase.

- **Study Participants**

Main inclusion criteria were:

Male or female aged ≥ 4 years with a confirmed diagnosis of NPC at the time of signing informed consent.

Patients had to have fallen within:

a. A SARA score of 7≤× ≤34 points (out of 40)

AND

b. Either:

i. Within the 2 to 7 range (0 to 8 range) of the Gait subtest of the SARA scale

OR

ii. Were able to perform the 9HPT-D (SCAFI subtest) in 20 ≤× ≤150 seconds.

Weight ≥15 kg at screening.

Patients were willing to disclose their existing medications/therapies for (the symptoms) of NPC, including those on the prohibited medication list. Non-prohibited medications/therapies (authorized medicines for NPC [e.g., miglustat], speech therapy, and physiotherapy) were permitted.

Main exclusion criteria were:

Patients who had any known hypersensitivity or history of hypersensitivity to:

- Acetyl-Leucine (DL-, L-, D-) or derivatives; excipients of the levacetylleucine sachet or of the placebo sachet

Simultaneous participation in another clinical study or participation in any clinical study involving administration of an IMP ("study drug") for at least 42 days prior to Visit 1.

Patients with severe vision or hearing impairment (that is not corrected by glasses or hearing aids) that, at the Investigator's discretion, interfered with their ability to perform study assessments.

Patients who had been diagnosed with arthritis or other musculoskeletal disorders affecting joints, muscles, ligaments, and/or nerves that by themselves affect patient's mobility and interfered with their ability to perform study assessments.

Patients unwilling and/or who were not able to undergo a 42-day washout period from any of the following prohibited medication prior to Visit 1 (Baseline 1) and remain without prohibited medication through Visit 6: N-Acetyl-DL-Leucine (e.g., Tanganil), levacetylleucine (prohibited if not provided as IMP in the IB1001-301 study), sulfasalazine, rosuvastatin

- **Treatments**

All patients were planned to receive levacetylleucine granules for oral suspension in a sachet, and a matching placebo in this double-blind, crossover study. Each sachet was suspended into 40 mL water, orange juice, or almond milk and mixed until the granules were fully suspended.

The study drug was formulated as 1000 mg levacetylleucine granules for oral suspension in a sachet10, to be suspended in 40 mL water, orange juice, or almond milk.

The placebo was formulated as matching granules for oral suspension in a sachet, to be suspended in 40 mL water, orange juice, or almond milk.

All patients were planned to receive their assigned study drug (levacetylleucine or placebo) for approximately 84 days (+7 days).

At Visit 2, patients were randomly assigned (1:1) to 2 randomization sequences:

1. Sequence 1: Starting Visit 2 (Baseline 2), patients received levacetylleucine during Treatment Period I, and immediately crossed over to receive placebo during Treatment Period II.
2. Sequence 2: Starting Visit 2 (Baseline 2), patients received placebo during Treatment Period I, and immediately crossed over to receive levacetylleucine during Treatment Period II.

During the treatment periods of this study, the dosing of the study drug was as follows:

Table 12: Dosing Regimen Study IB1001-301

Patient Population	Daily Dose	Morning Dose	Afternoon Dose	Evening Dose
Patients aged ≥13 years	4 g/day	2 g	1 g	1 g
Patients aged 4 to 12 years weighing ≥35 kg	4 g/day	2 g	1 g	1 g
Patients aged 4 to 12 years weighing 25 to <35 kg	3 g/day	1 g	1 g	1 g
Patients aged 4 to 12 years weighing 15 to <25 kg	2 g/day	1 g	No dose	1 g

The study drug was to be taken 30 minutes before, or at least 2 hours after a meal.

- **Objectives**

The primary objective was to evaluate the efficacy of levacetylleucine based on the Scale for the Assessment and Rating of Ataxia (SARA) for the chronic treatment of NPC.

The secondary objectives were:

o To assess the clinical efficacy of levacetylleucine on symptoms, functioning, and quality of life for patients with NPC;

o To evaluate the safety and tolerability of N-Acetyl-L-Leucine at 4 g/day in NPC patients aged ≥13 years, and weight-tiered doses in NPC patients aged 4 to 12 years of age

The exploratory objective was:

• To characterize the pharmacokinetics (PK) of levacetylleucine in patients with NPC.

- **Outcomes/endpoints**

Primary endpoint was:

Scale for the Assessment and Rating of Ataxia (SARA). The SARA scale includes 8 items that are related to gait, stance, sitting, speech, finger-chase test, nose-finger test, fast alternating movements and heel-shin test.

Secondary efficacy endpoints included the following:

Measurements of Neurological Signs and Functioning

Scale for Spinocerebellar Ataxia Functional Index (SCAFI). The SCAFI is composed of the 8-Meter Walk Test (8MWT), the 9-Hole Peg Test of the Dominant Hand (9HPT-D) and of the 9-Hole Peg Test of the Non-Dominant Hand (9HPT-ND), and the performance auditory-perceptual assessment (PATA) rate, a measure of speech performance

Measurement of Health-Related Quality of Life

European Quality of Life, 5-Dimension, 5-Level (EQ-5D-5L) for patients aged ≥18 years; European Quality of Life, 5-Dimension, Youth (EQ-5D-Y) for children aged 4 to 17 years (2 parts: the European Quality of Life visual analogue scale [EQ-VAS] and the European Quality of Life descriptive system).

Measurement of Overall Neurological Status

Modified Disability Rating Scale (mDRS) which score comprises 6 components (ambulation, language, manipulation, swallowing, seizures, and ocular movement).

Measurement of Global Impression

· Clinical Global Impression of Improvement (CGI-I) physician

· CGI-I caregiver

· CGI-I patient (if able)

The CGI-I assessment is based on a 7-point scale ranging from 'very much improved' to 'very much worse'.

Safety Endpoints

- Adverse events (AEs)
- Laboratory safety measurements: haematology, clinical chemistry, and urinalysis
- Vital signs
- Electrocardiogram (ECG)
- Physical examinations

- **Sample size**

Parent Phase

In a one-sided test at the 0.05 level a total sample size of 46 in a two-treatment, two-period placebo-controlled cross-over trial, achieves approximately 80% power for treatment comparisons in relation to the SARA/ modified scale for the assessment and rating of ataxia (mSARA) total score assuming a true mean difference of 1.0 / 0.85 (respectively) and a standard deviation for the total SARA/mSARA score between 7.5 and 8.5 and 6.375 and 7.225 (respectively) based on an analysis of covariance with the baseline SARA/mSARA score at the start of Period I as the covariate. These results, based on extensive simulations, assume a correlation of 0.95 between each of the pairwise outcomes: baseline for Period I, endpoint for Period I and endpoint for period II. The values for the standard deviation and the correlations used in the calculation are guided by what was observed in the IB1001-201 study. These levels of power are maintained when there is a positive or negative period effect of up to 0.5 or 0.425 units on the SARA/mSARA scale, respectively.

- **Randomisation and Blinding (masking)**

A central, computer-based randomization procedure was used.

To reduce the risk of breaking the blind, the appearance, colour, taste, and solubility properties of the IMP were identical for both treatments. In order to reduce bias, the study was double-blind, keeping all patients, their families/caregivers, the Sponsor, Investigators, and study site personnel involved in the study, including personnel carrying out study procedures, evaluating patients, entering study data and/or evaluating study data, blinded to treatment allocations until the database was locked for analysis.

- **Recruitment**

Parent study:

First patient, first visit: 30-Jun-2022

Last patient, last visit: 12-Jun-2023

Database lock: 12-Jun-2023

Study status: The DB Parent study is completed while the Open label extension is ongoing.

- **Conduct of the study**

Major amendments

Global Protocol Amendment dated 05-Dec-2021. This amendment was created due to the different non-US requirements regarding the primary endpoint and diagnosis of NPC. Key changes of the Global Protocol were:

- Utilization of SARA as the primary endpoint for non-US countries;
- An update of the inclusion criterion by the addition that patients with a confirmed diagnosis (4 categories) of NPC were eligible in non-US countries;
- The inclusion of mSARA as an exploratory endpoint for non-US countries.

Global amendment dated 08-Mar-2022. Key changes of Global Amendment were:

- Addition that 3 patients were required per age group 4 to 11 years, and 3 patients required 12 to 17 years to align with the requirements of the agreed Paediatric Investigational Plan;
- For patients aged 16 to 17 years, it was added that the EQ-5D-5L could be used based on the guidance of the EuroQol group if the Investigator determined it was appropriate.

- **Baseline data**

Table 13: Demographics – ITT/SAF Population

Parameter	Statistic/ Category	Age 4 to 12 Years 15 to <25 kg 2 g per Day (N=6)	Age 4 to 12 Years 25 to <35 kg 3 g per Day (N=3)	Age 4 to 12 Years ≥35 kg 4 g per Day (N=3)	Age ≥13 Years 4 g per Day (N=48)	Total (N=60)
Age	Mean (SD)	6.7 (1.0)	9.3 (1.2)	11.3 (1.2)	30.9 (14.6)	26.4 (15.9)
	Median	7.0	10.0	12.0	28.5	25.0
	Min, max	5, 8	8, 10	10, 12	13, 67	5, 67
Sex (n [%])	Female	3 (50.0)	2 (66.7)	1 (33.3)	21 (43.8)	27 (45.0)
	Male	3 (50.0)	1 (33.3)	2 (66.7)	27 (56.3)	33 (55.0)
Race (n [%])	Asian	0	1 (33.3)	0	1 (2.1)	2 (3.3)
	White	6 (100)	2 (66.7)	3 (100.0)	43 (89.6)	54 (90.0)
	Other	0	0	0	4 (8.3)	4 (6.7)
BMI (kg/m^2)	Mean (SD)	15.13 (1.19)	16.59 (0.64)	18.61 (2.24)	22.37 (4.23)	21.25 (4.53)
	Median	15.65	16.73	18.66	22.01	20.80
	Min, max	13.4, 16.3	15.9, 17.1	16.3, 20.8	14.6, 30.9	13.4, 30.9

Abbreviations: BMI = body mass index; ITT = Intention-to-Treat; Max = maximum; Min = minimum; N = number of patients; n = number of patients in the subgroup; SAF = Safety Analysis Set; SD = standard deviation.
Note: The ITT and SAF population are the same.

No participant under 6 years of age and weighing less than 20 kg was included. Only one participant below 6 years was included: participant ID 31-1-02 aged 5 (5y and 11 months at the time of enrolment) and weighing 22.7 kg.

Table 14: Baseline Characteristics – ITT Population

Parameter	Category	Total (N=60)
Naïve versus non-naïve (n [%])	Naïve	60 (100.0)
Age group (n [%])	Pediatric (<18 years)	23 (38.3)
	Adult (≥18 years)	37 (61.7)
Age group (n [%])	4 to 9 years old	7 (11.7)
	10 years and older	53 (88.3)
Dose group (n [%])	Age 4 to 12 years – 15 to <25 kg – 2 g per day	6 (10.0)
	Age 4 to 12 years – 25 to <35 kg – 3 g per day	3 (5.0)
	Age 4 to 12 years - ≥35 kg – 4 g per day	3 (5.0)
	Age ≥13 years – 4 g per day	48 (80.0)
Age at diagnosis group (n [%])	Early infantile (<2 years)	9 (15.0)
	Late infantile (2 to <6 years)	14 (23.3)
	Juvenile (6 to <15 years)	23 (38.3)
	Adolescent/adult (≥15 years)	14 (23.3)
SARA total score at Visit 1 (n [%])	>Median (14.50)	30 (50.0)
	≤Median (14.50)	30 (50.0)
Gender (n [%])	Male	33 (55.0)
	Female	27 (45.0)
Region (n [%])	United States	0 (0.0)
	Rest of the world	60 (100.0)
Miglustat at baseline (n [%])	Yes	51 (85.0)
	No	9 (15.0)
SARA total score at Visit 1 versus Visit 2 (n [%])[a]	>Median (0.00)	19 (31.7)
	≤Median (0.00)	41 (68.3)

Abbreviations: ITT = Intention-to-Treat; N = number of patients; n = number of patients in the subgroup; SARA = Scale for the Assessment and Rating of Ataxia. Nineteen patients had a difference between SARA total score at Visit 1 versus Visit 2 that was higher than the median of that difference, which is 0.00. That means that 19 patients had a higher SARA total score at Visit 2 than at Visit 1.

Medical History and Concomitant Diseases

Besides NPC, the most frequently reported (i.e., in 5 or more patients) medical history included ataxia (17 patients; 28.3%), epilepsy (13 patients; 21.7%), gaze palsy (10 patients; 16.7%), cognitive disorder, dysarthria, depression, dysphagia (each 8 patients; 13.3%), diarrhoea (7 patients; 11.7%), COVID-19, dystonia, tremor (each 6 patients; 10.0%), cataplexy, sleep disorder, splenomegaly, thrombocytopenia, and seasonal allergy (each 5 patients; 8.3%).

Prior and Concomitant Medications

A total of 34 patients (56.7%) of the SAF population reported prior medication. Acetylleucine (Acetyl-DL-Leucine [Tanganil] or levacetylleucine [as IMP in a previous Phase II study with IB1001]) was the

most frequently recorded prior medication (17 patients; 28.3%). All but 1 patient (98.3%) of the SAF population took concomitant medication. Miglustat was the most frequently used concomitant medication (51 patients; 85.0%).

- **Numbers analysed**

Table 25: Patients disposition

	Total (N=64)
Screened (n)	64
Treated (n (%))	60 (93.8%)
Reason for not treated (n (%))	
Screen failure	4 (6.3%)
Treated per country (n (%))	
Australia	6 (10.0%)
Czech Republic	4 (6.7%)
Germany	17 (28.3%)
Netherlands	9 (15.0%)
Slovakia	2 (3.3%)
Switzerland	12 (20.0%)
UK	10 (16.7%)
Completed parent study (n (%))	59 (98.3%)
Withdrawn from parent study (n (%))	1 (1.7%)
Physician decision	1 (1.7%)
Completed treatment period I (n (%))	59 (98.3%)
Withdrawn from treatment period I (n (%))	1 (1.7%)
Physician decision	1 (1.7%)
Started treatment period II (n (%))	59 (98.3%)
Completed treatment period II (n (%))	59 (98.3%)
Populations (n (%))	
ITT	60 (100.0%)
SAF	60 (100.0%)

Outcomes and estimation

Primary endpoint: SARA total score

Descriptive statistics of the SARA total and subscores at each visit and change from baseline per (pooled) treatment are presented below for the ITT population.

Table 36: Descriptive Statistics of the SARA Total Score – ITT Population

		ITT (N=60)			
		IB1001		Placebo	
		Observed Value	Change From Baseline	Observed Value	Change From Baseline
SARA – Total Score					
Baseline	n	60		59	
	Mean (SD)	15.88 (7.50)		15.68 (7.39)	
	Median	14.00		14.00	
	Min, Max	6.0, 33.0		6.0, 33.0	
Visits 3 or 5	n	58	58	56	56
	Mean (SD)	14.18 (7.75)	-1.78 (2.48)	14.71 (7.71)	-0.88 (1.97)
	Median	13.75	-1.50	12.50	-1.00
	Min, Max	1.5, 30.0	-8.0, 3.0	5.0, 32.0	-5.5, 6.5
Visits 4 or 6	n	59	59	58	58
	Mean (SD)	13.71 (7.68)	-1.97 (2.43)	15.21 (7.27)	-0.60 (2.39)
	Median	13.00	-1.50	13.25	-0.25
	Min, Max	1.0, 30.0	-8.5, 3.5	5.0, 32.0	-6.5, 5.0

Abbreviations: ITT = Intention-to-Treat; Max = maximum; Min = minimum; N = number of patients; N = number of patients in the subgroup; SARA = Scale for the Assessment and Rating of Ataxia; SD = standard deviation. Note: Baseline value was the Visit 2 value. No last observation carried forward approach was used.

A statistical analysis was performed, following method 2 of Mehrotra for crossover studies, to investigate statistical significance of the primary efficacy endpoint between levacetylleucine and placebo treatment.

Table 47: Primary Statistical Analysis Results for the SARA Total Score at the End of the Treatment Period - ITT Population

Effect/Variable	Estimate (SE)	90% Confidence Interval	95% Confidence Interval	p-Value*
Baseline value	0.95 (0.04)	(0.88, 1.03)	(0.86, 1.04)	<0.001
Treatment effect (IB1001 versus placebo)	-1.28 (0.31)	(-1.80, -0.76)	(-1.91, -0.65)	<0.001
LS mean IB1001	13.87 (0.31)	(13.34, 14.39)	(13.24, 14.50)	
LS mean placebo	15.15 (0.32)	(14.62, 15.68)	(14.52, 15.78)	

Abbreviations: ITT = Intention-to-Treat; LOCF = last observation carried forward; LS = least squares; SARA = Scale for the Assessment and Rating of Ataxia; SE = standard error. * One-sided p-value. For 1-sided p-values below 0.001, the 2-sided p-value was also below 0.001. =.

Analysis by Treatment Sequence

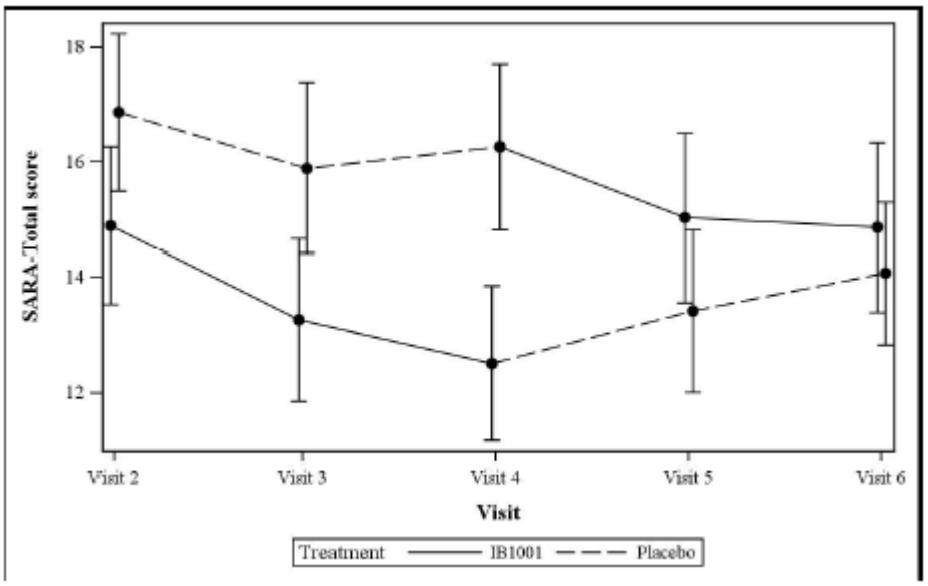
Descriptive statistics of the SARA total score at each visit and change from baseline at each visit per treatment sequence are presented below.

The mean baseline of the SARA score was higher in the placebo group than in the levacetylleucine group.

Table 58: Descriptive Statistics of the SARA Total Score at Each Visit per Treatment Sequence - ITT Population

		ITT (N=60)			
–	IB1001 - Placebo (N=30)		Placebo – IB1001 (N=30)		
		Observed Value	Change From Baseline	Observed Value	Change From Baseline
SARA – Total Score					
Baseline	n	30		30	
	Mean (SD)	14.90 (7.49)		16.87 (7.51)	
	Median	13.25		14.75	
	Min, Max	6.0, 33.0		7.0, 31.5	
Visit 3	n	28	28	29	29
	Mean (SD)	13.27 (7.45)	-1.71 (2.59)	15.90 (7.99)	-1.12 (1.56)
	Median	12.25	-1.25	14.00	-1.00
	Min, Max	1.5, 28.0	-8.0, 1.5	5.5, 32.0	-5.5, 1.0
Visit 4	n	29	29	30	30
	Mean (SD)	12.52 (7.19)	-1.93 (2.70)	16.27 (7.86)	-0.60 (1.88)
	Median	12.00	-1.50	13.75	-0.25
	Min, Max	1.0, 27.0	-8.5, 3.5	6.5, 32.0	-5.5, 2.0
Visit 5	n	27	27	30	30
	Mean (SD)	13.43 (7.33)	-0.63 (2.34)	15.03 (8.06)	-1.83 (2.41)
	Median	11.50	-1.00	14.00	-1.50
	Min, Max	5.0, 29.5	-4.5, 6.5	2.5, 30.0	-7.5, 3.0
Visit 6	n	28	28	30	30
	Mean (SD)	14.07 (6.53)	-0.61 (2.88)	14.87 (8.09)	-2.00 (2.18)
	Median	13.00	-0.25	13.50	-1.50
	Min, Max	5.0, 27.5	-6.5, 5.0	2.5, 30.0	-5.5, 2.0

Abbreviations: ITT = Intention-to-Treat; Max = maximum; Min = minimum; N = number of patients; n = number of patients in the subgroup; SARA = Scale for the Assessment and Rating of Ataxia; SD = standard deviation. Note: Baseline value is the Visit 2 value. No last observation carried forward approach was used.



Abbreviations: ITT = Intention-to-Treat; LOCF = last observation carried forward; SARA = Scale for the Assessment and Rating of Ataxia. Note: The dots represent the observed means, the vertical lines represent (+/-) the standard errors. No LOCF approach was used for this figure.

Figure 6: Mean (+/- Standard Error) Plot of the SARA Total Score Versus Time per Treatment Sequence - ITT Population

Secondary Analysis

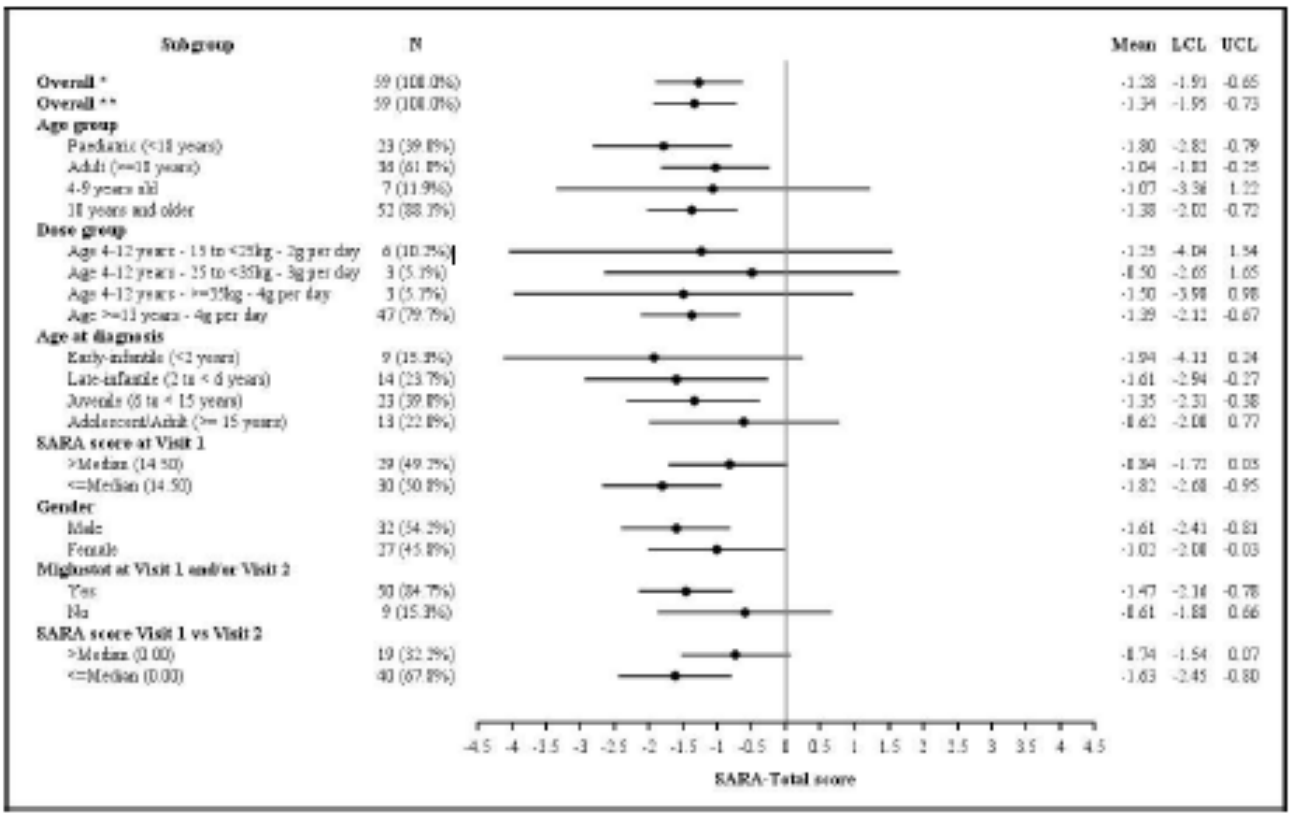
Table 69: Results of the Secondary and Exploratory Endpoints [Study IB1001-301 Parent Study – ITT]

	IB1001 Mean (SD) Change from Baseline	Placebo Mean (SD) Change from Baseline	IB1001 vs Placebo Difference	95% CI			P-value
Secondary Endpoints							
Spinocerebellar Ataxia Functional Index (SCAFI) total score	0.05 (SD=0.27)	-0.02 (SD=0.31)	0.07[1]	0.0	to	0.15	0.027
Euro-Quality of Life Visual Analogue Score (EQ-VAS)	1.2 (SD=16.2)	-1.1 (SD=21.4)	N/A	N/A		N/A	N/A
Modified Disability Rating Scale (mDRS)[2]	-0.030 (SD=0.060)	-0.001 (SD=0.061)	-0.021[1]	-0.040	to	-0.003	0.013
Investigator's Clinical Global Impression of Improvement (CGI-I)[3]	-0.7	-0.1	-0.6	N/A	to	N/A	0.008
Caregiver Clinical Global Impression of Improvement (CGI-I)[3]	-0.4	0.3	-0.7	N/A	to	N/A	0.003
Patient Clinical Global Impression of Improvement (CGI-I)[3]	-0.7	-0.2	-0.5	N/A	to	N/A	0.036
Exploratory Endpoints							
15-domain NPC Clinical Severity Scale (NPC-CSS)	-0.3 (SD=2.2)	0.1 (SD=2.1)	N/A	N/A		N/A	N/A
5-domain NPC Clinical Severity Scale (5-Domain NPC-CSS)	-0.2 (SD=1.8)	0.2 (SD= 1.5)	N/A	N/A		N/A	N/A
Modified Scale for the Assessment and Rating of Ataxia (mSARA)	-1.66 (SD=1.97)	-0.67 (SD=1.74)	-0.96[1]	-1.45	to	-0.46	<0.001[4]
Axial Scale for the Assessment and Rating of Ataxia (axial SARA)	-0.86 (SD=1.38)	0.02 (SD=1.66)	-0.85[1]	-1.24	to	-0.47	<0.001[4]

Abbreviations: CI = confidence interval; ITT = intention-to-treat; mDRS = modified disability rating scale; mSARA = modified scale for the assessment and rating of ataxia; N/A = not applicable; NPC-CSS = Niemann-Pick disease type C clinical severity scale; SARA = scale for the assessment and rating of ataxia; SD = standard deviation.
1 LS-Mean Difference is given
2 mDRS was scaled from 24 total points to a nought-to-one (0 to 1)
3 CGI-I was calculated for Treatment Period I using a 1-sided Wilcoxon Rank Sum test. The change from baseline is the reported change from a score of "4, no change", e.g. a mean score of 3.3 is defined as -0.7.
4 mSARA and axial SARA endpoint 2-sided p <0.001.

Analysis of Subgroups

Descriptive statistics of the SARA total score are presented for the subgroups age, age/weight/dosing group, age of diagnosis group, disease severity based on SARA total score at Visit, gender, miglustat, and intra-patient variability SARA Visits 1 and 2



Abbreviations: ITT = Intention-to-Treat; LCL = lower confidence limit; LOCF = last observation carried forward; N = number of patients; SARA = Scale for the Assessment and Rating of Ataxia; UCL = upper confidence limit.
* Least squares mean difference from the modelling analysis.
** Observed mean of the differences.
Note: The dots represent the mean of the differences between levacetylleucine and placebo, the horizontal lines represent the 95% confidence intervals. One patient discontinued before Visit 4 and has been omitted. One patient missed the Visit 6 assessment; therefore the Visit 5 result was used (LOCF).

Figure 7: Forest Plot of Mean Difference in SARA Total Score for Subgroups at Visit 4 or 6 with 95% Confidence Intervals - ITT Population

Extension study

In its response document, the Applicant has submitted data from the ongoing open-label extension phase (EP) in the form of an unpublished preprint that was not certified by a peer review (Patterson et al. Preprint 2024). No study report was provided.

At the end of the blinded part of the study, after a 42-day (+14 days) washout phase patients had the possibility to enter the extension period in which all patients received Aqneursa for at least 1 year.

The primary endpoint is based on the modified 5-domain NPC-CSS score with success defined as no change or a decrease in the 5-domain NPC-CSS score from Visit 7 to Visit 9.

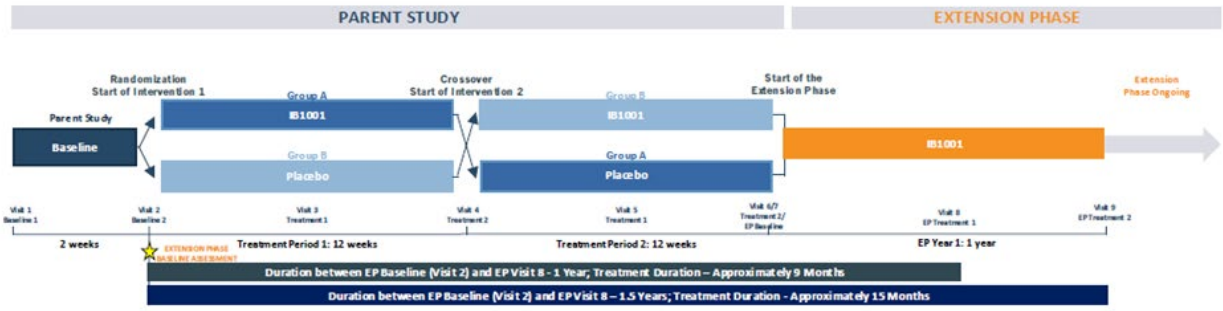


Figure 8

Baseline data

There were 54 patients enrolled in the extension phase, and 50 were included in the analysis.

Table 20: IB1001-301 Extension Phase (EP) Demographics and Baseline Characteristics

Parameter	Statistic/Category	Total (n = 54)
Age	Paediatric (<18 years)	22 (40.7%)
	Adult (>=18 years)	32 (59.3%)
Gender (n (%))	Female	24 (44.4%)
	Male	30 (55.6%)
Race (n (%))	American Indian or Alaska native	0 (0.0%)
	Asian	2 (3.7%)
	Black or African American	0 (0.0%)
	Native Hawaiian or other Pacific Islander	0 (0.0%)
	White	48 (88.9%)
	Other	4 (7.4%)
Age at diagnosis group (n (%))	Early-infantile (<2 years)	9 (16.7%)
	Late-infantile (2 to < 6 years)	13 (24.1%)
	Juvenile (6 to < 15 years)	22 (40.7%)
	Adolescent/Adult (>= 15 years)	10 (18.5%)
Dose group (n (%))	Age 4-12 years - 15 to <25kg - 2g per day	3 (5.6%)
	Age 4-12 years - 25 to <35kg - 3g per day	5 (9.3%)
	Age 4-12 years - >=35kg or age >=13 years - 4g per day	46 (85.2%)
Miglustat at baseline* (n (%))	Yes	47 (87.0%)
	No	7 (13.0%)

*Indicates concurrent miglustat use throughout the duration of the trial

Primary endpoint

In the preprint, the authors provided mean change from baseline of the 5 domain NPC-CSS and carried out a head-to-head comparison with historical cohorts, which was not planned in the initial protocol. These analyses were not pre specified in the protocol or SAP.

Table 21: Comparison of the 5-Domain NPC-CSS while receiving levacetylleucine with Historical & Clinical Trial Cohorts with levacetylleucine

	12 Months from Baseline			
	n=	Mean Change from Baseline*	% Change from Baseline **	Mean Difference vs IB1001-301 EP Cohort (NALL)
Study				
IB1001-301 Extension Phase (NALL)* **	50	-0.32 (SD=2.95)	-21%	N/A
IB1001-301 EP – NALL + Miglustat	42	-0.24 (SD=2.54)	-16%	N/A
IB1001-301 EP – NALL (no Miglustat)	8	-0.75 (SD=1.83)	-50%	N/A

Mengel et al. 2020 [6] (Natural History Cohort)	32	1.5 (SD=3.16)	+100%	+1.82
Mengel et al. 2021 [7] (Arimoclomol)****	34	0.76 (SD=2.4)	+51%	+0.96
Mengel et al. 2021 [7] – Arimoclomol + Miglustat	26	-0.06 (N/A)	-4%	+0.26
Mengel et al. 2021 [7] – Arimoclomol (no Miglustat)	8	4.2 (N/A)	+280%	+4.52
Mengel et al. 2021 [7] (Placebo)*****	16	2.15 (SD= 2.25)	143%	+2.27

* A higher score represents worse neurological status
**Calculated based on an annualized progression rate of 1.5 points (representing a +100% rate of annual progression); a positive value reflects disease progression; 0 reflects no change; a negative value reflects disease reversal.
***Includes patients treated with NALL (no Miglustat) (n=7) and patients treated with NALL + Miglustat (n=43)
****Includes patients treated with Arimoclomol (without miglustat) (n=8) and patients treated with Arimoclomol + miglustat combination therapy (n=26)
***** Includes patients treated with Placebo (no Miglustat) (n=4) and Placebo + Miglustat (n=12)

Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 22: Summary of efficacy for trial IB 1001-301

Title: Study IB 1001-301 Effects of N-Acetyl-L-Leucine on Niemann-Pick disease type C (NPC): A Phase III, randomized, placebo-controlled, double-blind, crossover study			
Study identifier	EudraCT number 2021-005356-10		
Design	Multicenter, double-blind, placebo-controlled, randomized, crossover Phase III study that enrolled male or female patients aged ⩾4 years with a confirmed diagnosis of NPC.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		12 weeks 2 weeks Approximately 2 years
Hypothesis	Superiority		
Treatments groups	Treatment		N-Acetyl-L-Leucine, 12 weeks, N= 60
	Placebo		placebo, 12 weeks, N= 60
Endpoints and definitions	Primary endpoint	SARA	Scale for the Assessment and Rating of Ataxia that includes 8 items that are related to gait, stance, sitting, speech, finger-chase test, nose-finger test, fast alternating movements and heel-shin test.
	Secondary endpoint	SCAFI	Scale for Spinocerebellar Ataxia Functional Index (SCAFI) is composed of the 8-Meter Walk Test (8MWT), the 9-Hole Peg Test of the Dominant Hand (9HPT-D) and of the 9-Hole Peg Test of the Non-Dominant Hand (9HPT-ND), and the performance auditory-perceptual assessment (PATA) rate, a measure of speech performance
	Secondary endpoint	EQ-5D	European Quality of Life, 5-Dimension, 5-Level
	Secondary endpoint	mDRS	Modified Disability Rating Scale (mDRS) this score comprises 6 components (ambulation, language, manipulation, swallowing, seizures, and ocular movement)
	Secondary endpoint	CGI-I	Clinical Global Impression of Improvement
Database lock	12-Jun-2023		

Title: Study IB 1001-301 Effects of N-Acetyl-L-Leucine on Niemann-Pick disease type C (NPC): A Phase III, randomized, placebo-controlled, double-blind, crossover study				
Study identifier	EudraCT number 2021-005356-10			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat			
Descriptive statistics and estimate variability	Treatment group	levacetylleucine	p*lacebo*	
	Number of subjects	60	59	
	SARA baseline mean	15.88	15.68	
	Standard deviation	7.50	7.39	
	SARA V3 or V5 mean	14.18	14.71	
	Standard deviation	7.75	7.71	
	SARA V4 or V6 mean	13.71	15.21	
	Standard deviation	7.68	7.27	
Effect estimate per comparison	Primary endpoint SARA	Comparison groups	Treatment effect (IB1001 versus placebo)	
		Estimate (SE)	-1.28 (0.31)	
		SE	(-1.80, -0.76)	
		P-value	<0.001	
Analysis description	**Secondary analysis:** All secondary efficacy endpoints were summarized using descriptive statistics and frequency tables due to the absence adjustment for multiple comparisons.			

2.6.5.3. Clinical studies in special populations

The Applicant did not conduct clinical studies in special populations.

2.6.5.4. In vitro biomarker test for patient selection for efficacy

Not applicable

2.6.5.5. Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable

2.6.5.6. Supportive study(ies)

Study IB1001-201

This study is a multinational, multi-center, open-label, rater-blinded single-arm study in paediatric and adult patients aged ≥6 years evaluating a 6-week treatment with levacetylleucine.

Patients were assessed during 3 study periods: a baseline period (with or without a study run-in), a 42-day (+7 days) treatment period followed by a 42-day (+7 days) and a washout period. Patients who completed the Parent Study were to be offered the opportunity to participate in an approximately 2-year Extension Phase. Patients received the same dose as in the pivotal study (i.e. approximately 0.1 mg/kg/d).

The primary efficacy objective was to assess the efficacy of levacetylleucine for the treatment of NPC, based on rater-blinded CI-CS of patient's change in performance over 6 weeks on either the 9HPT-D or the 8MWT (the choice of the 9HPT-D or 8MWT as the primary anchor around which the CI-CS assessment was based on patient's clinical symptoms).

The secondary objectives of the parent study were to evaluate the clinical efficacy of levacetylleucine on symptoms of ataxia, functioning, and quality of life for patients with NPC using Scale for Spinocerebellar Ataxia Functional Index (SCAFI), Scale for the Assessment and Rating of Ataxia (SARA), EQ-5D-5L, EQ-5D-Y, Modified Disability Rating Scale (mDRS), Annual Severity Increment Score (ASIS).

The primary objective in the Extension Phase (considered secondary to those of the parent study) was to evaluate the efficacy of levacetylleucine based on the 5-Domain Niemann-Pick Type C Clinical Severity Scale (NPCCSS) with success defined as no change or a decrease in the 5-domain NPC-CSS score.

Overall, 33 patients were included and 31 completed the parent study; 71.9% of the patients were adults and 90.9% took concomitant therapy with miglustat.

For the mITT population, the mean value (SD) CI-CS component for the treatment period was 0.48 (1.34) and - 0.38 (1.45) for the washout period showing an improvement in the treatment period and a deterioration at the end of the washout period. The primary endpoint reached statistical significance with a p value=0.029.

For the mITT population, the mean (SD) SARA score for the treatment period was - 1.19 (2.02) and 1.45 (2.56) for the washout period showing improvement in the treatment period and deterioration at the end of the washout period. Other secondary endpoints indicated a trend of improvement during the treatment period and deterioration during the washout period. However, it is difficult to draw conclusions since the secondary endpoints were measured in an open-label manner.

Among the 31 patients who completed the parent study, 19 patients were included in the Extension phase. The primary efficacy endpoint of the Extension Phase was the success rate based on the 5-domain NPC-CSS score, which is defined as the proportion of patients with no change or a decrease in the 5-domain NPC-CSS score after 12 months. For the mITTe population, the proportion of patients with success was 0.50 (90% CI: 0.31, 0.69) compared to a proportion of 0.10, which is the expected rate of success without treatment. But the changing of the primary endpoint from CI-CS to the 5-domain NPC-CSS score during the extension phase hampers the interpretation of these results.

2.6.6. Discussion on clinical efficacy

The initial development of levacetylleucine began with the racemate, N-acetyl-DL-leucine (NADLL), a molecule marketed for the treatment of acute vertigo in adults as it was observed that in compassionate use cases of patients with vertigo treated with NADLL, symptoms of cerebellar ataxia rapidly improved. This led to the hypothesis that NADLL may be an effective treatment for neurological disorders with cerebellar impairment. However, it seemed that the L-enantiomer (levacetylleucine) mediated most of the pharmacological effect leading to the development of levacetylleucine notably in NPC.

In order to support efficacy of levacetylleucine for the chronic treatment of Niemann-Pick type C disease (NPC) in adults and children from birth, the Applicant has provided a package with results from 2 studies

– one ongoing placebo-controlled crossover study followed by a long-term Extension Phase and one phase II open-label study followed by a long-term Extension Phase.

The proposed recommended dose is 4 grams per day for patients weighing more than 35 kg., 3 grams per day for patients weighing 25 to 34 kg and 2 grams per day for patients weighing 20 to 24 kg.

The Applicant did not conduct any dose finding study and justifies the proposed dosing based on the clinical use of the racemate and on non-clinical data.

It should be noted that the maximal recommended dose for N-acetyl-DL-leucine is 4 g/day corresponding to 2 g/day of the L enantiomer (levacetylleucine). As only the L enantiomer crosses the blood brain barrier, the exposure in L-enantiomer could be twice higher than with the maximal authorised dosage of the racemate.

The proposed recommended dose is considered acceptable.

Design and conduct of the clinical studies:

Study IB1001-301 (pivotal study)

The pivotal study IB1001-301 was an ongoing multicenter, randomized (1:1) double-blind, placebo-controlled, crossover phase III study (parent study) followed by an Extension phase. The duration of the treatment period during the cross-over was 12 weeks, which may be considered limited for a product intended for chronic use.

All patients were successively exposed to both treatments (levacetylleucine and placebo), randomization consisting of randomly assigning to patients the sequence of treatments in the 24-week exposure (levacetylleucine then placebo versus placebo then levacetylleucine) without wash out period.

The choice of a placebo arm is acknowledged and acceptable given that patients could continue their treatment with miglustat (only authorized product in the EU for the treatment of progressive neurological manifestations in patients with Niemann-Pick type C disease).

To ensure blinding, investigational medicinal products (IMPs) were administered as oral solution. However, the composition differed between the IMPs. Excipients of the levacetylleucine sachet were namely isomalt, hypromellose, and strawberry flavour. Excipients of the placebo sachet were isomalt, hypromellose, strawberry flavour, citric acid, microcrystalline cellulose, lactose, and denatonium benzoate.

The study aimed to include male or female patients with a confirmed diagnosis of NPC based on positive biomarker and/or filipin test and/or genetic tests.

Analyses were done in the intention-to-treat population (ITT; all participants who were randomly assigned) and were done with only the observed data (complete case analysis).

The sample size justification was based on an expected 1-unit difference in SARA total score in favour of levacetylleucine.

Patients <4 years of age have been excluded from the study. The lack of data in this population is a concern in regard to efficacy in younger patients.

Patients had to have a SARA score between 7 and 34 so that they could complete the study assessments meaning that patients who did not present neurological signs and the most severe patients were excluded. The exclusion of the most severe patients and asymptomatic patients is justified by the fact that these patients might not be able to complete the primary endpoint.

The study drug was to be taken 30 minutes before, or at least 2 hours after a meal. This is now reflected in the newly proposed product information and is in line with the missing information regarding the food effect.

At enrolment, patients were classified as either "naïve" or "non-naïve," based on their use within the past 42 days of prohibited medications such as N-Acetyl-D-Leucine and N-Acetyl-DL-Leucine (e.g., Tanganil). "Non naïve" patients had to undergo a minimum of 42 days pre-treatment washout period.

The primary endpoint for all regions outside of the USA was SARA (Scale for the Assessment and Rating of Ataxia) score which includes 8 items that are related to gait, stance, sitting, speech, finger-chase test, nose-finger test, fast alternating movements and heel-shin test. It is a validated clinical scale used to assess ataxic disorders, but it has not been validated to assess Niemann–Pick type C disease. Secondary endpoints included an ataxic scale (SCAFI) and a modified Disability Rating Scale and PROS. These scales are used to assess neurological symptoms, but it has not been conclusively established that they can be used to assess NPC disease.

The primary (SARA) and secondary endpoints used in this study aims to show an effect over placebo on neurological signs but not on the whole spectrum of symptoms of the NPC disease. The claimed indication is limited to the neurological signs of the NPC disease in order to reflect the results of the pivotal study.

The sample size justification was based on an expected 1-unit difference in SARA total score in favour of levacetylleucine, using a 10% type-1 error and 80% power. The two-sided 10% significance level was unusual and somewhat high for a phase III trial. However, given the context of a rare disease, this was retrospectively understandable for feasibility reasons. The primary endpoint p-value will have to be discussed if between 2.5% and 5% (one-sided).

No multiplicity testing procedure was implemented for the EU data analysis for which there was only one primary endpoint and no key secondary endpoint.

No wash-out was defined between the 2 intervention periods for the cross-over, this could increase the risk of ending up with notable difference of treatment effect at period 1 and period 2 (quantitative interaction between treatment and period factors). Similar treatment effect (levacetylleucine versus placebo difference) in period 1 (-1.33) and period 2 (-1.39) is observed. These results are consistent with the overall pooled difference (-1.28) which seems therefore well capturing the true treatment effect.

The statistical analysis of a crossover design is somewhat complex. The sponsor indicated that a mixed model was used, but the fixed and random factors were not clearly stated as well as the different possible interactions between factors. These interactions could be interesting to assess (notably treatment x period) given the absence of washout period between period 1 and 2. Even though the proposed primary endpoint analysis considering only 2 time-points per patient is acceptable (SARA total score at visit 4 and 6), it is not clear why the other time-points (visit 3 and 5) were not taken into account while the statistical model could handle them, whether data were collected or not. In their response, the Applicant clarified that there was no treatment x period interaction term in the model but there is no visible interaction and therefore no need to testing it. In addition, since the model captures adequately the data, there is no need to the complementary analysis requested.

The interim CSR of the pivotal study was limited to the analysis of the primary and secondary objectives of the Parent Study.

Extension phase

At the end of the blinded part of the study, after a 42-day (+14 days) washout period patients could enter the open label extension period in which all patients received Aqneursa for at least 1 year.

In their response document, the Applicant has submitted data from the ongoing open-label extension phase (EP) in the form of an unpublished preprint that was not certified by a peer review (Patterson et al. Preprint 2024). The final report will be provided in due time.

The primary endpoint for the EP was the 5-domain Niemann-Pick disease type C Clinical Severity Scale (NPC-CSS) and the SARA score is presented as an exploratory endpoint.

The primary endpoint was success, defined as no change or a decrease in the 5-domain NPC-CSS, comparing the Extension Phase baseline to the end of treatment in the Extension Phase.

Study IB1001-201 (supportive study)

This was an open label rater-blinded study that aimed to assess in the parent study the efficacy of levacetylleucine in adult and paediatric patients aged 6 years and above on neurological endpoints CI-CS score on either the 9-Hole Peg Test of the Dominant Hand (9HPT-D) or the 8-Meter Walk Test (8MWT) followed by an extension study.

Parent study

The parent study consisted of a run-in phase followed by a 6-week treatment phase and a 6-week wash-out period. The Extension study consisted in an extension treatment period I of approximately 365 days, followed by an extension washout of 42 days and an extension treatment period II of approximately 365 days.

In this study male or female patients aged ≥6 years in Europe with a confirmed diagnosis of NPC and weighed ≥15 kg at screening were included. The main inclusion criterion was SARA score of $5 \leq X \leq 33$ points (out of 40) and either the 2-7 range (0-8 range) of the Gait subtest of the SARA scale or ability to perform the 9HPT-D (SCAFI subtest) in $20 \leq X \leq 150$ seconds.

The primary endpoint was blinded raters' Clinical Impression of Change in Severity (CI-CS) score comparing either the 9-Hole Peg Test of the Dominant Hand (9HPT-D) or the 8-Meter Walk Test (8MWT) as the primary anchor. It was defined as:

> the CI-CS comparing videos of the patient's performance on the predefined anchor test at (i) the end of treatment versus (ii) baseline;
> minus
> the CI-CS comparing videos of the patient's performance on the predefined anchor test at (i) the end of washout versus (ii) end of treatment.

This chosen primary endpoint CI-CS has not been validated in this setting and does not fully reflect the condition studied.

The data validity and data integrity from the phase II study is questionable since there are issues which have not been adequately answered by the Applicant to the GCP inspectors. The scheduled visits were Visit 1 (baseline 1), Visit 2 (baseline 2), Visit 3 (after 4 weeks of treatment), Visit 4 (after 6 weeks of treatment), Visit 5 (4 weeks after start of washout) and Visit 6 (end of washout).

Additional efficacy endpoints included measurements of ataxia and functioning: SARA score, SCAFI score, Measurement of Health-Related Quality of Life, Measurement of Overall Neurological Status and Measurement of Global Impression scored by treating physician, caregiver, and patient. Secondary endpoints were assessed in an unblinded manner which could introduce a potential of bias and limits their interpretability.

Extension study

In the extension study, patients were followed approximately 2 years. Treatment has been administered approximately 365 days (treatment period I – Visit 7A/B, Visit 8), then followed extension phase

washout period (Visit 9A/B at the start of wash out period, Visit 10 at the end of wash out period) and then all patients were planned to continue an additional 365 days (treatment period II – Visit 11 during treatment and Visit 12 at the end of treatment period II).

The primary endpoint for the Extension study was the Modified 5- domain Niemann-Pick Disease type C Clinical Severity Scale (5-domain NPC-CSS) at the end of the Extension Phase treatment period 1 versus Extension Phase Baseline. There were several amendments during the study in the study conduct and planned analyses The major concern is that the primary endpoint for the extension study was changed from CI-CS of the primary anchor test to 5-domain NPC-CSS during the sixth amendment of the protocol dated 11-Oct-2022 few time before the end of the extension study dated which jeopardises the integrity of the results of this study.

Additional exploratory endpoints for the Extension study included measurements of Ataxia and Functioning: SARA total score and subscores, SCAFI total score and subscore, Measurement of Health-Related Quality of Life, Measurement of Overall Neurological Status and Measurement of Global Impression scored by treating physician, caregiver, and patient.

Primary efficacy endpoint differs between the parent and the extension phase of phase II study and does not correspond with chosen primary efficacy endpoint for phase III study IB1001-301; this makes difficult the interpretation of the long-term results.

Efficacy data and additional analyses:

Study IB1001-301 (pivotal study)

Parent study

The study included 60 patients across 11 sites in 7 countries in the EU, UK, Australia and Switzerland. None were included in the USA and 59 completed both periods of the cross-over. Overall, most patients were male (55%); the average age of the patients was 26.4 years (range 5 to 7). The mean weight was 58.68 kg (range 20.6 to 109.3). A total of 23 (38.3%) patients were <18 years at screening. The studied population did not reflect the initially proposed indication since paediatric population comprised only 6 patients under 12 years of age and children below 5 years of age were not included. The indication was updated during the procedure to include an age restriction (aged 6 years and older) in addition to the weight restriction (above 20 kg).

All patients had genetically confirmed diagnosis, and all patients had mutation in the NPC1 gene.

The sample size may be considered limited; however, this is acceptable given the rarity of the disease.

The respective minimum and maximum individual SARA scores were 6 and 33 out of a maximum of 40 points (median baseline score 14.5).

All but 1 patient (98.3%) of the SAF population took concomitant medication. miglustat was used as concomitant medication in 85.0% of the patients.

The majority of patients had received prior medication. Among them 9 previously received levacetylleucine [as IMP in the previous Phase II study with levacetylleucine]), 8 were reported to have previously received acetylleucine (Acetyl-DL-Leucine [Tanganil] and 8 previously received miglustat.

Regarding the SARA score, the mean (SD) change from baseline was -1.97 (2.43) after levacetylleucine treatment compared to -0.60 (2.39) after placebo. Two-sided statistical testing indicated a significant difference between (LS-mean difference: -1.28; 95% CI: -1.91, -0.65; p<0.001) showing an improvement.

Along with *Figure 4* shows that there is a noticeable numerical difference in treatment effect between period 1 and 2. Indeed, at visit 4, there is -3.76-unit difference between levacetylleucine and placebo (respectively 12.52 and 16.24) reduced to a -0.80 difference at visit 6 (14.07 and 14.87).

The first baseline (i.e. at inclusion) was used to calculate the change from baseline for both periods (V4 and V6). This method is acceptable when the washout period is short, which is the case here since there was no washout phase between the two treatment periods. The absence of a washout may result in a carryover effect, biasing the assessment of treatment efficacy. This risk of carryover does not appear to have been observed in the results, supporting the sponsor's choice. Indeed, after switching to placebo in the levacetylleucine arm, the change from baseline in this arm was similar to that of placebo (-0.61 vs. -0.60). The same was true in the placebo arm after switching to levacetylleucine, with the gain in efficacy similar to that observed in the levacetylleucine arm (-2.00 vs -1.93). However, the difference in SARA scores between arms at baseline remains questionable. According to the Applicant, this difference of -1.97 (14.90 vs. 16.87) is clinically significant because it is twice as large as that targeted in the trial as a treatment effect.

Predefined subgroup analyses based on age, disease severity, age of symptom onset, gender, and background miglustat use were performed showing an improvement. No clear pattern regarding a greater improvement for any subgroup can be drawn. However, the confidence intervals contained 0 the subgroup of patients without miglustat, in the subgroup of patients aged < 13 years which suggests no statistically significant difference. However, the low number of patients limits the interpretation of these data.

The Plot of the SARA Total Score Versus Time per Treatment Sequence shows that the effect of the product on the SARA score is rapid, but also that patients deteriorate rapidly when treatment is stopped, which in the context of a progressive disease could be attributable to a symptomatic effect of the product rather than a disease modifying effect.

Because there was no prespecified adjustment for multiple comparisons, all secondary efficacy endpoints were summarized using descriptive statistics and frequency tables, and no reliable conclusions can be drawn from these data.

Both SCAFI total score and mDRS composite score from baseline compared to placebo treatment showed improvement. The SCAFI score and the mDRS are scales used to assess neurological disorders, however the clinical relevance of the results observed in patients with NPC disease is not clear and should be justified.

Health-related quality of life measured with the EQ-5D-5L and EQ-5D-Y showed some improvement in mobility, self-care and pain discomfort. Measurements of Global Impression CGI-I measured by investigator, caregiver and patient showed some improvement while on levacetylleucine versus placebo.

The palatability of the finished medicinal product was evaluated as well, and this can be viewed as improvement in the compliance of NPC patients and benefit of the levacetylleucine treatment.

Biomarkers

No marker of pharmacodynamic activity of levacetylleucine (N-acetyl-L-leucine (NALL)) was included in the pivotal study and thus no biomarker assessment can be performed. There are several NPC-specific and non-specific biomarkers. These include Lyso-SM-509 (related to disease pathology), Heat shock protein 70 (Hsp70), cholestane-triol in serum, unesterified cholesterol in peripheral blood. These are used to measure the lipid burden in NPC, since e.g., Hsp70 stabilizes the lysosomal membrane independent of the NPC proteins.

Furthermore, the age of the neurological disease onset correlates (inversely) with the baseline concentration of Lyso-SM-509. According to the literature the Lyso-SM-509 levels correlate with the

serum cholestane-triol at baseline and should change over time. As reported in the literature, there is a reduction in plasma Lyso-SM-509 related to a 5-domain NPCCSS score change after 12 months of treatment (Mengel E, 2021)[1]. As part of the SA provided by the Spanish Medicines Agency (AEMPS), there was a clear recommendation to elucidate the mode of action of levacetylleucine as well as identify targeted neuronal brain structures and thus potential biomarkers.

The clinical meaningfulness of the primary endpoint (SARA) was not evaluated versus specific and non-specific biomarkers (e.g., Lyso-SM-509, Hsp70, cholestane-triol, unesterified cholesterol) with a clear mechanistic link to clinical outcome measure.

Extension study

In the preprint (Patterson et al. 2024) [2] the authors did not provide the rate of success as pre-specified but mean change from baseline on the 5-domain NPC-CSS as primary results. The observed mean decrease was -0.32 (SD=2.43) at month 12 and -0.067 (SD=2.94) at month 18.

The authors also provided descriptive comparison of the 5-domain NPC-CSS evolution in patients treated with levacetylleucine over history cohorts and clinical trial cohorts that show a mean (±SD) change of 1.5 (±3.16) at Month 12 and 2.25 (±4.74) at Month 18.

According to the authors, these results suggest improvement in neurological signs and symptoms after long-term treatment with levacetylleucine when compared to historical cohorts. However, the validity of the analysis which was not pre-specified in protocol and the validity of these indirect comparisons including the comparability of the populations between the pivotal study and the historical cohorts is not addressed by the Applicant. As clarified by the Applicant, the final study report is anticipated to be available in March 2029.

Different primary endpoints were evaluated in the two parts of the pivotal Study IB1001-301 (SARA score for the parent study and 5 domains NPC-CSS in the extension study). It is not clear how the results from the extension phase based on the 5-domain NPC-CSS scale (i.e. open-label 12 months and 18 months respectively) can support a long-term efficacy initially based on the SARA total score and the issue of deterioration after the treatment of levacetylleucine was stopped.

Study IB1001-201 (supportive study)

Parent study

The parent study included 33 patients with a diagnosis based on positive biomarker and/or filipin and/or genetic tests.

Overall, the mean (SD) age of the patients was 28.8 (15.1) years. A total of 10 (30.3%) patients were <18 years at screening and 5 patients were aged between 6 and 12 years old. Of the 33 treated patients, 23 (69.7%) were male and 10 (30.3%) were female. All patients of the SAF population took concomitant medication. Miglustat was the most frequently used concomitant medication (30 patients; 90.9%). This raises the question of how to properly determine the efficacy of levacetylleucine alone or in combination with miglustat.

Regarding the primary endpoint, the 9HPT-D was more often selected as the primary anchor test for the primary endpoint (20/32 patients; 62.5%) For the mITT population, the CI-CS component for the

[1] Mengel, Eugen et al. "Efficacy and safety of arimoclomol in Niemann-Pick disease type C: Results from a double-blind, randomised, placebo-controlled, multinational phase 2/3 trial of a novel treatment." Journal of inherited metabolic disease vol. 44,6 (2021): 1463-1480. doi:10.1002/jimd.12428

[2] Patterson M, Ramaswami U, Donald A, Foltan T, Gautschi M, Hahn A, Jones S, Kolnikova M, Arash-Kaps L, Park J, Reichmannová S, Walterfarng M, Wibawa P, Rohrbach M, Martakis K, Bremova-Ertl T, Gissen P. Disease-Modifying, Neuroprotective Effect of N-acetyl-L-leucine in Adult and Pediatric Patients with Niemann–Pick disease type C. Available at: https://www.medrxiv.org/content/10.1101/2024.10.11.24315318v1.

treatment period (Visit 4 versus Visit 2) had a mean value of 0.48 (SD=1.34, median=1.00) showing some improvement while the CI-CS component for the washout period (Visit 6 versus Visit 4) had a mean value of -0.38 (SD=1.45, median=-0.25) showing some deterioration.

A one-sided Wilcoxon signed-rank test was performed to investigate statistical significance of the primary efficacy endpoint as compared to a value of 0 for the mITT population. The (pseudo-) median of the difference in CI-CS using the Hodges- Lehmann estimator was 1.00 (90% CI: 0.25, 1.75). The CI-CS primary endpoint of the study reached statistical significance with p-value: 0.029

Statistical tests for the key secondary endpoint and all other secondary endpoints were exploratory and no correction for multiplicity was made. The results of the secondary endpoints indicate a trend towards improvement in neurological signs/symptoms with the product compared to placebo.

Extension study

A total of 19 patients continued into the Extension Phase of the 19 treated patients, 17 patients (89.5%) completed Visit 10 (Treatment period I) and 13 patients (68.4%) completed Visit 12 (Treatment period II). A majority of the patients (57.9%) were adults. A total of 18 patients (94.7%) of the SAF population took concomitant medication. Miglustat was the most frequently used concomitant medication (17 patients; 89.5%).

The primary endpoint for the Extension study was the Modified 5- domain Niemann-Pick Disease type C Clinical Severity Scale (5-domain NPC-CSS) at the end of the Extension Phase treatment period 1 versus Extension Phase Baseline. Success rate for the primary endpoint was based on the 5-domain NPC-CSS score, which is defined as the proportion of patients with no change or a decrease in the 5-domain NPC-CSS score from Visit 7 to Visit 9 versus 0.10 based on the assumption that under standard of care, 90% of patients would worsen. For the mITTe population, the proportion of patients with success was 0.50 (90% CI: 0.31, 0.69) compared to a proportion of 0.10, which is the expected rate of success without treatment.

The mean SARA total score for visit 9 versus visit 7 was 1.82 (SD=3.09, median=1.50), showing a deterioration of cerebellar sign and neurological symptoms under treatment.

Additional exploratory endpoints for the Extension study included measurements of Ataxia and Functioning: SARA subscores, SCAFI total score and subscore, Measurement of Health-Related Quality of Life, Measurement of Overall Neurological Status and Measurement of Global Impression scored by treating physician, caregiver, and patient.

No biomarker has been used for efficacy analysis although it was initially intended to be evaluated as secondary endpoint according to the AEMPS scientific advice for phase II/IIb study in NPC patients. The change in biochemical analysis of oxysterols, bile acids, and LysoSM509 was suggested as secondary parameters. In addition, the inclusion criteria are comprised from positive tested biomarkers besides the genetic tests. The Applicant has clarified that no validated biomarkers that could be used for measurement of the clinical efficacy, as there is no evidence that biomarkers correlate to clinical symptoms, functioning and life improving the quality of life.

The non-inclusion of patients under 6 years of age, the fact that the vast majority of patients had received concomitant miglustat, that this is an open-label study and that the primary endpoint does not cover all symptoms of the disease raise the same uncertainties as the pivotal study.

It should be noted that the results from the study Phase II - IB1001-201 should be interpreted with caution considering the design of the study (non-randomised, rater-blinded, single arm, open label study with the different setting of primary and secondary endpoints in comparison to the pivotal study IB1001-301) and unresolved GCP findings (violation).

2.6.7. Conclusions on the clinical efficacy

The main evidence on efficacy of levacetylleucine is derived from the interim results of an ongoing multicenter, randomized, double-blind, placebo-controlled, crossover study in adults and paediatric patients aged ≥ 4 years old followed by an extension phase. Overall, the conduct of a double-blinded randomised-controlled trial (RCT) in a sufficient number of NPC disease patients is positively recognized. The results showed a significant improvement of the SARA score (Scale for the Assessment and Rating of Ataxia) after 12 weeks of treatment and a deterioration when the treatment was stopped. Secondary endpoints that included an ataxic scale (SCAFI), a modified Disability Rating Scale and patient-reported outcomes (PROs) also indicated some improvement compared to placebo. The Applicant submitted data from the ongoing open-label extension phase (EP) in the form of an unpublished preprint that was not certified by a peer review (Patterson et al. Preprint 2024) which renders it difficult to evaluate this extension study. The final report will be provided by the Applicant in due time.

The Applicant also provided the results of a multinational, multi-center, open-label, rater-blinded single-arm study evaluating 6 weeks treatment with levacetylleucine in NPC adult and paediatric patients aged ≥6 years followed by an extension period. The results showed a significant improvement of the primary endpoint (however not validated for NPC) Clinical Impression of Change in Severity (CI-CS) score comparing either the 9-Hole Peg Test of the Dominant Hand (9HPT-D) or the 8-Meter Walk Test (8MWT) as the primary anchor. Additional efficacy endpoints showed some improvement; however, they were assessed in an unblinded manner which limits their interpretability. During the extension phase, the 5-domain NPC-CS score suggested that 50% of patient from 18 has improvement or no change in score however, this endpoint modified during the course of the study which undermines its interpretability. It should be noted that results of SARA score during the extension phase indicated a deterioration of cerebellar sign and neurological symptoms under long term treatment.

As primary efficacy endpoints of phase II study differ between the treatment periods (in parent and extension phase of phase II study) and do not correspond with chosen primary endpoint of the phase III study, the interpretation of the results of the phase II study particularly the long-term efficacy is difficult. In addition, the validity and integrity of the study IB1001-201 can be questioned due to GCP non-compliance.

2.6.8. Clinical safety

The assessment of levacetylleucine (or N-acetyl-L-leucine or NALL (named IB1001 in tables)) safety profile is based on data from two clinical studies: one pivotal phase III study 1001-301 (parent study completed and 60 patients dosed), and one phase II study 1001-201 plus the extension phase (completed, 33 patients dosed). For the appraisal of levacetylleucine safety profile, the Applicant has carried out a pooled safety data set for increasing the number of patients and total exposure in order to better detect any adverse reactions. Since 9 patients were enrolled in both studies they were counted once leading to a total number of 84 patients rather than 93. Such approach is relevant, and the safety AR is based on data observed from this safety pool. Besides, the Applicant has also displayed the safety outcomes from each study.

Of note, the extension phase of the pivotal study is ongoing, and no data have been submitted at this stage of the MAA procedure.

2.6.8.1. Patient exposure

Pooled studies in patients with NPC

The study IB1001-201 enrolled 33 patients, and the study IB1001-301 enrolled 60 patients. Nine patients participated in both studies IB1001-201 and IB1001-301. Therefore, in total, 84 patients were treated with levacetylleucine (IB1001 use or levacetylleucine use group) and 82 patients were not treated (wash-out or treated with the placebo) corresponding to the non-IB1001 use or non-levacetylleucine use. The table 23 summarize exposure data:

Table 23: IB1001 Exposure (ISS – SAF)

Parameter	Statistic/ Category	IB1001 use (N = 84)	Non-IB1001 (N = 82)
Total observation duration[a] [patient-years]	n	84	82
	Total	52.98	23.57
Observation duration[a] [days]	n	84	82
	Mean (SD)	230.4 (297.32)	105.0 (58.36)
	Median	86.00	87.00
	Min, max	36.0, 940.0	17.0, 321.0
IB1001 treatment duration[b] [days]	n	84	-
	Mean (SD)	229.3 (296.98)	-
	Median	86.00	-
	Min, max	36.0, 938.0	-
Maximum daily IB1001 dose [g]	n	84	-
	2	6 (7.14%)	-
	3	3 (3.57%)	-
	4	75 (89.29%)	-

Abbreviations: ISS = integrated summary of safety; max = maximum; min = minimum; N = number of patients; SAF = Safety Analysis Set; SD = standard deviation

[a] The observation duration (day) was defined as the sum of all durations (end period date – start period date + 1) of the combined periods.

[b] Treatment duration corresponds to the total time of exposure to IB1001 combined across periods.

▪ *Common Treatment Emergent Adverse Events-TEAEs*

The table 24 displays TEAEs from the safety pool:

Table 24: Overview Adverse Events (ISS – SAF)

	IB1001 Use (N = 84)		Non-IB1001 Use (N = 82)		Total (N = 84)	
	n (%)	m (r)	n (%)	m (r)	n (%)	m (r)
TEAE	55 (65.5)	241 (4.5)	38 (46.3)	103 (4.4)	61 (72.6)	344 (4.5)
Serious TEAE	10 (11.9)	31 (0.6)	2 (2.4)	2 (0.1)	12 (14.3)	33 (0.4)
TEAE by relationship*						
Not related	48 (57.1)	230 (4.3)	31 (37.8)	93 (3.9)	49 (58.3)	323 (4.2)
Related	7 (8.3)	11 (0.2)	7 (8.5)	10 (0.4)	12 (14.3)	21 (0.3)
Serious TEAE by relationship*						
Not related	10 (11.9)	31 (0.6)	2 (2.4)	2 (0.1)	12 (14.3)	33 (0.4)
Related	0 (0.0)	0 (0.0)	0 (0.0%)	0 (0.0)	0 (0.0)	0 (0.0)
TEAE events by severity*						
Mild	28 (33.3)	152 (2.9)	23 (28.0)	75 (3.2)	28 (33.3)	227 (3.0)
Moderate	17 (20.2)	60 (1.1)	12 (14.6)	23 (1.0)	21 (25.0)	83 (1.1)
Severe	10 (11.9)	29 (0.5)	3 (3.7)	5 (0.2)	12 (14.3)	34 (0.4)
TEAE leading to withdrawal	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
TEAE leading to death	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)

Abbreviations: ISS = integrated summary of safety; m = number of events; N = total number of unique patients; n (%) = number (percentage) of unique patients with at least 1 event, using N as denominator for the percentage; r = incidence rate per patient-year (calculated by dividing the number of events [m] by the total observation duration of the patients, expressed in years); SAF = Safety Analysis Set; TEAE = treatment-emergent adverse event

TEAEs were defined as an adverse event that started at or after any individual study drug exposure, including placebo for patients in IB1001-301 not previously enrolled in IB1001-201 study. TEAEs were assigned to ISS group labels as described in the integrated Statistical Analysis Plan, Section 5.1, based on the start date of the event. In early discontinuers, any AEs collected after the date of study discontinuation were included in the last period recorded for those patients.

The TEAE leading to death started 2 days after the last dose of IB1001 and was unrelated to the study drug.

* Only the maximum relationship and severity of all events reported by a patient was considered.

▪ *Treatments emergent adverse events by System Organ Class (SOC) and PTs (preferred terms):*

The table 25 displays TEAEs by SOC and PTs:

Table 25: Treatment-emergent Adverse Events Reported by at Least Two Patients During IB1001 Use (ISS – SAF)

	IB1001 Use (N = 84)		Non-IB1001 Use (N = 82)		Total (N = 84)	
System Organ Class Preferred Term	**n (%)**	**m (r)**	**n (%)**	**m (r)**	**n (%)**	**m (r)**
Any treatment-emergent adverse event	55 (65.5)	241 (4.5)	38 (46.3)	103 (4.4)	61 (72.6)	344 (4.5)
Infections and infestations	29 (34.5)	47 (0.9)	14 (17.1)	17 (0.7)	32 (38.1)	64 (0.8)
Upper respiratory tract infection	8 (9.5)	8 (0.2)	3 (3.7)	3 (0.1)	10 (11.9)	11 (0.1)
Nasopharyngitis	3 (3.6)	3 (0.1)	3 (3.7)	3 (0.1)	5 (6.0)	6 (0.1)
Pneumonia aspiration	4 (4.8)	5 (0.1)	1 (1.2)	1 (0.0)	5 (6.0)	6 (0.1)
Rhinitis	3 (3.6)	3 (0.1)	2 (2.4)	2 (0.1)	5 (6.0)	5 (0.1)
Coronavirus infection	4 (4.8)	5 (0.1)	0 (0.0)	0 (0.0)	4 (4.8)	5 (0.1)
COVID-19	2 (2.4)	2 (0.0)	2 (2.4)	2 (0.1)	4 (4.8)	4 (0.1)
Pneumonia	2 (2.4)	5 (0.1)	1 (1.2)	1 (0.0)	3 (3.6)	6 (0.1)
Gastroenteritis	2 (2.4)	2 (0.0)	1 (1.2)	1 (0.0)	3 (3.6)	3 (0.0)
Gastroenteritis viral	3 (3.6)	3 (0.1)	0 (0.0)	0 (0.0)	3 (3.6)	3 (0.0)
Lower respiratory tract infection	3 (3.6)	3 (0.1)	0 (0.0)	0 (0.0)	3 (3.6)	3 (0.0)
Influenza	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Gastrointestinal disorders	20 (23.8)	37 (0.7)	7 (8.5)	9 (0.4)	25 (29.8)	46 (0.6)
Diarrhoea	5 (6.0)	7 (0.1)	5 (6.1)	6 (0.3)	9 (10.7)	13 (0.2)
Dysphagia	7 (8.3)	8 (0.2)	0 (0.0)	0 (0.0)	7 (8.3)	8 (0.1)
Vomiting	4 (4.8)	4 (0.1)	1 (1.2)	1 (0.0)	5 (6.0)	5 (0.1)
Anal incontinence	3 (3.6)	3 (0.1)	2 (2.4)	2 (0.1)	4 (4.8)	5 (0.1)
Abdominal pain	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Nervous system disorders	21 (25.0)	31 (0.6)	7 (8.5)	11 (0.5)	24 (28.6)	42 (0.5)
Seizure	6 (7.1)	7 (0.1)	2 (2.4)	4 (0.2)	6 (7.1)	11 (0.1)
Epilepsy	2 (2.4)	2 (0.0)	1 (1.2)	2 (0.1)	2 (2.4)	4 (0.1)
Dyskinesia	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Dystonia	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Headache	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Memory impairment	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Injury, poisoning and procedural complications	20 (23.8)	62 (1.2)	12 (14.6)	35 (1.5)	22 (26.2)	97 (1.3)
Fall	16 (19.0)	42 (0.8)	12 (14.6)	32 (1.4)	19 (22.6)	74 (1.0)
Contusion	5 (6.0)	6 (0.1)	1 (1.2)	1 (0.0)	6 (7.1)	7 (0.1)
Traumatic haematoma	3 (3.6)	3 (0.1)	1 (1.2)	1 (0.0)	3 (3.6)	4 (0.1)
Psychiatric disorders	8 (9.5)	11 (0.2)	4 (4.9)	5 (0.2)	11 (13.1)	16 (0.2)
Aggression	2 (2.4)	2 (0.0)	1 (1.2)	1 (0.0)	3 (3.6)	3 (0.0)
Sleep disorder	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Respiratory, thoracic and mediastinal disorders	7 (8.3)	9 (0.2)	4 (4.9)	4 (0.2)	11 (13.1)	13 (0.2)
Cough	2 (2.4)	2 (0.0)	2 (2.4)	2 (0.1)	4 (4.8)	4 (0.1)
Epistaxis	3 (3.6)	3 (0.1)	0 (0.0)	0 (0.0)	3 (3.6)	3 (0.0)
Skin and subcutaneous tissue disorders	7 (8.3)	7 (0.1)	5 (6.1)	5 (0.2)	11 (13.1)	12 (0.2)
Rash	2 (2.4)	2 (0.0)	2 (2.4)	2 (0.1)	4 (4.8)	4 (0.1)
Rash pruritic	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
General disorders and administration site conditions	5 (6.0)	7 (0.1)	5 (6.1)	5 (0.2)	8 (9.5)	12 (0.2)
Fatigue	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Renal and urinary disorders	5 (6.0)	6 (0.1)	1 (1.2)	1 (0.0)	6 (7.1)	7 (0.1)
Urinary incontinence	2 (2.4)	3 (0.1)	0 (0.0)	0 (0.0)	2 (2.4)	3 (0.0)
Incontinence	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)

Abbreviations: COVID-19 = coronavirus disease 2019 ; m = number of events; MedDRA = Medical Dictionary for Regulatory Activities; N = total number of unique patients; n (%) = number (percentage) of unique patients with at least 1 event, using N as denominator for the percentage; r = incidence rate per patient-year (calculated by dividing the number of events [m] by the total observation duration of the patients, expressed in years); SAF = Safety Analysis Set

Adverse events were coded according to MedDRA, Version 24.1.

▪ *Severity of treatment emergent adverse events*

From the pooled safety data, the table 26 presents TEAEs of severe intensity by SOC and PT, overall and by treatment:

Table 26: Severe Treatment-emergent Adverse Events (ISS-SAF)

Table 19: Severe Treatment-emergent Adverse Events (ISS – SAF)

	IB1001 Use (N = 84)		Non-IB1001 Use (N = 82)		Total (N = 84)	
System Organ Class Preferred Term	**n (%)**	**m (r)**	**n (%)**	**m (r)**	**n (%)**	**m (r)**
Any TEAE	55 (65.5)	241 (4.5)	38 (46.3)	103 (4.4)	61 (72.6)	344 (4.5)
Any mild TEAE	28 (33.3)	152 (2.9)	23 (28.0)	75 (3.2)	28 (33.3)	227 (3.0)
Any moderate TEAE	17 (20.2)	60 (1.1)	12 (14.6)	23 (1.0)	21 (25.0)	83 (1.1)
Any severe TEAE	10 (11.9)	29 (0.5)	3 (3.7)	5 (0.2)	12 (14.3)	34 (0.4)
Infections and infestations	5 (6.0)	10 (0.2)	0 (0.0)	0 (0.0)	5 (6.0)	10 (0.1)
Pneumonia aspiration	4 (4.8)	5 (0.1)	0 (0.0)	0 (0.0)	4 (4.8)	5 (0.1)
Pneumonia	1 (1.2)	3 (0.1)	0 (0.0)	0 (0.0)	1 (1.2)	3 (0.0)
Lower respiratory tract infection	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Pneumonia viral	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Gastrointestinal disorders	4 (4.8)	4 (0.1)	0 (0.0)	0 (0.0)	4 (4.8)	4 (0.1)
Diarrhoea	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Dysphagia	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Gastrointestinal haemorrhage	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Gastric perforation	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Nervous system disorders	4 (4.8)	4 (0.1)	1 (1.2)	2 (0.1)	4 (4.8)	6 (0.1)
Seizure	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Epilepsy	1 (1.2)	1 (0.0)	1 (1.2)	2 (0.1)	1 (1.2)	3 (0.0)
Cataplexy	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Dystonia	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Injury, poisoning and procedural complications	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Spinal fracture	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Psychiatric disorders	1 (1.2)	1 (0.0)	1 (1.2)	2 (0.1)	2 (2.4)	3 (0.0)
Depression	0 (0.0)	0 (0.0)	1 (1.2)	2 (0.1)	1 (1.2)	2 (0.0)
Psychotic disorder	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Respiratory, thoracic and mediastinal disorders	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Aspiration	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Skin and subcutaneous tissue disorders	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Rash	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Investigations	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Weight decreased	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Renal and urinary disorders	1 (1.2)	2 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	2 (0.0)
Urinary incontinence	1 (1.2)	2 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	2 (0.0)
Metabolism and nutrition disorders	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Dehydration	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Surgical and medical procedures	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Hysterectomy	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Incisional hernia repair	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Uterine leiomyoma	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)

▪ *Treatments related adverse events*

From the pooled safety data, the table 27 presents treatment-related TEAEs by SOC and PT, overall and by treatment:

Table 27: Treatment-related Treatment-emergent Adverse Events (ISS-SAF)

	IB1001 Use (N = 84)		Non-IB1001 Use (N = 82)		Total (N = 84)	
	n (%)	m (r)	n (%)	m (r)	n (%)	m (r)
Any treatment-related TEAE	7 (8.3)	11 (0.2)	7 (8.5)	10 (0.4)	12 (14.3)	21 (0.3)
Gastrointestinal disorders	4 (4.8)	5 (0.1)	3 (3.7)	5 (0.2)	6 (7.1)	10 (0.1)
Diarrhoea	1 (1.2)	1 (0.0)	3 (3.7)	4 (0.2)	4 (4.8)	5 (0.1)
Anal incontinence	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	2 (0.0)
Dyspepsia	1 (1.2)	2 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	2 (0.0)
Flatulence	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Skin and subcutaneous tissue disorders	2 (2.4)	2 (0.0)	3 (3.7)	3 (0.1)	4 (4.8)	5 (0.1)
Drug eruption	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Rash	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Rash maculo-papular	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Rash pruritic	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Rosacea	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Psychiatric disorders	1 (1.2)	2 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	2 (0.0)
Aggression	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Restlessness	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Investigations	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Electrocardiogram QT prolonged	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Metabolism and nutrition disorders	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Increased appetite	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Nervous system disorders	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Restless legs syndrome[1]	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)

2.6.8.2. Adverse events

▪ *Common Treatment Emergent Adverse Events-TEAEs*

The table 28 displays TEAEs from the safety pool:

Table 28: Overview Adverse Events (ISS-SAF)

	IB1001 Use (N = 84)		Non-IB1001 Use (N = 82)		Total (N = 84)	
	n (%)	m (r)	n (%)	m (r)	n (%)	m (r)
TEAE	55 (65.5)	241 (4.5)	38 (46.3)	103 (4.4)	61 (72.6)	344 (4.5)
Serious TEAE	10 (11.9)	31 (0.6)	2 (2.4)	2 (0.1)	12 (14.3)	33 (0.4)
TEAE by relationship*						
Not related	48 (57.1)	230 (4.3)	31 (37.8)	93 (3.9)	49 (58.3)	323 (4.2)
Related	7 (8.3)	11 (0.2)	7 (8.5)	10 (0.4)	12 (14.3)	21 (0.3)
Serious TEAE by relationship*						
Not related	10 (11.9)	31 (0.6)	2 (2.4)	2 (0.1)	12 (14.3)	33 (0.4)
Related	0 (0.0)	0 (0.0)	0 (0.0%)	0 (0.0)	0 (0.0)	0 (0.0)
TEAE events by severity*						
Mild	28 (33.3)	152 (2.9)	23 (28.0)	75 (3.2)	28 (33.3)	227 (3.0)
Moderate	17 (20.2)	60 (1.1)	12 (14.6)	23 (1.0)	21 (25.0)	83 (1.1)
Severe	10 (11.9)	29 (0.5)	3 (3.7)	5 (0.2)	12 (14.3)	34 (0.4)
TEAE leading to withdrawal	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
TEAE leading to death	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)

Abbreviations: ISS = integrated summary of safety; m = number of events; N = total number of unique patients; n (%) = number (percentage) of unique patients with at least 1 event, using N as denominator for the percentage; r = incidence rate per patient-year (calculated by dividing the number of events [m] by the total observation duration of the patients, expressed in years); SAF = Safety Analysis Set; TEAE = treatment-emergent adverse event

TEAEs were defined as an adverse event that started at or after any individual study drug exposure, including placebo for patients in IB1001-301 not previously enrolled in IB1001-201 study. TEAEs were assigned to ISS group labels as described in the integrated Statistical Analysis Plan, Section 5.1, based on the start date of the event. In early discontinuers, any AEs collected after the date of study discontinuation were included in the last period recorded for those patients.

The TEAE leading to death started 2 days after the last dose of IB1001 and was unrelated to the study drug.

* Only the maximum relationship and severity of all events reported by a patient was considered.

▪ *Treatments emergent adverse events by System Organ Class (SOC) and PTs (preferred terms):*

The table 29 displays TEAEs by SOC and PTs:

Table 29: Treatment-emergent adverse events reported by at least 2 patients during IB1001 use (ISS-SAF)

	IB1001 Use (N = 84)		Non-IB1001 Use (N = 82)		Total (N = 84)	
System Organ Class Preferred Term	**n (%)**	**m (r)**	**n (%)**	**m (r)**	**n (%)**	**m (r)**
Any treatment-emergent adverse event	55 (65.5)	241 (4.5)	38 (46.3)	103 (4.4)	61 (72.6)	344 (4.5)
Infections and infestations	29 (34.5)	47 (0.9)	14 (17.1)	17 (0.7)	32 (38.1)	64 (0.8)
Upper respiratory tract infection	8 (9.5)	8 (0.2)	3 (3.7)	3 (0.1)	10 (11.9)	11 (0.1)
Nasopharyngitis	3 (3.6)	3 (0.1)	3 (3.7)	3 (0.1)	5 (6.0)	6 (0.1)
Pneumonia aspiration	4 (4.8)	5 (0.1)	1 (1.2)	1 (0.0)	5 (6.0)	6 (0.1)
Rhinitis	3 (3.6)	3 (0.1)	2 (2.4)	2 (0.1)	5 (6.0)	5 (0.1)
Coronavirus infection	4 (4.8)	5 (0.1)	0 (0.0)	0 (0.0)	4 (4.8)	5 (0.1)
COVID-19	2 (2.4)	2 (0.0)	2 (2.4)	2 (0.1)	4 (4.8)	4 (0.1)
Pneumonia	2 (2.4)	5 (0.1)	1 (1.2)	1 (0.0)	3 (3.6)	6 (0.1)
Gastroenteritis	2 (2.4)	2 (0.0)	1 (1.2)	1 (0.0)	3 (3.6)	3 (0.0)
Gastroenteritis viral	3 (3.6)	3 (0.1)	0 (0.0)	0 (0.0)	3 (3.6)	3 (0.0)
Lower respiratory tract infection	3 (3.6)	3 (0.1)	0 (0.0)	0 (0.0)	3 (3.6)	3 (0.0)
Influenza	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Gastrointestinal disorders	20 (23.8)	37 (0.7)	7 (8.5)	9 (0.4)	25 (29.8)	46 (0.6)
Diarrhoea	5 (6.0)	7 (0.1)	5 (6.1)	6 (0.3)	9 (10.7)	13 (0.2)
Dysphagia	7 (8.3)	8 (0.2)	0 (0.0)	0 (0.0)	7 (8.3)	8 (0.1)
Vomiting	4 (4.8)	4 (0.1)	1 (1.2)	1 (0.0)	5 (6.0)	5 (0.1)
Anal incontinence	3 (3.6)	3 (0.1)	2 (2.4)	2 (0.1)	4 (4.8)	5 (0.1)
Abdominal pain	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Nervous system disorders	21 (25.0)	31 (0.6)	7 (8.5)	11 (0.5)	24 (28.6)	42 (0.5)
Seizure	6 (7.1)	7 (0.1)	2 (2.4)	4 (0.2)	6 (7.1)	11 (0.1)
Epilepsy	2 (2.4)	2 (0.0)	1 (1.2)	2 (0.1)	2 (2.4)	4 (0.1)
Dyskinesia	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Dystonia	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Headache	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Memory impairment	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Injury, poisoning and procedural complications	20 (23.8)	62 (1.2)	12 (14.6)	35 (1.5)	22 (26.2)	97 (1.3)
Fall	16 (19.0)	42 (0.8)	12 (14.6)	32 (1.4)	19 (22.6)	74 (1.0)
Contusion	5 (6.0)	6 (0.1)	1 (1.2)	1 (0.0)	6 (7.1)	7 (0.1)
Traumatic haematoma	3 (3.6)	3 (0.1)	1 (1.2)	1 (0.0)	3 (3.6)	4 (0.1)
Psychiatric disorders	8 (9.5)	11 (0.2)	4 (4.9)	5 (0.2)	11 (13.1)	16 (0.2)
Aggression	2 (2.4)	2 (0.0)	1 (1.2)	1 (0.0)	3 (3.6)	3 (0.0)
Sleep disorder	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Respiratory, thoracic and mediastinal disorders	7 (8.3)	9 (0.2)	4 (4.9)	4 (0.2)	11 (13.1)	13 (0.2)
Cough	2 (2.4)	2 (0.0)	2 (2.4)	2 (0.1)	4 (4.8)	4 (0.1)
Epistaxis	3 (3.6)	3 (0.1)	0 (0.0)	0 (0.0)	3 (3.6)	3 (0.0)
Skin and subcutaneous tissue disorders	7 (8.3)	7 (0.1)	5 (6.1)	5 (0.2)	11 (13.1)	12 (0.2)
Rash	2 (2.4)	2 (0.0)	2 (2.4)	2 (0.1)	4 (4.8)	4 (0.1)
Rash pruritic	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
General disorders and administration site conditions	5 (6.0)	7 (0.1)	5 (6.1)	5 (0.2)	8 (9.5)	12 (0.2)
Fatigue	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Renal and urinary disorders	5 (6.0)	6 (0.1)	1 (1.2)	1 (0.0)	6 (7.1)	7 (0.1)
Urinary incontinence	2 (2.4)	3 (0.1)	0 (0.0)	0 (0.0)	2 (2.4)	3 (0.0)
Incontinence	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)

Abbreviations: COVID-19 = coronavirus disease 2019 ; m = number of events; MedDRA = Medical Dictionary for Regulatory Activities; N = total number of unique patients; n (%) = number (percentage) of unique patients with at least 1 event, using N as denominator for the percentage; r = incidence rate per patient-year (calculated by dividing the number of events [m] by the total observation duration of the patients, expressed in years); SAF = Safety Analysis Set

Adverse events were coded according to MedDRA, Version 24.1.

- *Severity of treatment emergent adverse events*

From the pooled safety data, the table 30 presents TEAEs of severe intensity by SOC and PT, overall and by treatment:

Table 30: Severe Treatment-emergent Adverse Events (ISS-SAF)

	IB1001 Use (N = 84)		Non-IB1001 Use (N = 82)		Total (N = 84)	
System Organ Class **Preferred Term**	**n (%)**	**m (r)**	**n (%)**	**m (r)**	**n (%)**	**m (r)**
Any TEAE	55 (65.5)	241 (4.5)	38 (46.3)	103 (4.4)	61 (72.6)	344 (4.5)
Any mild TEAE	28 (33.3)	152 (2.9)	23 (28.0)	75 (3.2)	28 (33.3)	227 (3.0)
Any moderate TEAE	17 (20.2)	60 (1.1)	12 (14.6)	23 (1.0)	21 (25.0)	83 (1.1)
Any severe TEAE	10 (11.9)	29 (0.5)	3 (3.7)	5 (0.2)	12 (14.3)	34 (0.4)
Infections and infestations	5 (6.0)	10 (0.2)	0 (0.0)	0 (0.0)	5 (6.0)	10 (0.1)
Pneumonia aspiration	4 (4.8)	5 (0.1)	0 (0.0)	0 (0.0)	4 (4.8)	5 (0.1)
Pneumonia	1 (1.2)	3 (0.1)	0 (0.0)	0 (0.0)	1 (1.2)	3 (0.0)
Lower respiratory tract infection	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Pneumonia viral	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Gastrointestinal disorders	4 (4.8)	4 (0.1)	0 (0.0)	0 (0.0)	4 (4.8)	4 (0.1)
Diarrhoea	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Dysphagia	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Gastrointestinal haemorrhage	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Gastric perforation	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Nervous system disorders	4 (4.8)	4 (0.1)	1 (1.2)	2 (0.1)	4 (4.8)	6 (0.1)
Seizure	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Epilepsy	1 (1.2)	1 (0.0)	1 (1.2)	2 (0.1)	1 (1.2)	3 (0.0)
Cataplexy	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Dystonia	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Injury, poisoning and procedural complications	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Spinal fracture	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Psychiatric disorders	1 (1.2)	1 (0.0)	1 (1.2)	2 (0.1)	2 (2.4)	3 (0.0)
Depression	0 (0.0)	0 (0.0)	1 (1.2)	2 (0.1)	1 (1.2)	2 (0.0)
Psychotic disorder	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Respiratory, thoracic and mediastinal disorders	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Aspiration	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Skin and subcutaneous tissue disorders	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Rash	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Investigations	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Weight decreased	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Renal and urinary disorders	1 (1.2)	2 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	2 (0.0)
Urinary incontinence	1 (1.2)	2 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	2 (0.0)
Metabolism and nutrition disorders	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Dehydration	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Surgical and medical procedures	2 (2.4)	2 (0.0)	0 (0.0)	0 (0.0)	2 (2.4)	2 (0.0)
Hysterectomy	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Incisional hernia repair	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Uterine leiomyoma	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)

▪ *Treatments related adverse events*

From the pooled safety data, table 31 presents treatment-related TEAEs by SOC and PT, overall and by treatment:

Table 31: Treatment-related Treatment-emergent Adverse Events (ISS-SAF)

	IB1001 Use (N = 84)		Non-IB1001 Use (N = 82)		Total (N = 84)	
	n (%)	m (r)	n (%)	m (r)	n (%)	m (r)
Any treatment-related TEAE	7 (8.3)	11 (0.2)	7 (8.5)	10 (0.4)	12 (14.3)	21 (0.3)
Gastrointestinal disorders	4 (4.8)	5 (0.1)	3 (3.7)	5 (0.2)	6 (7.1)	10 (0.1)
Diarrhoea	1 (1.2)	1 (0.0)	3 (3.7)	4 (0.2)	4 (4.8)	5 (0.1)
Anal incontinence	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	2 (0.0)
Dyspepsia	1 (1.2)	2 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	2 (0.0)
Flatulence	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Skin and subcutaneous tissue disorders	2 (2.4)	2 (0.0)	3 (3.7)	3 (0.1)	4 (4.8)	5 (0.1)
Drug eruption	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Rash	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Rash maculo-papular	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Rash pruritic	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Rosacea	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Psychiatric disorders	1 (1.2)	2 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	2 (0.0)
Aggression	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Restlessness	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Investigations	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Electrocardiogram QT prolonged	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Metabolism and nutrition disorders	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Increased appetite	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Nervous system disorders	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Restless legs syndrome[1]	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)

2.6.8.3. Serious adverse event/deaths/other significant events

▪ Serious AEs

From the pooled safety data, table 32 presents treatment-emergent SAEs by SOC and PT, overall and by treatment:

Table 32: Treatment-emergent Serious Adverse Events (ISS-SAF)

	IB1001 Use (N = 84)		Non-IB1001 Use (N = 82)		Total (N = 84)	
System Organ Class Preferred Term	**n (%)**	**m (r)**	**n (%)**	**m (r)**	**n (%)**	**m (r)**
Any SAE	10 (11.9)	31 (0.6)	2 (2.4)	2 (0.1)	12 (14.3)	33 (0.4)
Infections and infestations	6 (7.1)	12 (0.2)	0 (0.0)	0 (0.0)	6 (7.1)	12 (0.2)
Pneumonia aspiration	4 (4.8)	5 (0.1)	0 (0.0)	0 (0.0)	4 (4.8)	5 (0.1)
Pneumonia	1 (1.2)	4 (0.1)	0 (0.0)	0 (0.0)	1 (1.2)	4 (0.1)
Lower respiratory tract infection	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Pneumonia viral	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Upper respiratory tract infection	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Nervous system disorders	2 (2.4)	2 (0.0)	1 (1.2)	1 (0.0)	3 (3.6)	3 (0.0)
Cataplexy	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Epilepsy	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Seizure	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Surgical and medical procedures	3 (3.6)	3 (0.1)	0 (0.0)	0 (0.0)	3 (3.6)	3 (0.0)
Gastrostomy	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Hysterectomy	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Incisional hernia repair	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Gastrointestinal disorders	2 (2.4)	6 (0.1)	0 (0.0)	0 (0.0)	2 (2.4)	6 (0.1)
Gastrointestinal haemorrhage	1 (1.2)	4 (0.1)	0 (0.0)	0 (0.0)	1 (1.2)	4 (0.1)
Gastric perforation	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Melaena	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)
Psychiatric disorders	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)	2 (2.4)	2 (0.0)
Depression	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)	1 (1.2)	1 (0.0)
Psychotic disorder	1 (1.2)	1 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (0.0)

▪ Death

In the phase III IB1001-301 pivotal study, there was 1 death. One patient had a pre-planned implementation of a percutaneous endoscopic gastrostomy (PEG) feeding tube at his local hospital. The patient/family did not inform the study site or principal investigator (PI) until after the first visit of treatment Period I. The patient stopped study drug (levacetylleucine) 2 days before the procedure and planned to resume study drug following the procedure. The procedure had complications, leading to SAEs, including aspiration pneumonia, which ultimately proved fatal. The patient was withdrawn during the prolonged hospital stay (before death) when it was determined the patient would be unable to return to the study site for any further study assessments/visits.

No deaths were reported in the completed phase II study IB1001-201 as well as in the supportive IB1001-202 and IB1001-203 studies.

2.6.8.4. Laboratory findings

▪ In all studies, clinical laboratory variables did not show any clinically significant effects of levacetylleucine. Laboratory variables indicative of renal, hepatic, or haematological functions did not show any change over time during treatment with levacetylleucine.

▪ In all studies, vital signs and physical examinations did not change over time during treatment with levacetylleucine.

▪ Electrocardiogram, QT/QTc: An assessment of ECG findings was undertaken by the Applicant to support a formal waiver request for a thorough QT/QTC study for levacetylleucine. Based on the conducted analysis, the MAH claims that levacetylleucine does not have effects on QT/QTc or other cardiac parameters. Although the assessment of ECGs coming from clinical studies has its limitations (i.e., no

clear relation between the ECG recording time and dosing and associated C_{max}), there was no signal indicating that levacetylleucine affects ECG parameters.

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable

2.6.8.6. Safety in special populations

Intrinsic factors

No studies were done in patients with renal impairment. Patients with moderate and severe hepatic insufficiency were excluded from levacetylleucine clinical studies.

There were no relevant differences in safety variables across age groups in the pooled studies. More TEAEs, including more SAEs, were reported by male patients. A similar trend of higher reporting rates for males was also observed in "non- levacetylleucine use", indicating that this was not a levacetylleucine-specific effect. Other safety variables were generally similar across sex groups in the pooled studies.

No subgroup analyses by region or ethnicity/race were performed for the pivotal study due to the small sample size.

Extrinsic factors

▪ *Pregnancy and lactation:* There are no data from studies in pregnant women.

▪ *Overdose, drug abuse, withdrawal and rebound*

In accordance with ICH M3 (R2) guidance, further studies into the abuse potential of levacetylleucine are not warranted given the basic active moiety of levacetylleucine is a simple modified amino acid, and, therefore, the structure-activity of levacetylleucine does not suggest abuse potential. levacetylleucine is metabolized via deacetylation (cleavage of the acetic bond acetyl-group, releasing acetate) giving rise to a single metabolite, L-leucine. L-leucine is a naturally occurring amino acid that is endogenously present in humans (as is acetate) and human diets. AEs were evaluated consistently throughout the levacetylleucine studies by the data safety monitoring board (DSMB) for signs of abuse potential and no AEs have been determined to be related to abuse potential. In the phase III study IB1001-301, review of AEs matching the prespecified PTs for abuse potential determined that these did not indicate abuse potential. In addition, all AEs were evaluated for potential abuse potential relatedness, but none were considered indicative of abuse potential.

No signs of withdrawal or rebound have been observed in nonclinical or clinical studies with levacetylleucine.

▪ *Effect on ability to drive or operate machinery or impairment of mental ability:*

Levacetylleucine is not expected to affect the ability to drive or operate machinery or to impair mental ability.

2.6.8.7. Immunological events

No immunological events reported.

2.6.8.8. Safety related to drug-drug interactions and other interactions

The assessment of the drug-drug interaction profile of levacetylleucine is presented under the section 2.6.2 "Clinical Pharmacology".

2.6.8.9. Discontinuation due to adverse events

The Applicant claimed that there were no TEAEs leading to discontinuation during the pivotal phase III study 1001-301 and phase II study IB1001-201. In the Extension Phase of the phase II study, for 1 patient, ALT increased was reported as a TEAE of mild intensity in the treatment period I. This TEAE was not considered to be related to study treatment and did not lead to withdrawal of study drug but to interruption of study drug. This patient reported an ALT concentration of 100 U/L (reference range: 0 to 45 U/L) at Visit 8. At Visits 9 and 10, the reported ALT concentration was 52 U/L and 50U/L, respectively. At Visit 11, the reported ALT was within the reference range.

2.6.8.10. Post marketing experience

To date, levacetylleucine has not been marketed in any countries worldwide.

2.6.9. Discussion on clinical safety

The safety profile of levacetylleucine results from the assessment of a pooled safety data set with safety outcomes from the pivotal phase III study 1001-301 (completed, 60 patients dosed) and the phase II study 1001-201 plus the extension phase (completed, 33 patients dosed). The pooled safety data set has two groups: levacetylleucine use and non- levacetylleucine use (including data from placebo and from wash-out period). Since 9 patients were enrolled in both studies they were counted once leading to, the Safety database for levacetylleucine consists of 84 subjects who received at least 1 dose of levacetylleucine and were diagnosed with NPC. Additionally, the 2 studies were presented as part of the safety discussions by the Applicant.

In the pivotal phase III study 1001-301, placebo-controlled safety was evaluated by the Applicant by comprising data from the 12-week cross-over designed Phase III study. Thus, patients in the pivotal study were exposed to levacetylleucine 6 weeks in total. Placebo-controlled safety pool consisted of 60 patients (100%) who received at least one dose of levacetylleucine and 59 patients receiving at least one dose placebo. One patient withdrew.

The safety population of study IB1001-201 consisted of 33 patients, mean age of the patients was 28.8 years. Of the 33 treated patients, 71.9% were adults (≥18 years) and 28.1% were paediatric patients (≥6 years and ≤ 18 years). Majority of patients used miglustat at baseline. The duration of exposure for parent study ranged from 36 days (discontinuation due to an AE) to 134 days (Visit 4 was delayed by 91 days due to COVID-19). 31 patients completed the 6-week treatment period, two patients withdrew (unrelated AE - seizures and trauma, other). Out of the 33 patients, 19 patients pursued in the extension phase among them 6 withdrew (reasons of withdrawal: withdrawal of consent, lost to follow-up, absence of benefit, treatment considered not sufficient enough, patient's wish to withdraw from study participation). Thirteen (n=13) completed the extension phase. However, 40% (n=14) patients did not pursue for reasons regardless levacetylleucine treatment. From the pooling data, the total observation duration was 52.98 patient-years for the levacetylleucine use group and 23.57 patient-years for the non-levacetylleucine use group (placebo or wash-out period). The mean observation duration was 230.4 days and 105 days, respectively. The mean levacetylleucine treatment duration was 229.3 days, with a maximum of 938 days. A total of 29 patients (34.5%) were under 18 years at screening. The mean age

of the patients was 27.8 years. Out of the 84 patients, 50 (59.5%) were male and 34 (40.5%) were female.

Most patients, 75 patients (89.29%) were dosed with the maximum dose to be received 4 mg levacetylleucine per day.

Adverse events

Incidence rates were calculated for the events in the levacetylleucine exposure group and placebo group.

- Treatment emergent adverse events -TEAEs

▪ In the pooled safety data, two-third of patients who received levacetylleucine (65,5% n=55/84) experienced adverse events (AEs), corresponding to 241 events (Incidence rate [IR] = 4,5 per patient-year) of which 31 were serious. In the group of non-levacetylleucine use, 46,3% of patients (n=38/82) reported AEs, but corresponding to 103 events (IR= 4,4 per patient-year) and only two were serious.

Among AEs reported in the levacetylleucine use group, 8,3% were considered related to the studied treatment by the Applicant similarly to the non-levacetylleucine use group (8,5%). Most of these adverse events were mild to moderate in severity but 10 patients from the levacetylleucine use group experienced 29 severe AEs far higher than the non-levacetylleucine group where 5 AEs were reported as severe. No TEAEs leading to withdrawal or death were recorded in the non-levacetylleucine use group while one patient withdrew and one patient died in the levacetylleucine use group. These cases are further discussed thereafter under the section "Deaths" and "Discontinuations and interruptions related to AEs".

The most commonly-reported AEs in the levacetylleucine use group (≥ 5%) belong to the SOCs "Infections and Infestations", "Gastrointestinal Disorders", "Nervous System Disorders", "Injury, poisoning and procedural complications", "Psychiatric disorders", "Respiratory, thoracic and mediastinal disorders", "Skin and Subcutaneous Tissue Disorders", "General disorders and administration site conditions" and "Renal and urinary disorders".

According to the SOC, the incidence rates (IR per patient-year) differ from groups. As a matter of fact, the IR values were higher in the levacetylleucine use group compared to the non-levacetylleucine use group in the SOC "Infections and infestations" (IR=0.9 versus 0.7), "Gastrointestinal Disorders" (IR=0.7 versus 0.4), "Nervous System Disorders" (IR=0.6 versus 0.5) and "Renal and urinary disorders" (IR=0.1 versus 0). In other SOCs, IR values were equal or lower than IR values in non-levacetylleucine use group.

The PTs (preferred terms) that were more often reported during the levacetylleucine use (≧ 4 patients) and having higher incidence rate than during the non-levacetylleucine use were: upper respiratory tract infection (8 patients [9.5%] IR=0.2/patient-year; non-levacetylleucine use group: 3 patients [3.6%], IR= 0.1/patient-year), pneumonia aspiration (4 patients [4.8%] IR=0.1/patient-year; non-levacetylleucine use group: 1 patient [1.2%]) dysphagia (7 patients [8.3%] IR=0.2/patient-year; non-levacetylleucine use group: 0 patient), contusion (5 patients [6%], IR=0.1/patient-year); non-levacetylleucine use group: 1 patient [1.2%]), vomiting (4 patients [4.8%] IR= 0.1/patient-year; non-levacetylleucine use group: 1 patient [1.2%]) and Coronavirus infection (4 patients [4.8%] IR = 0.1/patient-year; non-levacetylleucine use group: 0 patient).

Besides, interestingly in the non-levacetylleucine use group, the AE of diarrhoea has been reported more frequently than in the levacetylleucine use group (5 patients [6.1%] IR = 0.3/patient-year; levacetylleucine use group: 5 patients [6%], IR= 0.1/patient-year). The appraisal of data on the SOC "Infections" does not allow any particular safety issues to be identified. However, three of the six AEs

more often reported in ≥ 4 patients receiving levacetylleucine versus non-levacetylleucine involved an infection (upper respiratory tract infection; pneumonia aspiration and Coronavirus infection). Based on the data from the combined dataset for NPC, the overall incidence rates of infection and infestation TAEs were similar. There was slight trend in increased number of infection and infestation in patients treated by levacetylleucine, however due to small sample size it is difficult to interpret any conclusion.

The AEs of vomiting, currently not considered related to levacetylleucine by the Applicant, raises a concern about levacetylleucine causality due to the higher incidence rate compared to non-levacetylleucine use and the occurrence of the event twice at different times, and no event under placebo or during the wash-out period. The Applicant provided additional explanations on vomiting AE on their clinical context. Current available cases of vomiting cannot allow a possible causal association with levacetylleucine treatment to be made.

Most TEAEs (310 of the 344 in total reported TEAEs) were of mild or moderate intensity. A total of 10 patients (11.9%) reported 29 severe TEAEs during levacetylleucine use and 3 patients (3.7%) reported 5 severe TEAEs during non-levacetylleucine use.

The incidence rates of TEAEs reported as severe was slightly higher during levacetylleucine use (IR=0.5/ patient-year) than during non-levacetylleucine use (IR=0.2/patient-year).

Safety -Study 301: Placebo-controlled safety was evaluated by the Applicant by comprising data from the 12-week cross-over designed Phase III study. Thus, patients in the pivotal study were exposed to levacetylleucine 6 weeks in total. Placebo-controlled safety pool consisted of 60 patients (100%) who received at least one dose of levacetylleucine and 59 patients receiving at least one dose placebo.

At least one TEAE was reported in 43 patients (71.7 %) and in total, 151 TEAEs were reported during the placebo-controlled 12-week period. Most of these TEAEs were mild and not related to levacetylleucine. Overall, slightly more levacetylleucine treated patients than placebo treated patients reported TEAEs (i.e., any TEAEs, non-fatal serious TEAEs, TEAEs leading to discontinuation of study treatment and TEAEs of special interest.

Overall, 8 patients reported at least one TEAE (Total of 12 TEAEs were reported as related): 13.3 % subjects for levacetylleucine and 10.2 % for placebo, respectively. Although, more patients treated with levacetylleucine reported more adverse events, the placebo had similar reporting of adverse events which is in line with the rather comorbid patient population (history of ataxia, epilepsy, cognitive disorder, depression, dysphagia, gaze palsy etc.). Non-fatal serious AEs were reported in 4 patients in total (6.7 %); 3.3 % and 3.4 % for levacetylleucine and placebo, respectively. None of these AEs were considered as related.

Severity and relatedness of TEAEs is difficult to assess as contrast between levacetylleucine and placebo, since the incidence rates were low. In most subjects experienced mild TEAEs related to the levacetylleucine treatment (112 TEAEs by 23 subjects, i.e. 38.3 % pats). Evaluation of levacetylleucine in younger children (above 1 month and up to 5 years) will be deferred until safety and efficacy data from the proposed Phase III trial are available.

Safety of study IB1001-201: Total 7 TEAEs in 4 patients were considered related to treatment – flatulence, diarrhoea, increased appetite, rash pruritic, rash, aggression and restlessness.

31 patients completed the 6-week treatment period, two patients were withdrawn (unrelated AE - seizures and trauma, other). 61 AEs were reported by 24 patients. Total 7 TEAEs in 4 patients were considered related to treatment – flatulence, diarrhoea, increased appetite, rash pruritic, rash, aggression and restlessness. There were no deaths, 4 patients reported a total of 6 unrelated SAEs

requiring hospitalisation – upper respiratory tract infection, lower respiratory tract infection, dehydration, fall, head injury, ribs fracture.

A total of 19 patients entered the extension phase, 6 withdrew from study (withdrawal of consent, lost to follow up, 2 patients did not receive any benefit, insufficient effect and withdrawal of consent). The duration of exposure to levacetylleucine during the Extension Phase ranged from 176 days to 816 days. 127 AEs were reported by 18 patients. Overall, 5 patients reported a total of 22 SAEs (required hospitalisation) – aspiration pneumonia, gastrointestinal haemorrhage of severe intensity, seizure, pneumonia, device dislocation, gastric perforation, cataplexy, pneumonia viral, pneumonia and gastrostomy, uterine leiomyoma, hysterectomy and aspiration of severe intensity.

- Treatment related adverse events

From the pooled safety data: Treatment-related TEAEs that were reported by more than 1 patient were diarrhoea, anal incontinence, and dyspepsia. Diarrhoea was reported by 1 patient (1.2%) during levacetylleucine use and by 3 patients (3.7%) during non-levacetylleucine use. Anal incontinence was reported by 1 patient (1.2%) each during levacetylleucine use and non-levacetylleucine use. Dyspepsia was reported by 1 patient (1.2%) during levacetylleucine use and by no patients during non-levacetylleucine use.

Treatment-related adverse events have been further discussed thereafter under "Adverse drug reactions in SmPC" part.

Safety of Study IB1001-301: In total, 12 TEAEs were considered as related by the Investigator during the 12-week placebo-controlled period. None of the SAEs were considered related to the levacetylleucine treatment. For levacetylleucine only 3 drug related AEs are reported. For placebo arm 9 drug related TEAEs were reported.

Safety of Study IB1001-201: Total 7 TEAEs in 4 patients were considered related to treatment flatulence, diarrhoea, increased appetite, rash pruritic, rash, aggression and restlessness. One patient reported a total of 2 TEAES related to the study drug in the extension – 2 cases of dyspepsia.

- Long-term safety data

In the pivotal study IB1001-301 60 patients (100%) with NPC received at least 1 dose of the study drug. The median duration of exposure to levacetylleucine was 85 days (range: 69, 97) with a mean duration of exposure of 86.2 days. Long-term exposure was not evaluated since the open-label extension phase of the pivotal study is still ongoing. No safety data are available.

From the study IB1001-201, out of the 33 patients, 19 patients pursued in the extensions phase among them 6 withdrew. Hence Thirteen (n=13) completed the extension phase of 1 year. This quite a low number of patients to adequately appreciate the long-term safety profile.

Moreover, no safety data are available in patients under 6 years old.

Therefore, as a missing information of the RMP safety concerns, "Long-term safety data" has been added.

- Serious adverse events

Ten (10) patients (11.9%) in the levacetylleucine use group and 2 patients (2.4%) in the non-levacetylleucine use group reported 31 and 2 SAEs, respectively, corresponding to an incidence rate = 0.6 /patient-year and 0.1/patient-year, respectively. The main SOCs retrieved, in at least 2 patients, were "Infections and infestations" (n=6 [7,1%], 12 AEs IR=0,2/patient-year; no case in the non-levacetylleucine use group), "Nervous system disorders" (n=2 [2,4%] 2 AEs; 1 case in the non-

levacetylleucine use group), "Surgical and medical procedure" (n=3 [3,6%], 3 AEs IR=0,1/patient-year; no case in the non-levacetylleucine use group), and "Gastrointestinal disorders" (n=2 [32,4%], 6 AEs IR=0,1/patient-year; no case in the non-levacetylleucine use group).

There were 5 SAEs of aspiration pneumonia which were reported by 4 patients (4.8%), IR=0.1/patient-year, during levacetylleucine use and none during non-levacetylleucine use. The assessment of these cases cannot allow any conclusion to be drawn on levacetylleucine causality due to either confounding factors or limited information.

Overall, none of the reported SAEs was considered to be treatment-related by the Applicant, and none resulted in withdrawal of study drug treatment.

▪ Death

One patient died during the phase III pivotal placebo-controlled study after enrolment in the pivotal study but the medical conditions around this death cannot allow any causal association to be made with levacetylleucine. The patient had a pre-planned implementation of a percutaneous endoscopic gastrostomy (PEG) feeding tube. The patient/family did not inform the study site/PI until after the first visit of treatment Period I. The patient stopped study drug (levacetylleucine treatment) 2 days before the procedure and planned to resume study drug following the procedure. The procedure had complications, leading to SAEs, including aspiration pneumonia, which ultimately proved fatal.

▪ Discontinuation or interruption due to adverse events

One patient withdrew for safety reason related to complication following the implementation of a percutaneous endoscopic gastrostomy (PEG) feeding tube at his local hospital, and regardless levacetylleucine treatment that had been stopped 2 days before.
Besides in the pivotal study, one patient discontinued treatment early from levacetylleucine and placebo, respectively during the 12-week period. The reason for early treatment discontinuation was related to other disease than NPC. No patient discontinued the treatment due to TEAE.

In the Extension Phase of the phase II study, for 1 patient, ALT increase was reported as a TEAE of mild intensity in the treatment period I. This TEAE was not considered to be related to study treatment and did not lead to withdrawal of study drug but to interruption of study drug. This patient reported an ALT concentration of 100 U/L (reference range: 0 to 45 U/L) at Visit 8. At Visits 9 and 10, the reported ALT concentration was 52 U/L and 50U/L, respectively. At Visit 11, the reported ALT was within the reference range.

Adverse drug reactions for the SmPC

This safety pool is considered as primary safety pool and should be used to justify the inclusion of ADRs into the list in Section 4.8 of the PI.

The Applicant list of Adverse Drug reactions (ADRs) for the section 4.8 is based on all TEAEs assessed as related by the investigator that occurred under levacetylleucine use. The number of patients contributing to the denominator for the frequency calculations is 84 and results from the pooled safety data set. The main issue is the assessment of the selected adverse drug reactions for the section 4.8 of the SmPC. Indeed, a further in-depth analysis of the corresponding narratives led to the following appraisal:

- *Increased appetite*: as claimed the Applicant, this event resolved without any action taken with the study medication. The patient subsequently enrolled in the IB1001-201 extension study and did not report any recurrence of this adverse event. Even though the investigator assessed the event of increased appetite as a non-serious mild adverse event and related to levacetylleucine (the data on which the investigator concluded are not detailed), the Sponsor assessed the event to be unrelated to

levacetylleucine considering that the event resolved without any change with the study medication. Moreover, the event did not recur with continued treatment with the study medication in the extension study.

From the CHMP point of view, based on the available data, and, since the event has not reoccurred whilst levacetylleucine treatment pursued, a causal relationship between levacetylleucine and the adverse event of increase appetite cannot be considered as "at least a reasonable possibility".

- *Aggressions*: Two cases of aggression that occurred in patient under levacetylleucine treatment have been described by the Applicant. For both cases, no change to the study medication was made during this time and levacetylleucine treatment pursued without any new adverse event of aggression. In one case, the NPC disease can explain the event. The Applicant indicated a positive dechallenge but the narrative does not mention any interruption of levacetylleucine, on the contrary no change to the study medication was made. In the second case, the NPC condition and the introduction of another treatment 1 month before whilst levacetylleucine was started 4 months ago, are confounding factors.

From the CHMP point of view, based on the available data, since the event did not reappear whilst levacetylleucine treatment pursued, a causal relationship between levacetylleucine and the adverse event of increase appetite cannot be considered as "at least a reasonable possibility".

- *Diarrhoea:* Seven events of diarrhoea reported by 5 patients were analysed and discussed thoroughly by the Applicant. The sponsor as well as the investigator assessed the events not related to levacetylleucine, except for one event, and for which the investigator concluded that it was related to levacetylleucine (but without providing criteria on which his appraisal is made). This event led to levacetylleucine interruption. The patient experienced diarrhoea during the period of levacetylleucine interruption, but also during the wash-out period and one year and 9 months after. For the two latter events, the investigator then concluded the events were not related to levacetylleucine.

For all other cases, no change to the study medication was made. levacetylleucine was pursued in the extension study without any recurrence of diarrhoea.

Of note, in non-levacetylleucine use group, a similar number of diarrhoea (n=6) was reported.

From the CHMP point of view, as regards the available data cannot allow a causal relationship between levacetylleucine and the adverse event of diarrhoea be considered as "at least a reasonable possibility".

- *Anal incontinence:* Two adverse events of anal incontinence occurred in two patients. In both cases the event resolved without any change of the study treatment and the investigator as well the sponsor considered this AE unrelated to levacetylleucine. Of note, in non-levacetylleucine use group, two patients also experienced anal incontinence. As reminded the Sponsor, the NP disease are prone to bowel dysfunction.

- *Dyspepsia*: One patient experienced two events of dyspepsia, 2 months and 13 months after starting levacetylleucine treatment. The investigator assessed them as related to levacetylleucine without providing the criteria behind this appraisal. The patient had a medical history of intermittent gastritis, levacetylleucine treatment was pursed and no further recurrence of dyspepsia reported during the extension study by the patient. Therefore, from the CHMP point of view, data appear limited to establish either at least a suspected causal relationship or at least a reasonable possibility of a causal relationship between the medicinal product and the adverse event.

- *Flatulence:* One patient experienced flatulence that resolved the day after the last dose of levacetylleucine. Both Sponsor and Investigator agree to consider this AE related to levacetylleucine. Even though the narrative of the case is limited, due the close temporal relationship and the positive dechallenge observed, the CHMP agrees to add the adverse event of flatulence in the section 4.8 of the SmPC and in the PL.

- *Rash pruritic:* Two patients reported rash pruritic, but the event was confounded by the medical history of the patients. Furthermore, the events resolved without any changes of the study medication. Therefore, from the CHMP view, data appear limited to establish either at least a suspected causal relationship or at least a reasonable possibility of a causal relationship between the medicinal product and this adverse event.

- *Rosacea:* one event of rosacea was reported in a patient who already had experience of rosacea before levacetylleucine initiation. No action was taken with levacetylleucine. The patient not recovered. Data actually are limited to adequately establish a suspected or at least possible relationship with levacetylleucine treatment.

Regarding *restlessness or restless legs syndrome*, the Applicant further analysed the two corresponding events and conclude by the deletion it from the list of ADR. The CHMP agrees that confounding factors impact the assessment of levacetylleucine causality (pre-existing medical condition, NP disease).

According to the Guideline on EU SmPC, the section 4.8 should include *all adverse reactions from clinical* trials, post-authorisation safety studies and spontaneous reporting for which, after thorough assessment, a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility, based for example, on their comparative incidence in clinical trials, or on findings from epidemiological studies and/or on an evaluation of causality from individual case reports. Adverse events, without at least a suspected causal relationship, should not be listed in the SmPC.

The Applicant agreed that it has not been established that there is at least a reasonable possibility of a causal relationship between levacetylleucine and the adverse events increased appetite, aggression, diarrhoea, anal incontinence, dyspepsia, rosacea and rash pruritic. These events have been removed from Section 4.8 of the SmPC and from the patient information leaflet (PIL). Restlessness has also been removed on the same basis. Only the adverse event of flatulence is now listed in Section 4.8 of the SmPC and in the PL.

Laboratory findings

Overall data on hematologic parameters (haemoglobin, erythrocytes, haematocrit, leukocytes and platelets), clinical biochemistry (sodium, potassium, lactate dehydrogenase, creatinine, urea nitrogen, bilirubin) and urinary chemistry appear consistent across studies. No particular trends have been identified from both the pivotal phase III and phase II/Extension phase studies.

Some patients had increased Alanine Aminotransferase, Aspartate Aminotransferase, alkaline phosphatase during the treatment period (Visit 3, 4, 8) in comparison to the normal value at baseline. These values have been assessed by the investigator as not related to the treatment.

However, in the phase III study 1001-301, regarding the hepatic transaminase ALT (alanine transaminase), , in the levacetylleucine use group, maximum observed values differ from the baseline to visit 4 or 6, 65 U/L and 80 U/L, respectively, whereas this is not observed in placebo group. Likewise, at visit 8 and 9 of the extension phase of the phase II study 1001-201, ALT increased (100 U/L and 95 U/L, respectively) up to an observed maximal value of 108 U/L at visit 10, while, at visit 1 of the phase II study, it was 54 U/L.

Furthermore, in the levacetylleucine use group of the phase III study, an increase in ALP (alkaline phosphatase) maximum values can be observed between baseline (321 U/L), visit 3 or 5 (465 U/L) and visit 4 or 6 (432 U/L) compared to placebo where maximum values increased but in a lesser extent (321 U/L, 360 U/L, 388 U/L, respectively). However, no TEAEs belonging to the SOC" Hepatobiliary disorders" were reported during IB1001-301 Parent study and IB1001-201 study. Presently available data cannot allow any liver toxicity potential to be identified. Besides, some patients experienced AST and ALT increase during visit 3 and 4 under levacetylleucine treatment that remains at visit 5 under placebo but

decrease at visit 6. One patient had normal values for ALT at baseline (visit 1 and visit 2). They increased to 61 U/L and 49 U/L at visit 3 and visit 4 under levacetylleucine treatment, for decreasing at visit 6 of the wash-out period. These changes, even though transient, are questioning. But data are limited and cannot allow a conclusion to be drawn.

One patient experienced a substantial increase of alkaline phosphatase at visit 6, 247 U/L, under levacetylleucine treatment, whilst at baseline, during placebo and at visit 5 under levacetylleucine, ALP values were ≦33 U/L. This value is of interest but since data on such changes are limited no conclusion can be drawn.

Therefore, in respect to liver enzymes changes and the uncertainties on levacetylleucine causality, the risk of hepatotoxicity has been added as an important potential risk in the list of safety concerns of the RMP.

Vital signs and physical

In all studies, vital signs did not change over time during treatment with levacetylleucine. No particular safety issues were identified

Electrocardiogram/QT/QTc

Based on publicly available information, the actual upper limit of solubility (525 µM) of levacetylleucine in aqueous solution is still considered implausible. Of note however, solubility of levacetylleucine as well as hERG testing itself was conducted in accordance with GLP and hence at the highest standard of reliability. A safety margin of ~10 is insisted to be low but can be considered acceptable if otherwise experimentally infeasible – this appears to be the case here. Furthermore, the other means of cardiac safety evaluation (e.g., telemetry) did not give reason for concern and that the racemic Tanganil, which is approved in France for over 60 years, is not associated with CV risk. According to the submitted clinical data, there is no signal or trend of pattern related to any TQT prolongation that could be due to levacetylleucine. Therefore, the Applicant request for a waiver for a thorough QT/QTC study with levacetylleucine is accepted.

Other safety information

No hypersensitivity reactions have been observed in clinical trials with levacetylleucine to date. Such reactions remain generally plausible also for levacetylleucine, given that NADLL is the racemic mixture of the enantiomer levacetylleucine and with NADLL events of hypersensitivity reactions have been observed in the clinical setting. Therefore, the proposed warning in the section 4.4 of the SmPC that anaphylactic and hypersensitivity reactions may occur after administration of levacetylleucine and immediate discontinuation of Aqneursa and necessary emergency treatment is required if such reactions occur, is supported.

2.6.10. Conclusions on the clinical safety

Majority of the patients with NPC treated with levacetylleucine reported at least one AE, but only the AE of flatulence can be listed as an ADR in section 4.8 due to a compatible TTO and a positive dechallenge.

Overall, even though two-third of patients who received levacetylleucine experienced adverse events (AEs), the assessment of the pooled safety data set does not evidence major adverse events that could be related to levacetylleucine. Likewise, none of the severe TEAEs was reported as related to the levacetylleucine administration by the investigator and no early discontinuation due to a TEAE was documented in exception to one case (1 patient reported a TEAE leading to withdrawal from the IB1001-201 Parent Study due to increased seizures considered unrelated to study treatment).

The long-term safety data are generated only from phase II study - the approximately one-year efficacy and safety data for 18 patients (up to visit 9) are presented and 13 patients completed the treatment period II (2 years of treatment). Additionally, long-term safety data from the pivotal Phase III Study 301 extension phase were not submitted as a study report. The safety in special populations (specifically paediatric population under 6 years) is a major concern.

2.7. Risk Management Plan

2.7.1. Safety concerns

Table 33: Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	Hepatotoxicity; Carcinogenicity; Developmental toxicity
Missing information	Long-term safety

2.7.2. Pharmacovigilance plan

Table 34: Part III.1: On-going and planned additional pharmacovigilance activities

Study Status	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
Not applicable				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
Not applicable				
Category 3 - Required additional pharmacovigilance activities				
IB1001-301 Extension Phase On-going	Evaluate long-term safety and tolerability in patients with NPC	Hepatotoxicity Long-term safety	Updated study protocol	3 months after marketing authorisation
			Final report	31 March 2029
Post-authorisation, non-interventional study using existing International Niemann Pick Disease Registry (INPDR) as data source Planned	Evaluate long-term safety and tolerability in patients with NPC	Hepatotoxicity Long-term safety	Study protocol	3 months after marketing authorisation
			Final report	To be confirmed

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
26-Week Oral (Gavage) Administration Carcinogenicity Study in the Transgenic rasH2 Mouse Planned	Evaluate the carcinogenic potential of IB1001	Carcinogenicity	Final report	30 June 2027
Intravenous (Slow Bolus) Preliminary Study of Embryo-Foetal Development in the Rabbit On-going	Study the effect of intravenous IB1001 on embryo-foetal development in the rabbit	Developmental toxicity	Final report	31 October 2025
8 Week Once Daily Oral (Gavage) Administration Dose Juvenile Toxicity Study with IB1001 in the Rat On-going	Assess the toxicity and toxicokinetic profile of IB1001 in juvenile rats	Developmental toxicity	Final report	31 December 2025

2.7.3. Risk minimisation measures

Table 35: Part V.3: Summary of risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Hepatotoxicity	Routine risk minimisation measures: Prescription only medicine Additional risk minimisation measures: None	Additional pharmacovigilance activities: IB1001-301 Extension Phase, due 31 March 2029 Post-authorisation, non-interventional study using existing International Niemann Pick Disease Registry (INPDR) as data source
Carcinogenicity	Routine risk minimisation measures: SmPC, Section 5.3 Prescription only medicine Additional risk minimisation measures:	Additional pharmacovigilance activities: 26-Week Oral (Gavage) Administration Carcinogenicity Study in the Transgenic rasH2 Mouse, due 30 June 2027

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	None	
Developmental toxicity	Routine risk minimisation measures: SmPC, Sections 4.6, 5.3; PIL, Section 2. Prescription only medicine Additional risk minimisation measures: None	Additional pharmacovigilance activities: Intravenous (Slow Bolus) Preliminary Study of Embryo-Foetal Development in the Rabbit, due 31 October 2025 8 Week Once Daily Oral (Gavage) Administration Dose Juvenile Toxicity Study with IB1001 in the Rat, due 31 December 2025
Long-term safety	Routine risk minimisation measures: Prescription only medicine Additional risk minimisation measures: None	Additional pharmacovigilance activities: IB1001-301 Extension Phase, due 31 March 2029 Post-authorisation, non-interventional study using existing International Niemann Pick Disease Registry (INPDR) as data source

2.7.4. Conclusion

The CHMP considers that the risk management plan version 0.8 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the Applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The Applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 24.09.2024. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the Applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on*

the readability of the label and package leaflet of medicinal products for human use.

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Aqneursa (levacetylleucine) is not included in the additional monitoring list.

3. Benefit-Risk Balance

3.1. Therapeutic Context

The Applicant submitted an application for Aqneursa (levacetylleucine (NALL)) for the treatment of Niemann-Pick Type C (NPC).

The agreed indication is as follows:

Aqneursa is indicated for the treatment of neurological manifestations of Niemann-Pick type C (NPC) disease, in combination with miglustat, or as a monotherapy in patients where miglustat is not tolerated, in adults and children aged 6 years and older and weighing at least 20 kg.

The mechanism of action of levacetylleucine is presented as rather non-specific and the active substance revealed no main pathway which would account for the mechanism of action at specific cell-target. Non-clinical studies demonstrated that levacetylleucine corrects energy metabolism, including improving adenosine triphosphate production. The recommended dose is based on the patient's body weight in kg ranging from 2 g per day (20 to 24 kg) to up to 4 g per day (35 kg or more).

3.1.1. Disease or condition

NPC is a rare, progressive, neurodegenerative, autosomal recessive, and inherited metabolic lysosomal storage disorder (LSD). Its estimated incidence is around 1:100,000 live births (Geberhiwot et al. 2018). Globally, miglustat is the only medicinal product authorised for NPC.

NPC is inherited in an autosomal-recessive manner. Diagnosis is established in probands with symptoms and signs suggestive of NPC and the presence of biallelic pathogenic variants in either NPC1 or NPC2 identified by molecular genetic testing, or the presence of clinical symptoms supported by biomarker and/or filipin testing [Patterson et al. 2000, updated 2020]. The clinical presentations of NPC disease are characterized by broad heterogeneity in serious and debilitating systemic, psychiatric, and neurological symptoms (which vary markedly depending on the age of onset of neurological symptoms) from a rapidly progressing neonatal form to an adult-onset chronic neurodegenerative condition. NPC is always fatal. The majority of NPC patients are children and die before the age of 20, with the median age of death being 12.5 years [Garver et al. 2007].

The course of the disease varies highly from patient to patient depending on the age of onset of neurological symptoms, from a rapidly progressing neonatal form to an adult-onset chronic neurodegenerative condition. Infantile and juvenile patients often experience the most severe symptoms, including epileptic seizures and cataplexy, while adult patients experience severe cognitive impairment, dementia, and psychosis [Patterson et al. 2013].

Systemic signs of liver, spleen and lung involvement typically precede the disease-defining neurodegeneration. This is particularly true for patients with onset during infancy and childhood. Neurological signs and symptoms include ambulation and walking difficulties, cognitive impairment,

swallowing difficulties, vertical supranuclear gaze palsy, seizures, and cataplexy. The progression of the neurological symptoms is responsible for disability and premature death in most cases (Vanier 2010).

The neurological symptoms in NPC include a delay in developmental motor milestones (early-infantile period) and problems at school, including difficulties in writing and impaired attention (late-infantile and juvenile period). A cardinal symptom is cerebellar ataxia (present in 70% of patients) where patients have problems with stance and gait ataxia and consequent falls, dizziness, clumsiness, dysmetria, and disdiadochokinesia. Other neurological signs are vertical supranuclear gaze palsy, dysphagia, gait disorders, and dementia, which can lead to a premature death. Cataplexy, seizures, and dystonia are also common symptoms [Patterson et al. 2013; Vanier 2010].

The initially claimed indication for Aqneursa was the following:

"Aqneursa is indicated in adults and children from birth for chronic treatment of Niemann-Pick Type C (NPC)."

Following comments and recommendations from the CHMP, the Applicant agreed to the final indication below:

"*Aqneursa is indicated for the treatment of neurological manifestations of Niemann-Pick type C (NPC) disease, in combination with miglustat, or as a monotherapy in patients where miglustat is not tolerated, in adults and children aged 6 years and older and weighing at least 20 kg*.".

3.1.2. Available therapies and unmet medical need

There are no curative therapies for NPC. Miglustat is the only medicinal product authorised for NPC in the EU. Miglustat is a compound first licensed for type 1 Gaucher disease that has received market authorisation for use in NPC patients in the European Union (EU), Japan, and other countries (but not in the United States [US]). Evidence in support of miglustat in NPC comes from a randomised clinical trial, long-term extension studies, and two retrospective surveys, demonstrating a reduction of the progression of clinically relevant neurological symptoms in patients with NPC. In NPC patients, miglustat is indicated for the treatment of progressive neurological manifestations and has been shown to slow the general progression of neurological symptoms in patients with NPC. However, miglustat has no curative effect. The Applicant claimed that levacetylleucine aims to address the unmet medical need in NPC by providing a novel mechanism of action that could lead to symptomatic improvement in neurological signs and symptoms.

3.1.3. Main clinical studies

Clinical data are mainly coming from the following two studies:

An ongoing pivotal ongoing phase III multinational, randomized, placebo-controlled, double-blinded, crossover study evaluating 12 weeks of treatment followed by an extension phase. The primary endpoint was the Scale for the Assessment and Rating of Ataxia (SARA) total score. This scale includes 8 items that are related to gait, stance, sitting, speech, finger-chase test, nose-finger test, fast alternating movements and heel-shin test. Secondary efficacy endpoints notably included the measurements of neurological signs and functioning using the Scale for Spinocerebellar Ataxia Functional Index (SCAFI), and measurement of Health-Related Quality of Life.

A phase II multinational, multi-centre, open-label, rater-blinded single-arm study in paediatric and adult patients aged ≥6 years evaluating 6weeks treatment followed by an extension phase. The primary

endpoint was blinded raters' Clinical Impression of Change in Severity (CI-CS) score comparing either the 9-Hole Peg Test of the Dominant Hand (9HPT-D) or the 8-Meter Walk Test (8MWT) as the primary anchor. Additional efficacy endpoints included measurements of ataxia and functioning: SARA score, SCAFI score, Measurement of Health-Related Quality of Life, Measurement of Overall Neurological Status and Measurement of Global Impression scored by treating physician, caregiver, and patient.

3.2. Favourable effects

In the pivotal study, the SARA total score showed an improvement after treatment with levacetylleucine over placebo treatment. After treatment with levacetylleucine, the mean (SD) change from baseline was -1.97 (2.43) compared to -0.60 (2.39) after placebo treatment. Two-sided statistical testing indicated a significant difference between levacetylleucine and placebo (LS-mean difference: -1.28; 95% CI: -1.91, -0.65; $p<0.001$). Patients who received levacetylleucine followed by placebo which effectively served as a washout from levacetylleucine had a significant worsening of symptoms when receiving placebo (difference in mean SARA total score = +1.55).

3.3. Uncertainties and limitations about favourable effects

As per the current consensus of clinicians, the progression and presentation of the NPC disease depend on the age at which the neurological symptoms onset. There are very distinct disease onsets (infantile, juvenile and late/adult onset). The Applicant initially sought an indication that would cover the whole population, with no limitations to this heterogeneity. This was not supported by the data from the clinical studies (Phase II and ongoing Phase III Study).

No dose-finding study was carried out. According to the non-clinical part of the dossier (PD *in vivo* studies in rats and cats), the dose that should have been investigated would be 6 g/ day for adults (based on the AUC). The clinical part of the dose development is based on the experience gained from the racemate. It should be noted that the racemate, L-enantiomer and D-enantiomer respectively can differ in the primary PK characteristics (C_{max}, AUC). According to the *in vitro* data the C_{max} and AUC was greater for the D-enantiomer when compared to the L-enantiomer.

The pivotal study excluded patients aged below 4 years old and no patients aged below 6 years old and weighting less than 20 kg were included. The lack of data in this population is a concern with regards to efficacy in this specific population. Following questions from the CHMP, the Applicant excluded this population from the indication.

The primary endpoint was SARA (Scale for the Assessment and Rating of Ataxia) which includes 8 items that are related to gait, stance, sitting, speech, finger-chase test, nose-finger test, fast alternating movements and heel-shin test. It is a validated clinical scale used to assess ataxic disorders, but it has not been validated to assess Niemann–Pick type C disease. Secondary endpoints included an ataxic scale (SCAFI), a modified Disability Rating Scale and PROs. These scales are used to assess neurological symptoms, but it has not been conclusively established that they can be used to assess NPC disease as a whole.

The duration of the treatment period during the cross-over was 12 weeks, which may be considered limited for a product intended for chronic use.

The Applicant has submitted data from the ongoing open-label extension phase (EP) in the form of an unpublished preprint that was not certified by a peer review (Patterson et al. Preprint 2024). No study report was provided which makes it difficult to assess the long-term efficacy.

The effect of the product on the SARA score was rapid, but patients deteriorated rapidly when treatment was stopped, which in the context of a progressive could be attributable to a symptomatic effect of the product rather than a disease modifying effect.

In both pivotal and supportive studies, a vast majority of patients (85% and 90.9% respectively) received levacetylleucine in combination with miglustat which raises an issue of how to properly determine the efficacy of levacetylleucine alone versus in combination with miglustat.

The overall long-term efficacy data are based on the 19 patients who entered the extension phase of the Phase II Study IB1001-201. Among them 18 patients were treated for one year and 13 completed the extension phase (two years treatment) However, the primary efficacy endpoint for the extension part (5-domain NPC-CSS) is different from the primary endpoint if the parent study (CGI – CS) and from the primary endpoint of pivotal study (SARA). These disparities preclude any conclusions regarding the long-term efficacy of the drug. The results from the study Phase II - IB1001-201 should be interpreted with caution considering the design of the study (non-randomised, rater-blinded, single arm, open label study with the different setting of primary and secondary endpoints in comparison to the pivotal study IB1001-301) and unresolved GCP findings (violation).

The Applicant committed to provide the study report of the extension phase data from the pivotal Study IB1001-301 to support the long-term efficacy and safety of levacetylleucine (MEA), which is due by 31 March 2029.

3.4. Unfavourable effects

Two-third of patients who received levacetylleucine (n=55/84) experienced adverse events (AEs), corresponding to 241 events with an incidence rate [IR] = 4,5 per patient-year, of which 31 were serious. Among AEs reported in levacetylleucine use group, 8,3% were considered related to the studied treatment by the Applicant. Most of these adverse events were mild to moderate in severity and 10 patients from levacetylleucine use group experienced 29 severe AEs.

Most TEAEs (310 / 344 reported TEAEs) were mild or moderate in intensity A total of 10 patients (11.9%) reported 29 severe TEAEs during levacetylleucine use. The incidence rates of TEAEs reported as severe was slightly higher during levacetylleucine use (IR=0.5/ patient-year) than with non-levacetylleucine use (IR=0.2/patient-year). This was mainly driven by respiratory events reported as severe. The appraisal of data on the SOC "Infections" does not allow any particular safety issue to be identified.

Twenty-one (21) treatment-related TEAEs were reported by 12 patients (14.3%). A total of 7 patients (8.3%) reported 11 treatment-related TEAEs during levacetylleucine use. The incidence rate of treatment-related TEAEs was 0.2/ patient-year during levacetylleucine use.

Treatment-related TEAEs for the list of adverse drug reactions for the section 4.8 of the SmPC is currently limited to the adverse drug reaction of flatulence. Two-third of patients who received levacetylleucine (n=55/84) experienced adverse events but only the adverse event of flatulence has been related to levacetylleucine treatment and is listed in the section 4.8 of the SmPC. No major adverse events have been identified.

3.5. Uncertainties and limitations about unfavourable effects

The Applicant did not identify initially any safety concerns for the RMP. Pertaining to the rarity of the disease, and then the low number of enrolled patients, the lack of sufficient long-term safety data is a concern. Long-term exposure is currently based only on the Phase II study 201. The pivotal Phase III study 301 is still ongoing. According to the ICH E1 guidance on population exposure, patients should be

exposed to the treatment for at least 12 months in case of chronic treatment regimen. Currently, only three months exposure was placebo controlled. This issue is particularly important for the paediatric population, where no data are available for children below 4 years of age and very limited in the age group 4 - 12 years (15 to 25 kg of weight). Therefore, the proposed list of safety concerns has been updated adding "long-term safety data", as missing information. There is also an uncertainty regarding hepatotoxicity that has been listed as an important potential risk in the list of the RMP safety concerns.

3.6. Effects Table

Regarding the, the mean (SD) change from baseline was after levacetylleucine treatment compared to after placebo. Two-sided statistical testing indicated a significant difference between (LS-mean difference: -1.28; 95% CI: -1.91, -0.65; p<0.001) showing an improvement.

Table 36. Effects Table for Aqneursa

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
SARA score mean	Scale for the assessment and rating of ataxia	SD	-1.97 (2.43)	-0.60 (2.39)	LS-mean difference: -1.28; 95% CI: -1.91, -0.65; p<0.001	Phase III 1001-301 study
Unfavourable Effects Treatment related TEAEs (percentage of patients)*						
Flatulence	Incidence of flatulence	%	1.2	Versus non-levacetylleucine use group	Outcomes from the pooled safety data set	Phase III 1001-301 study and phase II study/extension phase 1001-201

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The main evidence on efficacy of levacetylleucine is derived from the interim results of an ongoing multicenter, randomized, double-blind, placebo-controlled, crossover study in adults and paediatric patients aged ≥ 4 years old. The duration of the treatment period during the cross-over was 12 weeks, which may be considered limited for a product intended for chronic use. Overall, the conduct of a double-blinded RCT in a sufficient number of NPC disease patients is positively recognized.

Regarding the primary endpoint, the mean (SD) change from baseline of the SARA after treatment with levacetylleucine, was -1.97 (2.43) compared to -0.60 (2.39) after placebo treatment demonstrating a significant neurological improvement. The study also showed that patients who received levacetylleucine followed by placebo which effectively served as a washout from levacetylleucine had a significant worsening of symptoms when receiving placebo, (difference in mean SARA total score = +1.55).

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Long-term efficacy/safety data have been generated from the extension period of the phase II study for 18 patients and only 3 patients were of age 6 -12 years. The supporting data from study 201 can only be used for comparison of secondary endpoints used in the Pivotal Study 301. However, the primary endpoint of the pivotal study (SARA score) was not assessed in phase II study 201 as primary endpoint. Thus, the consistency in data cannot be established. In addition, SARA score did not reach significancy in the extension phase of the Phase II Study 201. The mean SARA total score for visit 9 versus visit 7 was 1.82 (SD=3.09, median=1.50), which demonstrated a deterioration of cerebellar sign and

neurological symptoms under treatment. However, the results from the study Phase II - IB1001-201 should be interpreted with caution considering the design of the study (non-randomised, rater-blinded, single arm, open label study with the different setting of primary and secondary endpoints in comparison to the pivotal study IB1001-301).

The Applicant also provided data from the extension phase of the pivotal study 301 with a cut-off date of 31 January 2025 (point at which all patients who entered after participation in the Parent Study had either completed Visit 9 or discontinued).

The data presented are based on unclean data without source document verification. This precludes a definitive conclusion regarding the long-term efficacy for NALL in NPC patients as currently proposed by the Applicant. However, preliminary results of the 5- / 15- domain NPC-CSS and the SARA suggest a slowing of disease progression with IB1001 treatment compared to what would be expected based on historical comparisons with patients receiving standard of care (with or without miglustat).

Preliminary safety data from the IB1001-301 Extension Phase demonstrate the same benign safety profile observed to date, with no new safety signals. The safety data suggest no treatment related ADRs, although 75% of the subjects experienced a TEAE and there are at least 2 events per subject (i.e. 47 subjects (74.6%) with 136 events). Furthermore, the TEAEs from the PT class Gastrointestinal disorders are in general high in frequency (14 subjects (22.2%) with 21 events), but no relationship was established.

It was agreed that the applicant could submit a summary of the preliminary results obtained in this study for support of their claim of treatment beyond 12 weeks. The Applicant committed to provide the study report of the extension phase data from the pivotal Study IB1001-301 to support the long-term efficacy and safety of levacetylleucine (MEA), which is due by 31 March 2029. This was considered acceptable.

On a safety aspect, two-third of patients who received levacetylleucine (n=55/84) experienced adverse events but only the adverse event of flatulence has been related to levacetylleucine treatment and is listed in the section 4.8 of the SmPC. No major adverse events have been identified.

However, although no important identified risk into the summary of safety concerns has been identified, in respect to liver enzymes changes and the uncertainties on levacetylleucine causality, the risk of hepatotoxicity is considered as an important potential risk in the summary of safety concerns of the RMP. There are also remaining issues concerning carcinogenicity and developmental toxicity and these issues have been added as safety concerns as important potential risks in the RMP.

3.7.2. Balance of benefits and risks

The main evidence on efficacy of levacetylleucine is derived from the interim result of an ongoing multicenter, randomized, double-blind, placebo-controlled, crossover study in 60 adults and paediatric patients aged ≥ 4 years old (Study IB1001-301). The duration of the treatment period during the cross-over was 12 weeks, which was considered limited for a product intended for chronic use. However, the Applicant submitted some preliminary data from the extension phase of the pivotal study 301 with a cut-off date of 31 January 2025 in support of the long-term use. No definitive conclusion can be drawn from these preliminary data, but the Applicant also committed to provide the study report of the extension phase data from the pivotal Study IB1001-301 to support the long-term efficacy and safety of levacetylleucine (MEA), which is due by 31 March 2029. This was considered acceptable. Overall, the conduct of a double-blinded RCT in a sufficient number of NPC disease patients is positively recognized.

Regarding the primary endpoint, the mean (SD) change from baseline of the Scale for the assessment and rating of ataxia (SARA) after treatment with levacetylleucine, was -1.97 (2.43) compared to -0.60 (2.39) after placebo treatment demonstrating a significant improvement. The study also showed that patients who received levacetylleucine followed by placebo which effectively is a washout from levacetylleucine had a significant worsening of symptoms when receiving placebo (difference in mean SARA total score = +1.55).

The initially claimed indication for Aqneursa was the following:

"Aqneursa is indicated in adults and children from birth for chronic treatment of Niemann-Pick Type C (NPC)".

The CHMP disagreed on several aspects of this indication, as described below:

Restriction to patients with neurological manifestations

Since the primary efficacy assessments of Studies IB1001-201 and IB1001-301 were for neurological signs and symptoms, e.g. neurological manifestations of NPC, the CHMP disagreed with a broad indication for chronic treatment of NPC, and requested the Applicant to reflect in the indication that the product is intended for the treatment of neurological manifestations of NPC disease. The Applicant agreed to modify the indication accordingly.

In response to the MO concerning the age limit and concomitant use with miglustat, the Applicant proposed the following indication:

"Aqneursa is indicated in combination with miglustat, or as a monotherapy in patients where miglustat is considered inappropriate, for the treatment of neurological manifestations of Niemann-Pick Type C (NPC) disease in adults and children weighing at least 20 kg."

Concomitant use with miglustat

The vast majority of patients (85%) in the pivotal study continued to receive miglustat throughout the study. This created a bias and a concern regarding the monotherapy indication and the Applicant was requested to consider a restriction to adjunctive therapy.

The Applicant argued that the restriction of the indication to adjunctive therapy is not appropriate. The Applicant's reasoning was based on results from *in vitro* and *in vivo* non clinical data showing an effect of levacetylleucine itself in a NPC mouse model, on the fact that the mechanisms of action of the two products are different, that patients were previously treated with miglustat and remained on a stable dose, results from a phase 2 study showing some efficacy of monotherapy in patients with GM2 gangliosidosis. The Applicant also argued that some patients did not tolerate miglustat due to adverse events.

However, the CHMP considered that the clinical evidence that efficacy is not dependent on the concomitant miglustat treatment is driven by very small subgroups of patients: 9 out of 51 in the pivotal study and 3 out of 29 in the phase 2 study and that further data are needed to be able to conclude on efficacy of levacetylleucine as monotherapy. The evidence provided by the Applicant was considered not comprehensive to conclude on the combination therapy or monotherapy with NALL (first line treatment in the NPC patients).

However, the argument regarding the similar efficacy (SARA score) in 15% of patients (N=9) who were not on miglustat prior to randomisation and during the trial involved in the pivotal study might be valid but the study was not powered to show this. A trend in the efficacy of Aqneursa in monotherapy might be supported, however considering the low number of patients, the data supports rather an assumption. Besides these issues, a supportive study (Study IB1001-201) conducted by the Applicant with an almost identical population indicated worsening of ataxia based on the SARA score. The efficacy (based on the

SARA score) was only formally supported by the double-blind part of the clinical programme in the pivotal study IB1001-301.

Even if the data are limited, the possibility to use Aqneursa as monotherapy in patients who cannot tolerate miglustat could be considered acceptable but as the term 'inappropriate' was not considered be precise enough to guide the prescriber, the CHMP requested to replace it with "not tolerated".

The Applicant agreed to modify the indication proposing use "*in combination with miglustat, or as a monotherapy in patients where miglustat is not tolerated*".

Restriction to children aged 6 years and older and weighing at least 20 kg.

Based on the available data from studies IB1001-201 and IB1001-301, only PK data from children aged 6 to 12 years and weighing at least 20 kg were available. No PK data from children aged below 6 years have not been submitted as part of this application, whereas children of at least 4 years were planned to be enrolled. Consequently, from a clinical PK perspective the restriction of children aged 6 years and weighing at least 20 kg is justified and levacetylleucine PK is considered unknown below these age/weight limits.

The Applicant accepted to restrict the indication to children weighing at least 20 kg but initially did not accept the age restriction of 6 years arguing that age was not found to be a significant covariate influencing the PK in the PopPK model.

However, according to the PK data provided, the CHMP considered that it is expected that age influences levacetylleucine PK and the CHMP reiterated their request to limit the use of Aqneursa to patients aged 6 years and older.

Following the oral explanation, the Applicant proposed a new indication that limits the use of Aqneursa to patients aged 6 years and older: *"Aqneursa is indicated in combination with miglustat, or as a monotherapy in patients where miglustat is not tolerated, for the treatment of neurological manifestations of Niemann-Pick type C (NPC) disease in adults and children aged 6 years and older and weighing at least 20 kg."*

The indication has been updated in line with the CHMP requests, and in line with the data from the clinical studies (Phase II and ongoing Phase III Study). This indication is acceptable.

The Applicant has demonstrated a significant improvement in the Scale for the assessment and rating of ataxia (SARA) after treatment with levacetylleucine in patients with NPC aged 6 years and older. The improvements seen in Study IB1001-301 are considered clinically relevant.

In terms of safety, the list of adverse drug reactions for the section 4.8 of the SmPC is limited to the adverse drug reaction of flatulence. Two-third of patients who received levacetylleucine (n=55/84) experienced adverse events but only the adverse event of flatulence has been related to levacetylleucine treatment and is listed in the section 4.8 of the SmPC. No major adverse events have been identified. The safety profile is considered to be manageable.

The Applicant committed to provide the study report of the extension phase data from the pivotal Study IB1001-301 to provide additional support of the long-term efficacy and safety of levacetylleucine, which is considered acceptable.

Overall, based on the submitted data, a positive benefit-risk can be concluded for the newly proposed indication.

3.7.3. Additional considerations on the benefit-risk balance

Input from patient organisations

Patient organisations are also involved in the procedure to share their perspective, experience and concerns about their conditions. The input has been obtained from the International Niemann-Pick Disease Alliance (INPDA) who provide collaborative forum for the sharing of information and experience regarding ASMD Niemann-Pick disease (types A, B and A/B) and Niemann-Pick type C (NPC) in term of best practice in care and support, the provision and information about research. There is no prospect of a curative therapy for NPC. Miglustat is recommended as the standard of care for NPC patients in many countries to slow the progression of NPC symptoms. However, some patients are unable to tolerate it due to side effects including peripheral neuropathy and severe diarrhoea / GI issues, and on its own is not enough to address the high level of unmet medical need in this patient community. If miglustat is not tolerated or deemed clinically appropriate, for these patients is the only option supportive care to relief symptoms and management of complications. A diagnosis of NPC is challenging for many reasons, including the rarity of the disease, which means most people, including non-expert health professionals, have no knowledge or clinical experience of it. Due to high degree of heterogeneity, it has been widely acknowledged that therapeutic approach to NPC require a combination of therapies, available to support patients at different stages of progression, with one therapeutic solution not being appropriate for all patients and that does not address all aspect of the disease, there remains significant unmet medical need for patient living with NPC.

NPC has a significant impact on the quality of life of patients and their family members, with numerous disabling symptoms including ataxia, dysphagia, dysarthria, gait imbalance, and cognitive deterioration. The loss of the ability to walk, dress, write, speak and eat has devastating consequences on quality of life, and imposes a tremendous burden on patients and families. Patients and carers report difficulties with fine motor skills, seizures. loss of swallow and problems with nutrition, hydration and administering of medication, loss of speech and mobility, loss of cognitive functions leading to the anxiety, stress and depressions and range of practical, emotional and psychological issues.

Disease stability, or slowing of progression, is widely seen as a meaningful and important outcome for patients. This is reflected in the opinion of expert clinicians, who recognize that whilst symptom reversal is unlikely, an achievable goal is to slow or halt progression. This would have a life changing impact for patients and their families, bringing better quality and quantity of life.

In addition, successful pregnancies have been reported in women with NPC, in many cases before diagnosis was made. For NPC patient at child-bearing potential is recommended birth control (miglustat).

Patients and carers often report exasperation in their efforts to get the right diagnosis, with their diagnostic journey stretching over many years. This in turn leads to delays in accessing expert care, practical support and symptomatic treatments, as well as impacting family planning decisions.

Patients and carers also report difficulties in accessing expert clinical care, with centres often located far from their home. This is especially so when the disease is more progressed and their burden of disease greater, or when there are family and/or financial constraints.

3.8. Conclusions

The overall benefit/risk balance of Aqneursa is positive.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Aqneursa is not similar to Xenpozyme within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000. See Appendix on Similarity.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Aqneursa is favourable in the following indication(s):

Aqneursa is indicated in combination with miglustat, or as a monotherapy in patients where miglustat is not tolerated, for the treatment of neurological manifestations of Niemann-Pick type C (NPC) disease in adults and children aged 6 years and older and weighing at least 20 kg.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that levacetylleucine is not to be qualified as a new active substance in itself.

Furthermore, based on the review of data on the quality, non-clinical and clinical properties of the active substance, the CHMP considers that levacetylleucine shares the same therapeutic moiety at the site of the biological activity as N-acetyl-DL-leucine, a previously authorised active substance in the European Union, notably in France. The CHMP considers that levacetylleucine in comparison with the previously authorised N-acetyl-DL-leucine is not to be qualified as a new active substance as insufficient evidence has been provided to demonstrate that it differs significantly in properties with

regard to safety and/or efficacy from the previously authorised active substance.

Refer to Appendix on new active substance (NAS).

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0043/2024 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.

5. Re-examination of the CHMP opinion of 24 July 2025

Following the CHMP conclusion that levacetylleucine is not a new active substance (NAS) the Applicant submitted detailed grounds for the re-examination of the CHMP recommendation to refuse the NAS request.

5.1. Detailed grounds for re-examination submitted by the Applicant

The Applicant presented their grounds for re-examination in writing and at an oral explanation.

CHMP Opinion and Applicant's Rationale for Re-examination

Throughout the initial assessment, the Applicant presented data from non-clinical and clinical studies showing what it argues to be compelling, clinically relevant differences between N-acetyl-L-leucine (NALL/levacetylleucine) and N-acetyl-DL-leucine (NADLL) and provided justification why it was not feasible to conduct head-to-head clinical trials in NPC patients (see summary below).

However, the CHMP final conclusion on NAS status of NALL was that ***"Based on the review of data on the quality, non-clinical and clinical properties of the active substance, the CHMP considers that levacetylleucine shares the same therapeutic moiety at the site of the biological activity as N-acetyl-DL-leucine, a previously authorised active substance in the European Union, notably in France. Furthermore, the CHMP considers that levacetylleucine in comparison with the previously authorised N-acetyl-DL-leucine is not to be qualified as a new active substance as insufficient evidence has been provided to demonstrate that it differs significantly in properties with regard to safety and/or efficacy from the previously authorised active substance."***

The Applicant strongly contests that there is insufficient evidence to demonstrate that NALL differs significantly in properties of safety and/or efficacy from NADLL.

First, the Applicant argues that there are compelling controlled clinical trials with NADLL and NALL in closely related indications treated for the same duration and with the same endpoints that can be used to compare the efficacy of NADLL vs NALL.

An additional head-to-head clinical trial comparing NADLL with NALL in patients with NPC for the sole purpose of substantiating NAS status is not feasible and ethical to conduct because:

- Tanganil (the racemate, NADLL) is not approved for NPC (only approved in France for acute use in acute vertigo) and hence "*the reference active substance is not authorised for the proposed indication*."
- It is unethical to expose NPC patients to an inert and toxicologically negative compound (NADL), or to conduct a trial that would not offer additional benefit to patients but rather

simply further substantiate NAS status.

- The conduct of a study solely aimed at obtaining NAS status from the EMA would violate several ICH GCP guidelines, including Principle 1.1: "*The rights, safety, and well-being of the participants are the most important considerations and should prevail over the interests of science and society*," as well as Principles 1.3 and 1.4. ICH E6 Good clinical practice - Scientific guideline | European Medicines Agency (EMA).

Secondly, the Applicant argues that there is compelling comparative non-clinical data which sufficiently distinguishes the effects of NALL vs the effects of NADLL to justify the NAS status of the L-enantiomer (NALL). This includes non-clinical data demonstrating that there are significant differences in the pharmacokinetics and pharmacodynamics of the two enantiomers, as well as potential differences in the safety of the L-enantiomer (NALL) vs the D-enantiomer (NADL) (50% of the racemate), namely:

- The L- and D-enantiomers have substantially different pharmacological activities. In all pharmacodynamic studies, the (L-enantiomer (NALL) is the effective, active enantiomer, whereas the D-enantiomer (NADL) is inactive and, at times, antagonistic. Administering the racemate (NADLL) therefore, exposes patients to an impurity (NADL) at the level of 50% of the active constituent. NADL is not an excipient or solvent and therefore would be considered an impurity; an impurity of 50% would never be tolerated and would be a serious violation of ICH guidelines for drug substance or drug product impurities.
- When administered as the racemate (NADLL), the D-enantiomer (NADL) substantially alters the pharmacokinetics of the L-enantiomer (NALL), which significantly complicates the dosing when the racemate (NADLL) is administered versus the L-enantiomer (NALL).
- When administered as the racemate (NADLL), the D-enantiomer (NADL) competes with the uptake and transport of the active L-enantiomer (NALL), which suppresses and restricts the beneficial activity of the active constituent (NALL) and thus renders a less effective treatment when administered as a racemate.
- Administration of NADL (and thereby NADLL) is associated with acute and long-term intolerances and safety/toxicity concerns which do not occur when NALL is administered.
    - NALL is rapidly metabolized (cleavage of the acetic bond releasing acetate and L-leucine), whereas NADL is not, and instead accumulates in the gut/ gut bacteria (where it is eventually excreted, e.g. through faeces and urine). Compounds which accumulate in gut bacteria are known to cause gastrointestinal side effects and intolerances directly consistent with those reported with Tanganil use, e.g. gas, stomach pain, bloating, incontinence, diarrhoea, side effects which have not been reported with NALL.
        - The Summary of Product Characteristics (SmPC) for Tanganil (NADLL) states the undesirable effects (at a low dose of 1.5 to 2 g per day, for up to 6 weeks) as classified by MedDRA System organ class are: **Immune system disorders**: Hypersensitivity reactions, anaphylactoid shock, and laryngeal oedema; **Skin and subcutaneous tissue disorders**: Pruritus (sometimes associated with rash), erythema, and urticaria; **Gastrointestinal disorders:** Abdominal pain [Tanganil SmPC 2022].
        - In contrast, the undesirable effects of Aqneursa (NALL), as determined by the CHMP, is flatulence, a significantly better side-effect profile than Tanganil (NADLL).
    - Long-term administration of the D-enantiomer (NADL) may cause accumulation, with deleterious effects.

Regarding the racemate (NADLL), the Applicant is of the view that those differences between the two enantiomers provide compelling evidence to suggest that the L-enantiomer (NALL) has a superior efficacy, superior safety profile, and neater pharmacokinetics[2] when administered independently rather than as the racemate (NADLL). Finally, the Applicant remains concerned that failure to recognize the NAS status of NALL risks generating substantial clinical and regulatory confusion. Indeed, the Applicant considered that such a decision will be interpreted as NALL and NADLL being therapeutically equivalent/interchangeable for the treatment of NPC, which will inadvertently promote the chronic use of the racemate (NADLL) for patients with NPC although:

- There has been no formal investigation into the efficacy or safety of NADLL in controlled clinical trials for NPC or complete pharmacokinetic or toxicology studies.
- There has been no regulatory assessment of the benefit-risk profile of NADLL in the NPC indication or any other similar indication.

Non-clinical Studies

Pharmacokinetic, Transport, and Safety Studies

This section summarises the Applicant's views on findings from comparative non-clinical in vivo pharmacokinetic and transport studies. These preclinical differences are conclusive, demonstrating the significant differences between the L-enantiomer (NALL) and the D-enantiomer (NADL) as well as how the co-administration of the isomers (as the racemate – NADLL) negatively impacts (is antagonistic to) the active L-enantiomer (NALL) and potentially contributes to off-target effects or toxicity.

Churchill et al. 2020: Pharmacokinetic Differences between NALL, NADL, and NADLL

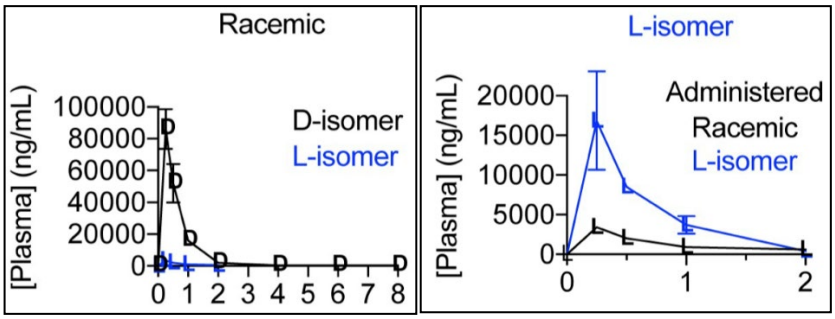
Churchill et al. 2020 conducted pharmacokinetic evaluations of NALL and NADLL, which revealed significant, unexpected and clinically relevant differences in the pharmacokinetics of both enantiomers after oral dosing. Those differences resulted in disproportionate total exposure (increase in the AUC) when the racemate (NADLL) is dosed, as NADL impedes the bioavailability of NALL.

NALL and NADLL were administered orally to mice. Plasma and tissue samples were collected at predetermined time points (0.25 to 8 h), quantified with liquid chromatography/mass spectrometry, and pharmacokinetic constants were calculated using a noncompartmental model. When the racemate (NADLL) was administered, both the maximum plasma concentration (C_{max}) and the area under the plasma drug concentration over time curve (AUC) were much greater for the D-enantiomer (NADL) than for the L-enantiomer (NALL) (Table 1). When the L-enantiomer (NALL) was administered, 2- to 3-fold higher C_{max} and AUC plasma values were observed compared to the dose-normalised NALL values after administration of the racemate (NADLL) (Figure 2A, B, C).

This is claimed by the Applicant to demonstrate a negative effect of the D-enantiomer (NADL) on systemic plasma levels of the L-enantiomer (NALL) when the racemate (NADLL) is administered. Elimination (ke and $T_{1/2}$) was similar for both enantiomers. These results demonstrate inhibition of uptake via an intestinal carrier of the L-enantiomer (NALL) by the D-enantiomer (NADL) when administered as a racemate (NADLL). This is a 2.5-fold difference in bioavailability between the single L-enantiomer (NALL) and the racemate (NADLL).

A **B**

[2] In that the pharmacokinetic profiles of the isomers are different, and co-administration impacts properties such as dose linearity and exposure of the active L-enantiomer.



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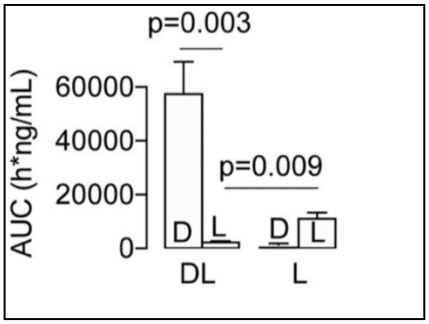


Figure 2: Plasma concentration of enantiomers versus time after administration of racemic N-acetyl-DL-leucine (NADLL) or purified N-acetyl-L-leucine (NALL) [Churchill et al. 2020]

A. Graph of plasma concentration of enantiomers versus time after administration of racemic N-acetyl-DL-leucine. Linear-linear plot. Values are the mean ± standard error of the mean with n = 3 (mice).

B. Replot of the data to facilitate direct comparison of the plasma concentration of N-acetyl-leucine enantiomers after oral administration of racemic N-acetyl-DL-leucine. Linear-linear plot. Values are the mean ± standard error of the mean with n = 3 (mice). C. Bar charts showing the pharmacokinetic parameters for the enantiomers of N-acetyl-L-leucine after administration of racemic N-acetyl-DL-leucine (denoted as DL) or N-acetyl-L-leucine (denoted as L). AUC calculated from the plasma concentration of drug. Values are the mean ± standard error of the mean with n = 3 (mice). Means were statistically analysed by pre-planned t tests. The means compared are indicated by the horizontal lines on the charts, and exact p values are provided for the comparisons.

Table 7: Calculated pharmacokinetic parameters for NADL and NALL plasma after oral administration of NADLL or NALL at 100 mg/kg

Compound Administered	**NADLL**		**NALL**	
Compound Qualified	**NADL**	**NALL**	**NADL**	**NALL**
Parmeter, unit				
r^2, -	0.91	0.93	0.85	0.72
k_e, -	2.2	2.8	1.7	2.4
T_{max}, h	<0.25	<0.25	<0.25	<0.25
C_{max}, ng/mL	86 100	3410	436	16 800
T_{last}, h	8.00	2.00	8.00	6.00
$C_{last,}$ ng/mL	247	623	16.2	168
$T_{1/2}$, h	0.31	0.4	0.25	0.29

Compound Administered	NADLL		NALL	
$AUC_{0\text{-last}}$, h x ng/mL	57 800	2 560	573	11 400
Ratio C_{max} L/D [#], -	0.04	-	38.5	-
Ratio AUC L/D [#], -	0.04	-	19.8	-

Churchill et al. 2020.

[#]The ratio of corresponding value between L and D enantiomers

In regard to the Churchill et al. 2020 data, the CHMP considered in the initial assessment of the application: ***"Purely phenomenological pharmacokinetic observations (e.g., competition for uptake) are not considered sufficient to rationalize development of only one enantiomer unless this enantiomer was proven to provide a benefit/risk-ratio superior to the counter-enantiomer or the racemate."***

First, the Applicant is confused by this comment. The Churchill et al. (2020) data are quantitative in vivo pharmacokinetic results that directly compare NADL, NALL, and NADLL and lead to concrete conclusions demonstrating the superior benefit/risk profile of NALL versus NADLL. The pharmacokinetic data are not descriptive observations but rather provide mechanistic/causal results that demonstrate the negative/antagonistic PK impacts of NADL on NALL when the racemate (NADLL) is administered, and are highly relevant for development, per FDA and EMA regulations.

Second, the major finding from Churchill et al 2020 was that the enantiomers of NADLL exhibit large, unexpected differences in pharmacokinetics due to both unique handling and/or inhibition of uptake of the L-enantiomer (NALL) by the D-enantiomer (NADL). The different pharmacokinetics of the enantiomers result in disproportionate total exposure (increase in the AUC) to the inert D-enantiomer (NADL) when the racemate (NADLL) is dosed, as the L-enantiomer (NALL) would be eliminated much faster. This finding is highly relevant for clinical dosing. A 2.5-fold difference in bioavailability between the single L-enantiomer (NALL) and the racemate (NADLL) obviously provided a manifest superior benefit/risk ratio for NALL versus NADLL, and a sufficient reason to develop the L-enantiomer (NALL) rather than the racemate (NADLL).

Third, the Applicant considered that it submitted compelling justification of the superior efficacy of the active L-enantiomer, and the inert/antagonist effects of the D-enantiomer. These pharmacokinetic studies demonstrated that the administration of NADL as part of the racemate supresses exposure to the active L-enantiomer (NALL), thereby reducing its efficacy. This contributes to a benefit/risk-ratio of NALL administered independently superior to the benefit/risk ratios of NADL and the racemate.

Thus, the Applicant was of the view that the CHMP opinion is not reflective of the data package presented.

Churchill et al. 2021: Uptake and Metabolism Differences between NALL, NADL, and NADLL

Churchill et al. 2021 demonstrated that adding an acetyl group to the nitrogen of the amino acid leucine (NADLL, NALL, NADL) converts the resulting molecules into substrates for the monocarboxylate transporters (MCTs), bypassing the leucine transporter (LAT). The Applicant considered that it was demonstrated that the interactions between the enantiomers of NADLL are competitive, and that the D-enantiomer interferes with the pharmacokinetics and metabolism of the L-enantiomer [see also Churchill et al. 2020].

The D-enantiomer (NADL) and the L-enantiomer (NALL) were tested in HEK-293 cells overexpressing the MCT1 transporter. The D-enantiomer (NADL) binds with greater affinity and is transported with

lower capacity than the L-enantiomer (NALL), so that when a racemate (NADLL) is administered, the D-enantiomer (NADL) competes with the pharmacologically active L-enantiomer (NALL) and greatly inhibits its uptake and distribution in target tissues. Moreover, the D-enantiomer (NADL) also competes with the acylase enzyme that converts the prodrug (NALL) to the intracellular active L-leucine. The net result is that when administered as the racemate (NADLL), the D-enantiomer (NADL) hampers the efficacy of the L-enantiomer (NALL) by interference with pharmacokinetic distribution and formation of the intracellularly active moiety (L-leucine).

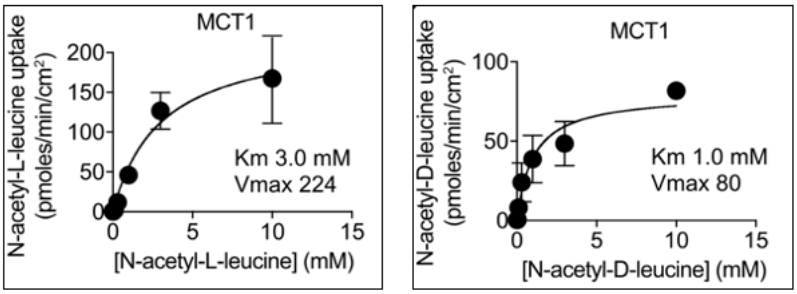
The extent of this interference is such that a dose of 4 g of NALL is equivalent to approximately 20 g of the racemate (NADLL). The dosage of NADLL and NALL that deliver equivalent amounts of plasma NALL can be calculated from pharmacokinetic results and the kinetics of the monocarboxylate transporter [Churchill et al. 2020; Churchill et al. 2021].

- From the pharmacokinetic study: if there was no interference by the D-enantiomer (NADL), when dosing with pure L-enantiomer (NALL), one would expect twice as much L in the plasma when administering the same amount in terms of grams. However, the interference of the D-enantiomer with the L-enantiomer results in a 5-fold difference in the dose proportionality. Dividing the expected ratio of 2 by the measured ratio of 5 gives 2.5, meaning that 2.5-fold more L-enantiomer was orally bioavailable when given as pure L-enantiomer than when given as the racemate. Or, inverting this, 1 divided by 2.5 gives 0.4, meaning that bioavailability was 40% of that expected with no interference from the D-enantiomer (Figure 3A, B, C, D).
- From measuring uptake by the monocarboxylate transporter [Churchill et al. 2021]: the relative occupancy of D-enantiomer or L-enantiomer at the transporter can be estimated based on the measured affinities using the equation below, where V_0 is the velocity of transport, V_{max} is the maximum transport, S is the concentration of the substrate (L-enantiomer in this case, and I is the concentration of the inhibitor (D-enantiomer).

$$V_0 = \frac{V_{\max}[\mathrm{S}]}{K_m\left(1+\frac{[\mathrm{I}]}{K_i}\right)+[\mathrm{S}]}$$

An example scenario taking the L-enantiomer at its K_m (3 mM), V_{max} (224 pmol/min) results in 672/(3 +3) = 112 pmol/min. In presence of the D-enantiomer at 3 mM (as would be in the racemate) and plugging in its K_m (1 mM), the scaling factor is (1+I/Ki) or 1 + 3/1 = 4, so V_0 = 45 pmol/min, giving a relative rate of 45/112 = 0.4 or 40%.

A **B**



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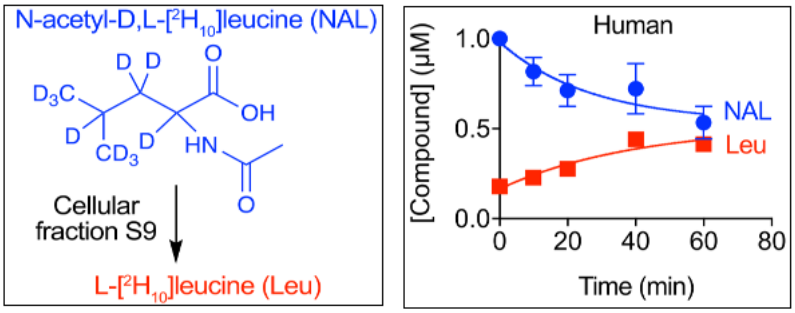


Figure 3: Both enantiomers of N-acetyl-leucine are transported by the monocarboxylate transporter (MCT1) but only the l-enantiomer is metabolized. [Churchill et al. 2021]

A. N-acetyl-l-leucine concentration-inhibition curves for the inhibition of uptake of the known substrate

B. N-acetyl-d-leucine concentration-inhibition curves for the inhibition of uptake of the known substrate

C. Chemical structure of deuterated N-acetyl-d,l-leucine incubated with cellular fraction S9 from liver to determine metabolism using liquid chromatography and mass spectrometry.

D. Time courses for loss of deuterated N-acetyl-d,l-leucine (1 µM initial concentration) and appearance of deuterated L-leucine for extracts derived from human livers. Data are colour-coded according to the chemical structures and names shown in e with deuterated N-acetyl-d,l-leucine in blue and deuterated l-leucine in red.

The CHMP considered that ***"The works by Churchill et al. 2020 and 2021, which demonstrate that NADL blocks absorption of NALL, is acknowledged but provide no immediate reason for the use of purified NALL."***

The Applicant strongly contests this as explained below.

1. The L-enantiomer (NALL) is the pharmacologically active enantiomer, while the D-enantiomer (NADL) is inert/ at times antagonistic. When the racemate (NADLL) is administered, the D-enantiomer (NADL) blocks the cellular uptake of the active L-enantiomer (NALL) via competitive inhibition of the transport monocarboxylate transporter 1 [MCT1]).

When administered as the racemate (NADLL), the inert D-enantiomer (NADL) was present at a much higher C_{max} and AUC relative to the active L-enantiomer (NALL), resulting in greater total exposure. When administered as purified N-acetyl-L-leucine (NALL), both the C_{max} and the AUC for NALL were higher compared to administration as the racemate (NADLL), even when scaled for the relative dose.

2. At the same time, the D-enantiomer (NADL) also interferes with the inner-cellular metabolism of the active L-enantiomer (NALL) (formation of the pharmacologically active L-leucine by cleavage of NALL to acetate and L-leucine) by interference with acylase, therefore further reducing its pharmacological activity.

The extent of this interference is such that a dose of 4 g of NALL would require approximately 20 g of the racemate (NADLL) to expose patients to 4 g of the active L-enantiomer (NALL). However, this does not mean that administering 20 g of the racemate would result in the same therapeutic effect as administering 4 g of the L-enantiomer[3]. Administering 20 g of the racemate would result in substantially altered pharmacokinetics, off-target effect, and also expose patients to 10 g of the inert/antagonist NADL with expected detrimental effects:

[3] 20 g of Tanganil is 10-13 times the recommended daily dose (1.5 g – 2 g), and 5 times the maximum daily dosage (4 g) permitted for only up to 6 weeks by the French Authority [Tanganil SmPC 2022].

- Oral intake of D-amino acids at high amounts results in oxidative stress as endogenous D-amino acid oxidases (DAAOs) degrade D-amino acids via oxidative deamination and release of hydrogen peroxide [Pollegiono et al. 2007; Friedman & Levin, 2012; Roskjaer et al. 2024; Yap et al. 2024].
- D-amino acids are not metabolized, so they accumulate in tissues and may provoke intolerances (e.g., gastrointestinal issues such as stomach pain, diarrhoea, incontinence) and serious damage e.g., suppression of the synthesis of other essential enzymes and inhibition of the growth rate of the animals.

Therefore, the Applicant concludes that Churchill et al. 2020 and 2021 provide conclusive evidence that administration of the racemate results in significant changes to the active L-enantiomer (NALL) at clinically relevant doses and do provide an immediate reason for the use of NALL versus NADLL or NADL.

Churchill et al. 2020 and 2021 demonstrated that changes (i.e., increase) to the dosing frequency of the racemate (NADLL) would be required to theoretically achieve exposure to the L-enantiomer (NALL) at 4 g/day (the dose demonstrated in all clinical trials to be the effective clinical dose)) due to the competition from the antagonistic D-enantiomer (NADL) which greatly inhibits NALL's uptake and distribution in target tissues (including CNS). Such an increase in dosing (1) cannot be guaranteed to have a therapeutic effect and (2) would be expected to result in clinically relevant changes that result in differences to contraindications, warnings or clinically significant adverse reactions at such a dose.

The EMA Reflection Paper on Enantiomers considers that the following may constitute a significant difference in safety and/ or efficacy to justify new active substance status:

- "Significant changes to the dosing frequency (e.g. bd to od) or another route of administration mandated by significant differences in safety and/or efficacy properties;
- Clinically relevant changes that result in differences to contraindications, warnings or clinically significant adverse reactions;"

Therefore, even assuming that 20 g NADLL has the same pharmacological effect as 4 g NALL (albeit the pharmacokinetics would be expected to be significantly altered, as well as off-target adverse effects), both the facts that different doses of NADLL and NALL (20 g NADLL vs 4 g NALL) would be required to have the same effect and that this difference in dosing would justify different contraindications, warnings or clinically significant adverse reactions, show a significant difference in safety and/ or efficacy between NALL and NADLL.

Churchill et al. 2020: Potential Toxicity Differences between NALL and NADLL due to NADLL

Another relevant finding from Churchill et al. 2020 was that the D-enantiomer lingered after single dosing and would accumulate, especially during chronic dosing. Unlike NALL, which is deacetylated and enters leucine pathways, the D-enantiomer is not deacetylated/metabolised and accumulates in the gut/ gut bacteria (and subsequently excreted, e.g., through faeces and urine). Compounds which accumulate in gut bacteria are known to cause gastrointestinal side effects and intolerances, e.g. gas, stomach pain, bloating, incontinence, diarrhoea [Zhang et al. 2015]. These same side effects have been directly reported by patients/ physicians monitoring Tanganil use.

Further, with long-term exposure (e.g. as required for the treatment of NPC), D-enantiomer accumulation could result in toxicity as reported for oral ingestion of D-amino acids (Figure 4) [D'Aniello et al. 1993; de Moraes 1987]. In contrast, the metabolism of the L-enantiomer (cleavage of the acetic bond releasing acetate and L-leucine) does not result in toxic metabolites. No long-term tissue retention of NALL or its endogenous metabolite resulting in local tissue reactions or other pathophysiological responses, is expected.

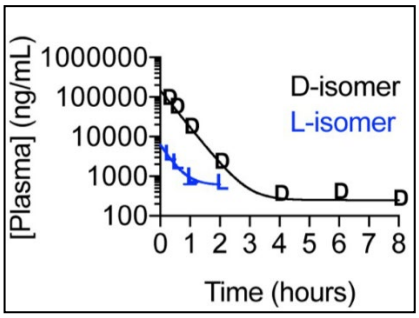


Figure **4***: Plasma concentration of enantiomers versus time after administration of racemic N-acetyl-DL-leucine [Churchill et al. 2020]*

Values are the mean ± standard error of the mean with n = 3 (mice).

The Applicant argued that the CHMP Opinion dismissed the concern of long-term toxicity with the D-enantiomer (NADL), considering that ***"the racemate has been widely administered off-label also to patients with NPC with a good safety profile".***

The Applicant acknowledges that the publications on the compassionate use clinical observational studies describe the racemate (NADLL) as being "well-tolerated". However, all those publications on the use of the racemate (NADLL) lack any systematic collection and documentation of adverse events, whether related or unrelated to study drug. The study reports also provide no reference to a safety monitoring plan, no data safety monitoring committee, and no routine collection or analysis of laboratory samples, physical examinations, vital signs, ECG recordings, or other clinical safety parameters. Therefore, these studies provide insufficient information to characterise the safety profile of NADLL or to even assume a lack of long-term toxicity.

The CHMP Opinion also considered that ***"Moreover, no direct evidence was provided for any long-term toxicity of N-acetyl-D-leucine"***. The Applicant does not agree.

The findings from Churchill et al. 2021 are directly consistent with a large body of literature that shows that D-amino acids accumulate in certain tissues, causing intolerances such as gastrointestinal side effects [Zhang et al. 2015] and serious damage such as suppression of the synthesis of glutamate oxaloacetate transaminase, glutamic pyruvic transaminase, and lactate dehydrogenase (Bardaweel et al. 2013). D'Aniello et al. concluded that: "If ingested, D-amino acids are not metabolized, they will accumulate in tissues and may provoke serious damage e.g. suppression of the synthesis of other essential enzymes and inhibition of the growth rate of the animals" (1993).

Studies directly comparing D-, DL-, and L- amino acids on growth and development show an inhibition of the synthesis of the enzymes glutamate oxaloacetate transaminase, glutamic pyruvic transaminase, and lactate dehydrogenase and a suppression of the growth rate in these animals when D-amino acids were ingested (drinking water at 10 mM) to an amount that exceeded the ability of D-AAO to oxidize them in a relatively short time (D'Aniello et al. 1993; Yap et al. 2024).

Growth rate of chicks was dramatically affected by the stereoisomers of amino acids fed; DL vs L amino acids in chick diet reduced growth by 2/3 compared to the growth of the L-only diet, with equal amounts of L-amino acids (Table 2, De Moraes et al. 1987). The D-isomer interfered with normal chick growth; "These results lend further evidence to toxicity of D-amino acids producing the growth depression observed with the DL-AA diet"

In contrast, L-amino acids are substrates and are metabolically cleared, and exhibit none of the deleterious effects on growth as the racemate amino acids (DL) or D.

No addition long-term toxicity studies with NADL or the racemate have been conducted because:

- the findings from Churchill et al. were directly supportive of all available literature on the accumulation of D amino acids, further reinforcing why the single active L-enantiomer (NALL), and not the racemate (NADLL), should be developed.
    - The Applicant is not developing the racemate (NADLL) due to the compelling, consistent body of evidence that demonstrates a superior benefit/risk profile of NALL over the racemate (NADLL) and thus strongly precludes development of the D-enantiomer (NADL) in any form (single enantiomer (NADL) or as a component of the racemate (NADLL).
    - The Applicant **was** responsible for conducting studies evaluating whether the racemate (NADLL) or an individual enantiomer (NALL or NADL) should be pursued. Once such evidence was established, the Applicant was not required to conduct a full battery of non-clinical studies with the enantiomer (NADL) and racemate (NADLL) that the Applicant is not developing.
    - Such studies (for a drug candidate not under, or planned to be under, development) would be costly, highly problematic in light of the 3R principle (e.g. conducting animal studies with a compound which is believed to have negative effects and, in addition, is never intended to be developed for humans, simply to prove that the animals indeed experienced negative effects), and useless since they do not inform the benefit risk profile of the drug intended to be marketed for patients with NPC (NALL).
- the recommended dose of Tanganil (NADLL) is 1.5-2 g daily, and the maximum recommended dose is 3-4 g daily. The recommended maximum duration of intake is 5-6 weeks. [Tanganil SmPC, 2022]. Accordingly, the marketing authorisation holder of Tanganil has not conducted any long-term toxicity studies or long-term clinical studies because this medicinal product is specifically not intended, or safely supported, for chronic use. The safety of NADLL has only been established for acute use.

Summary

The Applicant claims that its findings that NADL accumulates/ is not metabolised and will further accumulate with chronic dosing (including when administered as a component of the racemate (NADLL)) are directly consistent with the literature, reinforcing that NADL would be expected to (1) cause gastrointestinal intolerances such as gas, stomach pain, bloating, incontinence, diarrhoea as has been repeatedly reported during Tanganil use and (2) inhibit many enzymes, with predictably deleterious consequences [Zhang et al. 2015; Khoronenkova et al. 2008; Zhang et al. 2019]. For the reasons explained above, the Applicant does not agree with the CHMP's implied suggestion to conduct an additional study to demonstrate the long-term toxicity of NADL.

In the absence of additional GLP/GCP long-term safety studies (clinical or non-clinical) with the D-enantiomer (NADL) or the racemate (NADLL), the potential off-target effects or toxicity of the D-enantiomer (NADL) are said by the Applicant to be highly relevant because not only they contribute to the unanimous body of evidence which supported the development of the independent L-enantiomer (NALL) without the potentially harmful, inert D-enantiomer (NADL) in accordance with FDA and EMA requirements but also they currently are the only scientific data on potential safety of long term use of NADLL.

Non-clinical Pharmacology Studies

This section summarises findings from non-clinical comparative *in vitro* and *in vivo* pharmacology studies in relevant models of NPC and vestibular compensation. The Applicant considered that these preclinical differences are conclusive, demonstrating the positive pharmacological effects of the

isolated NALL and the inactivity of NADL, with at times antagonistic effects of NADL on NALL when administered as a racemate.

***In Vitro* Findings: Mechanism of Action**

In vitro studies from University of Oxford elucidating the mechanism of action for NALL show that NALL but not NADL or NADLL correct fundamental cellular defects of neurodegenerative disorders, including NPC.

Lysosome Calcium Ion (Ca2+) Levels

Lysosomes are calcium stores, and this calcium can be released by *Nicotinic acid adenine dinucleotide phosphate (NAADP*), a Ca^{2+}-mobilising intracellular messenger, evoking calcium signals that control specific cellular responses [Churchill et al. 2020]. Lysosomal calcium levels and calcium signalling are fundamental regulators of cellular processes including intracellular trafficking, membrane fusion, autophagy, transcription factor regulation, inflammasome priming and membrane excitability [Churchill et al. 2020]. Impaired lysosomal calcium levels have been identified in recent years to be critical to the pathophysiology of many neurodegenerative disorders [Feng and Yang 2016]. Deficits in lysosomal Ca^{2+} have been reported in genetic models of Parkinson's [Kilpatrick et al. 2016: GBA mutation] and Alzheimer's disease [Coen et al. 2012: PSEN mutation].

In 2008, Lloyd-Evans et al. showed NPC was associated with reduced levels of calcium in its lysosomes and impaired NAADP-evoked calcium release [Lloyd-Evans et al. 2008]. These cellular defects were shown to be fundamental to the aetiology of NPC, given lysosomal calcium release is a major regulator of vesicular fusion, trafficking, autophagy, inflammasome priming and other key cellular responses, which all go awry in NPC [Morgan et al. 2011].

The effects of NALL, NADL, and NADLL cellular treatment of NPC1-deficient cells (reflecting NPC disease) were assessed to determine whether they impact lysosomal calcium as a fundamental regulator of all these processes.

The *in vitro* study used human HeLa cells and compared wild-type cells and those in which the *NPC1* gene is knocked out (the cellular model for NPC disease). The calcium content of lysosomes was assessed with a combination of drug mimetics of NAADP and PI,3,5P2 (a coregulator of TPC2), termed TPC2-A1N and TPC2-A1P, which activate TPC2 channels and empty them of calcium [Gerndt et al. 2020]. The calcium fluxes from the lysosome were detected optically by use of a genetically-encoded calcium reporter functionally attached near the pore of the ion channel termed TPC2-G-GECO1.2 [Davis et al. 2020]. This gives an exquisitely selective report of calcium release *only* from lysosomes, only via TPC2, and not from other sources.

In wild-type HeLa cells, after 12-hours of treatment with NALL, there was a significant reduction in lysosomal calcium. This effect was specific to the NALL; NADL demonstrated no such effect (Figure 5).

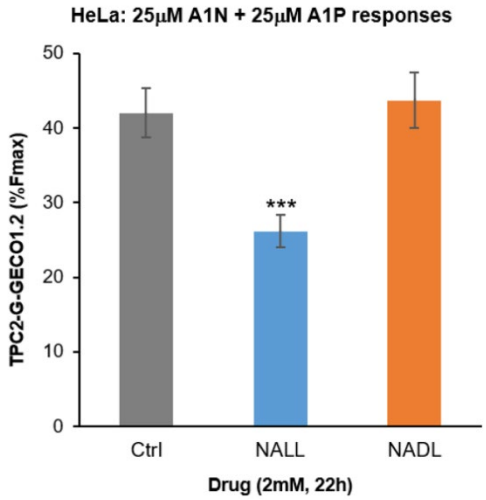


Figure 5: Levacetylleucine (NALL) treatment reduces lysosomal calcium in wild-type cells, but the D enantiomer (NADL) has no effect.

In *NPC1*$^{-/-}$ cells, earlier findings that there was significantly lower calcium in their lysosomes compared to wild-type control cells [Lloyd-Evans et al. 2008] were confirmed. However, after treatment of cells with NALL, the lysosomal calcium content normalised to higher levels, whereas NADLL treated cells had no effect, due to the presence of the antagonistic NADL (Figure 6).

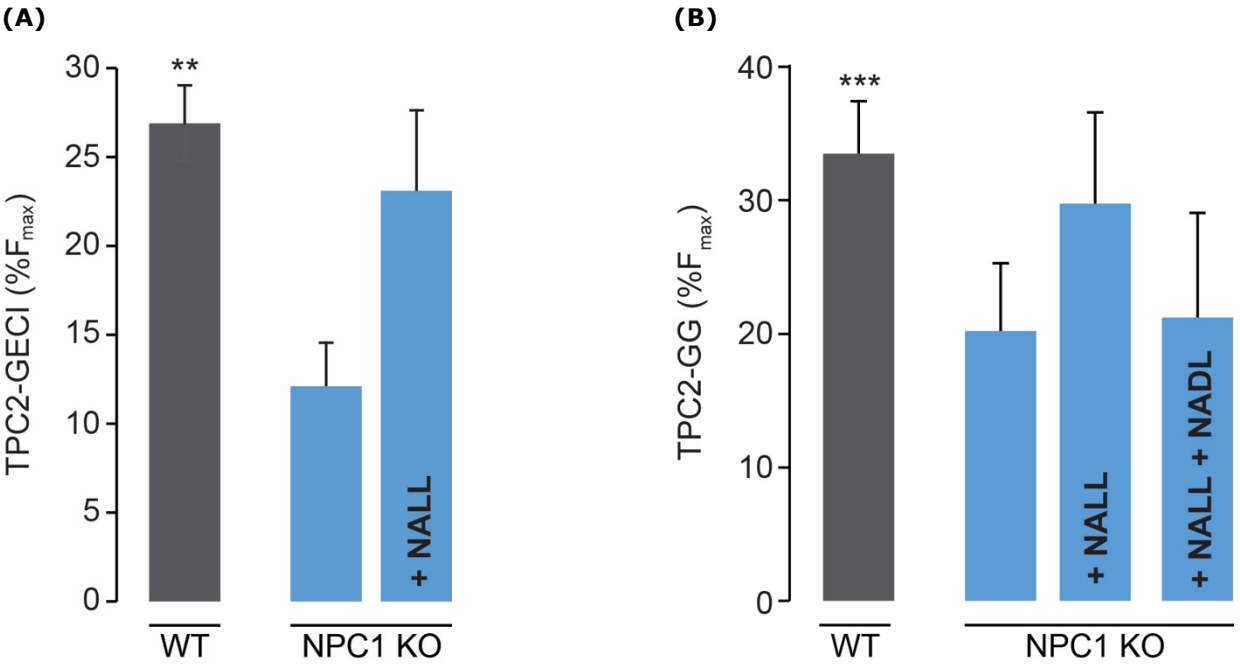


Figure 6: Impact of levacetylleucine (NALL) and racemate (NADLL) on lysosomal calcium

A. Lysosomal calcium content is significantly reduced in *NPC1*$^{-/-}$cells compared to wild type (middle blue bar). Notably, in NPC1$^{-/-}$ cells, levacetylleucine normalises lysosomal calcium by increasing it (right-hand blue bar) to levels similar to that seen in basal wild type cells (left hand grey bar). Collated mean ± SEM of the maximum Ca^{2+} amplitude upon TPC2 activation with 25 mM TPC2-A1-N and 25 mM TPC2-A1-P in HeLa (WT and NPC1 CRISPR-KO) in Ca^{2+}-free medium, detected using TPC2-G-GECO1.2. NPC1 KO HeLa were treated with 2 mM NALL in DMEM + 10% FCS for 22 hours at 37°C. Data are normalized to

the maximum G-GECO1.2 (F_{max}) response to 1 mM ionomycin plus 10 mM $CaCl_2$.

B. Levacetylleucine (NALL) treatment reduces lysosomal calcium in wild-type cells, but the racemate (NALL+NADL) has no effect. A smaller TPC2 Ca^{2+} nanodomain in NPC1 KO reflecting lower lysosomal Ca^{2+} storage may be restored by NALL but not racemate. Collated mean ± SEM of the maximum Ca^{2+} amplitude upon TPC2 activation with 25 mM TPC2-A1-N and 25 mM TPC2-A1-P in HeLa (WT and NPC1 CRISPR-KO) in Ca^{2+}-free medium, detected using TPC2-G-GECO1.2. NPC1 KO HeLa were treated with 2 mM NALL, or 2 mM NALL/ NADL racemic mixture, in DMEM + 10% FCS for 22 hours at 37°C. Data are normalized to the maximum G-GECO1.2 (F_{max}) response to 1 mM ionomycin plus 10 mM $CaCl_2$.

The findings that NALL normalizes lysosomal calcium ion (Ca2+) levels, but NADL and NADLL have no such effect, account for why the Applicant argues that NALL has beneficial therapeutic consequences such as the translocation of TFEB (see below) whereas NADL/ NADLL do not.

Transcription factor EB (TFEB) pathway

The Applicant claims that in vitro studies at the University of Oxford have further identified that NALL, but not NADL or NADLL, activates the Transcription factor EB (TFEB) pathway, resulting in robust translocation of TFEB into the nucleus.

TFEB orchestrates the expression of genes involved in lysosomal biogenesis and is one of the main transcriptional regulators of autophagy, as it promotes the expression of genes required for autophagosome formation, lysosome biogenesis, and lysosomal function [Napolitano and Ballabio, 2016]. It is also highly expressed in the central nervous system (CNS) [Cortes and La Spada, 2019].

TFEB contains basic helix-loop-helix-leucine zipper domains (bHLH-Zip) and belongs to the microphthalmia MIT-TFE family of transcription factors [Puertollano et al. 2018]. In its inactive state, TFEB resides in the cytoplasm; upon activation, it translocates to the nucleus to regulate gene expression.

Dysregulation of TFEB activity is implicated in various neurodegenerative and neurodevelopmental diseases. Activation of TFEB has been shown to correct pathological phenotypes in NPC models and proposed as a potential therapy to treatment NPC and other lysosomal storage disorders [Argüello et al. 2021]. Du et al. (2025) most recently demonstrated that overexpression of TFEB can mitigate cholesterol accumulation in NPC cells. Further, pharmacologically restored TFEB-nuclear translocation upregulated the expression of TFEB downstream genes, promoting lysosomal exocytosis and biogenesis and resulted in significantly increased cholesterol clearance in human and mouse NPC cell lines.

In the present study, HeLa cells were treated with NALL, NADL, or NADLL at different concentrations and visually scored as having nuclear localization if the nuclear abundance of TFEB exceeded those in the cytoplasm (Figure 7). NALL caused a robust translocation of TFEB into the nucleus, whereas the D-enantiomer (NADL) had no effect. The racemate NADLL also failed to activate TFEB translocation, indicating that the presence of the D-enantiomer (NADL) in the racemic mixture (NADLL) antagonizes the effect of the L-enantiomer (NALL).

NALL's mechanism of activation is therefore proposed to include activation of TFEB to upregulate lysosomal machinery and thereby promote the observed lipid and cholesterol clearance seen with NALL in the NPC model.

D-acetyl leucine does not activate the TFEB pathway and inhibits the action of levacetylleucine

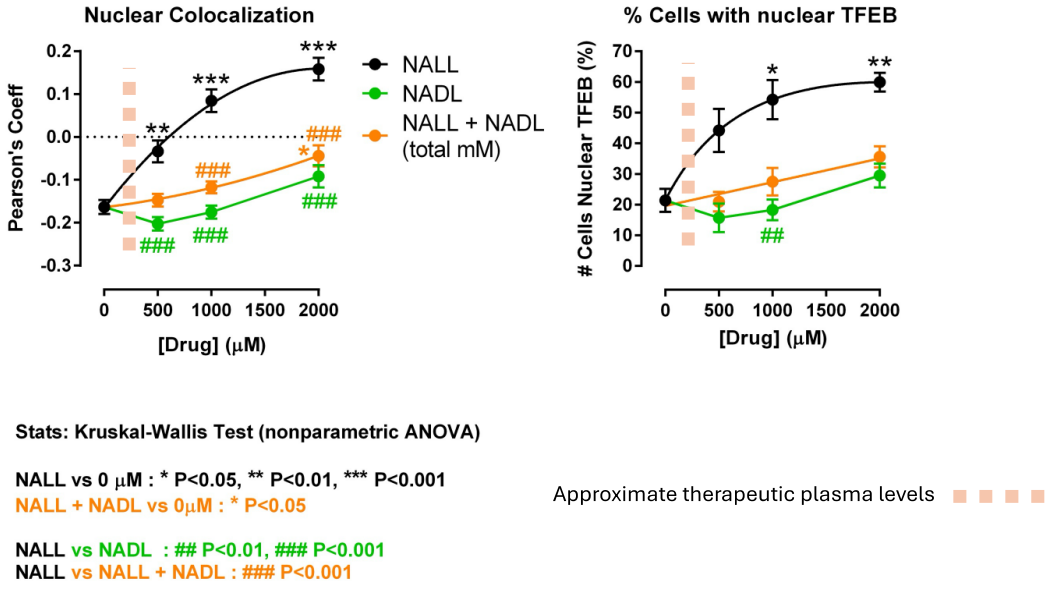


Figure 7: NALL, but not NADL, Activates Transcription factor EB

HeLa cells were treated with NALL and NADL and imaged using an Olympus IX71 microscope equipped with a 40Å~ UApo/340 objective. Cells were visually scored as having nuclear localization if the nuclear abundance of TFEB exceeded those in the cytoplasm.

The CHMP opinion considered that ***"The unpublished cell-culture data on the effect of NALL on the TFEB pathway is considered purely theoretical and premature to infer any in vivo or clinical relevance."***

The Applicant strongly contests this, as described below.

The elevation of transcription factor EB (TFEB) is an essential aspect of the pathophysiology of NPC. NPC is a lysosomal storage disease (LSD) characterised by abnormal cholesterol accumulation in lysosomes, impaired autophagy flux, and lysosomal dysfunction. The regulation of TFEB, a master lysosomal function regulator, reduces the accumulation of lysosomal substrates in LSDs where the degradative capacity of the cells is compromised [Argüello et al. 2021] and can dramatically ameliorate cholesterol accumulation in human and mouse NPC1 cell models. Thus, the regulation of TFEB is directly linked to NALL's ability to reduce the accumulation of substrates, including glycosphingolipid and cholesterol.

This finding is confirmed by the *in vitro* and *in vivo* studies with NALL, NADL, and NADLL that showed that NALL was significantly more effective at reducing the storage of these key substrates [te Vruchte et al. 2019]. This can be directly attributable to NALL's — but not NADLL or NADL — regulation of TFEB.

Therefore, the Applicant claims that these findings are not purely theoretical or too premature to infer in vivo or clinical relevance. To the contrary, they demonstrate a specific and beneficial effect in NPC patient fibroblasts. The Applicant does not agree that setting these studies aside in the NAS evaluation is justified.

In Vivo Findings: Niemann-Pick disease type C

In addition to the pharmacokinetic studies conducted to identify the optimal acetyl-leucine drug candidate (NALL, NADL, or NADLL) for development, in vivo studies were conducted in the mouse model of NPC to again determine if there were any benefits of administering either enantiomer independently, or the racemate [Kaya et al. 2021].

To directly compare the effects of treatment with NALL and NADL, *Npc1*$^{-/-}$ null mice were treated with daily doses of 0.1 mg/kg NALL or 0.1 mg/kg NADL, starting at an age of 3 weeks (at which point the *Npc1*$^{-/-}$ mouse is pre-symptomatic).

The major findings from this comparative in vivo study were that NALL, more than NADLL (and significantly more than NADL), improved the majority of parameters assessed, including both biochemical and functional assessments:

- Early treatment with NALL, but not NADL, slowed disease progression when treatment was initiated before symptom onset, consistent with a neuroprotective mechanism only with the L-enantiomer (NALL). NADL had no impact on survival, whereas NALL increased survival 200% more than the racemate (9.1% with NALL versus 4.5% with NADLL), which is consistent with NADL (50% of the racemate) impeding the efficacy of L-enantiomer (NALL)
- NALL helped maintain motor function and increased life span, whereas NADL had no effect (with the NADL-treated mice progressing in parallel trajectories to the untreated *Npc1*$^{-/-}$ controls). The L-enantiomer (NALL) is the neuroprotective isomer and thus has the following effects, whereas the D-enantiomer (NADL) has no effect:
    - to significantly delay the onset of functional decline (gait abnormalities, motor dysfunction) and the decline in general health and condition; and
    - to slow disease progression; and
    - to prolong survival.

The racemate (NADLL) was identified to have a therapeutic effect in a number of biochemical and functional parameters; however, it is argued by the Applicant that it was only NALL that consistently positively influenced all key outcomes.

Moreover, this in vivo study showed again that NADL was largely inactive, with no therapeutic benefit.

Based on the findings from Kaya et al. 2021 studies, the Applicant claims that:

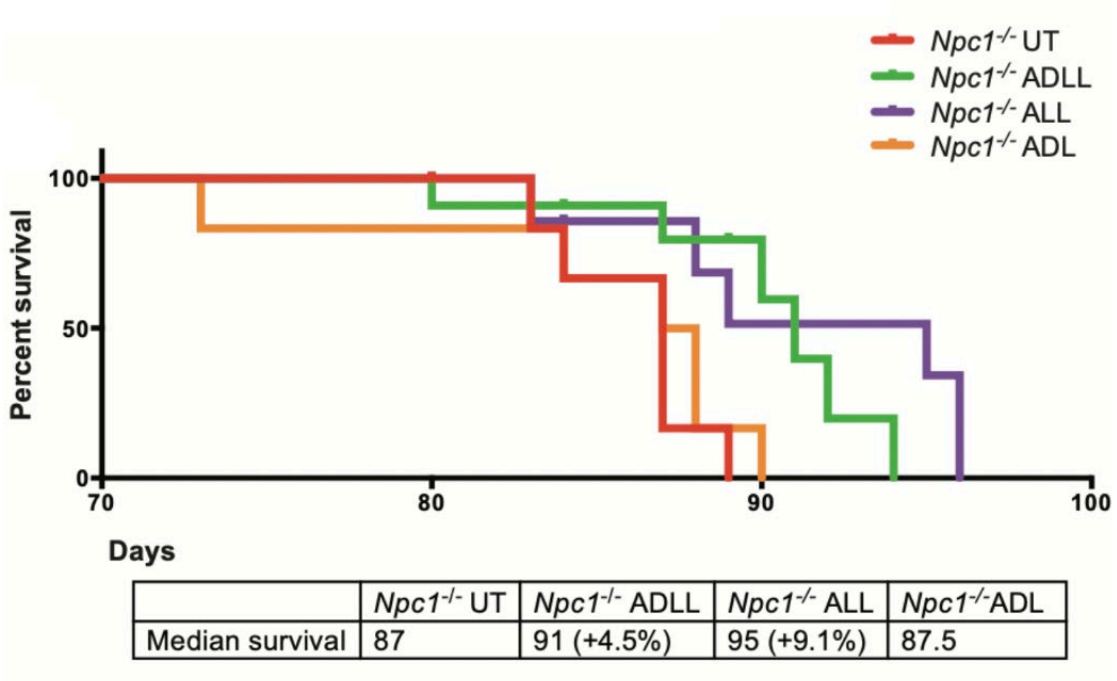
"The In vivo findings in the NPC mice with NALL, NADL, and NADLL strongly reinforce the use of NALL as a monotherapy. Looking at the totality of the data across all parameters, NALL was the maximally effective compound, and unequivocally the isomer where the activity of the disease-modifying, neuroprotective effects lie. For the long-term treatment, NADL can be considered at best an inert impurity. The findings from this study provide no reason, benefit, or rational to expose patients to the D-enantiomer, including as a racemate".

In regard to the *Kaya et al. 2021 data*, the CHMP Opinion disputed the overall findings on the grounds that the differences between NALL and NADLL would be negligible or that the parameter chosen by the Applicant would not be meaningful or that another parameter would negate the finding.

First, the Applicant contends the CHMP position and maintains its concern that the data is being selectively emphasized to discredit legitimate scientific findings. In reaching this conclusion, it is said that the CHMP highlighted four individual parameters (out of dozens presented in the publication) where the data were not conclusive of NALL being superiorly efficacious to NALL or NADL based on the p-values (including PDH-E levels, SOD2 levels, Purkinje cell loss).

The Applicant again wishes to emphasize that it is admittedly not known which of the cellular parameters examined in the NPC mice directly impact functional outcomes or survival. For this reason, the importance of individual parameters cannot be overemphasised and rather the data must be viewed in totality. It is the Applicant's view that, in its totality, the study clearly shows that on the majority of the parameters, NALL is superiorly efficacious.

Second, and critically, the finding from Kaya et al. 2021 was that only the L-enantiomer mediates a disease-modifying effect, causing a survival advantage, which is claimed to be relevant to clinical benefit in patients. When the racemate (NADLL) was administered, there was an improvement in survival due to the presence of the L-enantiomer (NALL), but this improvement was only half of the improvement noted when the L-enantiomer (NALL) was administered independently (Figure 8). The D-enantiomer (NADL) had no impact on survival (consistent with the racemate (NADLL) having half the impact on survival as the independent L-enantiomer (NALL).



	Npc1-/- UT	Npc1-/- ADLL	Npc1-/- ALL	Npc1-/-ADL
Median survival	87	91 (+4.5%)	95 (+9.1%)	87.5

Figure 8: AL analogues impact on Survival in NPC1 mice

For wild-type untreated (Npc1þ/þ UT), NPC1 untreated (Npc1 / UT), ADLL (Npc1 / ADLL), ALL (Npc1 / ALL), ADL (Npc1 / ADL) treatments minimum five, maximum seven animals for each group. Life expectancy percentages and median survivals (Gehan–Breslow–Wilcoxon test), n= 6

When reviewing this critical, clinically relevant parameter (survival), the CHMP commented: "NALL treatment but not NADL significantly increased survival: Although the effect for levacetylleucine (NALL) appears larger, the effect for NADLL was observed with even higher statistical certainty (p = 0.0334 for NALL vs. p = 0.0305 for NADLL)."

The Applicant contends this attempt to bias the interpretation of the data, relying on the p-value (which is simply a reflection of the spread of the data and thus does not speak to clinical benefit) instead of the number of days/ % change in survival. Kaya et al. 2021 demonstrated that NALL increases lifespan 200% to the racemate, a clear indication that the D-enantiomer (NADL) is negatively impacting survival in mice.

Irrespective of the precise mechanism, Kaya et al. 2021 established that the clinically relevant neuroprotective effects and increase in survival are driven by NALL; NADLL benefits from the activity of NALL, but the impact on survival is double with the L-enantiomer (NALL) when administered independently without the NADL.

Thirdly, as stated during the initial assessment, the Applicant does not claim that the Kaya et al. (2021) study demonstrated no beneficial effect of NADLL. It claims that the totality of the data robustly points – again - to the greatly superior efficacy of NALL (including, importantly, doubling the impact on survival when administered independently) as expressly concluded by the authors of the publication. In total, the Kaya et al. 2021 publication again provided evidence supporting the superior efficacy of NALL, and at minimum, demonstrated that there are indeed differences between the pharmacological effects of NALL and NADL/NADLL, which could reflect clinically relevant differences in humans.

Paradoxically, the CHMP Opinion also considered that *"on a general note, it should be reminded that NALL should be compared to the approved racemic NADLL rather than to NADL."*

However, the CHMP Opinion had previously indicated that *"As pointed out by the Applicant and according to the position of the EMA, if "new evidence emerges indicating a relationship between one enantiomer and a safety or efficacy issue…. supporting studies with the separate enantiomers will be required." Hence, safety and/or efficacy differences between enantiomers of the same compound are key for justifying clinical preference of one enantiomer over the other or over the racemate."*

The Applicant takes issue with what it considers to be the inconsistencies of the CHMP position and maintains that the comparison to the D-enantiomer is necessary, based on the legal requirements – which have been acknowledged by the CHMP - defined by both the US FDA and EMA, who stipulate that patients should not be unnecessarily exposed to an inactive or antagonistic isomer. Under the EMA guideline, if a racemate is to be administered, and one enantiomer is known to be inactive, the use of the racemate should be justified ("If only one enantiomer is active and the other has no useful contribution, development of the single active enantiomer should be considered.")

The Applicant claims that Kaya et al. 2021 provides data which reinforces the isolated use of NALL, and which in no way supports or justifies the use of NADLL.

***In Vivo* Findings: Vestibular Compensation**

In vivo experimental studies have demonstrated that NALL but not NADL facilitates the recovery process in vestibular deficits and accelerates the vestibular compensation process [Tighilet et al. 2015; Vibert and Vidal 2001].

Tighilet et al. (2015) studied and directly compared the effects of NADL and NALL in a cat model of unilateral vestibular loss. NALL significantly and strongly accelerated behavioural recovery as evaluated by assessments of static posture, vestibular nystagmus, and locomotor balance recovery ($p<0.001$) whereas NADL did not affect any parameter and showed the same recovery /behaved as the placebo-treated group receiving saline water. The conclusion was that NALL is the active enantiomer that significantly accelerates vestibular compensation, whereas NADL has no pharmacological effect.

Günther et al. (2015) directly compared the effects of NADL and NALL on vestibular compensation using behavioural testing and serial [^{18}F]-Fluoro-deoxyglucose ([^{18}F]-FDG)-µPET in a rat model of unilateral chemical labyrinthectomy. Again, NALL accelerated the postural compensation after unilateral vestibular damage, whereas NADL did not; the major conclusion was "Testing of the D- and L-enantiomer in our study revealed that N-acetyl-L-leucine is evidently the active component that accelerates postural compensation". Measurements of the regional cerebral glucose metabolic rate (rCGM) by means of µPET revealed that NALL, but not NADL, caused a significant increase of rCGM in

the vestibulocerebellum and a decrease in the posterolateral thalamus and subthalamic region. Moreover, NALL was found to be more effective than the racemate (NADLL), the activity of which was demonstrated to be inhibited by the inactive/antagonistic NADL.

The CHMP Opinion considered that *"The Applicant claims that NALL is the pharmacologically active enantiomer based on* Günther et al (2015)*,* Tighilet et al. (2015)*, and* Kaya et al. (2021)*. Of note, works by Günther et al. and Tighilet et al. address the effects N-acetyl-leucine on vestibular compensation, which is not considered to be of any proven relevance for NPC-disease."*

The Applicant contends that this is not only incorrect but also inconsistent with regulatory precedent.

First, it is the Applicant's view that the CHMP's conclusion is scientifically incorrect. Indeed, the NALL specificity of the therapeutic effect in models of vestibular compensation, indicates a stereospecific pharmacological mode of action that is directly relevant for NPC given the phylogenetic and electrophysiological similarities and close interactions between vestibular and deep cerebellar neurons.

Testing of the D- and L-enantiomers in Günther et al. 2015 and Tighilet et al. 2015 revealed that NALL is the active component of NADLL that accelerates postural compensation in a dose-dependent manner. The behavioural improvement with NALL treatment follows an increase of regional cerebral glucose metabolism in the vestibulocerebellum, showing that NALL (but not NADLL or NADL) augments plasticity mechanisms via complex molecular and cellular modulations with different time courses. The imaging studies clearly show an activation of the cerebellum, which is relevant for NPC, given that cerebellar dysfunction is the hallmark of the disease, leading to classic symptoms such as ataxia, dysmetria, dysphagia, cognition/language impairment, disdiadochokinesia, and dysmetria.

These *in vivo* studies also demonstrated that NALL improved multisensory integration namely in the thalamus which is also impaired in NPC since this disease affects all neurons of the brain [Bense et al. 2004].

In summary, these non-clinical findings revealed a mechanism of NALL which the Applicant claims is relevant for NPC, in that it can modulate glutamate neurotransmission in the cerebellum via the branched-chain amino acid transferases and thereby induces changes in long-term potentiation or depression via changes in glutamatergic neurotransmission or cellular calcium levels in the cerebellum. Further, activation of metabotropic glutamate receptors is required for neuronal plasticity and is highly relevant for maintaining cellular homeostasis in NPC.

Secondly, the CHMP Opinion is not consistent with regulatory precedent. The evaluation of NAS status is not tied to a disease state – it is tied to evidence of significant safety and/or efficacy differences between NALL and NADLL. These publications clearly and robustly demonstrate the pharmacological activity of the L-enantiomer (NALL) versus the racemate (NADLL), and this information is considered to be highly supportive of NAS status as it again demonstrates the activity of the L-enantiomer (NALL) and the inhibitory effects of the D-enantiomer (NADL) when it is co-administered as a racemate (NADLL).

Applicant's conclusion on Non-clinical Data

The CHMP Opinion concluded that: *"In summary, insufficient non-clinical evidence was provided to demonstrate that levacetylleucine is superior to the racemic NADLL, which is approved as Tanganil and which was already used successfully for clinical off-label treatment of NPC. Moreover, no concrete evidence was provided that the NADL component of Tanganil causes any adverse clinical effects. In conclusion, from a non-clinical efficacy and safety perspective, levacetylleucine cannot be regarded a new active substance for use in the applied indication."*

However, as it clearly results from the developments above:

- The Applicant did provide sufficient compelling comparative non-clinical evidence to demonstrate that NALL is superior to the racemic NADLL. Based on this data, per both EMA and FDA regulations, it would not be justifiable to pursue the development of the racemate because NADL inhibits the efficacy of the active NALL, changes the pharmacokinetics of NALL with significant dosing implications, is antagonistic for key pharmacological activities of NALL, and has potential toxicity concerns.
    - This required the Applicant to commence, from scratch, a new development program with the L-enantiomer (NALL), including complete non-clinical, clinical efficacy, and pharmacokinetic studies.
    - Note - the CHMP Opinion also considered that: *"The Applicant is also referred to EMA/651649/2010 2.3.2, which clearly states that changes in pharmacokinetics alone as well as preclinical differences that are inconclusive or unlikely to result in significant changes in clinical efficacy or safety, are unlikely to be sufficient to justify a new active substance status."* The Applicant does not agree with this comment. The Applicant submitted more non-clinical studies, beyond pharmacokinetic data. The non-clinical data submitted by the Applicant is conclusive since it led to conclude that the maximally efficacious, safe analogue of N-acetyl-leucine was the L-enantiomer (NALL) administered independently and that the administration of the racemate (NADLL) would not only unnecessarily and unjustifiably expose to the inactive/ antagonistic D-enantiomer (NADL) but also significantly complicate the dosing and pharmacokinetics of the drug, with less efficacy, i.e., that NALL was superior to NADLL.
- Given that this non-clinical data established that it was not justifiable to pursue the development of the racemate, by the same rationale, this evidence is sufficient to support that the L-enantiomer (NALL) is superior to the racemate.
    - The Applicant highlights that the CHMP's implied request for additional non-clinical studies to further demonstrate the toxicology of the D-enantiomer (NADL) is inconsistent with the 3R principle. The only reason for the Applicant to complete additional non-clinical studies (e.g. long-term toxicity studies) with NADLL would be if the Applicant were to identify benefits of administering both the D- and L- enantiomers (NADL and NALL). As described throughout the document, not only is there evidence that the D-enantiomer (NADL) has no discernible benefit or activity, there also is compelling evidence that it is antagonistic and has potential detrimental effects. Therefore, the sole reason to conduct additional toxicity studies with NADLL would be to further demonstrate the negative aspects of the D-enantiomer (NADL), and this type of study is not only long and costly but also incompatible with the 3R principle.
- It is scientifically inappropriate and unjustifiable to rely on compassionate use studies of Tanganil for treatment of NPC to draw any conclusions about the safety, efficacy, or benefit-risk profile of NADLL for NPC or other disorders like cerebellar ataxia (especially when formal, placebo-controlled, randomized controlled studies with NADLL for cerebellar ataxias failed).
- The therapeutic indication applied for is not relevant for the determination of the NAS status of the active substance.

The comparative non-clinical data submitted by the Applicant is compelling as it is statistically robust, reproducible, well-controlled, supports the mechanism of action and claims made for NALL, and reduces uncertainty about the safety and efficacy of NALL.

The CHMP Opinion also concluded that: *"It is not clear from the arguments presented by the Applicant how the pharmacological and pharmacokinetic differences observed between the 2 enantiomers in vitro*

and in non-clinical models can translate into a significant difference in term of efficacy and safety."
Again, the Applicant contests this position. The non-clinical pharmacological and pharmacokinetic differences observed between the two enantiomers were compelling, significant and conclusive enough that all experts (clinicians, medicinal chemists, pharmacologists, toxicologists) and regulators concluded that the maximally efficacious, safe analogue of N-acetyl-leucine was the L-enantiomer (NALL) administered independently, and that the administration of the racemate (NADLL) would not only unnecessarily and unjustifiably exposed to the inactive/ antagonistic D-enantiomer (NADL), but also significantly complicate the dosing and pharmacokinetics of the drug, with less efficacy.

As described below, head-to-head clinical studies in patients with NPC – a rare, debilitating, fatal disease—with NALL and NADLL simply to further illuminate these significant differences in terms of efficacy and safety, are not feasible and not ethical per ICH-GCP. The Applicant continues to take issue with the fact that the CHMP Opinion seemingly requests the Applicant to conduct unethical clinical trials on vulnerable populations, where risks and detrimental effects are expected based on the significant non-clinical evidence. Such a request is even more unjustifiable given that it arises because the CHMP is relying on compassionate use observational data to dismiss the result of a valid comparison of randomised, controlled clinical data between NALL and NADLL. The burden of proof seems unfairly balanced where the Applicant is required to support its position with head-to-head comparative clinical trial data, but the CHMP may dismiss controlled NALL and NADLL clinical data due to findings from compassionate use observational data.

Clinical Data

Clinical Trials

The Applicant performed a literature review for completed clinical trials performed with the racemate or L-enantiomer for neurological disorders.

Table 2 provides an overview of the identified trials, including 3 clinical trials with NALL ("IB1001 clinical studies") and 1 clinical trial with NADLL.

Table 8: Comparison of clinical trials conducted with NADLL and NALL for neurological conditions

Trial ID	**ALCAT** [Feil et al. 2021]	**IB1001-201** [Study IB1001-201 CSR; Bremova-Ertl et al. 2022]	**IB1001-202** [Study IB1001-202 CSR; Martakis et al. 2023]	**IB1001-301** [Study IB1001-301 interim CSR; Bremova-Ertl et al. 2024]
Title	Effects of Acetyl-DL-Leucine on cerebellar ataxia - a multinational, multicenter, randomized, double-blind, placebo-controlled, 2-way crossover phase III trial (EudtraCT 2015-000460-34)	Effects of NALL on Niemann-Pick disease type C (NPC): A multinational, multicenter, open-label, rater-blinded Phase II study (EudtraCT 2018-004331-71)	Effects of NALL on GM2 Gangliosidosis (Tay-Sachs and Sandhoff Disease): A multinational, multicenter, open-label, rater-blinded Phase II study (EudtraCT 2018-004406-25)	Effects of NALL on Niemann-Pick disease type C (NPC): A Phase III, randomized, placebo-controlled, double-blind, crossover study (EudraCT: 2021-005356-10)
Compound	NADLL (racemate)	NALL (L-enantiomer)	NALL (L-enantiomer)	NALL (L-enantiomer)
Phase	II/III	IIb	IIb	III
Study Type	Investigator-Initiated	Industry Sponsor (IntraBio)	Industry Sponsor (IntraBio)	Industry Sponsor (IntraBio)
Design	Randomized, placebo-controlled, crossover	Open-label, rater-blinded	Open-label, rater-blinded	Randomized, placebo-controlled, crossover
Indication	Inherited Cerebellar Ataxias (CA)	Niemann-Pick type C (NPC)	GM2 Gangliosidosis (GM2)	Niemann-Pick type C (NPC)
Objective	Determine if NADLL is effective at treating the neurological symptoms (e.g. ataxia) in patients with inherited CA	Determine if NALL is effective at treating the neurological symptoms (e.g. ataxia) in patients with NPC	Determine if NALL is effective at treating the neurological symptoms (e.g. ataxia) in patients with GM2	Determine if NALL is effective at treating the neurological symptoms (e.g. ataxia) in patients with NPC
Study Design/ Duration	Multinational, randomized, placebo-controlled, double-blinded, crossover study evaluating 12 weeks of treatment with NADLL versus 12 weeks of placebo. Patients were assessed during 3 study periods: a baseline period, followed by two treatment sequences, each consisting of a 6-week treatment period, followed by a 4-week washout period. Patients were randomized 1:1 to one of two different treatment sequences, either NADLL (up to 5 g per day) followed by placebo or vice versa.	Multinational, multi-centre, open-label, rater-blinded single-arm study evaluating 6 weeks' treatment with NALL versus 6-week post treatment washout. Patients were assessed during 3 study periods: baseline period, a 6-week treatment period, and a 6-week post-treatment washout period.	Multinational, multi-centre, open-label, rater-blinded single-arm study evaluating 6 weeks' treatment with NALL versus 6-week post treatment washout. Patients were assessed during 3 study periods: baseline period, a 6-week treatment period, and a 6-week post-treatment washout period.	Multinational, randomized, placebo-controlled, double-blinded, crossover study evaluating 12 weeks of treatment with NALL versus 12 weeks of placebo. Patients were assessed during 3 study periods: baseline period, the first 12-week treatment period ("Period I"), and a second 12-weel second period ("Period II"). Patients were randomized 1:1 to receive either NALL in Period I followed by Placebo in Period 2 or vice versa.
Overlapping Endpoints	• Primary: Scale for the Assessment and Rating of Ataxia (SARA) • Secondary: Spinocerebellar Ataxia Functional Index (SCAFI)	• Secondary: Scale for the Assessment and Rating of Ataxia (SARA)	• Secondary: Scale for the Assessment and Rating of Ataxia (SARA)	• Primary: Scale for the Assessment and Rating of Ataxia (SARA) • Secondary: Spinocerebellar Ataxia Functional Index (SCAFI)
Population	108 Patients enrolled; 105 patients analysed per ITT set	33 patients enrolled; 30 patients analysed per mITT set	32 patients enrolled; 30 patients analysed per mITT set	60 patients enrolled and analysed per ITT set

Trial ID	ALCAT [Feil et al. 2021]	IB1001-201 [Study IB1001-201 CSR; Bremova-Ertl et al. 2022]	IB1001-202 [Study IB1001-202 CSR; Martakis et al. 2023]	IB1001-301 [Study IB1001-301 interim CSR; Bremova-Ertl et al. 2024]
SARA Results	***Treatment Period:*** After 6-weeks NADLL treatment, mean SARA change -0.29 vs -0.52 with placebo (mean treatment difference: 0.23 points [95% CI, −0.40 to 0.85 points]; P = 0.48), no statistical or clinically relevant difference in cerebellar signs (e.g. ataxia)/ neurological symptoms with NADLL (in contrast, patients performed numerically better in the placebo treated arm) ***Washout Period:*** Washout Period: Following the 6-week post-treatment washout from NADLL, mean SARA change +0,38 (with 95% CI (-0.03,0.79), *p* = 0.066), summary	***Treatment period:*** After 6 weeks NALL treatment, mean SARA change − 1.19 ((95% CI (-1.93, -0.45) *p* = 0.003), a statistically significant and clinically meaningful improvement in cerebellar signs (e.g. ataxia)/ neurological symptoms with NALL ***Washout Period:*** Following the 6-week post-treatment washout from NALL, mean SARA change +1.45 (with 95% CI (0.54, 2.36), *p* = 0.003), a statistically significant and clinically meaningful worsening (deterioration)/ return to baseline status in cerebellar signs (e.g. ataxia)/ neurological symptoms after NALL was stopped	***Treatment Period***: After 6 weeks NALL treatment, mean SARA change -1.41 (95% CI (-2.06, -0.76), p<0.001), a statistically significant and clinically meaningful improvement on cerebellar signs and neurological symptoms with NALL ***Washout Period:*** Following the 6-week post-treatment washout from NALL, mean SARA change +1.43(95% CI (0.60, 2.25), p<0.001),), a statistically significant and clinically meaningful worsening (deterioration)/ return to baseline status in cerebellar signs (e.g. ataxia)/ neurological symptoms after NALL was stopped	***Treatment Period:*** After 12-weeks NALL treatment NALL, mean SARA change -1.97 vs with -0.60 (with placebo (Least Squares mean difference -1.28; 95% Confidence Interval, -1.91 to -0.765; p<0.001), a statistically significant and clinically meaningful improvement on cerebellar signs and neurological symptoms with NALL ***Washout Period:*** For patients treated with NALL in period 1 followed by placebo in period II (effectively, post-treatment washout), following the 12-week post-treatment washout from NALL, mean SARA change +1.55 (95% Confidence Interval, 0.71 to 2.39; p=0.002)) a statistically significant and clinically meaningful worsening (deterioration)/ return to baseline status in cerebellar signs (e.g. ataxia)/ neurological symptoms after NALL was stopped
Summary	In this multi-centre clinical trial, NADLL had no effect no benefit on cerebellar signs (e.g. ataxia) or neurological symptoms. / was not superior to placebo.	In this multi-centre clinical trial, NALL demonstrated a statistically significant and clinically meaningful improvement on cerebellar signs and neurological symptoms, the effects of which were lost during the post-treatment washout period, reinforcing the robustness of NALL's treatment effect.	In this multi-centre clinical trial, NALL demonstrated a statistically significant and clinically meaningful improvement on cerebellar signs and neurological symptoms, the effects of which were lost during the post-treatment washout period, reinforcing the robustness of NALL's treatment effect.	In this multi-centre clinical trial, NALL had a statistically significant and clinically meaningful benefit on cerebellar signs (e.g. ataxia) or neurological symptoms compared to placebo. When treatment with NALL was stopped, there was a significant worsening (return to baseline) reinforcing the robustness of NALL's treatment effect. The -1.97 change was significantly above the threshold of a clinically relevant change, reflecting a clinically meaningful change for the patient with NALL.

Comparability of Studies

The ALCAT trial investigated NADLL for inherited cerebellar ataxia, and the IB1001 clinical studies investigated NALL for GM2/NPC. Inherited cerebellar ataxia and GM2/NPC are diseases characterized by cerebellum dysfunction/cerebellar denegation, with shared clinical phenotypes and identical hallmark neurological symptoms.

The findings from the ALCAT trial (NADLL) and the IB1001 clinical studies (NALL) can be directly compared, especially due to the following:

- **The aetiology and pathogenesis causing neurological manifestations in inherited CA, GM2 and NPC are analogous.** Despite different underlying gene defects, the cellular aetiology and pathogenesis of CAs, GM2, and NPC are characterised by shared patterns of lysosomal calcium / inter-ion signalling dysfunction, mitochondrial dysfunction (leading to impaired glucose metabolism) and synaptic dysfunction, culminating in cellular dysfunction, neuroinflammation, cell death and neurodegeneration.
- **Inherited CAs, GM2 and NPC are characterised by the same phenotype and neurological manifestations.** Due to the same patterns of cellular pathogenesis leading to neurological dysfunction and neurodegeneration throughout the brain, CAs, GM2 and NPC feature the same hallmark neurological manifestations, including cerebellar ataxia, dysarthria, dysphagia, dystonia, tremor, impaired balance and gait, upper and lower extremity dysfunction, loss of fine motor skills, impaired speech and swallowing, and cognitive impairment.
- **The mechanism of action by which NALL or NADLL could hypothetically work for these disorders would be the same.** Rather than targeting distinct aspects of their respective diseases' aetiologies, the trials' hypotheses were that the investigated drug would work on the same overlapping features that converge on a shared final common pathway of neurodegeneration.
- **The clinical trials use highly analogous designs with identical objectives.** Both the ALCAT trial and the IB1001 clinical studies were designed to investigate (1) the effect of NADLL or NALL on neurological manifestations (e.g., ataxia) after a 6 or 12-week treatment period, as well as any deterioration during a 6-12 week post-treatment washout period; and (2) identical endpoints, the SARA and SCAFI, across the trials.

The findings from each trial are presented below.

ALCAT Trial

Study Design [Feil et al. 2017]

The ALCAT trial was a randomised, double-blind, placebo-controlled, 2-treatment 2-period crossover phase 3 clinical trial where patients were randomised 1:1 to one of two different treatment sequences, either NADLL (up to 5 g per day) followed by placebo or vice versa. Each sequence consists of two 6-week treatment periods, separated by a 4-week washout period (Figure 9).

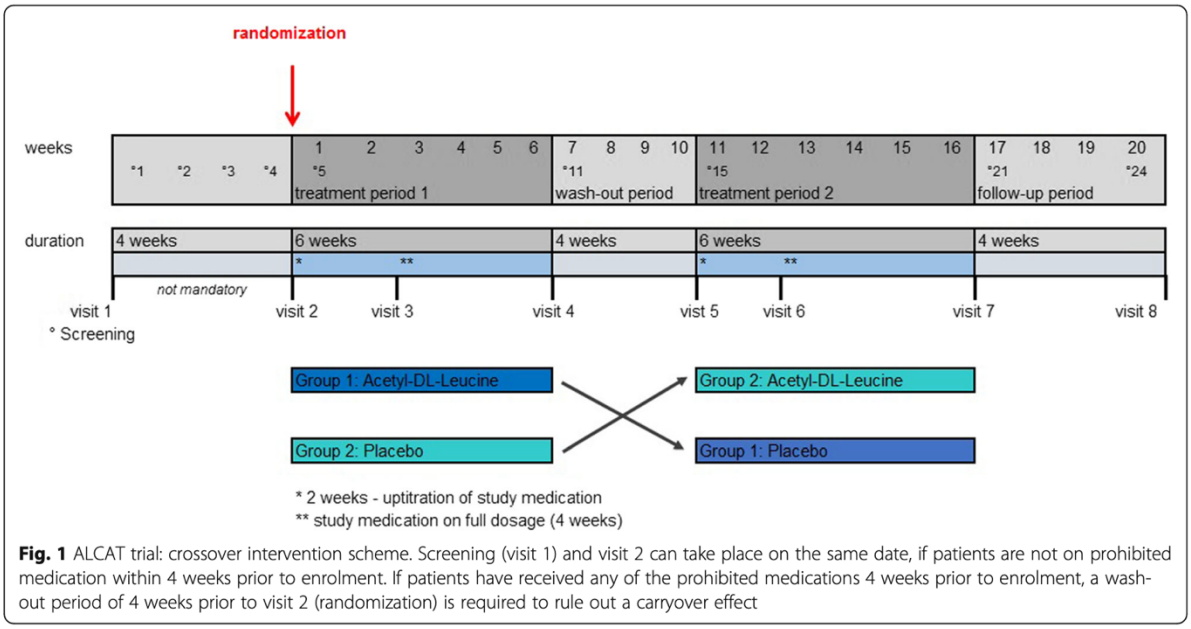


Fig. 1 ALCAT trial: crossover intervention scheme. Screening (visit 1) and visit 2 can take place on the same date, if patients are not on prohibited medication within 4 weeks prior to enrolment. If patients have received any of the prohibited medications 4 weeks prior to enrolment, a wash-out period of 4 weeks prior to visit 2 (randomization) is required to rule out a carryover effect

Figure ***9****:* *ALCAT trial design [Feil et al. 2017]*

Results [Feil et al. 2021]

The principal analysis found no evidence of a treatment benefit of NADLL compared to placebo after 6-weeks of treatment. The mean absolute change from baseline to week 6 in SARA total scores did not differ significantly between NADLL and placebo (mean treatment difference: 0.23 points [95% CI, −0.40 to 0.85 points]; P = 0.48) (Table 3). Changes over time within periods and between both periods were not considered clinically relevant because this improvement in disease symptoms was far less than a clinically meaningful score of 1 point or greater. At Week 6, an overall mean reduction in SARA total score values of −0.40 points (95% CI, −0.78 to −0.03 points; P = 0.03) compared with the period-dependent baseline was observed, whereas at week 2, the overall mean difference was −0.19 points (95% CI, −0.56 to 0.18 points; P = 0.45) (Feil et al. 2021).

The results were identical on the secondary SCAFI endpoint - no treatment benefit of NADLL compared with placebo could be found – mean difference 0.01 (95% CI −0.05 to 0.06); p=83). Further, there was no evidence for a clinically relevant effect of NADLL on the subjective health rating EQ visual analogue scale compared with placebo at week 6 with no evidence of a period effect.

*Table **9**:* *Summary for the Primary Outcome in the Full Analysis Set of 105 Patients [Feil et al. 2021]*

	Marginal means (95% CI)		Acetyl-DL-leucine – placebo, mean difference (95% CI)[a]	*P* value[b]
	Acetyl-DL-leucine	Placebo		
SARA total score				
Baseline[c]	13.11 (12.03 to 14.18)	13.35 (12.27 to 14.42)	−0.24 (−0.68 to 0.20)	.28
Week 2	13.13 (12.06 to 14.21)	12.94 (11.87 to 14.02)	0.19 (−0.25 to 0.63)	.39
Week 6	12.82 (11.74 to 13.90)	12.83 (11.75 to 13.91)	−0.01 (−0.47 to 0.44)	.95
Changes in SARA total score from baseline				
Week 2	0.03 (−0.41 to 0.46)	−0.40 (−0.84 to 0.03)	0.43 (−0.18 to 1.05)	.17
Week 6	−0.29 (−0.74 to 0.16)	−0.52 (−0.95 to −0.08)	0.23 (−0.40 to 0.85)	.48

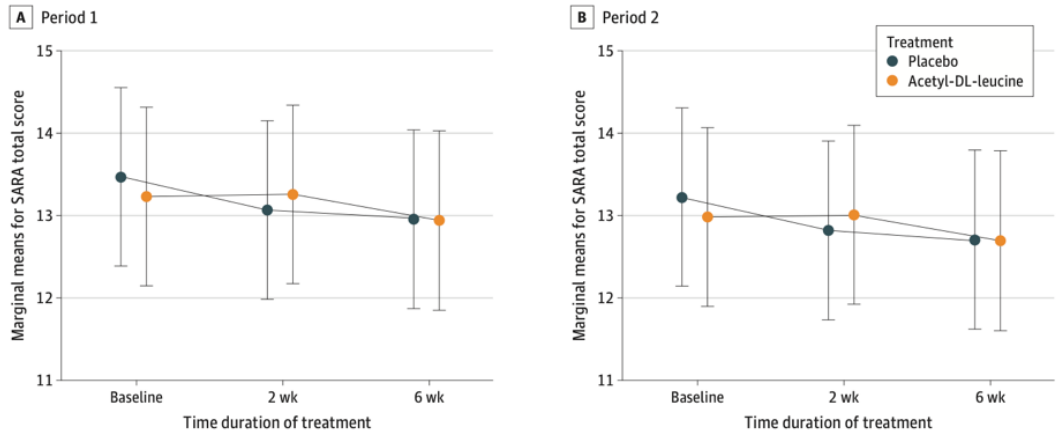


Figure 10: *Results of the SARA in the ALCAT Trial [Feil et al. 2021]*

NALL Phase II Trials – IB1001-201 and IB1001-202

Study Design [Fields et al. 2021]

The IB1001-201 and IB1001-202 trials were rater-blinded, open label Phase IIb studies. The trials utilized the same protocol design and consisted of three consecutive study periods: a 2-week (+7 day) baseline period, a 6-week (+7 day) treatment period (in which all patients were to receive NALL), and a 6-week (+7 day) post-treatment washout period (Figure 11).

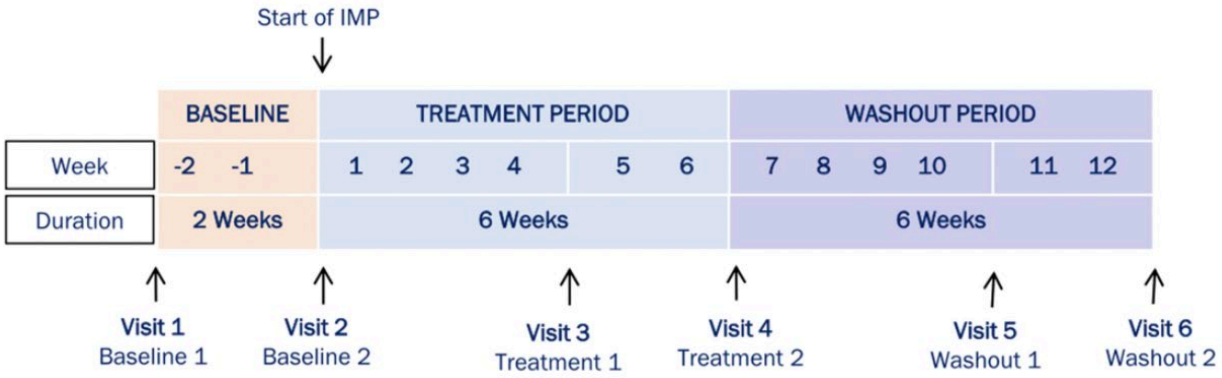


Figure 11: IB1001-201 and 202 Study Design [Fields et al. 2021]

Results

In both studies, NALL has demonstrated a statistically significant and clinically meaningful improvement in the SARA after 6 weeks of treatment, followed by statistically significant and clinically meaningful worsening in the post-treatment washout period.

- **IB1001-201 (NPC) [Bremova-Ertl et al. 2022]**
  - After 6 weeks of treatment with NALL, the mean change in SARA − 1.19 (SD = 2.02, median = − 1.00, *n* = 31) with 95% CI (-1.93, -0.45) *p* = 0.003, demonstrating a statistically significant and clinically meaningful improvement on cerebellar signs and neurological symptoms (Figure 12A)
  - Following the 6-week post-treatment washout period, the mean change in SARA score was 1.45 (SD = 2.56, median 0.75, *n* = 28) with 95% CI (0.54, 2.36), *p* = 0.003, a statistically significant and clinically meaningful worsening (deterioration) and return to baseline status (Figure 12A)
  -
- **IB1001-202 (GM2) [Martakis et al. 2023]**
  - After 6 weeks of treatment with NALL, the mean change in SARA was -1.41 (95% CI (-2.06, -0.76), p<0.001), demonstrating a statistically significant and clinically meaningful improvement on cerebellar signs and neurological symptoms (Figure 12B)
  - After the post-treatment washout, the mean change in SARA score was 1.43 (95% CI (0.60, 2.25), p=0.001), a statistically significant and clinically meaningful worsening (deterioration) and return to baseline status (Figure 12B)

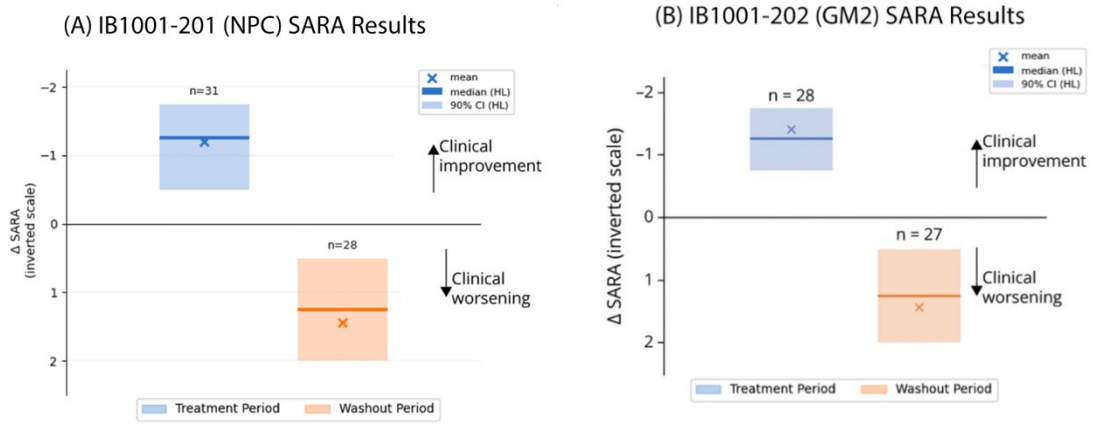


Figure **12***: Results of the SARA in the IB1001-201 and 202 trial [Bremova-Ertl et al. 2022; Martakis et al. 2023]*

(**A**) Results from IB1001-201 Niemann-Pick disease type C (**B**) Results from IB1001-202 – GM2 Gangliosidosis. For each test, the results comparing baseline to end of treatment (left-hand column, blue) are compared with the results comparing the end of the treatment period to the end of the washout period (right-hand column, orange). The vertical length of the column represents the 90% Hodges-Lehmann (HL) CI. Solid lines are used to denote the Hodges-Lehmann Median Estimator, and cross symbols are used to denote the mean response.

NALL Phase III Trial – IB1001-301

Study Design [Fields et al. 2023]

The IB1001-301 trial was a randomised, double-blind, placebo-controlled, crossover trial.

The Phase III trial consisted of a baseline period followed by two consecutive 12-week treatment periods. Eligible patients were randomly assigned in a 1:1 ratio to receive NALL in period 1 for 84 to 91 days and then matching placebo in period 2 for 84 to 91 days (sequence 1) or to receive placebo in period 1 for 84 to 91 days and then NALL in period 2 for 84 to 91 days (sequence 2). NALL or placebo was immediately switched at the end of period 1 (Visit 4) (Figure 13).

Figure **13***: IB1001-301 Study Design [Fields et al. 2023]*

Results [Bremova-Ertl et al. 2024]

The IB1001-301 trial was directly consistent with the Phase II studies, the IB1001-201 and 202 trials.

- NALL demonstrated a statistically significant and clinically meaningful improvement in the SARA after 12 weeks of treatment
    - After 12 weeks treatment with NALL, the mean change on the SARA was -1.97 (2.43) compared with -0.60 (2.39) with placebo (Least Squares mean difference -1.28; 95% Confidence Interval, -1.91 to -0.765; p<0.001) (Figure 14)
- Patients who received treatment with NALL during the first 12-week treatment period followed by placebo in the second 12-week treatment period had a significant worsening of symptoms under placebo, which effectively served as a washout from NALL
    - Difference in mean SARA total score between Visits 4 (end of treatment) and Visit 6 (end of washout) was +1.55 (95% Confidence Interval, +0.71 to +2.39; p=0.002), reflecting a statistically significant and clinically meaningful deterioration in neurological signs and symptoms when treatment with NALL was stopped.
- In addition, the secondary SCAFI total score showed an improvement in neurological signs/symptoms and functioning after treatment with NALL compared to deterioration after placebo treatment. After treatment with NALL, the mean (SD) change from baseline was 0.05 (0.27) compared to -0.02 (0.31) after placebo treatment (least squares mean difference: 0.07; 95% CI: 0.00, 0.15; p=0.027).

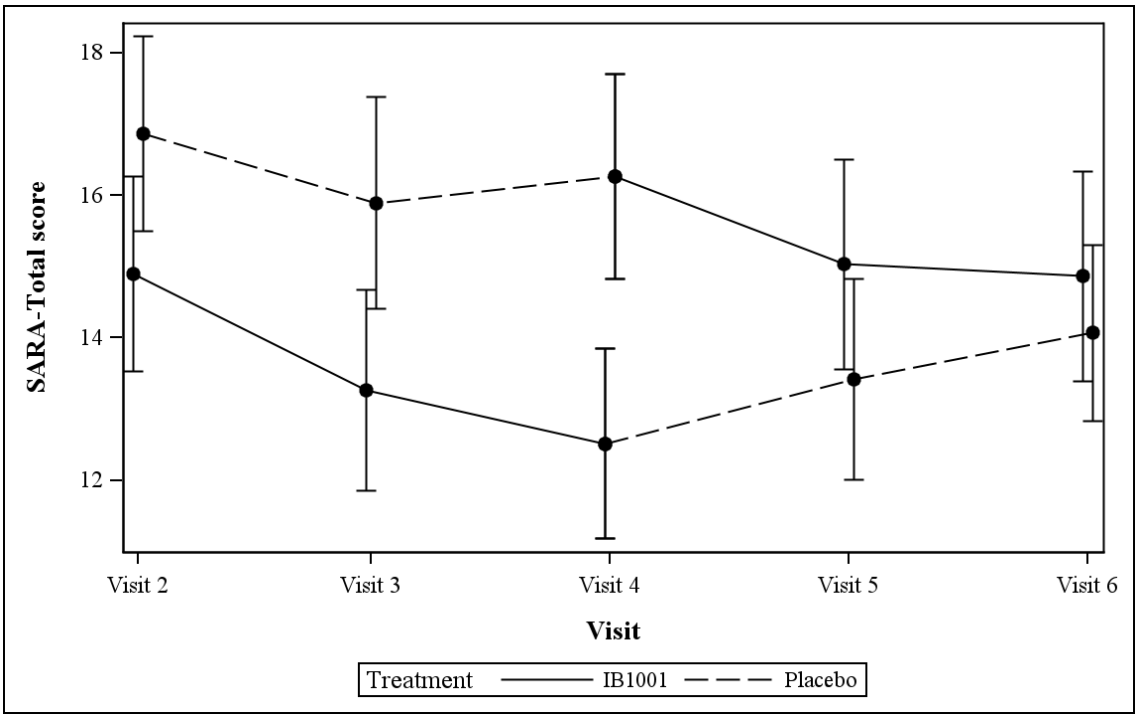


Figure 14: Results of the SARA in the IB1001-301 trial [Bremova-Ertl et al. 2024]

Abbreviations: ITT = intention-to-treat; LOCF = last observation carried forward; SARA = Scale for the Assessment and Rating of Ataxia.

Note: The dots represent the observed means, the vertical lines represent (+/-) the standard errors. No LOCF approach was used for this figure.

Summary of NADLL and NALL Clinical Data

The ALCAT trial with NADLL and the IB1001-201, 202, and 301 clinical studies with NALL, were highly comparable clinical trials investigating NADLL/NALL for cerebellar disorders (characterized by overlapping disease pathology leading to neurological symptoms, the same hallmark neurological manifestations) and aiming to demonstrate the effectiveness of NADLL/NALL for treatment of cerebellar ataxia based on the SARA scale after 6/12 weeks.

Although the ALCAT trial (NADLL) was a significantly larger and therefore a much more robustly powered clinical trial than the IB1001 (NALL) clinical studies, the ALCAT trial failed to show any signal of a treatment effect (including any benefit when treatment was introduced or effect when treatment was stopped) or any difference between NADLL versus placebo. In contrast, the three IB1001 clinical studies with NALL demonstrated robust statistical significance on the SARA, including both a significant and clinically meaningful improvement in patients' cerebellar ataxia under treatment (Table 4), and a deterioration (return to baseline status) when treatment was stopped in the post-treatment washout/versus placebo (Figure 15, Table 5).

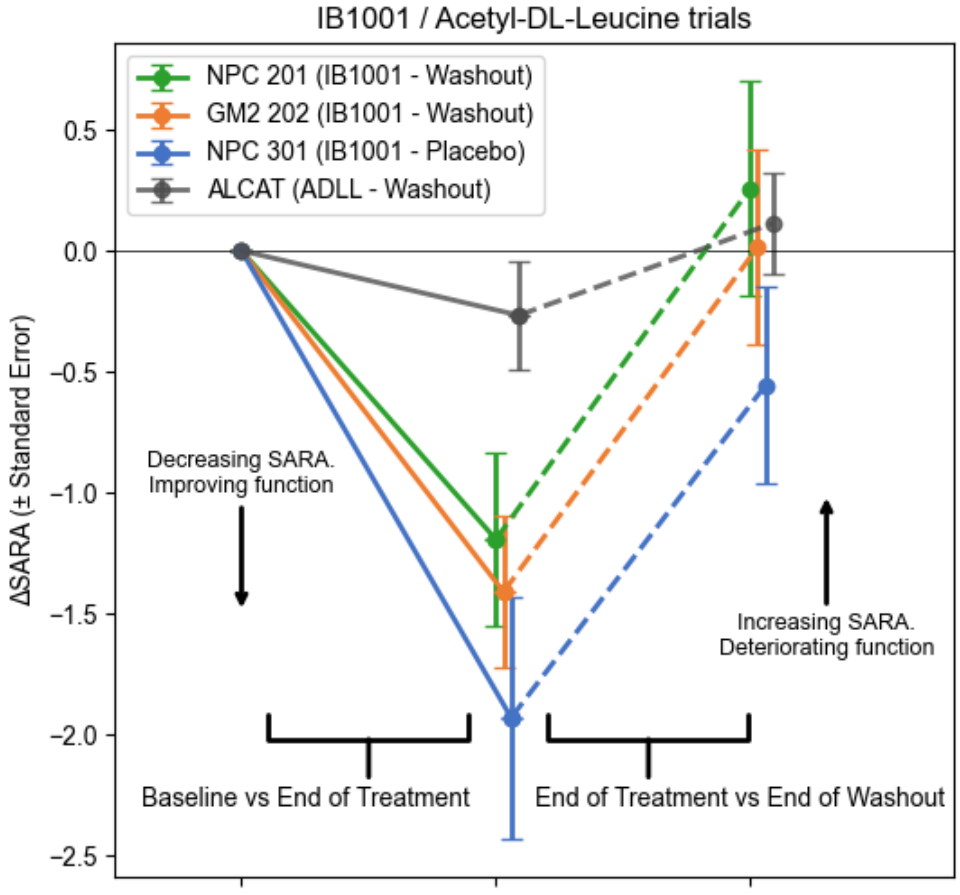


Figure 15: Comparative results of the SARA in clinical trials with NALL/NADLL

The solid line demonstrates the mean SARA change comparing the baseline SARA score with the end of washout SARA score. The dashed line demonstrates the mean SARA change comparing the end of treatment SARA score with the end of washout SARA score.

This clearly illustrates that in the IB1001 clinical studies (NALL), there was a significant improvement in neurological symptoms [as captured on the SARA] after NALL treatment compared to baseline (indicated by the solid green, orange, and blue lines in Figure 15, where the mean SARA change after NALL treatment showed below a -1 point improvement[4] - see Table 4). In comparison, in the ALCAT trial (NADLL) (ALCAT – grey), there was no meaningful change in neurological symptoms compared to baseline [as captured on the SARA] after NADLL treatment (indicated by the solid grey line in Figure 15, where the mean SARA change after NADLL treatment did not surpass a -1 point improvement – see Table 4).

Table 10: Comparative results of the SARA in clinical trials with NALL/ NADLL: treatment

	NPC 201	**GM2 202**	**NPC 301**	**ALCAT**
SARA change from baseline visit vs end of treatment visit	-1.19	-1.41	-1.97	-.029

Additionally, in the IB1001 clinical studies (NALL), there was a significant worsening in neurological symptoms [as captured on the SARA] comparing the end of NALL treatment with the end of post-NALL treatment washout (indicated by the dashed green, orange, and blue lines in Figure 15, where the mean SARA change after NALL washout showed above a +1 point worsening – see Table 5). In comparison, in the ALCAT trial (NADLL), there was no meaningful difference in neurological symptoms [as captured on the SARA] comparing the end of NADLL treatment with the end of post-NADLL treatment washout (indicated by the dashed grey line in Figure 15, where the mean SARA change after NADLL washout did not surpass a +1 point worsening – see Table 5).

Table 11: Comparative results of the SARA in clinical trials with NALL/ NADLL: washout

	NPC 201	**GM2 202**	**NPC 301**	**ALCAT**
SARA change from end of treatment visit vs end of post-treatment washout visit	+1.45	+1.43	+1.55	+0.38

The CHMP Opinion considered: *"In order to support the relevant impact of the administration of the L-enantiomer in terms of efficacy compared to N-acetyl-DL-leucine the applicant refers to the ALCAT study (Feil K 2021). This was a randomised, double-blind, crossover study where patients were randomised 1:1 to one of two different treatment sequences, either N-acetyl-DL-leucine (up to 5 g per day) for 6 weeks followed by placebo or vice versa in 108 participants with cerebellar ataxia of hereditary (suspected or genetically confirmed) or nonhereditary or unknown type. This study failed to show a difference in mean SARA total change scores at week 6 compared to baseline between the active vs placebo treatment. However, it is difficult based on this indirect comparison to conclude that there is a*

[4] Park et al, 2024

different between the L-enantiomer compared to N-acetyl-DL-leucine in terms of efficacy as both studies were conducted in different populations and with different treatment duration".

The Applicant strongly contests this:

1. The populations in the ALCAT trial and the IB1001 clinical studies suffered from closely related disorders – cerebellar ataxia for NADLL and GM2/NPC for NADLL - with the same pathology of disease leading to the same, shared neurological manifestations.
2. The treatment durations (6 weeks ALCAT, 6 or 12 weeks NALL) are identical or closely comparable.

Moreover, the studies had an identical objective - to evaluate the impact of a drug substance (NADLL or NALL) on cerebellar signs (e.g., ataxia) and neurological symptoms in these disorders. The studies further employed the identical SARA endpoint.

Therefore, the Applicant maintains that these adequately controlled studies can be compared, demonstrating clinical efficacy with the L-enantiomer (NALL) but not with the racemate (NADLL). The negative findings of the ALCAT trial underscore the limitations of the racemic compound and highlight the importance of stereospecificity in drug development. The lack of efficacy observed with NADLL likely reflects both the pharmacologically inactive nature of the D-enantiomer and its potential to interfere with the desired biological effects of the L-isomer (NALL). This unsuccessful trial provided another pivotal rationale for the development and investigation of the enantiomerically pure NALL, which has since shown more favourable pharmacokinetics, better blood-brain barrier penetration, and preliminary clinical efficacy in inherited and acquired neurological disorders.

Concerningly, the CHMP Opinion also considered that: *"Moreover, some publications have shown that the racemate is associated with a clinical improvement based on the SARA score:*

- *Strupp 2013 showed an improvement in the SARA score in 13 patients with cerebellar ataxia treated with N- acetyl DL leucine during 1 week.*
- *Schniepp 2016 showed improvement in the SARA score in 18 patients with cerebellar ataxia treated with N- acetyl DL leucine during at least 4 weeks*
- *One case series (Bremova 2015) in 12 patients with NPC disease treated for 3 weeks with a subsequent washout period of 1 month. Showed a significant between baseline and on medication and between on medication and washout."*

The Applicant highlights that:

- These observational studies were all single-site, compassionate use, non-ICH-GCP studies with no formal procedures for data collection, blinding, pharmacovigilance, or controls. The results of such studies may not be used to deny the results of a legitimate randomized controlled clinical trial (ALCAT trial). That the CHMP Opinion emphasizes observational studies of cerebellar ataxia to undermine the conclusions from the formal, RCT ALCAT trial in cerebellar ataxia, is not justifiable.
  - Of note – the ALCAT trial demonstrates why findings from observational studies should be read with caution and must be confirmed in adequate, well-controlled studies, as the results could not be recapitulated in a controlled clinical setting.
- Strupp and Schniepp were conducted in patients with cerebellar ataxia (the same population as the ALCAT trial). So, the CHMP dismissed the findings from the well-controlled RCT ALCAT trial because it was conducted "in different populations [to NPC]" but subsequently cited observational, non-controlled compassionate use studies as evidence supporting the efficacy of NADLL although those studies were also conducted in different populations. This inconsistency is not justifiable.

Clinical Data with Tanganil

A systematic literature review conducted by Vanderkam et al. (2019) found no substantial evidence of the efficacy of NADLL in vertigo/dizziness and recommended that randomised trials be carried out to assess its efficacy. The major finding was that there is no substantial evidence of efficacy of NADLL in vertigo/dizziness from any study. These included (1) a study in patients who had undergone neurotomy, where no improvement in dizziness was observed in patients who received NADLL, (2) a non-randomised study in patients that found no improvement in vertigo symptoms, and (3) a study in healthy volunteers undergoing vestibular training, where no improvement in symptoms was observed with NADLL over placebo [Vanderkam et al. 2019].

The conclusions from the systematic review were:

- "There is no solid evidence of the efficacy of [NADLL] in vertigo/dizziness. Given its frequent prescription and the cost generated for the French social security system, high-quality randomised trials should be carried out to assess its efficacy."
- "[NADLL] is an unassessed drug and until proven otherwise can be considered an impure placebo."
- "NADLL has a marketing authorization and is reimbursed in France for the symptomatic treatment of vertigo/dizziness, although there is no evidence of its efficacy."
- "Until sufficient data is gathered, evidence-based clinicians should remember that it is at best an impure and costly placebo."

Similar conclusions were reported as early as 1958: "In contrast, acetyl-DL-leucine, like other amino acids tested, was ineffective when administered orally, even at high doses (400 mg/kg per day)." [Celice et al. 1958].

Applicant's conclusion on Clinical Data

The CHMP Opinion considered that *"the Applicant did not provide new data supporting a relevant difference in terms of clinical efficacy and safety between the L-enantiomer and the racemate."*

The Applicant strongly contests this.

1. The Applicant did provide clinical data indirectly comparing the clinical efficacy of the racemate (NADLL) and NALL. This included:
   a. clear data (including placebo-controlled data) with both NALL and NADLL on identical endpoints, for identical time frames and for highly related indications that demonstrate that NALL, but not NADLL, is clinically effective (directly consistent with the non-clinical findings).
   b. a literature review which demonstrates that there are no published positive clinical studies (randomised or non-randomised) to confirm the efficacy of the racemate (NADLL) for any indication, including acute vertigo. In contrast, there are multiple, robustly statistically significant clinical studies with the L-enantiomer (NALL).

The Applicant has not conducted further clinical studies comparing NADLL with NALL for the following reasons:

1. Based on the totality of the compelling comparative non-clinical data, there is no rationale to expose patients to a toxicologically negative compound (NADL, 50% of the racemate) by conducting head-to-head studies for the sole purpose to further substantiate a NAS status. In vivo studies demonstrate that the L-enantiomer (NALL) is the pharmacologically active enantiomer [Günther et al. 2015; Kaya et al. 2021; Tighilet et al. 2015], whereas in vivo studies have demonstrated a potential for negative

toxicological effects of the D-enantiomer (NADL), in that the D-enantiomer (NADL) negatively competes/interferes with the L-enantiomer (NALL) for uptake and negatively influences the pharmacokinetics. Given that the L-enantiomer (NALL) is the pharmacologically active enantiomer, and there are possibly toxicity and safety concerns with the administration of the D-enantiomer (NADL), there is no justification to expose patients to the inert/antagonistic D-enantiomer (NADL) [FDA 1992; EMA 1993].

2. The Declaration of Helsinki (version 2024) clearly restricts the conduct of clinical trials in human subjects to trials that may benefit patients:
   - Article 7: The primary purpose of medical research involving human participants is to generate knowledge to understand the causes, development and effects of diseases; improve preventive, diagnostic and therapeutic interventions; and ultimately to advance individual and public health.
   - Article 16: In medical practice and in medical research, most interventions involve risks and burdens. Medical research involving human participants may only be conducted if the importance of the objective outweighs the risks and burdens to the research participants.
   - Article 33: The benefits, risks, burdens, and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:
     - If no proven intervention exists, the use of placebo, or no intervention, is acceptable; or
     - If for compelling and scientifically sound methodological reasons the use of any intervention other than the best proven one(s), the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention; and the participants who receive any intervention other than the best proven one(s), placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention. Extreme care must be taken to avoid abuse of this option.

     Those principles, which have been translated into ICH clinical guidelines, preclude both the conduct of clinical trials simply to prove the NAS status of an active substance, and the use of a medicinal product with less efficacy and potentially less safety as the standard of care, especially for rare diseases.

   - Head-to-head clinical trials are not feasible. Even assuming that a comparative clinical trial between NALL and NADLL is authorised by national ethics committees in the EU, in practice, conducting such clinical trials with NALL (Aqneursa) and NADLL (Tanganil and generic copies) is not feasible because the reference active substance (NADLL) and its generic copies are only approved in France for acute vertigo – they are not authorised for the proposed indication NPC.

In any event, a head-to-head comparative clinical trial should not be required. Pursuant to the EMA's own Reflection Paper on Enantiomers, head-to-head clinical studies are preferred but not necessary where (1) head-to-head studies are not feasible; and (2) sufficient and compelling non-clinical data demonstrate that the two substances differ significantly in properties with regard to safety and efficacy. Therefore, direct comparative clinical data between NALL and NADLL should not be required since (1) the Applicant explained above the reasons why head-to-head clinical trials with NALL and NADLL are neither feasible nor ethical; and (2) the Applicant provides compelling not only non-clinical data but also indirect comparative clinical data that demonstrate that the NALL and NADLL differ significantly in properties with regard to safety and efficacy.

- ***Patient and Physician reports of direct experience of patients who have taken both Tanganil (NADLL) and IB1001 /AQNEURSA (NALL)***

In the IB1001 clinical trials[5], medication history, including previous Tanganil use, was recorded at screening. Direct comparisons of patients' experience on Tanganil (NADLL) and IB1001 (NALL) were therefore possible at clinical trial sites where patients had been recorded as using Tanganil prior to the IB1001 studies.

In addition, in the United States, many patients and families reported using Tanganil in an unlicensed setting prior to the approval of AQNEURSA, again enabling direct comparisons of patients' experience with Tanganil (NADLL) and AQNEURSA (NALL).

The major, consistent conclusions from physicians and patients who have taken both NADLL and NALL are the following.

- Patients and physicians reported significantly higher adverse events/intolerances with Tanganil (NADLL), including gastrointestinal issues, tremor, heartburn, incontinence, diarrhoea, stomach pain, which did not occur when they received IB1001/AQNEURSA (NALL), demonstrating that NALL has a superior safety profile than NADLL.
    - Expert Physicians and toxicologists believe that these AEs are directly due to the NADL. Unlike NALL, which is deacetylated and enters leucine pathways, the D-enantiomer is not deacetylated/metabolized and accumulates in the gut/ gut bacteria (and subsequently excreted, e.g., through faeces and urine). Compounds which accumulate in gut bacteria are known to cause gastrointestinal side effects and intolerances directly consistent with those reported, e.g., gas, stomach pain, bloating, incontinence, diarrhoea [Zhang et al. 2015].
- NALL was consistently described as significantly more efficacious than NADLL.

Other Considerations

The Applicant maintains that failure to recognize NAS status for NALL will have negative consequences and potential risks for NPC patients.

It is the Applicant's contention that, in deciding <u>not</u> to recognize NALL as a distinct active substance from NADLL, the EMA implicitly suggests to healthcare professionals and patients (Tanganil and its generics are OTC products) that NALL and NADLL are therapeutically equivalent, with no differences with regard to safety and/or efficacy, although:

- The Applicant has clearly demonstrated differences in the pharmacokinetics, pharmacodynamics, and uptake between NALL and the NALL due to the D-enantiomer (NADL).
    - The co-administration of NALL and NADL in the racemic mixture (NADLL) fundamentally transforms the pharmacokinetic and pharmacodynamic properties of NALL, resulting in different therapeutic and pharmacokinetic effects.
- The administration of the racemate (NADLL) exposes patients to the inactive/ antagonistic D-enantiomer (NADL) with potentially detrimental effects (the presence of the D-enantiomer in NADLL introduces a material and unresolved safety concern regarding gastrointestinal intolerances, long-term accumulation, and potential neurotoxicity).

[5] Including IB1001-201 for NPC, IB1001-202 for GM2 Gangliosidosis, IB1001-203 for Ataxia-Telangiectasia, and IB1001-301 for NPC.

- First hand-reports from patients who have used both NADLL (Tanganil) and NALL (IB1001) as well as physicians' clinical observations directly identify risks and intolerances with Tanganil which can be directly attributed to the poor metabolization of NADL, which do not occur with NALL, as well as superior efficacy with NALL versus Tanganil (NADLL) both in symptomatic improvement and for long-term, disease-modifying treatment.

Declining to recognize NALL as a NAS will generate clinical and regulatory confusion, as well as promoting the chronic use of the racemate (NADLL) for patients with NPC (including potentially at doses multiples of the approved maximum). Unlike AQNEURSA (NALL), NADLL has not undergone formal clinical development for NPC (or any other indication, including acute vertigo since approval was granted before the modern standards for clinical development), nor has it demonstrated safety or efficacy in controlled studies.

In other words, a CHMP negative opinion on the NAS status of NALL would undermine the clear scientific, pharmacological, and regulatory distinctions between the two substances, and would be interpreted as the EMA's endorsement of Tanganil (NADLL) as an appropriate alternative for the treatment of neurological symptoms of NPC, despite the comprehensive data demonstrating the superior benefit/risk profile of NALL versus NADLL, the absence of any legitimate regulatory assessment of the benefit-risk profile of Tanganil in this indication or any other similar indication, and a failed clinical trial.

Granting NAS status to NALL is therefore essential to protect NPC patients from inappropriate extrapolation and potential harm.

The risk is not theoretical. The off-label use of Tanganil is well-documented and persistent throughout Europe. A shortage of NADLL has been reported in France for several months, which coincides not only with the discontinuation of two generics of Tanganil but also with the approval of AQNEURSA in the U.S. Off-label use of NADLL will most likely increase after the approval of AQNEURSA in the EU, especially if NALL is not recognized a NAS status. Although AQNEURSA will be protected by data exclusivity (the Applicant has developed their own data package to support the authorization of AQNEURSA), the Applicant will not be able to oppose off-label prescriptions (written under the erroneous assumption there is no difference between the two compounds) since it is the physicians' prerogative.

Applicant's conclusion

The CHMP has issued a positive opinion in favour of the approval of AQNEURSA (NALL) for the treatment of patients with NPC, but a negative opinion on the NAS status of NALL. The Applicant maintains that they have submitted sufficient evidence that the racemate (NADLL) and the L-enantiomer (NALL) differ significantly in properties with regard to efficacy and potentially to safety to warrant NAS status be granted for NALL.

Specifically, the Applicant has submitted:

Clinical

A comparison between the results of a controlled clinical trial with NADLL and the results of three controlled clinical studies with NALL. This comparison clearly shows that NALL significantly improves neurological symptoms (e.g., ataxia) whereas NADLL does not.

Such comparison can legitimately be made because those clinical trials have been conducted:

- in closely related indications and thus patient populations (cerebellar ataxia and NPC have a same pathology of disease leading to the same, shared neurological manifestations),
- for a very similar duration (6 weeks NADLL and 6 or 12 weeks NALL),

- with the same objective (to evaluate the impact of NADLL/NALL on cerebellar signs (e.g. ataxia) and neurological symptoms in these disorders), and
- with identical endpoints (SARA and SCAFI).

An additional head-to-head clinical trial comparing NADLL with NALL in patients with NPC for the sole purpose of substantiating NAS status, is not ethical and feasible under current law and guidelines. Therefore, pursuant to the EMA's own Reflection Paper on Enantiomers, direct comparative clinical data should not be required as the Applicant already submitted not only compelling non-clinical data but also compelling indirect comparative clinical data.

Furthermore, the data available from patients who have experience using both NADLL and NALL highlight a better tolerance of NALL compared to NADLL, as well as superior efficacy.

Non-Clinical

- Compelling and conclusive comparative non-clinical data demonstrating significant differences in the pharmacokinetics and pharmacodynamics of NALL and NADL and potential significant differences in the safety of NALL and NADL:
    - The two enantiomers of the racemate (NADLL) have substantially different pharmacological activities, and the L-enantiomer (NALL) is the effective, active enantiomer in all pharmacodynamic studies, whereas the D-enantiomer (NADL) is inactive, and at times, antagonistic when administered as the racemate (NADLL).
    - When the racemate (NADLL) is administered, the inert D-enantiomer NADL substantially alters the pharmacokinetics of the active NALL, resulting in substantially lower levels of exposure for the active L-enantiomer (NALL).
    - When the racemate (NADLL) is administered, the inert D-enantiomer NADL competes and suppresses with the uptake and transport of the active L-enantiomer (NALL), reducing its pharmacological effect.
    - When the racemate (NADLL) is administered, the inert D-enantiomer NADL may accumulate, with deleterious effects.

Regarding the racemate (NADLL), those differences between the two enantiomers reasonably suggest that the L-enantiomer (NALL) has superior efficacy, superior safety profile, and neater pharmacokinetics when administered independently than as the racemate (NADLL). This is confirmed by the indirect comparative clinical data submitted by the Applicant, the patients' head-to-head experience with both Tanganil (NADLL) and NALL (IB1001) and the fact that FDA and EMA supported the development of NALL over NADL and NADLL in accordance with their guidance on development of chiral substances/racemic mixtures.

Finally, the Applicant is concerned that failure to recognize the NAS status of NALL will generate substantial clinical and regulatory confusion and put NPC patients at risk. The CHMP failing to grant NALL the NAS status will be interpreted as NALL and NADLL being therapeutically equivalent and thus NADLL being a suitable treatment for NPC, which will inadvertently promote the chronic use of NADLL for patients with NPC although (1) there has been no formal investigation into the efficacy or safety of NADLL in controlled clinical trials or complete toxicology studies, and (2) there is no regulatory assessment of the benefit-risk profile of NADLL in the NPC indication or any other similar indication.

For all these reasons, the Applicant maintains that NALL is a new active substance and that the CHMP should recognise its NAS status.

5.2. CHMP position on NAS status claim under Indent 2

EMA note: Tables and Figures numbering in sub-section 5.2 and 5.3 is aligned with the numbering included in the Applicant's detailed grounds for re-examination documents.

Indent 2 of Annex 1 of chapter 1 of volume 2A of the European Commission's Notice to Applicants (NtA), on the procedures for marketing authorisation, defines a new active substance as including:

'an isomer, mixture of isomers, a complex or derivative or salt of a chemical substance previously authorised in a medicinal product for human use in the European Union but differing significantly in properties with regard to safety and/or efficacy from that chemical substance previously authorised'.

The *Reflection paper on considerations given to designation of a single stereo isomeric form (enantiomer), a complex, a derivative, or a different salt or ester as new active substance in relation to the relevant reference active substance* (EMA/651649/2010) provides some examples of what might constitute a significant difference in safety and/or efficacy to justify new active substance status. It includes, among others, "Compelling preclinical data where it is not feasible to conduct head to head clinical studies, e.g. differences in reproductive toxicity or carcinogenicity, or the reference active substance is not authorised for the proposed indication" as an example of evidence likely to be sufficient. It also includes, among others, "Preclinical differences that are inconclusive or unlikely to result in significant changes in clinical efficacy or safety" as an example of evidence unlikely to be sufficient.

The Applicant requested re-examination of the NAS status of levacetylleucine because it considers that there is sufficient compelling comparative non-clinical and clinical evidence to demonstrate the significant differences in the safety and/or efficacy profiles of the L-enantiomer (levacetylleucine) and the racemate (NADLL). The Applicant reaffirmed the claim that levacetylleucine qualifies as a NAS under indent 2 of the specified definition. The Applicant's argumentations in support of its position are summarised in the following points:

- Mechanism of action studies (including in NPC models) that demonstrate:
  - NALL normalises the Transcription factor EB (TFEB) pathway, resulting in increased autophagosome formation and ameliorating lysosomal function; however, neither the racemate NADLL nor the D-enantiomer ("NADL") has any effect on this pathway, which demonstrates the antagonistic effect of the D-enantiomer when administered as a racemate (NADLL).
  - NALL normalises levels of lysosomal calcium ions necessary for cellular signalling and lysosomal function; however, neither the racemate NADLL nor the D-enantiomer (NADL) has any effect on this pathway, which demonstrates the antagonistic effect of the D-enantiomer when administered as a racemate (NADLL).
  - NALL reduces substrate accumulation; however, neither the racemate NADLL nor the D-enantiomer (NADL) has any effect, which demonstrates the antagonistic effect of the D-enantiomer when administered as a racemate (NADLL).
  - NALL is the active isomer responsible for the neuroprotective, disease-modifying effects, whereas the D-enantiomer (NADL) is, at best, inert but antagonistic in the treatment of various pathways.
- Pharmacokinetic differences by which the D-enantiomer inhibits transport of the active L-enantiomer in NADLL, leading to an approximately 250% difference in the uptake of the L-enantiomer administered individually into neuronal cells compared with NADLL.

- Controlled clinical trial data demonstrating that NALL, but not NADLL, improves cerebellar signs and symptoms (on identical endpoints after the same duration of treatment).
- Patients and physicians reports on the back-to-back use of Tanganil (NADLL) and levacetylleucine (NALL) that demonstrate a substantially superior benefit-risk ratio with NALL. These reports include risks and intolerances with Tanganil that do not occur with NALL, as well as superior efficacy with NALL versus Tanganil both in symptomatic improvement and for long-term, disease-modifying treatment.

5.2.1. CHMP position on NAS status claim under Indent 2 based on non-clinical data

- **Mechanism of action studies (including in NPC models)**

In vitro findings: Mechanism of action

Lysosome Calcium Ion (Ca2+) Levels

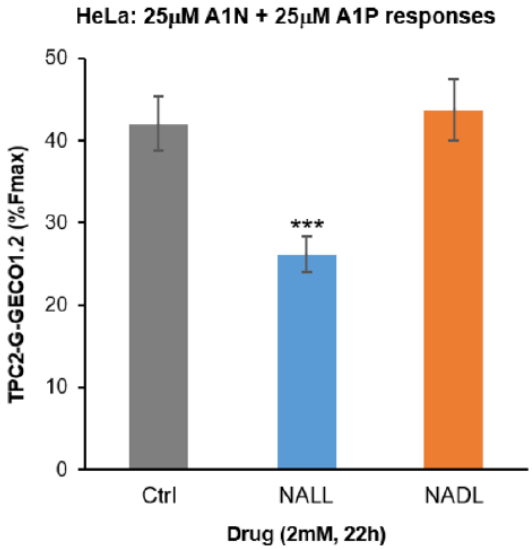


Figure 16: Levacetylleucine (NALL) treatment reduces lysosomal calcium in wild-type cells, but the D enantiomer (NADL) has no effect.

The *in vitro* experiment in wild-type HeLa cells shows that NALL reduces lysosomal calcium after 12-h treatment, while NADL shows no such effect. The meaning of this different NALL *vs*. NADL effect in wild-type HeLa cells for the diseases with reduced lysosomal calcium level is not clear.

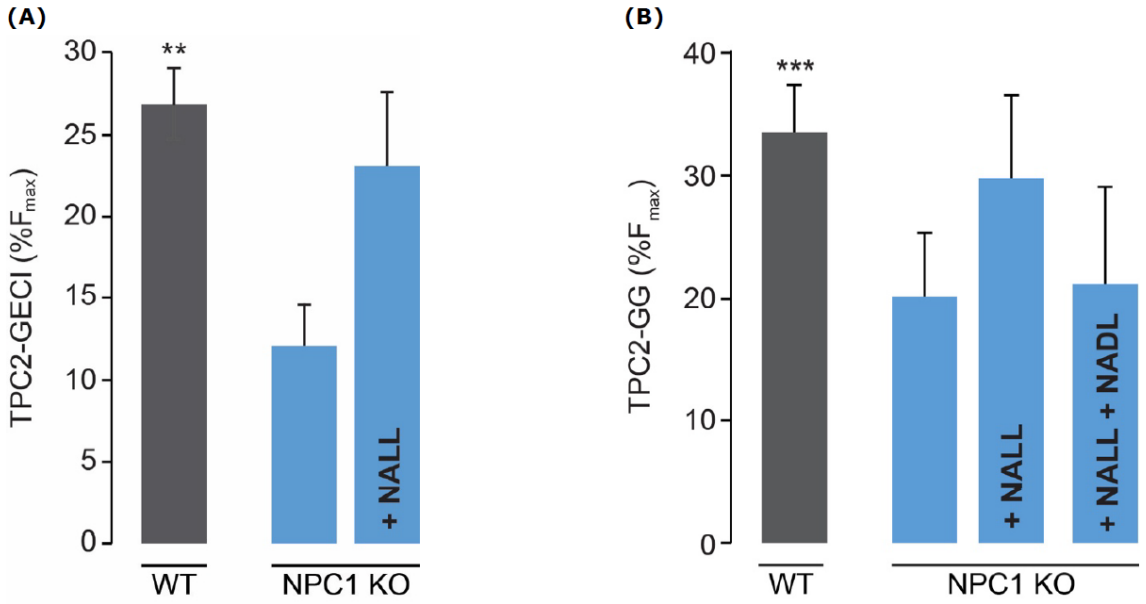


Figure 17: Impact of levacetylleucine (NALL) and racemate (NADLL) on lysosomal calcium

In figures showing results obtained in the ***NPC1-/- cells***, the statistical significance marks are not placed in relation between groups or explained in figure caption. Therefore, no conclusion can be derived except from visibly lower calcium level in lysosomes of *NPC1-/-* cells compared to wild-type control cells. In figure A, it seems that NALL significantly restores the calcium to similar levels as in wild type cells, while from figure B, the chances are impossible that any effect from treatment either with NALL or with the racemate might be significant based on the magnitude of variability (reference is made to the SEM bars).

Transcription factor EB (TFEB) pathway

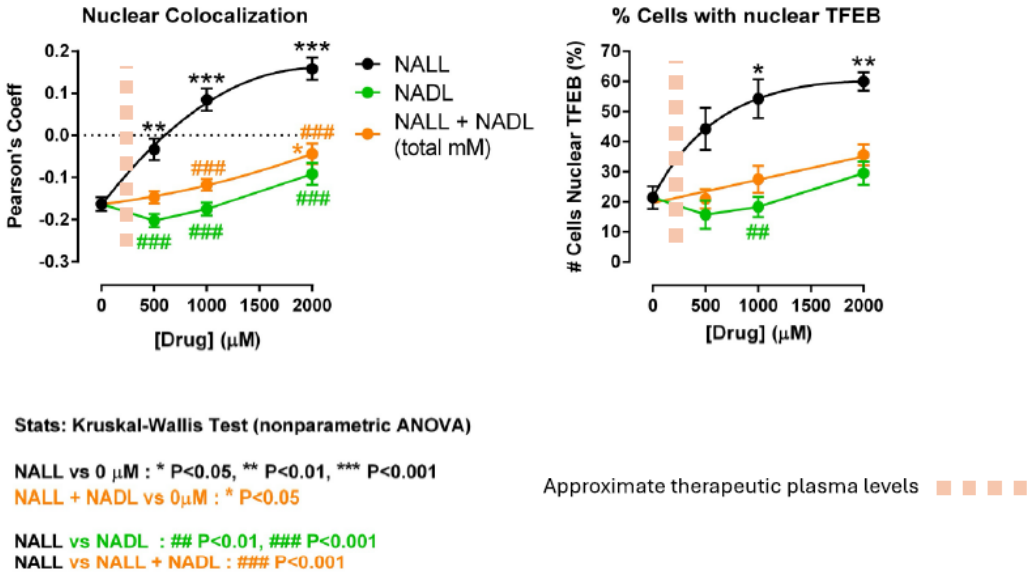


Figure 18: NALL, but not NADL, Activates Transcription factor EB

The above figure shows that NALL increases the percent of cells with nuclear TFEB, while NADL has no such effect. Moreover, it inhibits the effect of NALL within the NADLL. These results are obtained in HeLa

wild-type cells. As it was shown based on lysosomal calcium levels, the effects of NALL in HeLa cells and NPC1-/- cells may not be comparable. Additionally, the concentrations used in this assay are significantly above therapeutic plasma levels.

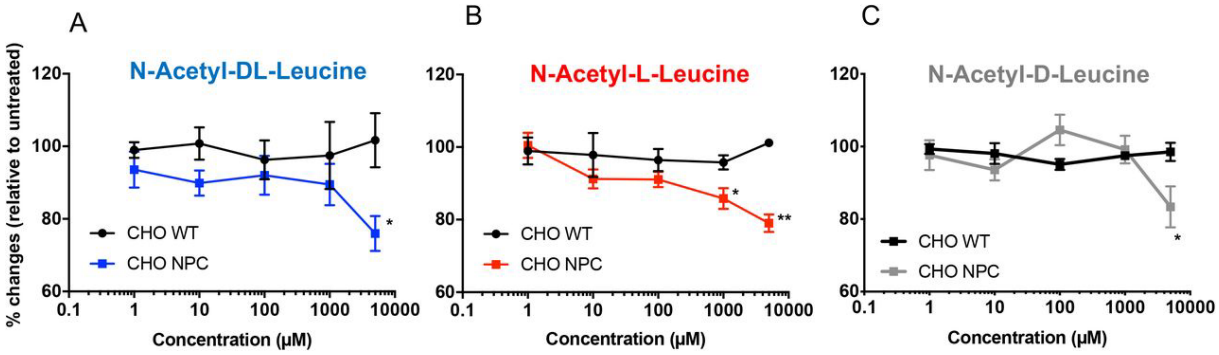
The novel research is indeed focused on TFEB as a target in various neurodegenerative and neurodevelopmental diseases, including Niemann-Pick type C disease. It is not agreed that TFEB is causal defect in NPC disease. Causal defect is the lack of NPC1 protein due to genetic mutation in the NPC1 gene. Without functional NPC1 or NPC2 proteins, cholesterol and other lipids accumulate in the lysosomes of cells leading to cellular malfunction.

The newest manuscript on the TFEB topic (Du et al., 2025) concludes that: "*…upregulating lysosome machinery via targeting TFEB represents a promising approach to treat NPC and related lysosomal storage diseases, and provides the possibility of TFEB agonists ie SFN as potential NPC therapeutic candidates.*" Therefore, based on this manuscript (published this year), the TFEB as a target would need further research to determine its clinical relevance in Niemann-Pick type C disease and the observed effect of NALL on TFEB may not be considered as crucial for the mechanism of action in NPC disease and is not, at this stage, considered relevant enough to carry the compelling non-clinical evidence required for NAS status.

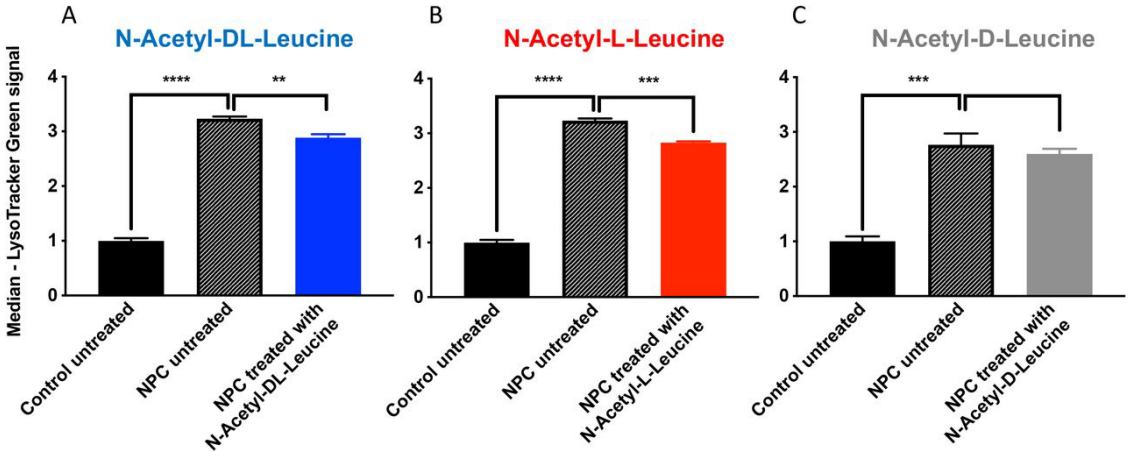
Next, the Applicant connects the TFEB pathway activation and the lysosomal volume reduction with NADLL/NADL observed in study published by te Vruchte et al. (2019) with a following conclusion:

„This finding (i.e., TFEB activation) *is confirmed by the in vitro and in vivo studies with NALL, NADL, and NADLL that showed that NALL was significantly more effective at reducing the storage of these key substrates [te Vruchte et al. 2019]. This can be directly attributable to NALL's — but not NADLL or NADL — regulation of TFEB."*

Te Vruchte et al. (2019) is a non-peer-reviewed paper published in bioRxiv (open access preprint repository), in which the effects of NADLL, NALL and NADL are compared on relative lysosomal volume in fibroblasts or wild type (WT) and NPC1-null CHO cell lines and in fibroblasts from NPC1 patients. The Applicant only shortly discussed the paper but did not present the figures from this paper (see figures below).

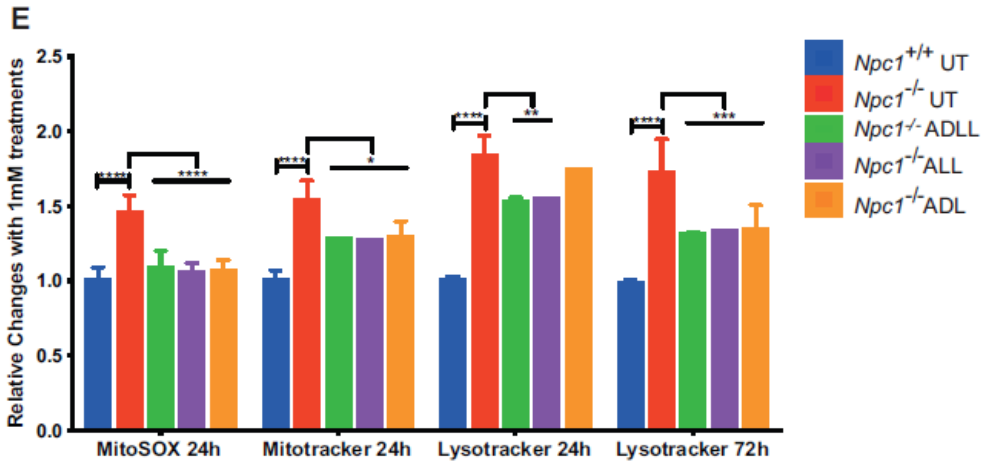


Interestingly, the above figure shows that all treatments reduced lysosomal volume at 5 000 µM. Only NALL had a statistically significant effect at 1000 µM. Nevertheless, these are all extremely high concentrations, of questionable clinical relevance. From Aqneursa prescribing information: *„Levacetylleucine maximum concentration (C_{max}) and area under the curve from time 0 to 24 hours ($AUC_{0\text{-}24hrs}$) were 8.3 (3.3) µg/mL and 33.2 (12.5) h*µg/mL."*



The above figure shows the results from human fibroblasts from NPC1 patients. Incubating NPC1 patient fibroblasts for 7 days with 1 mM NADLL and NALL led to a statistically significant decrease in fluorescent signal (A and B). Although 1 mM N-NADL showed a trend of reduced fluorescent signal, it failed to reach statistical significance (C). Note the similar values for *control untreated* bar and note the difference in *NPC untreated* bar at NADLL and NALL *vs*. NADL which has lower mean value.

In contrast to te Vruchte et al. (2019), Kaya et al. (2021) reported no difference in lysosomal volume between the treatments at 1 mM in NPC1-deficient CHO cells by utilizing lysosomal and mitochondrial probes.

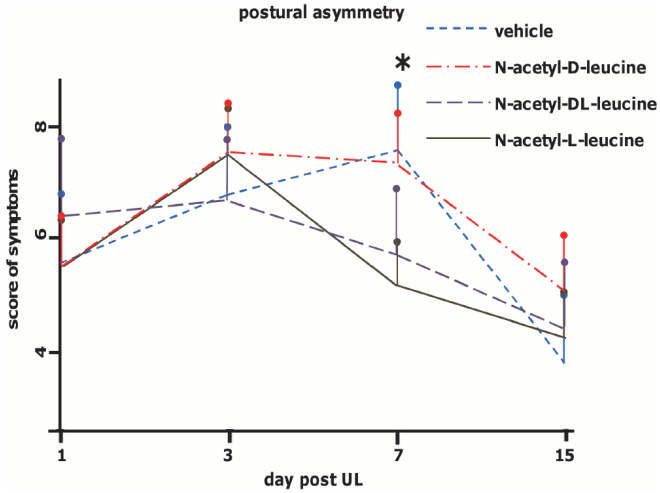


<u>*In vivo* findings: Vestibular compensation</u>

Although previous assessments disregarded these findings since they do not concern the intended indication, they are re-considered in this re-examination since they contain direct *in vivo* pharmacological comparisons of the racemate and separate enantiomers and since the potential for the clinical utility of N-Acetyl-DL-Leucine in the treatment of cerebellar disorders such as NPC, (e.g. in the reduction of ataxic symptoms), were hypothesized to occur through a potentially similar mechanisms observed in models of vertigo. This hypothesis was based on phylogenetic and electrophysiological similarities and close interactions between vestibular and deep cerebellar neurons. Two papers are discussed in this regard, in which it was demonstrated *in vivo* that the L-enantiomer is pharmacologically active. There were several hypotheses presented for the ineffectiveness of the D-enantiomer: the potential enzymatic degradation (e.g., potentially increased expression of D-amino-acid oxidase by astrocytes), differences

in isomer transport or incompatibility to stereospecific binding site. However, no differences between the effects of the L-enantiomer and the racemate could be observed (see the following figures below).

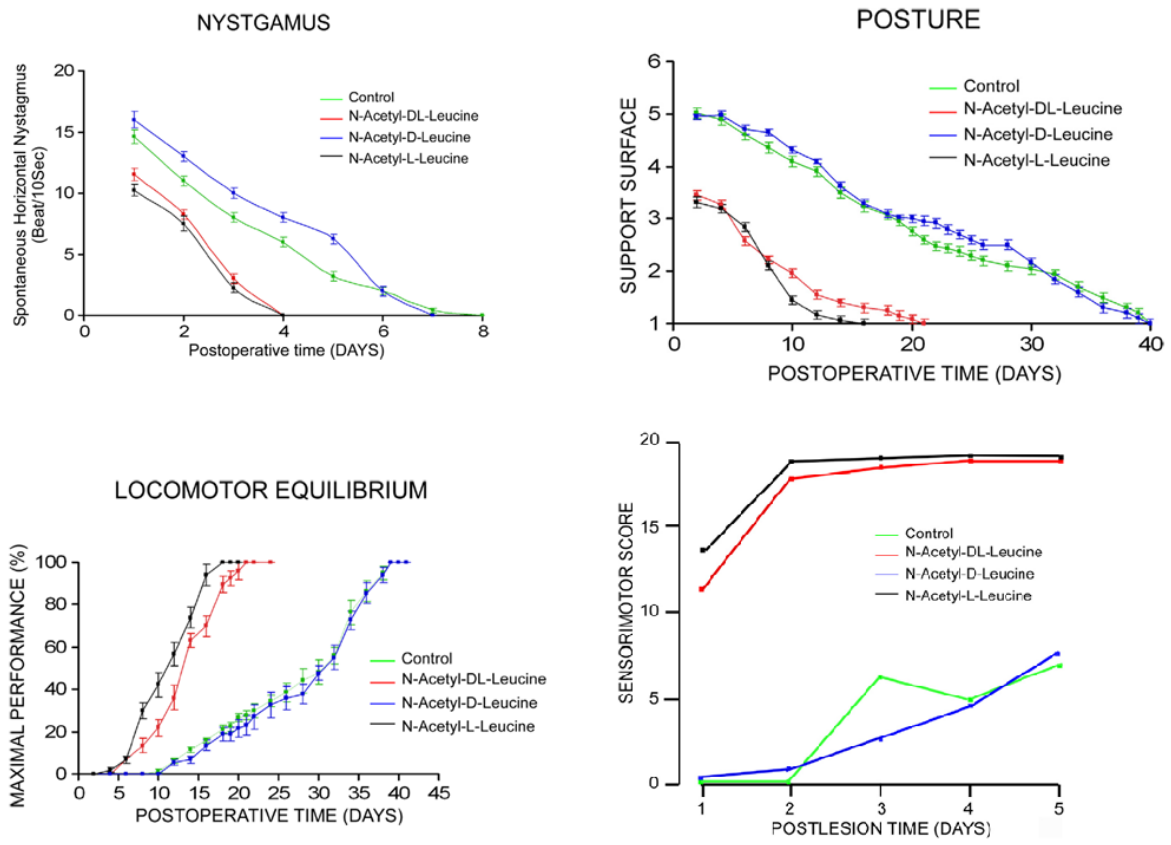
Gunther et al. (2015)



The above figure shows postural imbalance scores after unilateral labyrinthectomy in the control and treatment groups of Sprague-Dawley rats. Sham treatment (vehicle), N-acetyl-DL-leucine, N-acetyl-L-leucine or N-acetyl-D-leucine (60 mg/kg) were administered i.v. on days 1, 2 and 3 (six animals in each group). Postural imbalance scores were analysed in all groups on days 1, 3, 7 and 15. Postural imbalance scores were significantly decreased in the N-acetyl-DL-leucine- ($p < 0.03$) and N-acetyl-L-leucine-group ($p < 0.01$) on day 7.

Note that no difference in effect can be observed between acetyl-DL-leucine (ADLL) and acetyl-D-leucine (ADL), even though they are applied at the same dose and even though the study in later experiments, in which the effects of acetyl-L-leucine (ALL) and ADL were investigated, revealed that the L-enantiomer is pharmacologically active (results not shown here).

Tighilet et al. (2015)



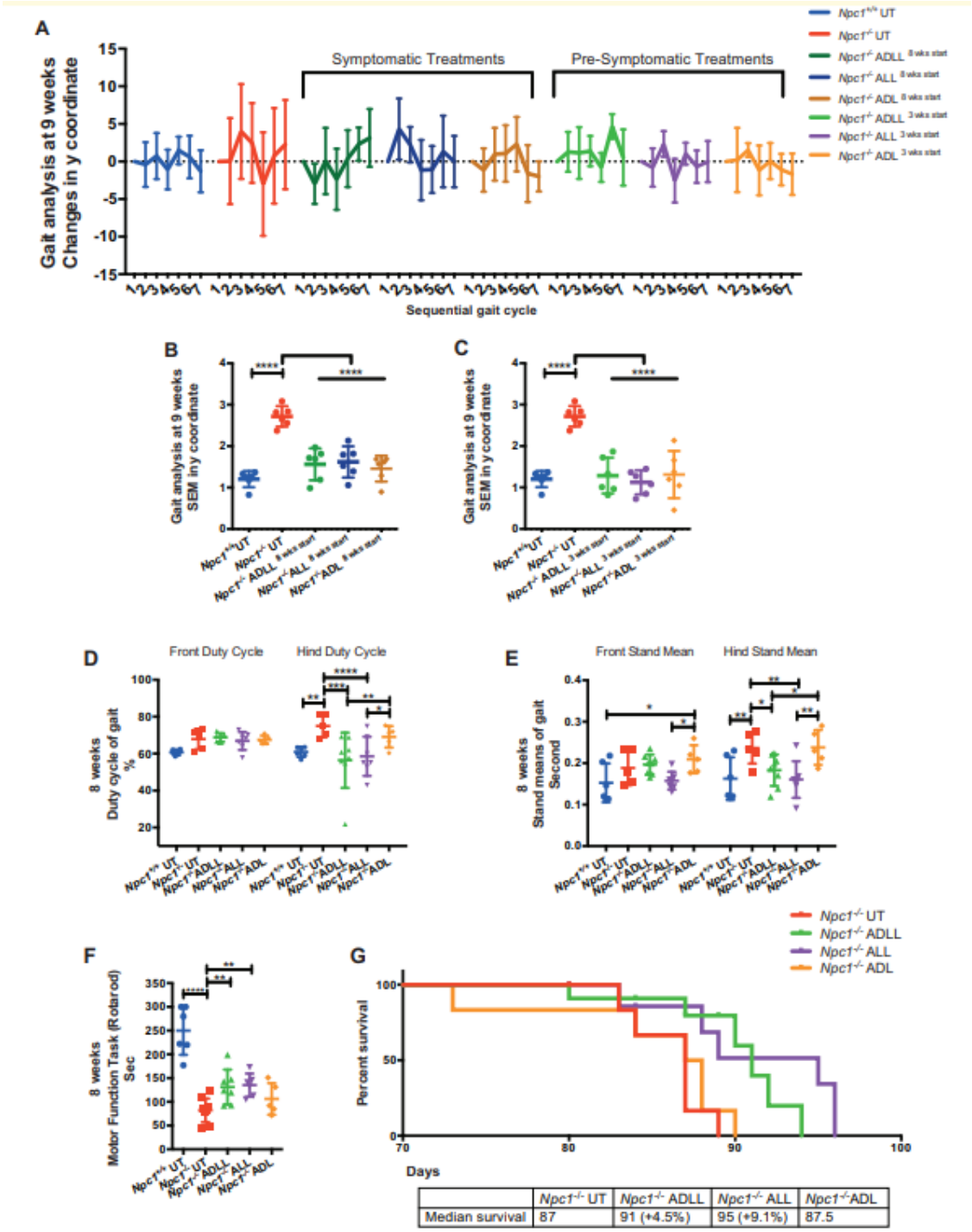
The above figures show ineffectiveness of the D-enantiomer on investigated parameters, while no significant difference can be observed for the racemate and the L-enantiomer. However, in contrast to previous study, in this experiment on unilateral vestibular loss model in cats, the racemate was applied at the dose of 30 mg/kg/day for the i.v. route, and then 60 mg/kg/day for the p.o. route. For the isomers, the doses were 15 mg/kg/day, and then 30 mg/kg/day, for the i.v. and p.o. routes, respectively.

In vivo findings: Niemann-Pick disease type C (Kaya et al., 2020)

The Applicant expressed the concern that the data from paper Kaya et al., 2020 have been selectively emphasized to discredit legitimate scientific findings: "*the CHMP highlighted four individual parameters (out of dozens presented in the publication) where the data were not conclusive of NALL being superiorly efficacious to NALL or NADL based on the p-values (including PDH-E levels, SOD2 levels, Purkinje cell loss)*".

Even if all data are taken into account, there is still not enough evidence to clearly note the superiority of the L-enantiomer over the racemate even though, in this study, all treatments are applied to animals in the same dose (see below).

The behavioural parameters evaluated in the study are presented in the following figure:



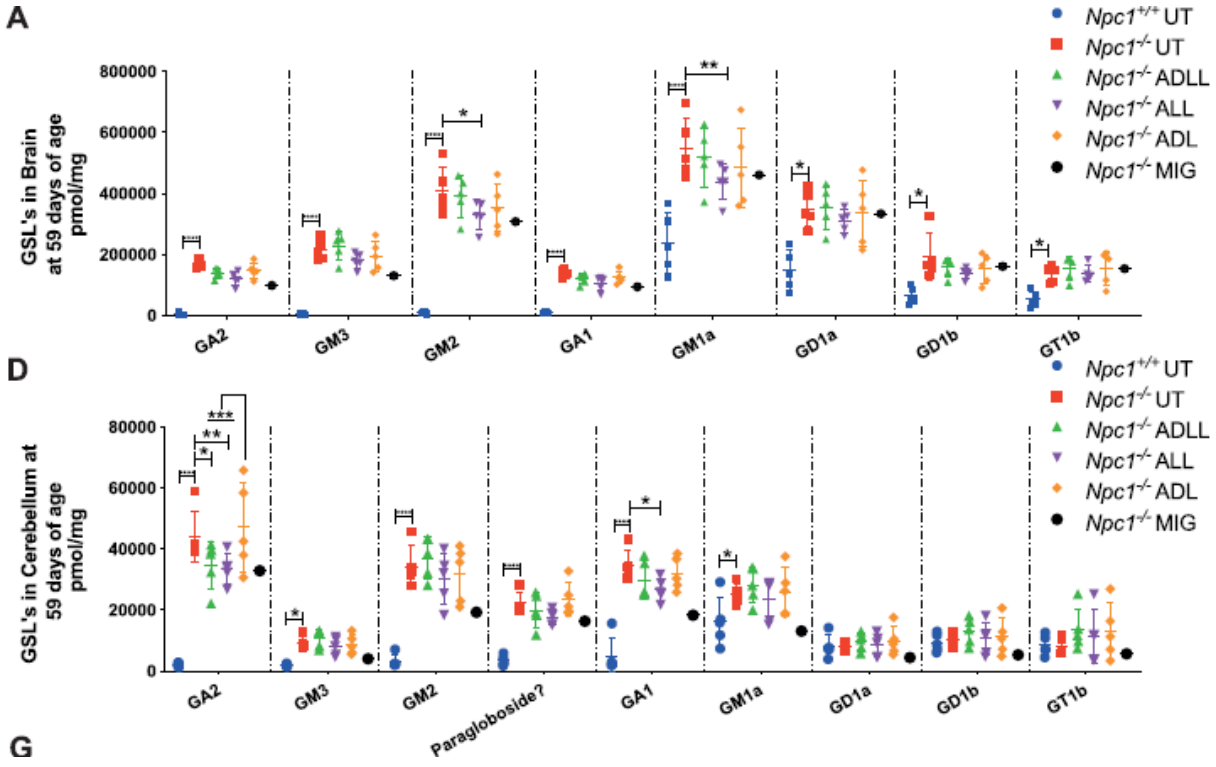
A-C) Anti-ataxic effect is stereoisomer independent, since all three treatments (0.1 g/kg/day) applied either in pre-symptomatic (A, C) or symptomatic (A, B) settings displayed significantly reduced ataxia. The individual enantiomers provided similar benefit to the racemic mixture for the symptomatic treatment of ataxia.

D-F) Pre-symptomatic treatment with ADLL and ALL, but not ADL, improves gait abnormalities (D-E) and motor function (F) in Npc1/ mice. It is hard to note any difference between the ADLL and ALL applied at the same dose.

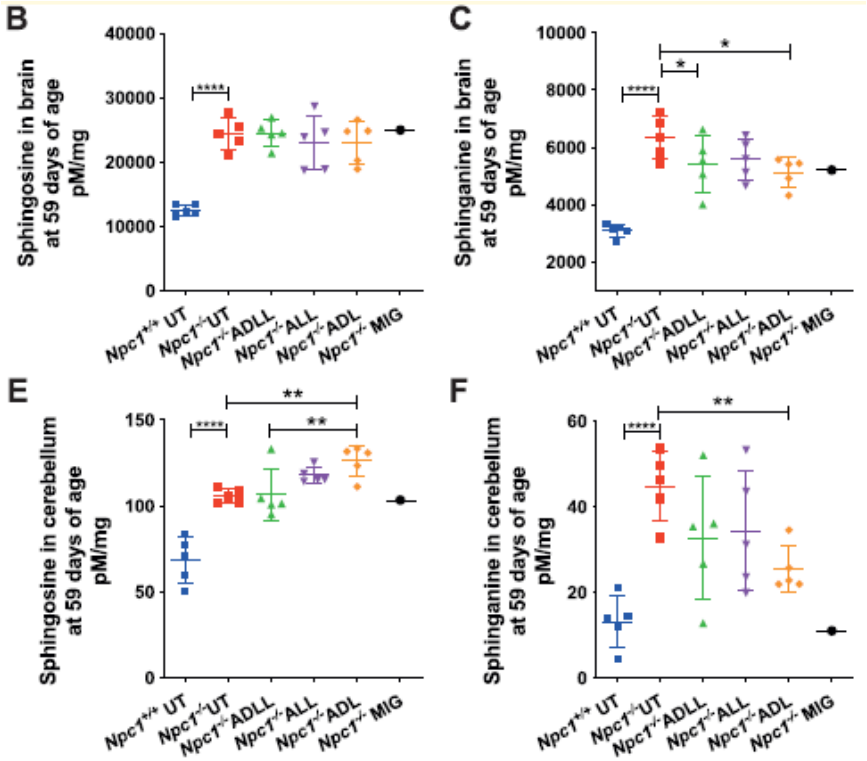
G) Pre-symptomatic treatment with ADLL and ALL, but not ADL, modestly extends survival in Npc1/ mice. Relative to untreated Npc1/ mice, the life span of animals treated from weaning was modestly but significantly increased by 8 days (9.1%) with ALL treatment (P = 0.0334), 4 days with ADLL (P = 0.0305) (4.5%) and was not changed with ADL (P = 0.6908). This effect displays the L-enantiomer selectivity and a better effect for ALL *vs*. ADLL can be seen at first. However, it is not clear how medians were obtained and how raw data look. In the graph, median survival is easy to verify for all groups except for the ALL group. It cannot be easily seen if the long horizontal ALL curve is near or at median line (50%). If this line is at 50%, then the median survival should be reported as the average of the first and last times at which survival is 50%. In this case, median survival would be very close to median survival of ADLL. Moreover, the question is how relevant the median is in situations where two curves intersect multiple times, as is the case here for the ADLL and ALL curves. Lastly, the choice of the method for evaluation of survival is not explained in the paper. Therefore, the interpretation of this figure is not straight-forward.

Effects on biochemical parameters

Reduction in lipid species in brain and cerebellum

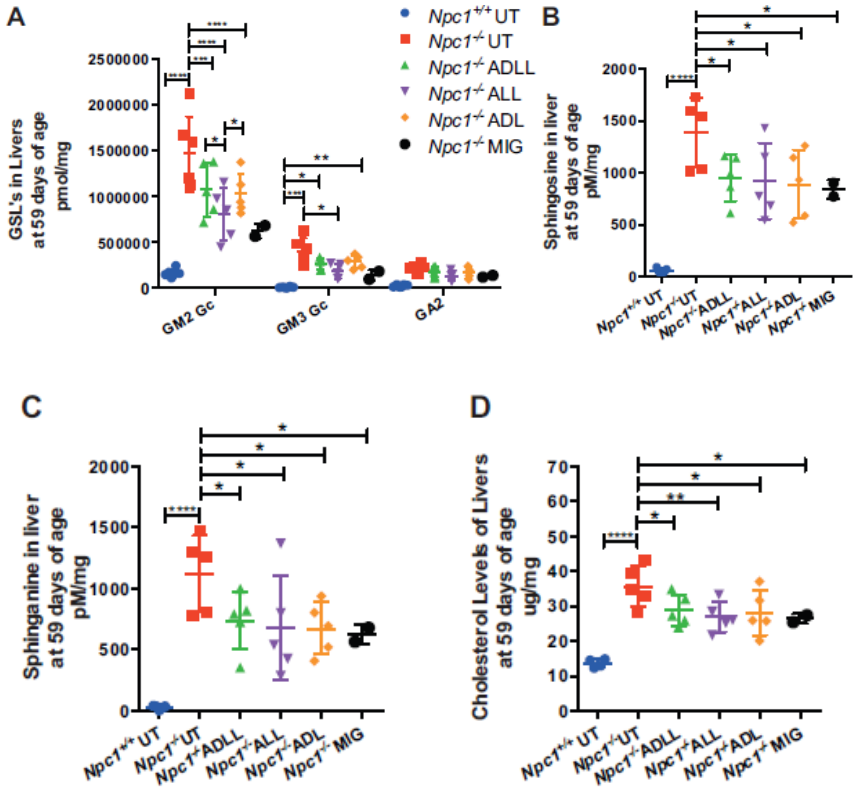


Glycosphingolipids in A) brain, B) cerebellum. Total GSLs in the forebrain and cerebellum were not significantly altered by any of the AL treatments (result not shown). Significant reduction of GM2 and GM1a only by ALL was observed in brain, while in cerebellum, a reduction of GA2 by ADLL and ALL treatment and a reduction of GA1 was observed with ALL treatment only. Total brain cholesterol levels are not changed in the NPC brain.



No effects on sphingosine in brain at any treatment, while increase in sphingosine in cerebellum by ADL. Decrease of sphinganine in brain and cerebellum by ADL was seen, while ADLL reduced sphinganine in brain.

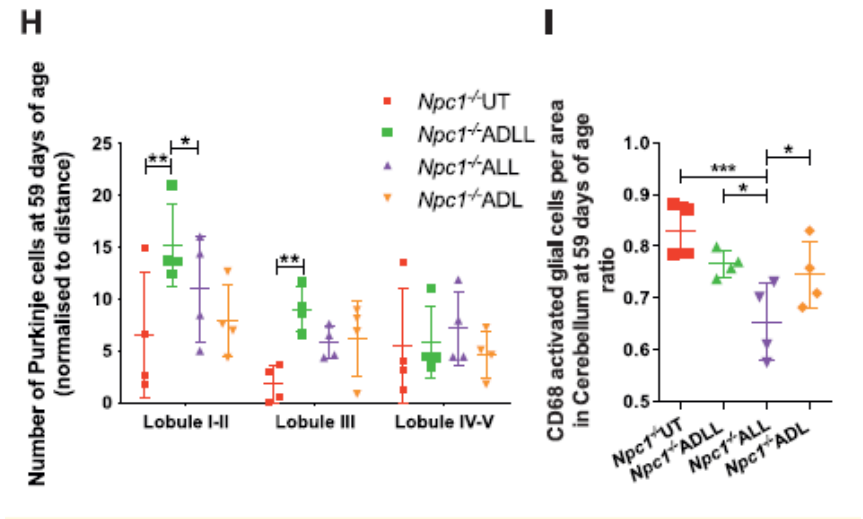
Reduction of lipid species in liver



In contrast to brain, all treatments alleviated lipid storage in liver (total GSL and major GSL species GM1Gc, GM3Gc and GA2). The effect of ALL achieved statistical significance only for GM3Gc (see figure A). The effect on all other lipids appears very similar between the treatments.

To summarize, ADLL and ALL had no effects on total sphingolipids and sphingosine in brain. In cerebellum, ADLL and ALL had no effects on total sphingolipids, sphingosine and sphinganine. ADLL reduced sphinganine in brain. ALL only was effective in reduction of several specific GSLs, but the targets were different in brain and in cerebellum. The meaning of these effects in the mechanism of action of ALL/ADLL are not clear and do not provide consistency. In contrast to brain treated Npc1-/- mice, AL analogues were observed to reduce the levels of stored lipids in the liver (see above) as well as in CHO cells (results not shown). Additionally, there were no differences in reduction of lysosomal volume (measured by several lysosomal and mitochondrial probes) by all AL treatments in NPC1-deficient CHO cells. Moreover, in the Kaya et al. paper, it is stated that: *„The mechanism that underpins this 'substrate reduction' action of ALs currently remains unclear, but in view of the high degree of synergy when combined the substrate reduction therapy drug miglustat, it may not be a major contributor to AL's therapeutic effect in NPC1 disease."* Therefore, any differences in observed parameters in therapeutic effect in NPC1 disease are questionable.

Purkinje cell loss and neuroinflammation in the cerebellum

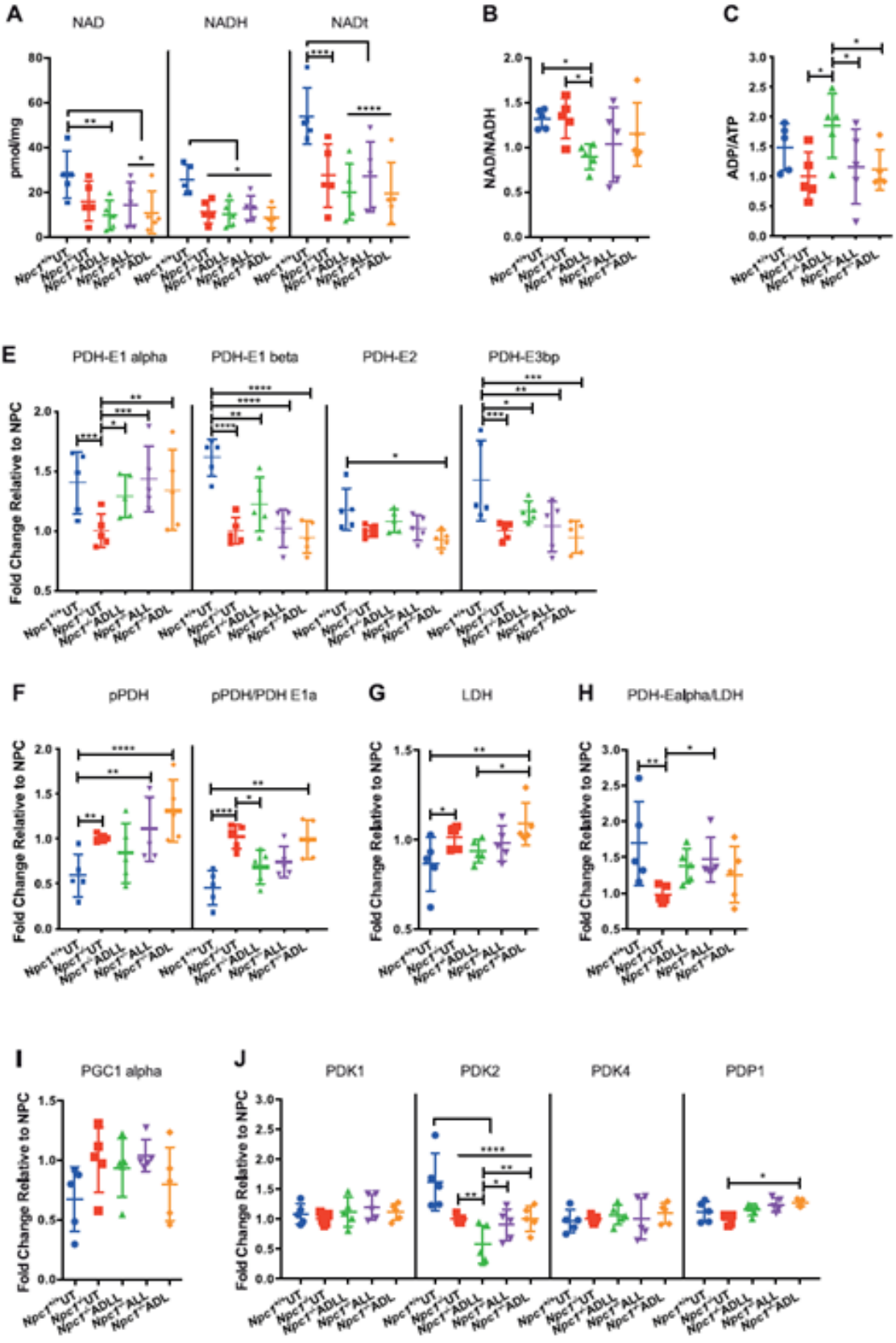


Only ADLL significantly increased Purkinje cell survival, while only ALL decreased the number of activated microglia. ADL did not mediate any long term, neuroprotective effects assessed with Purkinje cell count and CD68 staining.

In addition to an unclear mechanism of action on symptomatic improvement of ataxia which appears stereoisomer independent, based on the observed effect of ADLL and ALL on Purkinje cell survival and reduction of activated microglia, it was hypothesized that the L-enantiomer has neuroprotective effect in NPC1. However, current results do not allow to draw conclusions on mechanisms of potential neuroprotective effects. Regarding the changes observed on the glucose/energy metabolism, even the Applicant claims:

"The Applicant again wishes to emphasize that it is admittedly not known which of the cellular parameters examined in the NPC mice directly impact functional outcomes or survival."

Although any further discussion on potential superiority of the L-enantiomer over the racemate in the context of these effects seems pointless, the figures containing all parameters related to metabolic changes in cerebellum are listed below. None of them points to the L-enantiomer superiority. On the contrary, most Npc1-/- effects are reversed by the racemate, while ALL normalized only altered levels of PDH (figure E) and LDH (figure G), but not more effectively than the racemate.


A
NAD
NADH
NADt
pmol/mg
B
NAD/NADH
C
ADP/ATP
E
PDH-E1 alpha
PDH-E1 beta
PDH-E2
PDH-E3bp
Fold Change Relative to NPC
F
pPDH
pPDH/PDH E1a
G
LDH
H
PDH-Ealpha/LDH
I
PGC1 alpha
J
PDK1
PDK2
PDK4
PDP1
Npc1+/+UT
Npc1-/-UT
Npc1-/-ADLL
Npc1-/-ALL
Npc1-/-ADL

In conclusion on *in vitro* and *in vivo* pharmacology, deficiencies are identified at both levels. The study by Kaya et al. (2021), in which the efficacy of ADLL and its distinct enantiomer components was examined *in vivo*, in a mouse model of NPC1, is considered of highest importance in the present case. In this study, the same doses of the racemate and the separate enantiomers were used. Similar effects between the racemate and ALL were observed in almost all or majority of parameters, even though there was 50% less "active" L-enantiomer applied to the animals within the racemate and even though significant PK differences in the L-enantiomer exposure when applied alone or as racemate were reported (i.e. the antagonistic effect on absorption of the L-enantiomer by the D-enantiomer when applied orally). Potential "ceiling" effect of the L-enantiomer was not considered and discussed up to now and dose-response, which might solve this issue, has not been performed. Some dose-response experiments were performed *in vitro*, but the findings are questionable due to high concentrations that were examined, in comparison to concentrations of ALL that are therapeutic.

- **Pharmacokinetic differences by which the D-enantiomer <u>inhibits</u> transport of the active L-enantiomer in NADLL, leading to an approximately 250% difference in the uptake of the L-enantiomer administered individually into neuronal cells compared with NADLL.**

It is agreed that there are PK differences between the racemate and the L-enantiomer and that non-clinical PK data suggest that the D-enantiomer negatively impacts the uptake of the L-enantiomer. Additionally, it is agreed that these PK differences observed in mouse may translate to humans based on highly conserved MCT1 and acylase activities across mammals.

- **<u>Potential toxicity differences between ALL and ADLL due to ADL</u>**

There are no experimental data available to compare the long-term toxicity profile of the racemate and enantiomers. From the 3R aspects, these studies are not warranted in the light of unresolved issues at pharmacological level. However, the absence of substance-specific toxicity data must be emphasized to be clear that hypothetical considerations cannot be accepted as compelling evidence. The hypotheses on toxic effects of ADL based on discussion on potential clinical implications of "unexpected" PK data from Churchill et al. (2020) as well as a few selectively picked references are unsubstantiated and only speculative. Only one single-dose PK study was performed, while no data are available to determine PK following repeat-dosing to verify claims on accumulation of the D-enantiomer and associated toxicity.

The theoretical considerations developed by the Applicant cannot be accepted from non-clinical perspective as compelling evidence.

Conclusion based on non-clinical data

It can be agreed that there are PK differences between the racemate and the L-enantiomer and that non-clinical PK data suggest that the D-enantiomer negatively impacts the uptake of the L-enantiomer.

The next step would be to determine if these PK differences affect the pharmacological effects between the L-enantiomer and the racemate. Even if the CHMP could agree that non-clinical pharmacology studies in totality (but not in all individual assays) may suggest the superiority of the L-enantiomer over the D-enantiomer, there is insufficient evidence to conclude that the L-enantiomer differs significantly from the racemate.

Based on a detailed review of the submitted data, it is understood that the molecular target(s) of NALL in NPC disease are currently not know. This interpretation can also be confirmed with the currently approved labelling for NALL, which states that:" *The distinct molecular target for levacetylleucine in the*

treatment of NPC is unknown." *(FDA, section 12.1 of the Full Prescribing Information)*. Therefore, deriving any conclusion from *in vitro* and *in vivo* biochemical data is difficult. Nevertheless, even this type of data (*in vitro*) does not provide sufficient evidence to conclude on significant differences between the L-enantiomer and the racemate. *In vivo* data, which investigate the concrete effects on several symptoms of NPC disease model are considered the most informative for the evaluation of potential differences between the two treatments. However, *in vivo* data in NPC model not only suggest that the racemate and the L-enantiomer are equally effective (although applied in the same doses) but suggest that the D-enantiomer is effective at least in reduction of ataxia. Although these findings are in contrast to findings from vestibular compensation models, in which the D-enantiomer is totally ineffective, it is not clear why the Applicant claims that the D-enantiomer is antagonistic, when a clear therapeutic anti-ataxic effect can be observed with the D-enantiomer as well, while other behavioural parameters examined in mouse NPC model are not worsened by the D-enantiomer. The potential neuroprotective effects that can be attributed only to ALL (and not to ADL) require further investigation for potential impact on functional level.

Additionally, there are no comparative toxicity data to substantiate the claims on potential deleterious effects of the D-enantiomer to justify the superiority of the L-enantiomer over the racemate in the view of safety.

It is acknowledged that the Applicant conducted the development program for the isolated L-enantiomer in line with the guidance on development of chiral substances/racemic mixtures. Early non-clinical data provided input that the L-enantiomer may be superior candidate for further development. However, it does not automatically mean that early investigative data (e.g., *in vitro* data or *in vivo* animal data) will be replicated and confirmed in the clinic.

In the present case there are no comparative clinical data available on efficacy or safety. Therefore, compelling non-clinical data is a prerequisite as evidence to show differences. The presented non-clinical data alone still do not provide compelling evidence to support the NAS status for levacetylleucine since the comparative pharmacological data between the L-enantiomer and the racemate do not allow to conclude that the L-enantiomer differs significantly from the racemate. Although PK studies demonstrated that administration of NADL as part of the racemate supresses exposure to NALL, pharmacological data do not suggest that efficacy of the racemate is reduced because of this.

The in-depth interpretation and re-assessment of the submitted data only strengthened previous concerns that non-clinical evidence is not compelling enough to fulfil regulatory requirements for granting the NAS status for levacetylleucine according to the *Reflection paper on considerations given to designation of a single stereo isomeric form (enantiomer), a complex, a derivative, or a different salt or ester as new active substance in relation to the relevant reference active substance* (EMA/651649/2010).

5.2.2. CHMP position on NAS status claim under Indent 2 based on clinical data

- **Controlled clinical trial data**

According to current EMA guidelines for NAS designation, where a previously authorised medicinal product for human use in the European Union includes a racemate and a new application for only one of the two enantiomers of the racemate is submitted, this enantiomer would have been a substantial part (50 %) of the racemate and would therefore be considered as the same active substance as the racemic mixture, unless the Applicant provides evidence that the two substances differ significantly in properties

with regard to safety and/or efficacy. Though the decision is made on a case-by-case basis, the preferred type of evidence to show significant differences justifying NAS status is a direct comparison via head-to-head clinical studies of both active substances, unless there is compelling evidence derived from pre-clinical and/or clinical data. Although indirect, non-comparative evidence may be acceptable, it is noted that this type of evidence may be less compelling (EMA/CHMP/QWP/104223/2015, EMA/651649/2010).

A direct head-to-head comparison between NALL (N-acetyl-L-leucine, L-enantiomer) and the racemate, NADLL (N-acetyl-DL-leucine), to compare their efficacy and safety profiles has not been performed in patients with Niemann-Pick disease type C or any other similar indication, due to feasibility/ethical issues, which are acknowledged. To overcome this important gap in the evidence, the Applicant has provided an indirect comparison between trials performed with NALL (IB1001-201, IB1001-202, and IB1001-301) and NADLL (ALCAT), concluding that clinical efficacy with the L-enantiomer but not with the racemate has been demonstrated. Notably, no statistical method for indirect comparison has been used to arrive at that conclusion and therefore, important uncertainties arise as to the acceptability of that interpretation of the available clinical data. Even though all the compared clinical trials assessed the effects of the studied treatment on ataxia, the high heterogeneity between the studies (population, study design, interventions and outcomes) precludes a conclusion regarding the comparison between the active substances.

To illustrate the heterogeneity between studies, an analysis of the most significant differences is provided below:

Design of studies:

ALCAT: double-blind cross-over study with two 6-week treatment periods separated by a 4-week washout; IB1001-201 and IB1001-202: open-label studies, IB1001-301: double-blind cross-over study with two 3-month treatment periods with no washout period in between.

Patient population:

- Disease:

The Applicant's argumentation regarding the clinical aspects is mostly based on an indirect comparison of 4 scientific publications: 1) Feil et al (2021) assessing the efficacy of acetyl-DL-leucine in the treatment of cerebellar ataxias (ALCAT study); 2) Bremova-Ertl et al (2022) assessing the efficacy of acetyl-L-leucine in the treatment of Niemann-Pick type C disease (study IB1001-201); 3) Bremova-Ertl et al (2024) assessing the efficacy of acetyl-L-leucine in the treatment of Niemann-Pick type C disease (study IB1001-301 study); and 3) Martakis et al (2023) assessing the efficacy of acetyl-L-leucine in the treatment of GM2 gangliosidosis (study IB1001-202).

Below is the assessment of the validity of that comparison, using as the starting point the only study provided by the Applicant that assesses the efficacy of the racemate, which is on a different patient population with a different disease (and disease group) that was used in the studies and development plan of the L- enantiomer (Feil et al (2021) study population).

In table 1 of the Feil 2021 JAMA study, the characterization of the baseline patient population is presented, indicating that Cerebellar ataxia subtypes were hereditary (77.8-75.9%) and nonhereditary (22.2-24.1%), leading to subgroups of Spinocerebellar Ataxia (SCA - autosomal dominant), Autosomal recessive, other SCA types and Sporadic (SAOA).

When consulting the paper's supplementary information, the table describing the patient's conditions further detail the following diagnosis: Spinocerebellar Ataxia, Friedreich's ataxia, SYNE1 ataxia, CANVAS and SAOA. It should be noted that this table specifically describes primary cerebellar ataxias — diseases defined by their cerebellar degeneration as the core feature. Niemann-Pick and GM2 are systemic

lysosomal diseases where ataxia is secondary and therefore are classified separately (in metabolic or lysosomal categories, not SCA/ARCA/CANVAS classes).

Disease core pathophysiology

- *Cerebellar ataxias* are primary neurodegenerative diseases affecting the cerebellum and its afferent/efferent pathways.

This is often due to neuronal loss, synaptic dysfunction, or impaired cerebellar circuitry in Purkinje cells, dentate nucleus, brainstem connections, or spinocerebellar tracts.

This can result from genetic mutations (e.g., SCAs), sporadic neurodegeneration (e.g., MSA-C), or acquired causes.

- Niemann-Pick type C disease is a lysosomal lipid trafficking defect caused by mutations in NPC1 or NPC2 genes, leading to intracellular accumulation of unesterified cholesterol and glycosphingolipids in neurons, hepatocytes, and other cell types.

This results in widespread neuronal dysfunction, axonal degeneration, and cell death, as well as visceral organ damage (liver, spleen).

- GM2 gangliosidosis is a lysosomal storage disease due to deficiency of β-hexosaminidase A (Tay-Sachs) or A + B (Sandhoff), leading to accumulation of GM2 gangliosides in neurons and glia, causing neuronal swelling, dysfunction, and death.

It affects both central and peripheral nervous systems and, in Sandhoff, visceral organs.

Disease course / clinical manifestations

- *Cerebellar ataxias*, as a disease group classification is a heterogeneous group (SCA, MSA-C, idiopathic, etc.), characterized by a CNS-limited pathology, largely restricted to the cerebellum and brainstem, with rare or secondary peripheral or systemic involvement.

- This is opposed to *NPC* where manifestations are multisystemic, involving the liver, spleen, lung, and CNS. Neurological manifestations dominate later, but systemic signs may precede them.

- For *GM2 gangliosidosis*, severe forms involve liver, spleen, bone marrow, and skeletal system, while juvenile/adult forms are predominantly neurological but still multisystemic.

- Regarding the ataxia, in *cerebellar ataxias*, ataxia is the primary and defining feature of the disease, with ataxia being the central clinical manifestation, often with accompanying features like dysarthria, oculomotor deficits, and gait imbalance.

- In *NPC* ataxia is common but not a primary feature. Cerebellar ataxia in NPC results from progressive Purkinje cell degeneration but is part of a broader neurodegenerative picture including cognitive decline, vertical supranuclear gaze palsy, dystonia, seizures, and psychiatric symptoms.

- The same happens for *GM2 gangliosidosis*, in which ataxia is a frequent manifestation, but not a defining one. Cerebellar ataxia occurs due to secondary cerebellar and spinal tract degeneration, often alongside spasticity, hypotonia, seizures, vision/hearing loss, and cognitive decline.

- Inclusion criteria: age (ALCAT: ⩾18 years, IB1001-201 and IB1001-202: ⩾6 years, IB1001-301: ⩾4 years), weight (ALCAT: not included as inclusion criteria; IB1001-201, IB1001-202 and IB1001-301: ⩾ 15 kg), Scale for the Assessment and Rating of Ataxia (SARA) score (ALCAT: ⩾3 points, IB1001-201 and IB1001-202: ⩾5 and ⩽33, IB1001-301: ⩾7 and ⩽34).

- Baseline demographic/disease characteristics: mean age in years (ALCAT: 54.8, IB1001-201: 28.8, IB1001-202: 27, IB1001-301: 26.4); median SARA score (ALCAT: 12.25; IB1001-201: 12.0, IB1001-202: 11.75, IB1001-301: 14.50).

With regard to age, the typical adult onset of cerebellar ataxias is reflected in the baseline characteristics of the Feil 2021 study (ALCAT study), with a mean age at diagnosis of 55 years. This opposed to a much earlier age of manifestation for Niemann-Pick type C disease (commonly associated with Progressive neurovisceral systemic disease with premature death) and GM2 gangliosidosis (in the case of infantile form can lead to death by 4yrs). This is also reflected in the mean age at diagnosis of 27 years and 20.5 years for the NPC studies (studies IB1001-201 and IB1001-301, Bremova-Ertl 2022 and 2024) and 23 years for the GM2 gangliosidosis study (study IB1001-202, Martakis, 2023), significantly different from the Feil 2021 study.

- Background treatment:

Also, to be noted as an additional factor that adds another layer of differences between these studies is the fact that a very significant part of the Niemann-Pick type C disease study population was under an active treatment with an approved medicine and not just supportive care. In the studies assessing the efficacy of acetyl-L-leucine in the treatment of Niemann-Pick type C disease (IB1001-201 and IB1001-301), 70-75% of patients have miglustat as a background treatment, which not only adds a bias in assessing efficacy but also adds arguments to the fact that the patient populations and respective diseases are significantly different and cannot be used in bridging exercises to compare efficacies.

Interventions:

- Formulations: powder for oral suspension (NALL) *versus* tablets (NADLL), which can influence PK and bioavailability and therefore pharmacological activity.

- Dosing regimens: ALCAT study: 5 g per day after a 2-week up-titration period (1.5 g per day taking the first week, 3 g per day taking the second week). IB1001-201, IB1001-202 and IB1001-301: 2 g per day, 3 g per day or 4 g per day, depending on age and body weight.

Primary efficacy endpoint:

SARA in ALCAT and IB1001-301; Clinical Impression of Change in Severity in IB1001-201 and IB1001-202.

Conclusion

The comparison performed between the Feil study on Cerebellar Ataxias and the Bremova-Ertl / Martakis studies does not allow for a proper efficacy comparison of acetyl-DL-leucine and acetyl-L-leucine.

The diseases differ in: group classifications (CA as an heterogeneous group of several CA manifestations) *vs* well-characterised Lysosomal Storage Diseases; pathophysiology and clinical course/manifestations (CNS-limited *vs* Visceral/Multisystemic, different central and peripheral neurologic manifestations, ataxia as primary vs secondary manifestation); age of onset, reflected in the very significant difference in mean age of diagnosis between studies (55 yrs for the Cerebellar Ataxias study *vs* 20-23 yrs for the NPC and GM2 gangliosidosis study); and on the background treatment, since for the case of Niemann-Pick type C disease, there is a treatment approved and in both the studies provided miglustat was an active treatment in 70-75% of participants, which clearly constitutes not only a bias in the comparison analysis but also highlights the differences in the diseases.

Regarding the Applicant's argument on similar pathophysiology, the common aspects are secondary when compared to core pathophysiology of the three diseases. Stating that all three diseases culminate in "cellular dysfunction, neuroinflammation, cell death and neurodegeneration" is a statement that can be applied to the majority, if not all, neurodegenerative diseases. The core pathophysiology and the

molecular pathways associated with onset and development of the diseases are closely correlated to the efficacy of different pharmacological approaches and their respective targets.

Furthermore, heterogeneity between studies is also noted in the design of the studies, interventions (formulations and dosing regimens), primary efficacy endpoint and inclusion criteria.

The lack of evidence of acetyl-DL-leucine efficacy in the treatment of vertigo cannot be used to establish superior efficacy of acetyl-L-leucine since there is no study of acetyl-L-leucine in the same condition to compare efficacy.

Taking all these factors into consideration, an efficacy comparison between acetyl-DL-leucine and acetyl-L-leucine using a bridging strategy to extrapolate efficacy data from different conditions and patient populations cannot be supported.

- **Lack of comparative efficacy data**

Regarding point 1. of the argumentation stating that "The Applicant did provide clinical data indirectly comparing the clinical efficacy of the racemate (NADLL) and NALL", the issue is not if data was provided but rather the inability for that data to support the Applicant's claim of superior efficacy. The comparison of clinical studies in different diseases cannot be used to establish a proper comparison and therefore cannot support a conclusion of significant differences, either in efficacy or safety.

In Kaya et al (2021), Niemann-Pick disease type C patients were evaluated after 12 months of acetyl-DL-leucine treatment and rates of disease progression were slowed, with stabilization or improvement in multiple neurological domains. In Bremova et al (2015) 12 patients with NP-C disease were treated with acetyl-DL-leucine 3 g/d for 1 week and then with 5 g/d for 3 weeks with a subsequent washout period of 1 month. Treatment improved SARA, SCAFI, mDRS score, and VAS scores.

However, despite both studies were associated with clinical improvements, it should be noted that studies were smaller in size when compared to Bremova 2022 and Bremova 2024 and with considerable limitations associated with the observational type of study and its wide standard deviations in the clinical endpoints. Therefore, it can be agreed that these studies are insufficient to accurately characterize the efficacy profile of acetyl-DL-leucine in NPC.

Nevertheless, given the observation from the sponsor that "no clinical study existed to show clinical benefit of NADLL" it is therefore important to mention here that it is not completely true.

Furthermore, the Applicant has also used argumentation points in the safety discussion that includes tolerability data from informal, non-blinded, not standardised reports, which in this case cannot even be considered an observational study.

Regarding the Applicant's suggestion that a direct head-to-head comparison was suggested and references to the Helsinki Declaration were used, the initial CHMP remark merely asked for "data supporting a relevant difference in terms of clinical efficacy and safety between the L-enantiomer and the racemate."

This does not imply that a head-to-head study should be performed, but rather that a proper comparison should be made. This comparison could be performed, in principle, by indirect comparison. The limitation with the data provided by the Applicant is that the data is unable to support the existence of significant differences in safety and/or efficacy due to differences in patient populations.

<u>Safety argument</u>

Comparing safety data from acetyl-DL-leucine *vs* acetyl-L-leucine cannot be considered adequate. Pharmacovigilance data from acetyl-DL-leucine is related to a different exposed population given the

very different indications. Even in this setting, given the limited exposure of the NPC population with acetyl-L-leucine, the comparison would have serious limitations.

- **Patients and physicians reports on the back-to-back use of Tanganil (NADLL) and levacetylleucine (NALL)**

Furthermore, the tolerability data provided by the Applicant from patients and physicians is originated from informal, non-blinded, not standardised reports and cannot be used to support a claim of significant differences in safety.

Conclusion based on clinical data

Regarding clinical data, reassessment of the Applicant's grounds for re-examination led to the following conclusions:

1. The Applicant's argumentation regarding the clinical aspects is mostly based on an indirect comparison of four scientific publications: 1) Feil et al (2021) assessing the efficacy of acetyl-DL-leucine in the treatment of cerebellar ataxias; 2) Bremova-Ertl et al (2022) assessing the efficacy of acetyl-L-leucine in the treatment of Niemann-Pick type C disease; 3) Bremova-Ertl et al (2024) assessing the efficacy of acetyl-L-leucine in the treatment of Niemann-Pick type C disease; and 3) Martakis et al (2023) assessing the efficacy of acetyl-L-leucine in the treatment of GM2 gangliosidosis.
2. Feil et al (2021) is the only study provided by the Applicant that assesses the efficacy of the racemate, but it focuses on a different patient population with a different disease (and disease group) than the one used in the studies and development plan of the L- enantiomer, with different disease core pathophysiology; different disease course/clinical manifestations; different age onset; different background treatment. The comparison performed between the Feil study on Cerebellar Ataxias and the Bremova-Ertl / Martakis studies does not allow for a proper efficacy comparison of acetyl-DL-leucine and acetyl-L-leucine.
3. On the clinical data with Tanganil, the lack of evidence of acetyl-DL-leucine efficacy in the treatment of vertigo cannot be used to establish superior efficacy of acetyl-L-leucine since there is no study of acetyl-L-leucine in the same condition to compare efficacy,
4. On the arguments provided related with different safety profile, comparing the safety data from acetyl-DL-leucine *vs* acetyl-L-leucine cannot be considered adequate as the pharmacovigilance data from acetyl-DL-leucine is related to a different exposed population given the very different indications. In addition, the limited exposure of the NPC population with acetyl-L-leucine, raises the serious limitations on the comparison made.
5. Furthermore, the tolerability data provided by the Applicant from patient/physician is originated from informal, non-blinded, not standardised reports and cannot be adequately used for regulatory support of superior safety.

In conclusion, on the basis of the totality of clinical data provided by the Applicant in this re-examination procedure, it is not possible to conclude that levacetylleucine (N-acetyl-L-leucine) differs significantly in properties with regard to safety and/or efficacy from N-acetyl-DL-leucine.

5.3. Overall conclusion on the grounds for re-examination

The CHMP assessed all the detailed grounds for re-examination and argumentations presented by the Applicant in writing and during the oral explanation in front of the CHMP held on 10 November 2025.

Based on the review of available data on the active substance, the CHMP considers that levacetylleucine is not to be qualified as a new active substance in itself.

Based on the review of data on the non-clinical and clinical properties of the active substance, the CHMP considers that levacetylleucine in comparison to the racemate, N-Acetyl-DL-Leucine, previously authorised as a medicinal product in the European Union, is not to be qualified as a new active substance as insufficient evidence has been provided to demonstrate that it differs significantly in properties with regard to safety and/or efficacy from the previously authorised substance.

6. Benefit-risk balance following re-examination

EMA note: the Applicant's request for re-examination of the CHMP opinion dated 24 July 2025 was limited to the recommendation of the CHMP to refuse IntraBio Ireland Limited's NAS status claim. Therefore, with regard to Aqneursa benefit-risk balance, the CHMP opinion of July 2025 (and the text in the following sections) is unchanged.

6.1. Therapeutic Context

The Applicant submitted an application for Aqneursa (levacetylleucine (NALL)) for the treatment of Niemann-Pick Type C (NPC).

The agreed indication is as follows:

Aqneursa is indicated for the treatment of neurological manifestations of Niemann-Pick type C (NPC) disease, in combination with miglustat, or as a monotherapy in patients where miglustat is not tolerated, in adults and children aged 6 years and older and weighing at least 20 kg.

The mechanism of action of levacetylleucine is presented as rather non-specific and the active substance revealed no main pathway which would account for the mechanism of action at specific cell-target. Non-clinical studies demonstrated that levacetylleucine corrects energy metabolism, including improving adenosine triphosphate production. The recommended dose is based on the patient's body weight in kg ranging from 2 g per day (20 to 24 kg) to up to 4 g per day (35 kg or more).

6.1.1. Disease or condition

NPC is a rare, progressive, neurodegenerative, autosomal recessive, and inherited metabolic lysosomal storage disorder (LSD). Its estimated incidence is around 1:100,000 live births (Geberhiwot et al. 2018). Globally, miglustat is the only medicinal product authorised for NPC.

NPC is inherited in an autosomal-recessive manner. Diagnosis is established in probands with symptoms and signs suggestive of NPC and the presence of biallelic pathogenic variants in either NPC1 or NPC2 identified by molecular genetic testing, or the presence of clinical symptoms supported by biomarker and/or filipin testing [Patterson et al. 2000, updated 2020]. The clinical presentations of NPC disease are characterized by broad heterogeneity in serious and debilitating systemic, psychiatric, and neurological symptoms (which vary markedly depending on the age of onset of neurological symptoms) from a rapidly progressing neonatal form to an adult-onset chronic neurodegenerative condition. NPC is always fatal. The majority of NPC patients are children and die before the age of 20, with the median age of death being 12.5 years [Garver et al. 2007].

The course of the disease varies highly from patient to patient depending on the age of onset of neurological symptoms, from a rapidly progressing neonatal form to an adult-onset chronic neurodegenerative condition. Infantile and juvenile patients often experience the most severe

symptoms, including epileptic seizures and cataplexy, while adult patients experience severe cognitive impairment, dementia, and psychosis [Patterson et al. 2013].

Systemic signs of liver, spleen and lung involvement typically precede the disease-defining neurodegeneration. This is particularly true for patients with onset during infancy and childhood. Neurological signs and symptoms include ambulation and walking difficulties, cognitive impairment, swallowing difficulties, vertical supranuclear gaze palsy, seizures, and cataplexy. The progression of the neurological symptoms is responsible for disability and premature death in most cases (Vanier 2010).

The neurological symptoms in NPC include a delay in developmental motor milestones (early-infantile period) and problems at school, including difficulties in writing and impaired attention (late-infantile and juvenile period). A cardinal symptom is cerebellar ataxia (present in 70% of patients) where patients have problems with stance and gait ataxia and consequent falls, dizziness, clumsiness, dysmetria, and disdiadochokinesia. Other neurological signs are vertical supranuclear gaze palsy, dysphagia, gait disorders, and dementia, which can lead to a premature death. Cataplexy, seizures, and dystonia are also common symptoms [Patterson et al. 2013; Vanier 2010].

The initially claimed indication for Aqneursa was the following:

Aqneursa is indicated in adults and children from birth for chronic treatment of Niemann-Pick Type C (NPC).

Following comments and recommendations from the CHMP, the Applicant agreed to the final indication below:

"*Aqneursa is indicated for the treatment of neurological manifestations of Niemann-Pick type C (NPC) disease, in combination with miglustat, or as a monotherapy in patients where miglustat is not tolerated, in adults and children aged 6 years and older and weighing at least 20 kg*.".

6.1.2. Available therapies and unmet medical need

There are no curative therapies for NPC. Miglustat is the only medicinal product authorised for NPC in the EU. Miglustat is a compound first licensed for type 1 Gaucher disease that has received market authorisation for use in NPC patients in the European Union (EU), Japan, and other countries (but not in the United States [US]). Evidence in support of miglustat in NPC comes from a randomised clinical trial, long-term extension studies, and two retrospective surveys, demonstrating a reduction of the progression of clinically relevant neurological symptoms in patients with NPC. In NPC patients, miglustat is indicated for the treatment of progressive neurological manifestations and has been shown to slow the general progression of neurological symptoms in patients with NPC. However, miglustat has no curative effect. The Applicant claimed that levacetylleucine aims to address the unmet medical need in NPC by providing a novel mechanism of action that could lead to symptomatic improvement in neurological signs and symptoms.

6.1.3. Main clinical studies

Clinical data are mainly coming from the following two studies:

An ongoing pivotal ongoing phase III multinational, randomized, placebo-controlled, double-blinded, crossover study evaluating 12 weeks of treatment followed by an extension phase. The primary endpoint was the Scale for the Assessment and Rating of Ataxia (SARA) total score. This scale includes 8 items that are related to gait, stance, sitting, speech, finger-chase test, nose-finger test, fast alternating movements and heel-shin test. Secondary efficacy endpoints notably included the measurements of

neurological signs and functioning using the Scale for Spinocerebellar Ataxia Functional Index (SCAFI), and measurement of Health-Related Quality of Life.

A phase II multinational, multi-centre, open-label, rater-blinded single-arm study in paediatric and adult patients aged ≥6 years evaluating 6weeks treatment followed by an extension phase. The primary endpoint was blinded raters' Clinical Impression of Change in Severity (CI-CS) score comparing either the 9-Hole Peg Test of the Dominant Hand (9HPT-D) or the 8-Meter Walk Test (8MWT) as the primary anchor. Additional efficacy endpoints included measurements of ataxia and functioning: SARA score, SCAFI score, Measurement of Health-Related Quality of Life, Measurement of Overall Neurological Status and Measurement of Global Impression scored by treating physician, caregiver, and patient.

6.2. Favourable effects

In the pivotal study, the SARA total score showed an improvement after treatment with levacetylleucine over placebo treatment. After treatment with levacetylleucine, the mean (SD) change from baseline was -1.97 (2.43) compared to -0.60 (2.39) after placebo treatment. Two-sided statistical testing indicated a significant difference between levacetylleucine and placebo (LS-mean difference: -1.28; 95% CI: -1.91, -0.65; $p<0.001$). Patients who received levacetylleucine followed by placebo which effectively served as a washout from levacetylleucine had a significant worsening of symptoms when receiving placebo (difference in mean SARA total score = +1.55).

6.3. Uncertainties and limitations about favourable effects

As per the current consensus of clinicians, the progression and presentation of the NPC disease depend on the age at which the neurological symptoms onset. There are very distinct disease onsets (infantile, juvenile and late/adult onset). The Applicant initially sought an indication that would cover the whole population, with no limitations to this heterogeneity. This was not supported by the data from the clinical studies (Phase II and ongoing Phase III Study).

No dose-finding study was carried out. According to the non-clinical part of the dossier (PD *in vivo* studies in rats and cats), the dose that should have been investigated would be 6 g/ day for adults (based on the AUC). The clinical part of the dose development is based on the experience gained from the racemate. It should be noted that the racemate, the L-enantiomer and the D-enantiomer respectively can differ in the primary PK characteristics (C_{max}, AUC). According to the *in vitro* data the C_{max} and AUC was greater for the D-enantiomer when compared to the L-enantiomer.

The pivotal study excluded patients aged below 4 years old and no patients aged below 6 years old and weighting less than 20 kg were included. The lack of data in this population is a concern with regards to efficacy in this specific population. Following questions from the CHMP, the Applicant excluded this population from the indication.

The primary endpoint was SARA (Scale for the Assessment and Rating of Ataxia) which includes 8 items that are related to gait, stance, sitting, speech, finger-chase test, nose-finger test, fast alternating movements and heel-shin test. It is a validated clinical scale used to assess ataxic disorders, but it has not been validated to assess Niemann–Pick type C disease. Secondary endpoints included an ataxic scale (SCAFI), a modified Disability Rating Scale and PROs. These scales are used to assess neurological symptoms, but it has not been conclusively established that they can be used to assess NPC disease as a whole.

The duration of the treatment period during the cross-over was 12 weeks, which may be considered limited for a product intended for chronic use.

The Applicant has submitted data from the ongoing open-label extension phase (EP) in the form of an unpublished preprint that was not certified by a peer review (Patterson et al. Preprint 2024). No study report was provided which makes it difficult to assess the long-term efficacy.

The effect of the product on the SARA score was rapid, but patients deteriorated rapidly when treatment was stopped, which in the context of a progressive could be attributable to a symptomatic effect of the product rather than a disease modifying effect.

In both pivotal and supportive studies, a vast majority of patients (85% and 90.9% respectively) received levacetylleucine in combination with miglustat which raises an issue of how to properly determine the efficacy of levacetylleucine alone versus in combination with miglustat.

The overall long-term efficacy data are based on the 19 patients who entered the extension phase of the Phase II Study IB1001-201. Among them 18 patients were treated for one year and 13 completed the extension phase (two years treatment) However, the primary efficacy endpoint for the extension part (5-domain NPC-CSS) is different from the primary endpoint if the parent study (CGI – CS) and from the primary endpoint of pivotal study (SARA). These disparities preclude any conclusions regarding the long-term efficacy of the drug. The results from the study Phase II - IB1001-201 should be interpreted with caution considering the design of the study (non-randomised, rater-blinded, single arm, open label study with the different setting of primary and secondary endpoints in comparison to the pivotal study IB1001-301) and unresolved GCP findings (violation).

The Applicant committed to provide the study report of the extension phase data from the pivotal Study IB1001-301 to support the long-term efficacy and safety of levacetylleucine (MEA), which is due by 31 March 2029.

6.4. Unfavourable effects

Two-third of patients who received levacetylleucine (n=55/84) experienced adverse events (AEs), corresponding to 241 events with an incidence rate [IR] = 4,5 per patient-year, of which 31 were serious. Among AEs reported in levacetylleucine use group, 8,3% were considered related to the studied treatment by the Applicant. Most of these adverse events were mild to moderate in severity and 10 patients from levacetylleucine use group experienced 29 severe AEs.

Most TEAEs (310 / 344 reported TEAEs) were mild or moderate in intensity A total of 10 patients (11.9%) reported 29 severe TEAEs during levacetylleucine use. The incidence rates of TEAEs reported as severe was slightly higher during levacetylleucine use (IR=0.5/ patient-year) than with non-levacetylleucine use (IR=0.2/patient-year). This was mainly driven by respiratory events reported as severe. The appraisal of data on the SOC "Infections" does not allow any particular safety issue to be identified.

Twenty-one (21) treatment-related TEAEs were reported by 12 patients (14.3%). A total of 7 patients (8.3%) reported 11 treatment-related TEAEs during levacetylleucine use. The incidence rate of treatment-related TEAEs was 0.2/ patient-year during levacetylleucine use.

Treatment-related TEAEs for the list of adverse drug reactions for the section 4.8 of the SmPC is currently limited to the adverse drug reaction of flatulence. Two-third of patients who received levacetylleucine (n=55/84) experienced adverse events but only the adverse event of flatulence has been related to levacetylleucine treatment and is listed in the section 4.8 of the SmPC. No major adverse events have been identified.

6.5. Uncertainties and limitations about unfavourable effects

The Applicant did not initially identify any safety concerns for the RMP. Pertaining to the rarity of the disease, and then the low number of enrolled patients, the lack of sufficient long-term safety data is a concern. Long-term exposure is currently based only on the Phase II study 201. The pivotal Phase III study 301 is still ongoing. According to the ICH E1 guidance on population exposure, patients should be exposed to the treatment for at least 12 months in case of chronic treatment regimen. Currently, only three months exposure was placebo controlled. This issue is particularly important for the paediatric population, where no data are available for children below 4 years of age and very limited in the age group 4 - 12 years (15 to 25 kg of weight). Therefore, the proposed list of safety concerns has been updated adding "long-term safety data", as missing information. There is also an uncertainty regarding hepatotoxicity that has been listed as an important potential risk in the list of the RMP safety concerns.

6.6. Effects Table

Regarding the, the mean (SD) change from baseline was after levacetylleucine treatment compared to after placebo. Two-sided statistical testing indicated a significant difference between (LS-mean difference: -1.28; 95% CI: -1.91, -0.65; p<0.001) showing an improvement.

Table 36. Effects Table for Aqneursa

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
SARA score mean	Scale for the assessment and rating of ataxia	SD	-1.97 (2.43)	-0.60 (2.39)	LS-mean difference: -1.28; 95% CI: -1.91, -0.65; p<0.001	Phase III 1001-
Unfavourable Effects Treatment related TEAEs (percentage of patients)*						
Flatulence	Incidence of flatulence	%	1.2	Versus non-levacetylleucine use group	Outcomes from the pooled safety data set	Phase III 1001-301 study and phase II study/extension phase 1001-201

6.7. Benefit-risk assessment and discussion

6.7.1. Importance of favourable and unfavourable effects

The main evidence on efficacy of levacetylleucine is derived from the interim results of an ongoing multicenter, randomized, double-blind, placebo-controlled, crossover study in adults and paediatric patients aged ≥ 4 years old. The duration of the treatment period during the cross-over was 12 weeks, which may be considered limited for a product intended for chronic use. Overall, the conduct of a double-blinded RCT in a sufficient number of NPC disease patients is positively recognized.

Regarding the primary endpoint, the mean (SD) change from baseline of the SARA after treatment with levacetylleucine, was -1.97 (2.43) compared to -0.60 (2.39) after placebo treatment demonstrating a significant neurological improvement. The study also showed that patients who received levacetylleucine followed by placebo which effectively served as a washout from levacetylleucine had a significant worsening of symptoms when receiving placebo, (difference in mean SARA total score = +1.55).

Long-term efficacy/safety data have been generated from the extension period of the phase II study for 18 patients and only 3 patients were of age 6 -12 years. The supporting data from study 201 can only be used for comparison of secondary endpoints used in the Pivotal Study 301. However, the primary

endpoint of the pivotal study (SARA score) was not assessed in phase II study 201 as primary endpoint. Thus, the consistency in data cannot be established. In addition, SARA score did not reach significancy in the extension phase of the Phase II Study 201. The mean SARA total score for visit 9 versus visit 7 was 1.82 (SD=3.09, median=1.50), which demonstrated a deterioration of cerebellar sign and neurological symptoms under treatment.

However, the results from the study Phase II - IB1001-201 should be interpreted with caution considering the design of the study (non-randomised, rater-blinded, single arm, open label study with the different setting of primary and secondary endpoints in comparison to the pivotal study IB1001-301).

The Applicant also provided data from the extension phase of the pivotal study 301 with a cut-off date of 31 January 2025 (point at which all patients who entered after participation in the Parent Study had either completed Visit 9 or discontinued).

The data presented are based on unclean data without source document verification. This precludes a definitive conclusion regarding the long-term efficacy for NALL in NPC patients as currently proposed by the Applicant. However, preliminary results of the 5- / 15- domain NPC-CSS and the SARA suggest a slowing of disease progression with IB1001 treatment compared to what would be expected based on historical comparisons with patients receiving standard of care (with or without miglustat).

Preliminary safety data from the IB1001-301 Extension Phase demonstrate the same benign safety profile observed to date, with no new safety signals. The safety data suggest no treatment related ADRs, although 75% of the subjects experienced a TEAE and there are at least 2 events per subject (i.e. 47 subjects (74.6%) with 136 events). Furthermore, the TEAEs from the PT class Gastrointestinal disorders are in general high in frequency (14 subjects (22.2%) with 21 events), but no relationship was established.

It was agreed that the applicant could submit a summary of the preliminary results obtained in this study for support of their claim of treatment beyond 12 weeks. The Applicant committed to provide the study report of the extension phase data from the pivotal Study IB1001-301 to support the long-term efficacy and safety of levacetylleucine (MEA), which is due by 31 March 2029. This was considered acceptable.

On a safety aspect, two-third of patients who received levacetylleucine (n=55/84) experienced adverse events but only the adverse event of flatulence has been related to levacetylleucine treatment and is listed in the section 4.8 of the SmPC. No major adverse events have been identified.

However, although no important identified risk into the summary of safety concerns has been identified, in respect to liver enzymes changes and the uncertainties on levacetylleucine causality, the risk of hepatotoxicity is considered as an important potential risk in the summary of safety concerns of the RMP. There are also remaining issues concerning carcinogenicity and developmental toxicity and these issues have been added as safety concerns as important potential risks in the RMP.

6.7.2. Balance of benefits and risks

The main evidence on efficacy of levacetylleucine is derived from the interim result of an ongoing multicenter, randomized, double-blind, placebo-controlled, crossover study in 60 adults and paediatric patients aged ≥ 4 years old (Study IB1001-301). The duration of the treatment period during the cross-over was 12 weeks, which was considered limited for a product intended for chronic use. However, the Applicant submitted some preliminary data from the extension phase of the pivotal study 301 with a cut-off date of 31 January 2025 in support of the long-term use. No definitive conclusion can be drawn from

these preliminary data, but the Applicant also committed to provide the study report of the extension phase data from the pivotal Study IB1001-301 to support the long-term efficacy and safety of levacetylleucine (MEA), which is due by 31 March 2029. This was considered acceptable. Overall, the conduct of a double-blinded RCT in a sufficient number of NPC disease patients is positively recognized.

Regarding the primary endpoint, the mean (SD) change from baseline of the Scale for the assessment and rating of ataxia (SARA) after treatment with levacetylleucine, was -1.97 (2.43) compared to -0.60 (2.39) after placebo treatment demonstrating a significant improvement. The study also showed that patients who received levacetylleucine followed by placebo which effectively is a washout from levacetylleucine had a significant worsening of symptoms when receiving placebo (difference in mean SARA total score = +1.55).

The initially claimed indication for Aqneursa was the following:

"Aqneursa is indicated in adults and children from birth for chronic treatment of Niemann-Pick Type C (NPC)".

The CHMP disagreed on several aspects of this indication, as described below:

Restriction to patients with neurological manifestations

Since the primary efficacy assessments of Studies IB1001-201 and IB1001-301 were for neurological signs and symptoms, e.g. neurological manifestations of NPC, the CHMP disagreed with a broad indication for chronic treatment of NPC, and requested the Applicant to reflect in the indication that the product is intended for the treatment of neurological manifestations of NPC disease. The Applicant agreed to modify the indication accordingly.

In response to the MO concerning the age limit and concomitant use with miglustat, the Applicant proposed the following indication:

"Aqneursa is indicated in combination with miglustat, or as a monotherapy in patients where miglustat is considered inappropriate, for the treatment of neurological manifestations of Niemann-Pick Type C (NPC) disease in adults and children weighing at least 20 kg."

Concomitant use with miglustat

The vast majority of patients (85%) in the pivotal study continued to receive miglustat throughout the study. This created a bias and a concern regarding the monotherapy indication and the Applicant was requested to consider a restriction to adjunctive therapy.

The Applicant argued that the restriction of the indication to adjunctive therapy is not appropriate. The Applicant's reasoning was based on results from *in vitro* and *in vivo* non clinical data showing an effect of levacetylleucine itself in a NPC mouse model, on the fact that the mechanisms of action of the two products are different, that patients were previously treated with miglustat and remained on a stable dose, results from a phase 2 study showing some efficacy of monotherapy in patients with GM2 gangliosidosis. The Applicant also argued that some patients did not tolerate miglustat due to adverse events.

However, the CHMP considered that the clinical evidence that efficacy is not dependent on the concomitant miglustat treatment is driven by very small subgroups of patients: 9 out of 51 in the pivotal study and 3 out of 29 in the phase 2 study and that further data are needed to be able to conclude on efficacy of levacetylleucine as monotherapy. The evidence provided by the Applicant was considered not comprehensive to conclude on the combination therapy or monotherapy with NALL (first line treatment in the NPC patients).

However, the argument regarding the similar efficacy (SARA score) in 15% of patients (N=9) who were not on miglustat prior to randomisation and during the trial involved in the pivotal study might be valid

but the study was not powered to show this. A trend in the efficacy of Aqneursa in monotherapy might be supported, however considering the low number of patients, the data supports rather an assumption. Besides these issues, a supportive study (Study IB1001-201) conducted by the Applicant with an almost identical population indicated worsening of ataxia based on the SARA score. The efficacy (based on the SARA score) was only formally supported by the double-blind part of the clinical programme in the pivotal study IB1001-301.

Even if the data are limited, the possibility to use Aqneursa as monotherapy in patients who cannot tolerate miglustat could be considered acceptable but as the term 'inappropriate' was not considered be precise enough to guide the prescriber, the CHMP requested to replace it with "not tolerated".

The Applicant agreed to modify the indication proposing use "*in combination with miglustat, or as a monotherapy in patients where miglustat is not tolerated*".

Restriction to children aged 6 years and older and weighing at least 20 kg.

Based on the available data from studies IB1001-201 and IB1001-301, only PK data from children aged 6 to 12 years and weighing at least 20 kg were available. No PK data from children aged below 6 years have not been submitted as part of this application, whereas children of at least 4 years were planned to be enrolled. Consequently, from a clinical PK perspective the restriction of children aged 6 years and weighing at least 20 kg is justified and levacetylleucine PK is considered unknown below these age/weight limits.

The Applicant accepted to restrict the indication to children weighing at least 20 kg but initially did not accept the age restriction of 6 years arguing that age was not found to be a significant covariate influencing the PK in the PopPK model.

However, according to the PK data provided, the CHMP considered that it is expected that age influences levacetylleucine PK and the CHMP reiterated their request to limit the use of Aqneursa to patients aged 6 years and older.

Following the oral explanation, the Applicant proposed a new indication that limits the use of Aqneursa to patients aged 6 years and older: *"Aqneursa is indicated in combination with miglustat, or as a monotherapy in patients where miglustat is not tolerated, for the treatment of neurological manifestations of Niemann-Pick type C (NPC) disease in adults and children aged 6 years and older and weighing at least 20 kg."*

The indication has been updated in line with the CHMP requests, and in line with the data from the clinical studies (Phase II and ongoing Phase III Study). This indication is acceptable.

The Applicant has demonstrated a significant improvement in the Scale for the assessment and rating of ataxia (SARA) after treatment with levacetylleucine in patients with NPC aged 6 years and older. The improvements seen in Study IB1001-301 are considered clinically relevant.

In terms of safety, the list of adverse drug reactions for the section 4.8 of the SmPC is limited to the adverse drug reaction of flatulence. Two-third of patients who received levacetylleucine (n=55/84) experienced adverse events but only the adverse event of flatulence has been related to levacetylleucine treatment and is listed in the section 4.8 of the SmPC. No major adverse events have been identified. The safety profile is considered to be manageable.

The Applicant committed to provide the study report of the extension phase data from the pivotal Study IB1001-301 to provide additional support of the long-term efficacy and safety of levacetylleucine, which is considered acceptable.

6.7.3. Overall, based on the submitted data, a positive benefit-risk can be concluded for the newly proposed indication.Additional considerations on the benefit-risk balance

Input from patient organisations

Patient organisations are also involved in the procedure to share their perspective, experience and concerns about their conditions. The input has been obtained from the International Niemann-Pick Disease Alliance (INPDA) who provide collaborative forum for the sharing of information and experience regarding ASMD Niemann-Pick disease (types A, B and A/B) and Niemann-Pick type C (NPC) in term of best practice in care and support, the provision and information about research. There is no prospect of a curative therapy for NPC. Miglustat is recommended as the standard of care for NPC patients in many countries to slow the progression of NPC symptoms. However, some patients are unable to tolerate it due to side effects including peripheral neuropathy and severe diarrhoea / GI issues, and on its own is not enough to address the high level of unmet medical need in this patient community. If miglustat is not tolerated or deemed clinically appropriate, for these patients is the only option supportive care to relief symptoms and management of complications. A diagnosis of NPC is challenging for many reasons, including the rarity of the disease, which means most people, including non-expert health professionals, have no knowledge or clinical experience of it. Due to high degree of heterogeneity, it has been widely acknowledged that therapeutic approach to NPC require a combination of therapies, available to support patients at different stages of progression, with one therapeutic solution not being appropriate for all patients and that does not address all aspect of the disease, there remains significant unmet medical need for patient living with NPC.

NPC has a significant impact on the quality of life of patients and their family members, with numerous disabling symptoms including ataxia, dysphagia, dysarthria, gait imbalance, and cognitive deterioration. The loss of the ability to walk, dress, write, speak and eat has devastating consequences on quality of life, and imposes a tremendous burden on patients and families. Patients and carers report difficulties with fine motor skills, seizures. loss of swallow and problems with nutrition, hydration and administering of medication, loss of speech and mobility, loss of cognitive functions leading to the anxiety, stress and depressions and range of practical, emotional and psychological issues.

Disease stability, or slowing of progression, is widely seen as a meaningful and important outcome for patients. This is reflected in the opinion of expert clinicians, who recognize that whilst symptom reversal is unlikely, an achievable goal is to slow or halt progression. This would have a life changing impact for patients and their families, bringing better quality and quantity of life.

In addition, successful pregnancies have been reported in women with NPC, in many cases before diagnosis was made. For NPC patient at child-bearing potential is recommended birth control (miglustat).

Patients and carers often report exasperation in their efforts to get the right diagnosis, with their diagnostic journey stretching over many years. This in turn leads to delays in accessing expert care, practical support and symptomatic treatments, as well as impacting family planning decisions.

Patients and carers also report difficulties in accessing expert clinical care, with centres often located far from their home. This is especially so when the disease is more progressed and their burden of disease greater, or when there are family and/or financial constraints.

6.8. Conclusions

The overall benefit/risk balance of Aqneursa is positive.

7. Recommendations following re-examination

Outcome

Based on the arguments of the Applicant and all the supporting data on quality, safety and efficacy, the CHMP re-examined its initial opinion and in its final opinion concluded by consensus that the benefit-risk balance of Aqneursa is favourable in the following indication(s):

Aqneursa is indicated in combination with miglustat, or as a monotherapy in patients where miglustat is not tolerated, for the treatment of neurological manifestations of Niemann-Pick type C (NPC) disease in adults and children aged 6 years and older and weighing at least 20 kg.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that levacetylleucine is not to be qualified as a new active substance in itself.

Furthermore, based on the review of data on the quality, non-clinical and clinical properties of the active substance, the CHMP considers that levacetylleucine shares the same therapeutic moiety at the site of the biological activity as N-acetyl-DL-leucine, a previously authorised active substance in the European Union, notably in France. The CHMP considers that levacetylleucine in comparison with the previously authorised N-acetyl-DL-leucine is not to be qualified as a new active substance as insufficient evidence has been provided to demonstrate that it differs significantly in properties with regard to safety and/or efficacy from the previously authorised active substance.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0043/2024 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.

8. Appendices

8.1. CHMP AR on similarity dated 22 December 2025

8.2. CHMP AR on New Active Substance (NAS) dated 24 July 2025