25 May 2023
EMA/267469/2023
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Ztalmy

International non-proprietary name: ganaxolone

Procedure No. EMEA/H/C/005825/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

ACTH	adrenocorticotropic hormone
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
ASM	anti-seizure medication
ASMF	Active Substance Master File = Drug Master File
AUC	area under the concentration-time curve
AUCss	area under the concentration-time curve at steady state
AUC0-inf	area under the drug-concentration vs time curve from time zero to infinity
AUC0-24	area under the drug-concentration vs time curve from time zero to 24 hours
BCRP	breast cancer resistance protein
BIS	bispectral index
BR	bromide
BSEP	bile salt export pump
CBZ	carbamazepine
CD	cyclodextrin
CDD	cyclin-dependent kinase-like 5 deficiency disorder
CDKL5	cyclin-dependent kinase-like 5
CFU	Colony Forming Units
CGI-C	Caregiver Global Impression of Change
CGI-CSID	Caregiver Global Impression of Change in Seizure Intensity/Duration
CGI-I	Clinical Global Impression of Change – Improvement
CGICA	Caregiver Global Impression of Change in Attention
CL	clearance
CLB	clobazam
CLIP170	cytoplasmic linker protein 170
CLN	clonazepam
Cmax	maximum concentration
CMC	chemistry, manufacturing, and controls
Cmin	minimum concentration

CNS	central nervous system
cASMS	concomitant anti-epileptic agents
CP	Centralised Procedure
CQA	Critical Quality Attribute
CSR	clinical study report
CSWS	continuous spike wave in sleep
CYP	cytochrome P450 isoenzyme
DAD	diode array detector
DB	double-blind
DRF	Dose range finding
ECG	electrocardiogram
eDiary	electronic seizure diary
EEG	electroencephalogram
EMA	European Medicines Agency
Emax	maximum effect
EOP	End of Phase
ESM	ethosuximide
EU	European Union
FBM	felbamate
FDA	Food and Drug Administration
FOS	focal onset seizures
FT-IR	Fourier Transform Infrared Spectroscopy
FXS	fragile X syndrome
GABA	γ-aminobutyric acid
GABAA	γ-aminobutyric acid type A
GC	Gas Chromatography
GCP	Good Clinical Practice
GNX	ganaxolone
HDPE	High Density Polyethylene
HP	hydroxypropyl
HPLC	High performance liquid chromatography
ICDD	International CDKL5 Disorder Database
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IEC	Independent Ethics Committee
Impr.	improvement
IND	Investigational New Drug application
IQGAP1IQ	motif containing GTPase activating protein 1
IRB	Institutional Review Board
IS	infantile spasms
ISS	Integrated Summary of Safety
ITT	intent-to-treat
IUPAC	International Union of Pure and Applied Chemistry
IV	intravenous(ly)
KF	Karl Fischer titration
LAR	Legally Authorized Representative
LCM	lacosamide
LDPE	Low Density Polyethylene
LEV	levetiracetam
LGS	Lennox Gastaut Syndrome
LoI	Letter of Intent
LSLV	last subject last visit
LTG	lamotrigine
MAA	Marketing Authorisation Application
MATE	multidrug and toxin extrusion transporter
max	maximum
MedDRA	Medical Dictionary for Regulatory Activities
min	minimum
MSX	mesuximide
MTD	Maximum Tolerated Dose
NDA	New Drug Application
NOEL	No Observed Effect Level
NRG	Name Review Group
OAT	organic anion transporters
OATP	organic anion transporting polypeptides
OCT	organic cation transporters

OL	open-label
OLE	open-label extension
OXC	oxcarbazepine
PB	phenobarbital
PBO	placebo
PCDH	protocadherin
P-gp	P-glycoprotein
PHT	phenytoin
Ph. Eur.	European Pharmacopoeia
PIP	Paediatric Investigation Plan
PK	pharmacokinetic(s)
POS	partial-onset seizures
PP	per protocol
PP	Polypropylene
PPD	postpartum depression
PRM	primidone
PT	preferred term
PTSD	posttraumatic stress disorder
Q1	first quartile
Q3	third quartile
QT	interval longation
QTc	corrected QT interval
QTPP	Quality target product profile
REM	rapid eye movement
RH	Relative Humidity
RUF	rufinamide
SAE	serious adverse event
SAP	statistical analysis plan
SE	status epilepticus
SmPC	Summary of Product Characteristics
SOC	system organ class
STP	stiripentol
SUDEP	sudden-unexpected-death-in-epilepsy

SULT1E1	sulfotransferase family 1E member 1
t½	terminal half-life
TAMC	Total Aerobic Microbial Count
TEAE	treatment-emergent adverse event
tmax	time of maximum concentration
TQTc	thorough QT corrected
TPM	topiramate
TSC	tuberous sclerosis complex
TYMC	Total Combined Yeasts/Moulds Count
US	United States
USP	United States Pharmacopoeia
UV	Ultraviolet
VAS	visual analog scale
VGB	vigabatrin
VNS	vagus nerve stimulation
VPA	valproic acid
WoE	weight of evidence
ZNS	zonisamide

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Marinus Pharmaceuticals Emerald Limited submitted on 9 October 2021 an application for marketing authorisation to the European Medicines Agency (EMA) for Ztalmy, 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 10 December 2020.

Ztalmy was designated as an orphan medicinal product EU/3/19/2224 on 13 November 2019 in the following condition: Treatment of CDKL5 deficiency disorder.

The applicant applied for the following indication: *Adjunctive treatment of epileptic seizures associated with cyclin-dependent kinase-like 5 deficiency disorder (CDD) in patients 2 years of age and older*.

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/0171/2021 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0171/2021 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Ztalmy 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/Ztalmy

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 not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's request(s) for consideration

1.5.1. Accelerated assessment

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

1.5.2. New active substance status

The applicant requested the active substance ganaxolone contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.6. Protocol assistance

The applicant received the following protocol assistance on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
22 March 2018	EMEA/H/SA/3732/1/2018/PED/SME/III	Caroline Auriche, Minne Casteels

The protocol assistance pertained to the following nonclinical and clinical aspects:

- Nonclinical: The overall nonclinical development programme to support a Marketing Authorisation Application (MAA), including acceptability to perform a 13-week juvenile toxicity study in rat and 2-year carcinogenicity study in rat post-approval, and to limit withdrawal assessment to non-clinical studies
- Clinical: Agreement whether cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder is a distinct clinical condition with a significant unmet medical need. Sufficiency of completed package of studies of the drug interaction and drug transporter potential in support of MAA. Acceptance to formulate [14C]-ganaxolone for oral dosing as a solution in a human mass balance/metabolism study. Acceptance to study ganaxolone in patients with hepatic or renal impairment post-approval. Strategy to decide whether a human Thorough QT (TQT) study is necessary based on outcome of additional non-clinical cardiovascular studies with ganaxolone in parallel with the Phase 3 study. Plan to construct a paediatric PK model to determine the dose of GNX in the various paediatric age ranges that will produce a Cmax and AUC exposure similar to that achieved following an efficacious dose determined in the adult epilepsy population.

  Agreement whether Study 1042-CDKL5-3001 could serve as the single pivotal efficacy study to support approval. Design of study 1042 CDKL5 3001, including dose selection, age range, primary and secondary endpoints, including seizure types to be included, statistical methods, the safety monitoring plan, and proposal to conduct an open-label safety extension study. Sufficiency of the extent and duration of patient exposure from Study 1042-CDKL5-3001 in addition to the safety experience with ganaxolone acquired through the completed clinical studies in various indications conducted with ganaxolone to support a MAA.

1.7. Steps taken for the assessment of the product

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

Rapporteur: Johann Lodewijk Hillege Co-Rapporteur: Armando Genazzani

The application was received by the EMA on	9 October 2021
Accelerated Assessment procedure was agreed-upon by CHMP on	19 August 2021
The procedure started on	28 October 2021
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	29 December 2021
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	29 December 2021
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	4 January 2022
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	13 January 2022
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	25 January 2022
The Accelerated Assessment timetable was reverted to a standard assessment timetable on	25 January 2022
The applicant submitted the responses to the CHMP consolidated List of Questions on	22 November 2022
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	8 January 2023
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	26 January 2023
The applicant submitted the responses to the CHMP List of Outstanding Issues on	27 March 2023
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	14 April 2023
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	26 April 2023
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	12 May 2023
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 Ztalmy on	25 May 2023
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)	25 May 2023

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

CDKL5 deficiency disorder (CDD) is a developmental encephalopathy caused by pathogenic variants in the gene cyclin-dependent kinase-like 5 (CDKL5). The clinical characteristics commonly associated with a CDKL5 mutation include early-onset medication-refractory seizures, severe intellectual and gross motor impairment, and severe sleep disturbances. Since 2013, CDD is characterised as a separate disorder, whereas before that time it was classified as an atypical variant of Rett Syndrome.

2.1.2. Epidemiology

CDD is a rare, genetically defined paediatric epilepsy with an estimated incidence of 1:40,000 to 1:60,000 live births (Olson 2019; Demarest 2019). The disorder is more common in females than in males (ratio 4:1; Olson 2019).

2.1.3. Biologic features

The CDKL5 gene is located on the short arm of the X chromosome and encodes a serine-threonine kinase essential for neuronal maturation and development (Jakimiec et al., 2020). CDKL5 is widely expressed in the brain. A wide variety of pathogenic variants in the CDKL5 gene have been reported, of which most are *de novo* mutations. In addition, cases of parental mosaicism have been reported (Orphanet).

2.1.4. Clinical presentation, diagnosis

The severity of CDD is variable, although most children experience treatment-resistant seizures and severe neurodevelopmental impairment. Patients may experience one or more of several different seizure types, including tonic-clonic, atonic, clonic, tonic, myoclonic, absence, and focal seizures, as well as infantile spasms (IS) (Kilstrup-Nielsen et al., 2012). Three stages of seizures in the course of CDD have been proposed: Stage I, early-onset epilepsy (onset 1 to 10 weeks); Stage II, epileptic encephalopathy with IS and hypsarrhythmia; Stage III, tonic seizures with myoclonia and variable level of control with ASMs (Bahi-Buisson et al., 2008). Frequent myoclonic jerks are a key component of epileptic manifestations at this later stage. Some patients show a peculiar seizure pattern with prolonged generalized tonic-clonic events lasting 2 to 4 minutes gradually transitioning to repetitive, distal myoclonic jerks.

Other neurologic manifestations include deficits in speech or language, limited or complete loss of functional hand use, stereotypies, hypotonia, cortical blindness, autistic features, and sleep disturbances. Children may also have dysautonomia, impaired swallowing, or constipation.

The overall life-expectancy for a CDD patient is likely shorter than for age-matched healthy individuals. Due to the rarity of CDD, little is known about long-term prognosis and life expectancy. Most individuals who have been identified with this condition are under 18 years of age.

2.1.5. Management

There are currently no treatments approved specifically for seizures associated with CDD. Seizures in CDD are treated with anti-seizure medications, corticosteroids, a ketogenic diet, vagal nerve stimulation and neurosurgery.

2.2. About the product

Ganaxolone (GNX) is a methyl-substituted analogue of the endogenous neurosteroid allopregnanolone, a derivative of progesterone. The structure of GNX is identical to allopregnanolone, with a methyl substitution at the 3β position. This structural modification prevents back-conversion to intermediates active at steroid receptors and increases GNX bioavailability (Lyden et al., 2000).

GNX elicits its anti-seizure effects by targeting allosteric sites of the gamma-aminobutyric acid type A (GABAA) receptor to positively modulate and enhance the function of both synaptic and extrasynaptic GABAA receptors.

The sought indication for Ztalmy (GNX) was for the adjunctive treatment of epileptic seizures associated with CDD in patients 2 years of age and older.

Ztalmy is an oral solution and should be titrated gradually to achieve the recommended daily dose based on clinical response and tolerability. It is given 3 times per day. In patients weighing ≤ 28 kg, the recommended daily dose is 63 mg/kg/day. In patients weighing > 28 kg, the recommended daily dose is 1800 mg/day.

2.3. Type of application and aspects on development

The CHMP agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on:

- Seizures in CDD are abundant and serious
- The efficacy of the existing anti-epileptic treatment options in seizures in CDD is modest
- Ganaxolone could be a welcome addition to the existing arsenal of treatment options for seizures in CDD, based on the effect of ganaxolone on seizures in CDD observed in the global Phase 3, randomised, placebo-controlled, double-blind (DB) study 1042-CDD-3001, which appears larger than usually seen with ASMs.
- The safety of ganaxolone appears acceptable

However, during assessment, a number of Major Concerns had to be addressed and it was no longer appropriate to pursue the accelerated assessment.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as an oral suspension containing 50 mg/ml of ganaxolone as active substance.

Other ingredients are: hypromellose (E464), polyvinyl alcohol (E1203), sodium lauryl sulfate (E487), methyl parahydroxybenzoate (E218), propyl parahydroxybenzoate (E216), sodium benzoate (E211), citric acid, anhydrous (E330), sodium citrate dihydrate (E311), artificial cherry flavour (including

propylene glycol [E1520] and benzyl alcohol [E1519]), sucralose (E955), simethicone emulsion (simethicone, polysorbate 65, methylcellulose, polyethylene glycolmonostearate, glycerol monostearate, xanthan gum, benzoic acid [E210], sorbic acid, and purified water), and purified water

The product is available in high-density polyethylene (HDPE) bottles with polypropylene (PP) child resistant (CR) caps lined with induction foil liners as described in section 6.5 of the SmPC.

A press-in adaptor and 3 ml and 12 ml oral syringes are included in the packaging.

2.4.2. Active substance

2.4.2.1. General information

The chemical name of ganaxolone is 1-[(3*R*, 5*S*, 8*R*, 9*S*, 10*S*, 13*S*, 14*S*, 17*S*)-3-hydroxy-3, 10, 13-trimethyl-1, 2, 4, 5, 6, 7, 8, 9, 11, 12, 14, 15, 16, 17-tetradecahydrocyclopenta[a]phenanthren-17-yl]ethenone corresponding to the molecular formula $C_{22}H_{36}O_2$. It has a relative molecular mass of 332.53 g/mol and the following structure:

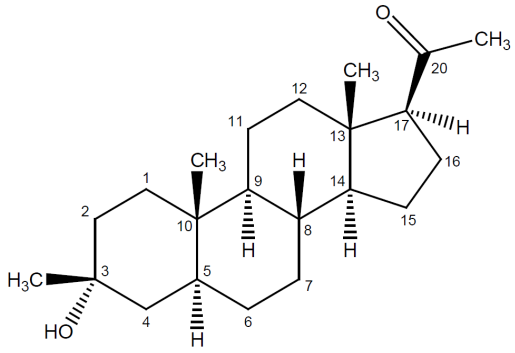


Figure 1: Active substance structure

The chemical structure of ganaxolone was elucidated by a combination of elemental analysis, ultraviolet spectroscopy (UV), infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR), mass spectrometry and X-ray crystallography. The solid-state properties were investigated by X-ray powder diffraction (XRD).

The active substance is a crystalline white to off-white powder, which is non-hygroscopic and practically insoluble in water. Ganaxolone exhibits stereoisomerism due to the presence of 8 chiral centres. Ganaxolone has a steroid core structure and derives its absolute configuration from the starting material which is synthesised using established procedures. The downstream diastereomeric impurities from epimerization are routinely controlled in the active substance specification as specified impurities. In addition, the correct enantiomer of the active substance is routinely confirmed by optical rotation.

The polymorphic nature of ganaxolone was investigated in detail. Only a single crystalline form, Form A, was identified under all studied conditions.

The risk of presence of elemental impurities was evaluated in line with ICH Q3D. Based on the risk assessment provided, it is acceptable that only Palladium is routinely controlled in the active substance specification.

2.4.2.2. Manufacture, characterisation and process controls

The active substance is manufactured by one manufacturing site.

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory. The ASMF holder is the single supplier of the active substance.

Ganaxolone is synthesised in two main steps using a well-defined starting material with an acceptable specification.

Particle size is controlled in the specification of the active substance.

During the procedure, a Major Objection was raised in relation to the designation of the starting material. In response, further justification regarding the designation and the control strategy were presented. The responses were satisfactory and resolved the Major Objection. The starting material is acceptable.

During the procedure, a further Major Objection was raised in relation to the control of a potentially genotoxic impurity which is used in the last step of the manufacturing process of the starting material. In response, control of the impurity was added to the active substance starting material specification with a suitable limit. The limit is supported by results of spiking experiments. Responses were sufficient to resolve the Major Objection. The starting material specification is acceptable.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, the starting material and reagents have been presented.

The characterisation of the active substance and its impurities is in accordance with the EU guideline on chemistry of active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

The active substance is packaged in double low-density polyethylene (LDPE) bags which are then placed in a HDPE drum. The primary packaging material complies with EU 10/2011 and Ph. Eur. 3.1.3.

2.4.2.3. Specification

The active substance specification includes tests for appearance, identity (FT-IR, HPLC), melting range (Ph. Eur.), assay (HPLC), purity (HPLC), residual solvents (GC headspace), specific rotation (Ph. Eur.), water content (KF), residue on ignition (Ph. Eur.), particle size (laser diffraction), elemental impurities (ICP-MS), and microbial enumeration (Ph. Eur.).

The proposed specification is acceptable and in line with ICH Q6A requirements. The particle size limit is 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 assay and impurities testing has been presented.

Batch analysis data for six batches representative of the proposed commercial process and six process validation batches are provided. In addition, batch data was also provided for earlier batches manufactured during development. The results are within the specifications and consistent from batch to batch.

2.4.2.4. Stability

Stability data from 9 batches (including four registration batches and three process validation batches) of active substance from the proposed manufacturer stored in a container closure system representative of that intended for the market for up to 60 months under long term conditions (25°C / 60% RH) and for up to six months under accelerated conditions (40°C / 75% RH) according to the ICH guidelines were provided. The following parameters were tested*:* appearance, crystallinity, assay, purity, water content, microbial enumeration and bacterial endotoxins.

All tested parameters were within the specifications and no significant trends were observed.

Photostability testing following the ICH guideline Q1B was performed on one batch. Photostability was studied in solution as well as in the solid state and the active substance is classified as light sensitive. Forced degradation studies were also carried out in both solid and solution phase demonstrating the stability-indicating nature of the analytical methods.

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 36 months without special temperature storage conditions. The container adequately protects the active substance from light.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Ztalmy 50 mg/ml is a white to off-white suspension for oral use. Each bottle contains 110 ml of suspension.

Pharmaceutical development focused on obtaining a product suitable for the target population (indication: treatment of rare paediatric epilepsies). The quality target product profile (QTPP) for the finished product was defined and CQAs were also defined following a question during the procedure (CQAs: appearance, identification, assay, related substance, preservative assay, re-suspendability, uniformity of dosage units, pH, particle size distribution, dissolution, deliverable volume, viscosity, preservative effectiveness/microbial tests, weight loss, elemental impurities, nitrosamines and residual solvents).

The active substance ganaxolone is practically insoluble in water and therefore the main challenge in product development was to obtain an adequate dissolution profile. A high surface area facilitates dissolution in the gastrointestinal tract following oral administration.

The selection of excipients, including functions and rationale for quantities, is described. Excipients were selected based on experience from previous products. Sodium laurilsulfate is used to stabilise the formation of the active substance particles and hypromellose is used to stabilise the active substance particles formed during the process and to stabilize the diluted drug product. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards, except for artificial cherry flavour, which complies with in-house standards and the 30% simethicone emulsion, which complies with USP. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.4.1 of this report. The safety of the selected excipients in view of use in the paediatric population and acceptability/ palatability issues have been sufficiently addressed and justified. During the procedure, a Major Objection was raised on the content of preservatives and in particular, the use of sodium benzoate and benzyl alcohol, which is a component of artificial cherry flavour. The applicant provided satisfactory responses to resolve the Major Objection. As the level of the preservatives is safe and acceptable for

the target population, the formulation is accepted with a commitment by the applicant to developing a sodium benzoate free formulation (see recommendations). Development work has already started (refer to PIP EMEA-002341-PIP01-18-M01).

Excipient compatibility studies were not conducted during development, however no physical or chemical incompatibilities were observed on assessment of available stability data for clinical batches. 29 finished product batches have been manufactured using different batches of each excipient and 19 batches of active substance. The specifications were met in all cases. The information provided is sufficient.

Compatibility of the product with commonly used food and beverages and enteral feeding tubes is being investigated, however the results are not available yet (see recommendations). The applicant committed to investigating these issues further and this is acceptable.

The formulation used during clinical studies is the same as that intended for marketing. A short overview of the early formulations used in clinical studies is provided. The relevant clinical studies relevant for this Marketing Authorisation Application have all been performed with the same formulation as proposed for commercial use.

Formulation development is described with a focus on the development of a suitable milling process. The relevant physico-chemical characteristics have been identified (particle size distribution, dissolution, re-suspendability, viscosity, foam formation) and are adequately discussed. The test methods and limits for these characteristics have been justified. The specification limits for particle size distribution are acceptable in view of the available results for batches obtained by the milling process and equipment intended for the commercial manufacturing both at release and during stability studies. The proposed shaking procedure for administration of the suspension is acceptable but will be re-evaluated or adjusted, if needed based on ongoing re-suspendability experiments with shorter shaking/standing times. This work will be completed post-approval (see recommendations).

The development of the dissolution method is described. The solubility of ganaxolone in different media with varying concentrations of surfactants has been investigated and the medium with the lowest possible concentration of surfactant capable to achieve dissolution has been selected. The selection of the dissolution method conditions is adequately discussed and justified. The dissolution method for release testing uses a compendial apparatus. The impact of deviations in manufacturing or the composition were studied. Qualitative changes in formulation did not result in significant differences in dissolution profiles. Based on the results of the tests and considering the solubility of the active substance, the discriminatory power of the method is adequately demonstrated.

The manufacturing process development is discussed, with a focus on the milling and dilution steps. Although the batches obtained show difference, especially upon storage, they have both been used alongside each other in clinical studies and no significant difference *in vivo* was observed. The studies performed to select the process parameters of the milling step have been adequately described. The critical process parameters are media load, chiller set point and agitator speed. Control of these parameters is critical in order to control the suspension, which has to be limited to avoid precipitation of an excipient. The critical process parameters have been studied by testing combinations of investigated settings. Manufactured batches have also been tested after storage at long term and accelerated conditions up to 6 months. The results confirm that the parameters ranges selected are all suitable and that the process is adequately controlled by monitoring of outlet temperature and particle size distribution. The suitability of the proposed administration devices has been confirmed in terms of accuracy of dosing, microbial contamination, readability of the graduation and syringeability throughout the proposed period of use. The selected 3 ml and 12 ml syringes are considered adequate for the intended dosing regimen (see also chapter on stability for further details).

To confirm the suitability of the primary packaging, a leachables simulation study was conducted where the finished product in the proposed commercial container closure system was aged. Results confirmed that leachables at significant levels are not expected in the finished product in normal use.

The primary packaging is a high-density polyethylene (HDPE) bottle with a polypropylene (PP) child resistant (CR) cap lined with an induction foil. The materials comply with Ph. Eur. and 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. Dosing syringes of 3 ml and 12 ml are co-packaged with the medicinal product.

2.4.3.2. Manufacture of the product and process controls

The finished product is manufactured at one manufacturing site, with further sites involved for secondary packaging and QC testing.

The manufacturing process consists of four main steps: compounding of solutions, homogenisation and milling of the slurry, filtration and dilution of the milled slurry and bottle filling. The process is considered to be a non-standard manufacturing process.

The manufacturing process is described in sufficient detail. There is only one proposed commercial batch size.

A critical process time is identified for the harvested milled slurry. Holding times at other stages of the process have also been adequately defined.

The target fill weight corresponds to an approximate overfill of 5.5 ml ensuring that the label claim volume can been extracted.

During the procedure, a Major Objection was raised in relation to the need to provide process validation data from 3 full production scale batches. In response, the required information was presented to resolve the Major Objection. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

2.4.3.3. Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance, identification (HPLC and HPLC (DAD), assay (HPLC), impurities (HPLC), uniformity of dosage units (Ph. Eur.), re-suspendability (HPLC), preservative assay (HPLC), dissolution (Ph. Eur.), pH (Ph. Eur.), deliverable volume (USP), particle size analysis (Ph. Eur.), burkholderia cepacia (USP), microbial enumeration (Ph. Eur.), specific organisms (Ph. Eur.), viscosity (Ph. Eur.) and uniformity of mass delivered doses (Ph. Eur.). Efficacy of antimicrobial preservation (Ph. Eur.) and weight loss (weight determination) are only tested at shelf-life.

Release and shelf-life specifications are acceptable and include all relevant parameters in line with ICH Q6A.

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. All ICH Q3D class 1 and 2A elements were evaluated. In addition, elements which could possibly be present in the finished product due to the manufacturing process of active substance (catalyst), or the finished product have also been considered. The relevant elements have been monitored in ten finished product batches manufactured using the proposed commercial manufacturing. Batch analysis data demonstrated that

each relevant elemental impurity was not detected above 30% of the respective PDE. Based on the presented data it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product has been performed as requested by the CHMP as a Major Objection 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 "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided, it is accepted that there is no risk of nitrosamine impurities in the active substance or the related finished product. Therefore, no specific control measures are deemed necessary. The Major Objection raised during the procedure is resolved.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for all batches of the proposed formulation manufactured to date and include batches manufactured with investigated milling systems. This comprises 28 batches of which 10 were manufactured at the proposed commercial manufacturing site and of which 5 were designated as registration batches. The registration batches were manufactured using the proposed commercial active substance and manufacturing process and were released using the proposed commercial finished product specifications. Results confirm the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing.

2.4.3.4. Stability of the product

Stability data is available from five batches of finished product manufactured by the proposed commercial process, of which four were pilot scale and one was full commercial scale. All batches were packed in the packaging proposed for marketing and were stored both upright and inverted. Samples were stored for up to 24 months under long term conditions (25°C / 60% RH) and for up to 6 months under accelerated conditions (40°C / 75% RH) according to the ICH guidelines. The batches of medicinal product are representative of those proposed for marketing. In addition, supportive data is available from several batches, which were stored for up to 36 months under long term conditions (25°C / 60% RH) and for up to 6 months under accelerated conditions (40°C / 75% RH).

Samples were tested for the same parameters as for release and in addition, for weight loss and efficacy of antimicrobial preservation. The analytical methods used were the same as for release and are stability-indicating. The results show no significant changes or trends.

Forced degradation studies were conducted on one batch and confirmed the stability indicating nature of the analytical methods for assay and impurities testing. Samples were exposed to acid hydrolysis, base hydrolysis, heat, oxidation and photolytic conditions. The highest level of degradation was observed under acid-hydrolysis conditions. Minor degradation was observed upon exposure to 3 times the exposure recommended in ICH guidance. An acceptable mass balance was obtained under all stressed conditions.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Results confirm that the finished product in the commercial packaging is not sensitive to degradation by light.

In-use stability study

An in-use stability study was conducted using aged finished product that was within or had exceeded its two year shelf-life. Testing included microbiological testing in accordance with USP and Ph. Eur. and was conducted over 31 days which is the proposed in use shelf-life. All tested parameters complied with their limits. Analytical results are comparable from the first dose from a full bottle to the last dose from a depleted bottle. No trends related to the age of the finished product were observed. The results demonstrate that the preservative systems inhibit bacterial and yeast/mould challenges (inoculation) for up to 24 months under long-term storage conditions and for the in-use shelf-life.

Temperature excursions and freeze thaw studies

Temperature excursions have been studied between 5°C ±3°C /ambient RH and 25°C ±2°C /60% ±5%RH (24 hours each, three cycles) to simulate the finished product encountering cold conditions, between 25°C ±2°C /60% ±5% RH and 40°C ±2°C /75% ±5% RH (24 hours each, three cycles) to simulate brief exposure to warmer temperature and a freeze/thaw study has been conducted (2 days at -20°C and 2 days at 25°C/60% RH, one or three cycles) to determine the effects of freezing of the finished product. Results show that the suspension is stable across the studied temperatures.

Dosing devices and repeated use

During the procedure, a Major Objection was initially raised on the proposed dosing device (12 mL oral syringe) as the suitability was not considered adequately justified. The "in-use" microbiological study conducted did not adequately reflect real-world use and results therefore could not be extrapolated to expected patient use. In response, the applicant proposed new dosing devices, which are the 3 ml and 12 ml syringes (differently graduated) now co-packed with the product. According to the supplier's specifications, the syringes should not be used for more than 50 cycles. For the 12 ml syringes, considering the minimal dose that is to be measured with these syringes, the content of one bottle is spent after 36 cycles. The SmPC clearly indicates that for each new bottle, a new syringe should always be used. For the 3 ml syringe, the SmPC clearly indicates that it should be replaced with a new one after 16 days (48 cycles) of use and two syringes are provided with each bottle. These instructions are considered adequate.

A study was conducted simulating the normal use of the product and syringes as per the SmPC (maximum repeat use of new syringes i.e., 36 cycles for the 12 ml syringe and 60 cycles for the 3 ml syringe). The study addressed the readability of the graduation marks, the risk of microbial contamination and the accuracy of dosage. For all three aspects, results adequately demonstrate that the syringes are suitable for the intended use, the graduation does not change and the tests for microbial contamination and uniformity of delivered doses comply with the requirements until the end of the study. The suitability of the dosing devices has been adequately confirmed and the syringes comply with Ph. Eur. 2.9.27 requirements. The Major Objection is resolved.

Based on available stability data, the proposed shelf-life of 2 years without any special temperature storage conditions and in-use instructions to 'use within 30 days of first opening the bottle' as stated in the SmPC (section 6.3) are acceptable.

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

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. During the procedure, two Major Objections were raised on the active substance relating to the designation of the starting material and the control of an impurity in the starting material. Satisfactory responses were received to resolve the Major Objections. In addition, four Major Objections were raised on the finished product relating to the preservative content, the need to provide process validation data, the nitrosamine risk assessment and the dosing device. Satisfactory responses were received on the issues raised and the Major Objections are resolved. 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 the need to develop a sodium benzoate-free formulation and to continue and finalise further development studies related to the compatibility of the oral suspension with food, drinks, enteral tubes as well as to further study shake time and stand time. These points are put forward and agreed as recommendations for future quality development (see recommendations).

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:

Description	Recommended within Proc. No
Quality: develop a sodium benzoate-free suspension (Q4 2024)	**EMEA/H/C/005825/0000**
Quality: assess the compatibility of the oral suspension with food, drinks, enteral tubes, shake time and stand time (Q4 2024)	**EMEA/H/C/005825/0000**

The applicant has committed to completing the work by the end of 2024.

2.5. Non-clinical aspects

2.5.1. Introduction

The applicant provided an adequate non-clinical package with studies covering primary and secondary pharmacodynamics, safety pharmacology, pharmacokinetics and toxicology.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

In vitro primary pharmacodynamics

The *in vitro* primary PD studies consisted of radioligand binding assays, the evaluation of $GABA_A$ receptor-mediated currents and chloride uptake experiments.

The radioligand binding assays performed in rat brain cortical membrane suspension demonstrated that GNX was able to inhibit the binding of TBPS (chloride channel antagonist) and potentiate the binding of flunitrazepam (benzodiazepine site) and muscimol (GABA recognition site). GNX exhibited modulatory activity with an IC_{50} of 80 nM for TBPS, an EC_{50} of 125 nM for flunitrazepam, and 86 nM for muscimol. GNX was demonstrated to be stereoselective and was a positive modulator of the chloride channel site and the benzodiazepine site. In addition, GNX had positive allosteric modulatory effects at $GABA_A$ receptors similar to those of the endogenous steroid allopregnanolone (3α,5α-P).

In Xenopus laevis oocytes expressing human $GABA_A$ receptors (α1β1γ2L, α2β1γ2L and α3β1γ2L), GNX concentration-dependently enhanced chloride currents evoked by GABA, with an EC_{50} of 213, 94 and 122 nM, respectively. GNX directly activated the $GABA_A$ receptor at relatively high concentrations (1–10 µM).

In rat brain cortical synaptoneurosomes, GNX potentiated GABA-stimulated chloride uptake in a concentration-dependent manner (at 1 µM and 10 µM) up to 43%, indicating that GNX enhances $GABA_A$ receptor function.

In vivo primary pharmacodynamics

The anticonvulsant activity of GNX was characterized in chemically-induced convulsion models (pentyletetrazol (PTZ), TBPS, aminophylline, bicuculline, strychnine), in the 4-aminopyridine (4-AP)-induced lethality model, in the maximal electroconvulsive shock (MES) model and in the cornea-kindled convulsion model.

In mice, the maximal protection against PTZ-induced clonic seizures was 10 minutes after GNX administration, whereas in rats, GNX was also maximally protective after 30 minutes. In both mice and rats, the efficacy of GNX decreased over time. GNX administered to mice increased the dose of PTZ required to produce clonic convulsions, thus the seizure threshold, in a dose-dependent manner, before reaching a dose sufficient to produce ataxia in the rotarod test. GNX also dose-dependently protected against PTZ-induced seizures in rats. After oral administration in rats, the GNX dose that was protective for 50% of the animals tested (ED_{50}) was 21.0 mg/kg.

GNX demonstrated protective effects in mice against convulsions induced by bicuculline, TBPS and aminophylline, with ED_{50} values of 4.6 mg/kg, 11.7 mg/kg and 11.5 mg/kg. There was no protective effect against convulsions induced by strychnine, probably because strychnine is an antagonist of glycine and acetylcholine receptors.

In rats kindled to stage 5 seizures by daily electrical stimulation of the cornea, IP administration of GNX exhibited potent anticonvulsant effects, with an ED_{50} of 4.5 mg/kg.

In the MES model, GNX (IP) dose-dependently inhibited seizures in mice, with an ED_{50} of 29.7 mg/kg and an onset of 30 minutes. The efficacy of GNX against MES significantly decreased over time. After oral administration in rats, GNX dose-dependently inhibited MES-induced seizures, with an ED_{50} of 58.4 mg/kg. In both mice and rats, the effective doses caused drug-related behavioural effects in the rotarod test.

In the 4-AP model, doses that induced protection were compared to doses that caused general behavioural toxicity in the same mice, as measured by loss of traction reflex. The protective dose without causing general behavioural toxicity was 30 mg/kg; higher doses (56 and 100 mg/kg) also caused a loss of traction, whereas lower doses (10 and 17 mg/kg) were not protective.

2.5.2.2. Secondary pharmacodynamic studies

In vitro secondary pharmacodynamics

The selectivity of GNX was evaluated in binding screens of receptors, ion channels, and enzymes. In addition, GNX and major human metabolite M2 activity on the progesterone receptor and oestrogen receptors α and β was assessed. The functional activity of M2 at the $GABA_A$ receptor was also investigated, in addition to a screen of 44 pharmacological targets.

In a screen of CNS receptors, the activity of GNX as a competitive inhibitor of the binding of several radioligands was investigated for 37 types of receptors or recognition sites. At 10 µM, GNX had limited activity (not more than 40%) on inhibiting or stimulating ligands for the GABA recognition-, benzodiazepine-, and chloride channel sites. This is probably due to the absence of exogenously added GABA and/or the absence of chloride ions in the incubation mixture, which are thought to be necessary for GNX' allosteric action.

GNX had a minimal affinity for cytosolic steroid receptors (IC_{50} > 10 µM), including soluble oestrogen, glucocorticoid, progesterone and testosterone receptors.

In an *in vitro* binding assay screen with receptors, transporters, and ion-gated channel systems that have CNS sites of action, GNX (10 µM) inhibited (95.8%) binding of the antagonist radioligand for the GABA-gated chloride channel.

The applicant also provided an evaluation of M2 and GNX activity as agonist and antagonist of the human progesterone receptor and oestrogen receptors (PGR, ERα and Erβ, respectively). Neither M2 nor GNX demonstrated activity as agonist or antagonist of the human progesterone receptor and oestrogen receptors (PGR, ERα and Erβ, respectively) except for antagonist activity of M2 at the ERβ receptor.

In an *in vitro* off-target radioligand assay, M2 (10 µM) was evaluated against 44 pharmacological targets. M2 showed no significant effects (>50%), except for agonist activity against the adenosine A_{2A} (human) receptor (61% inhibition). Since the free fraction is about 0.3 µM, this is sufficiently below the concentration required to elicit activation of the A_{2A} receptor.

In vivo secondary pharmacodynamics

To evaluate the dependence potential of GNX, GLP-compliant drug abuse liability studies in rats were conducted that evaluated self-administration, drug discrimination and physical dependence.

In self-administration studies, GNX showed reinforcing characteristics from 0.1 mg/kg.

GNX also generalised to midazolam in drug discrimination studies, suggesting that GNX may produce midazolam-like psychoactive effects, such as the risk of tolerance and abuse.

In a physical dependence study, rats demonstrated signs of withdrawal and tolerance to the behavioural and physiological effects of GNX at clinically relevant exposures, indicating that GNX may cause the development of physical dependence. However, these effects were less pronounced compared to diazepam.

2.5.2.3. Safety pharmacology programme

The safety pharmacology package comprised GLP-compliant cardiovascular (dogs), respiratory (rats) and CNS (rats) safety studies, and non-GLP CNS studies (rodents), including a rotarod test, locomotor test, cognitive function study and a sedation study.

In a GLP-compliant female rat CNS study, preformulated GNX (10, 20 and 40 mg/kg) was evaluated for clinical observations, body weights and the modified Irwin battery. In line with ICH S7A, the modified Irwin battery evaluated motor activity, behavioural changes, coordination, sensory/motor reflex responses and body temperature. A transient, GNX-related decrease in body temperature (<5%) was observed in all dose groups. At 20 mg/kg onwards, the only GNX-related CNS effects were abnormal visual responses and ataxia. Ataxia was considered to be dose-dependent. Mild to moderate grasping loss, abnormal righting reflex and low carriage were observed in animals in the high-dose group. All behavioural effects ranged from mild to moderate between 2-6 hours post-dose and were noted at the mid and high dose in a dose- and time-dependent manner, most likely due to the sedative, pharmacological effect of GNX.

Locomotor activity (both mice and rats) and cognitive function (mice) studies were dose-dependently influenced by GNX. In mice, dose-dependent effects of GNX on motor function started from 20 mg/kg onwards and in rats at 5 mg/kg. The lowest dose, which produced a significant decrease in locomotor activity for GNX was 16-fold lower than valproate (GNX 50 mg/kg vs valproate 800 mg/kg) and 18-fold lower than ethosuximide (GNX 50 mg/kg vs ethosuximide 900 mg/kg) in mice. In rats, on the contrary, the minimum sedative dose for GNX (15 mg/kg) was 53-fold lower than valproate and ethosuximide (800 mg/kg), suggesting that GNX has more potent sedative effects compared to controls.

Higher doses (40 mg/kg in mice and 20 mg/kg in rats) also caused a loss of righting reflex, which was considered adverse. Cognitive function was significantly impaired at a dose of 30 mg/kg. These GNX effects were presented as deficits in motor function or in the latency to learn a response and were attributed to the sedative effect of GNX. GNX had strong sedative properties when administered IV to rats as a nanosuspension formulation or a Captisol formulation. In both formulations, GNX transiently induced dose-dependent, deep sedation but not full anaesthesia, starting from the low dose.

In two GLP-compliant *in vitro* hERG inhibition studies, no significant hERG inhibition was observed with GNX at concentrations up to 10 µM, which is approximately 11-fold the total GNX concentration at the clinical C_{max} of ~300 ng/ml. Assuming that 1% of GNX is available as a free fraction, the safety margin for unbound GNX becomes 1100 times the anticipated clinical C_{max} and no hERG-related effects would be expected in humans.

In a GLP-compliant telemetry study in male dogs, it was reported that orally administered doses of preformulated GNX up to 15 mg/kg did not affect body temperature, blood pressure, heart rate (HR), or qualitative or quantitative ECG parameters (QRS duration and RR, PR, and QT(c) intervals), but it was associated with ataxia.

Notably, HR appeared to be increased up to 140 bpm in all dose groups, including controls, when the drug was administered. In addition, in GNX-treated animals, the HR remained elevated in a dose-related manner for up to five hours past dosing, while in control animals, the HR returned to baseline quickly after dosing. However, there were no signs of cardiovascular effects in the clinical studies.

In a GLP-compliant respiratory safety study in female rats, no notable changes were related to preformulated GNX in respiratory rate, tidal volume, or minute volume at doses up to 40 mg/kg.

2.5.2.4. Pharmacodynamic drug interactions

Pharmacodynamic drug interactions of GNX with ethanol and diazepam were investigated in non-GLP rodent studies.

Ethanol potentiation studies in mice and rats suggested that GNX may increase the sedative effect of ethanol in a dose-dependent manner.

A combination study of diazepam and GNX was conducted in the rat lithium-pilocarpine model of status epilepticus (SE). Diazepam (5 mg/kg) or GNX (3 or 6 mg/kg) administered (IV) individually had modest effects on suppressing EEG activity. It should be noted that there were only 2 animals in the GNX alone groups. In contrast, the combination of diazepam with GNX suppressed EEG activity in a dose-dependent manner. One animal in the diazepam + GNX 6 mg/kg group died post-dosing at the time when the EEG activity was most severely reduced. The other animals in this group appeared deeply sedated. In a separate group of rats, plasma levels of GNX and diazepam alone and in combination were measured. The C_{max} and half-life of these compounds were not influenced by co-administration, suggesting that the synergistic effect was not due to a PK interaction.

2.5.3. Pharmacokinetics

Fifteen single-dose PK studies were conducted in mice, rats, rabbits, dogs, and monkeys. The single-dose study in rabbits demonstrated low and highly variable exposure, which precluded rabbits from being used in an assessment of embryofoetal toxicity.

2.5.3.1. Absorption

The single-dose PK of GNX was studied in male and female CD-1 mice at different doses. Following IV dosing at 10 mg/kg (n = 4 mice/time point), the plasma concentration of GNX declined quickly (~4-fold from 0.5 – 1 hour after dosing) in a bidirectional fashion (0-8h). For both male and female mice, distribution volume (Vss) was very high (21 L/kg), i.e. ~29-fold more than body water. Clearance (plasma) was 263 and 298 mL/min/kg for male and female mice, respectively, which is ~3-fold the liver blood flow. The terminal half-life ($t_{1/2}$) value after dosing was 1.3 and 1.6 h in male and female mice, respectively.

Upon oral administration of 40 mg/kg (n = 4 mice/time point), the absorption in plasma was fast, with a Tmax of 1.5 h, but present at the first timepoint (15 min) and extending up to 2h. A terminal elimination half-life ($t_{1/2}$) value of 1.9 and 3.7 h was found in female and male, respectively, but this is underestimated given the short follow-up. Absolute oral bioavailability (Fpo) in mice was only 6.2% (M) and 8.0% (F), with exposure (AUC) in females (179 ng*h/mL) higher than in males (156 ng*h/mL). In a different study, mice were orally dosed (male, 500 and 1000 mg/kg; female, 250 and 500 mg/kg), yielding a similar (dose normalised) exposure, but absorption in male mice appeared to be slower than in female mice.

The PK of GNX in plasma and brain tissue was determined in male CF1 mice following a single dose IV administration (10 mg/kg, n = 8/time point, 0-24h), and it was found that GNX rapidly entered the brain. At the first time point (1 min), the highest brain exposure (Cmax 8223 ng/g) was found, which was 4-fold lower than in plasma (36331 ng/mL).

GNX exposure in the brain declined quickly thereafter (distribution half-life of 15 min), but less fast than in plasma, leading to 5-fold higher levels in the brain than in plasma at 5 min and ~9-fold higher at 15 – 30 min post-dosing. Total GNX brain exposure (AUCinf) was about 2-fold higher than in plasma. Similar results were found upon intraperitoneal (IP) GNX dosing (20 mg/kg), which gave a

comparable exposure in brain and plasma at the first timepoint (5 min) and 1.5 (at plasma Tmax 0.5 h) and 4-fold at 2 h post-dosing.

In a third study with ^{3}H-GNX (single IV dose, 10 mg/kg) administered to male CF1 mice, it was found that radioactivity declined biphasic in plasma with a rapid distribution phase and a slower elimination phase (plasma t½ 20h, brain t½ 15h). For unchanged GNX, the elimination t½ for plasma and brain were 0.9 and 1.2, respectively. Following a single oral administration of 20 mg/kg ^{14}C-GNX to CD-1 mice (male/female), ^{14}C-GNX related radioactivity showed a Tmax at 1 – 4h and declined thereafter up to 72h after dosing with a terminal half-life ($t_{1/2}$) value of 26-40 h.

The single-dose PK of ganaxolone was studied in male Sprague Dawley rats following IV dosing at 2 mg/kg and oral gavage dosing at 40 or 80 mg/kg GNX (n = 3/time point). Following IV administration, the plasma concentration of GNX declined in a bidirectional fashion (0-8h). Distribution to the tissues was very fast, with a half-life of 3 minutes, and distribution volume (Vss) was high (7.9 L/kg), i.e. ~12-fold more than body water. Clearance from plasma was 77 mL/min/kg, which is 1.4-fold the liver blood flow. Terminal half-life ($t_{1/2}$) value up to 8h after dosing was ~1.5 h.

Upon oral administration to male rats, the absorption in plasma was fast with a Tmax of 1.5 to 2 h for the 40 - 80 mg/kg (50% HPβCD) and 40 mg/kg (20% HPβCD) groups, respectively. Thereafter, the plasma concentration of GNX declined, but this was slower with increasing doses or lower HPβCD formulation. A terminal elimination half-life ($t_{1/2}$) value of 5.5 – 8.6 h was found, but this is underestimated, given the short follow-up. Upon oral dosing of GNX, blood exposure ($AUC_{0-\infty}$) increased more (~8-fold) than dose-proportional over the dose range of 40 to 80 mg/kg (in 50% HPβCD). Absolute oral bioavailability (Fpo) was stated to be 11.3% for 40 mg/kg (50% HPβCD), 44.7% for 80 mg/kg (50% HPβCD), and 17.3% for 40 mg/kg (20% HPβCD). The formula used to calculate the Fpo was corrected as the Fpo is the ratio of the dose-normalised AUCpo/AUCiv.

Following a single oral administration of 10 mg/kg ^{14}C-GNX to SD rats (male/female), ^{14}C-GNX related radioactivity showed a Tmax at 1 – 4h and declined thereafter up to 72h after dosing with a terminal half-life ($t_{1/2}$) value of ~13 h. In a second study given ^{14}C-GNX (IV 2 mg/kg, PO 40 mg/kg) to male SD rats, a similar biphasic PK profile of the ^{14}C-related radioactivity was seen upon IV administration with a fast biphasic distribution of label to the tissues but a longer terminal elimination than with non-labelled GNX (T½ 11-12h). Upon oral administration, a Tmax of radioactivity in plasma was seen at ~2 h and a terminal elimination T½ of 31 h. Absolute oral bioavailability, calculated using ^{14}C-related $AUC_{0\text{-}inf}$ values (and without the CL in the formula, using the actual dose), was 67 and 77% for blood and plasma. The blood-to-plasma (B/P) ratio, based on [^{14}C]-GNX related oral exposure (AUC) was found to be 0.56, indicating a preferential partitioning in plasma.

The PK of [^{14}C]-GNX was evaluated in female rabbits after single oral (10, 40, 60, 80 mg/kg, n=2-4) and IV (2, 4 mg/kg, n=1) doses. Upon IV administration, GNX plasma concentration showed a biphasic, rapid decline, which was by 2-fold in 10 min and about 20-fold in the first 2 h, thereafter, the terminal elimination half-life was 7-10 h. However, the [14C]-GNX related radioactivity showed a much slower decline, i.e. only 2-fold in the first 6 h after dosing, indicating fast and extensive metabolism. At 2 h after dosing, the plasma [^{14}C]-radioactivity concentration was ~20-fold higher than GNX, suggesting substantial metabolism. The terminal elimination half-life of [^{14}C]-radioactivity was >24 h.

In fasted male Beagle dogs (n = 3), the single-dose PK was studied following IV dosing at 2 mg/kg and oral gavage dosing at 40 or 60 mg/kg GNX. Following IV administration in dogs, the plasma concentration of GNX declined in a bidirectional fashion (0-8h). Distribution to the tissues was very fast with a half-life of 6 minutes, and distribution volume (Vss) was high (3.5 L/kg), i.e. ~6-fold more than body water. Plasma clearance was 29 mL/min/kg, similar to the liver blood flow. Terminal half-life ($t_{1/2}$) value up to 8h after dosing was 1.7 h. Upon oral administration to male dogs, the absorption in plasma was fast but extended with Tmax ranging from 0.75 to 8 h. Thereafter, the plasma concentration of

GNX declined with finally a terminal half-life ($t_{1/2}$) value was 34 - 60 hrs but measured only up to 24-52 hrs. Upon oral dosing of GNX, blood exposure ($AUC_{0-\infty}$) increased more (3-fold) than dose-proportional over the dose range of 40 to 60 mg/kg (in 50% HPβCD). For the 40 mg/kg dose, two different formulations were used (20% and 50% HPβCD), which both gave a similar exposure ($AUC_{0-\infty}$), but with the 20% HPβCD formulation, a 2-fold higher Cmax was found, and a steeper decline thereafter. Absolute oral bioavailability (Fpo) was updated using the correct formula and found to be 64.6 for 40 mg/kg (50% HPβCD), 116% for 60 mg/kg (50% HPβCD), and 54.9% for 40 mg/kg (20% HPβCD).

Following a single oral administration of 10 mg/kg ^{14}C-GNX to Beagle dogs (male/female), ^{14}C-GNX related radioactivity showed a Tmax at 1 – 1.5h, was found to be 2-fold higher in females than in males and declined thereafter slowly with terminal half-life ($t_{1/2}$) value of 30-47 h. In a second study given ^{14}C-GNX (IV 2 mg/kg, PO 10 mg/kg) to male Beagle dogs (n=6), a similar biphasic PK profile of the ^{14}C-related radioactivity was seen upon IV administration with a fast distribution of label to the tissues but a longer terminal elimination than with GNX. Upon oral administration, a Tmax of radioactivity in plasma was seen at 84 min and a terminal elimination of 29 h. Absolute oral bioavailability, calculated using ^{14}C-related $AUC_{0\text{-}inf}$ values, was >90% for blood or plasma. The blood to plasma (B/P) ratio, based on [^{14}C]-GNX related concentration (AUC), was found to be 0.57, indicating a preferential partitioning in plasma.

The single-dose PK of ganaxolone in fasted male cynomolgus monkeys (n=4) was studied following IV dosing at 2 mg/kg and oral dosing at 10 mg/kg ganaxolone with and without food. Following IV administration, the plasma concentration of ganaxolone declined in a multidirectional fashion characterized by a systemic clearance of 1.4 L/h/kg (50% of liver flow) and a high volume of distribution (Vss) of 3.4 L/kg, which is ~5-fold more than body water. Following oral administration, absorption was slow in plasma in fasted condition (Tmax 7.4 hrs) and slightly faster with food (Tmax 4.9 hrs). Based on AUC, in plasma, exposure was 20% lower with food than in fasted condition and Cmax 29% lower with food. Oral bioavailability was low (4.0%) and slightly lower with food (3.2%).

2.5.3.2. Distribution

In vitro: The equilibrium dialysis method showed that GNX was highly protein-bound, generating 1.1% of unbound GNX in human plasma and that more than 10 hr were needed to reach equilibrium.

Protein binding studies with GNX were also performed *in vitro* using plasma from mice, rats and humans at 50 or 500 ng/ml (n=2) after incubation at 37°C for 4 h. GNX plasma protein binding (PPB) was found to be >86.3%, >94.6%, >84.4% at 50 ng/ml and >99.1%, >99.3%, and >99.3% at 500 ng/ml for mouse, rat and human, respectively. In a later supplied study report CYP2472 R2, which described the determination of the extent of protein binding of ganaxolone in plasma of humans, rats, and dogs using equilibrium dialysis (up to 8 hrs), ganaxolone at 5 µM (1662.5 ng/ml) was found to be very highly protein bound with PPB values ≥99.9% in rat, dog and human plasma.

In vitro studies with the human putative metabolite (M2) indicates that M2 is very highly protein-bound in mice, rats and dogs. M2 appeared highly permeable in human Caco-2 cells.

The partitioning of GNX into plasma and blood was determined after single-dose oral administration of [^{14}C]-GNX to mouse, rat and dog. The blood-to-plasma (B/P) ratio, based on [^{14}C]-GNX related concentration (Cmax), was found to be 0.56, indicating a preferential partitioning in plasma. This B/P ratio increased over time up to 0.87 at 72 hrs after dosing, indicating some red blood cell (RBC) partitioning over time.

In vivo: The *in vivo* tissue distribution of a single dose of labelled [^{3}H]-ganaxolone was investigated in the male CF1 mice (IV, 10 mg/kg [^{3}H]-GNX) in a mass balance study up to 24 hrs post-dose. At the earliest time point (15 min) radioactivity was highest in the liver and intestines, followed by the kidney, brain, lung, spleen, testis, and heart and declined thereafter in all tissues except the intestines (highest at 6 hrs).

The *in vivo* tissue distribution of a single dose of labelled [^{14}C]-ganaxolone was investigated in the male SD albino rat (IV 2 mg/kg, PO 40 mg/kg [^{14}C]-GNX) in a mass balance study up to 48 hrs post-dose. At the earliest time point (1 h) after IV or PO administration, radioactivity was detected in all 23 tissues, indicating a fast and broad distribution, and declined thereafter in generally all tissues except intestines upon IV administration (highest at 8 hrs). Upon PO administration, the highest radioactivity, except in the gastro-intestinal tract, was found in the liver, adrenal glands, fat pancreas, lymph gland and mammary glands. At 48 hrs post-dose total tissue radioactivity declined to ~2% of the dose as compared to 87% at 1h. Label in brain and testes indicates passage of the respective barriers by GNX.

The *in vivo* tissue distribution of a single dose of labelled [^{14}C]-ganaxolone was investigated in the partially-pigmented male Long Evans rat (PO 5 mg/kg [^{14}C]-GNX) using quantitative whole-body autoradiography (QWBA) at 1h, 4h, 24h, 72h and 168 h post-dose. Tissue distribution was wide in pigmented rats and comparable to that in the non-pigmented male rats. The highest ^{14}C-related radioactivity at 1 h post-dose was found in bile and liver. The earliest time point (1h) was for most tissues, the Tmax. Tissue to plasma ratio (T/P) was generally found to be between 0.2 – 1.5 for all tissues during the first 4 hours post-dose, except for the liver (T/P=19). No radioactivity was found in abdominal fat, bones, and eye lens. At 72h post-dose, radioactivity in all tissues was not detectable, except for the liver. As pigmented and non-pigmented skins were comparably labelled, it is concluded that there is no melanin binding. Radioactivity was found to cross the testis, and blood-brain barrier, and exposure was about 2-fold lower than in blood.

After a single oral administration of [^{14}C]ganaxolone to lactating SD rats, [^{14}C]-GNX-derived radioactivity was readily secreted as GNX and metabolites into milk. GNX was both in plasma (16%) and milk (71%) the largest component of radioactivity. The milk to plasma ratio ranged from 2.2 – 4.2 for [^{14}C]-GNX-derived radioactivity and 3.6 – 14 for GNX, indicating a selective distribution to milk.

2.5.3.3. Metabolism

In vitro metabolism studies using human liver microsomes and recombinant CYP P450 enzymes are consistent with ganaxolone metabolism by CYP3A4, and to a much lesser extent by 3A5, 2B6, 2C19 and 2D6 enzymes. The metabolism of GNX was further evaluated in rat and human liver microsomes, using labelled ([^{3}H]- or [^{14}C]-) GNX. The primary metabolite observed with human liver microsomes was identified as 16α-hydroxyganaxolone (M1). This metabolite was also formed during incubation of [^{14}C]-GNX with the mouse, rat, and dog liver microsomes. The initial metabolites in the proposed pathway, 16α-hydroxyganaxolone, 20β-hydroxyganaxolone, and 3β-hydroxymethylganaxolone (all mono-hydroxylated), when isolated from microsomal incubates and re-introduced into microsomal incubations, were all further metabolized to 20-keto reduced di-hydroxylated and tri-hydroxylated metabolites.

In vivo metabolism with metabolite identification was evaluated in mice, rats and dogs, providing information on possible metabolic pathways. Gender differences in the metabolic profiles were observed, caused by a sex difference in hepatic expression of the metabolising (CYP) enzymes yielding different metabolite levels in female as compared to male rodents.

Following a single oral administration of 20 mg/kg ^{14}C-GNX to CD-1 mice (male/female, M/F), radio-HPLC profiles showed that unchanged GNX was the minor source of circulating radioactivity in

plasma (1.8%/1.5%). The largest of the >10 circulating radioactive metabolites in plasma (M/F) were M39 (22%/12%), M35 (11%/7%) and M45 (1.5%/8.8%). M44 was a minor circulating component in male mouse plasma, while it was at 12.7% in female mouse plasma. Most of the metabolites were formed via Phase I biotransformation, e.g. multiple (mono-, di-, tri- and tetra-) hydroxylation (16alpha-OH, 3beta-OH) and ketone reduction (20beta-OH).

In mouse faeces (0-72h), containing 93% of the radioactive dose, most of the excreted radioactivity was associated with unchanged GNX (5-8%) and 10 metabolites, of which M40/M41 (~11%), M36 (~9%), M35 (12%, male-only), and the rest were 1-6% of the radioactive dose. In urine, containing about 4-8% of the radioactive dose, unchanged GNX (0.01%) and 13 metabolites were found, of which M44 was excreted as the largest radioactive component (~0.4% of the radioactive dose), while the others were <0.2%.

Following a single oral administration of 20 mg/kg ^{14}C-GNX to SD rats (male/female), radio-HPLC profiles showed that unchanged GNX was the minor source of circulating radioactivity in male plasma but large in female (M/F, 1.0%/8.5%). The largest of the >15 circulating radioactive metabolites in male rat plasma (M/F) were M31 (25%/1.5%), M30 (8.3%/0.4%) and M28/M29 (7.0%/0.5%), while in female rat plasma the largest were M23 (1%/16%), M1 (1%/12%), and M26/M27 (0.1%/8.3%). Most of the metabolites were formed via Phase I biotransformation, e.g. multiple (mono-, di-, tri- and tetra-) hydroxylation (16alpha-OH, 3beta-OH) and 20-ketone reduction (20beta-OH). Glucuronidation was the only observed Phase II metabolic reaction.

In rat faeces (0-72h), most of the excreted radioactivity (58% of dose) in male rats was mainly associated with unchanged GNX (21%), and >15 metabolites, of which M33 (~5%) was the largest and the rest were 0.1-2.6% of the radioactive dose, while in female rat faeces, containing 75% of radioactivity dose, unchanged GNX (~3%) were minor. In female faeces, M23 (~8%) and M24/M25 (10%) were the major metabolites, and the rest of the >15 metabolites were 0.1-4% of the radioactive dose. In urine containing 16% (M) and 8% (F) of the radioactive dose, ~12 metabolites were found, of which in male M20 and M21/M22 were excreted at about ~4% of the radioactive dose, while the others were <1.5%. Unchanged GNX was less than 0.1% in urine.

Following a single oral administration of 10 mg/kg ^{14}C-GNX to Beagle dogs (male/female), radio-HPLC profiles showed that unchanged GNX was a large part of circulating radioactivity in dog plasma (M/F, 21%/34%). The largest of the 14 circulating radioactive metabolites was M1 (28%/21%). Of the other metabolites in dog plasma (M/F), M15 (7%/5%) and M5 (2%/3%) were identified, while the remaining metabolites were 0.2-6.4% of the radioactivity in plasma. These others in plasma were 42%/36% of total radioactivity from 0-48h. Most of the metabolites were formed via Phase I biotransformation, e.g. multiple (mono-, di-, tri- and tetra-) hydroxylation (16alpha-OH, 3beta-OH) and 20-ketone reduction (20beta-OH).

In dog faeces (0-168h/216h), most of the excreted radioactivity (~70% of dose) in male and female dogs was associated with unchanged GNX (M/F, 24%/27%) and ~20 metabolites, of which M1 (32%/22%) was the largest, followed by M5 (6%/3%) and the rest of the metabolites were less than 6% of the radioactive dose. In urine containing 3% (M) and 5% (F) of the radioactive dose, ~24 metabolites were found, each excreted at less than 0.4% of the radioactive dose. Unchanged GNX was less than 0.1% in urine.

A comparison of ganaxolone metabolites in human plasma upon multiple dosing was made with metabolites in mouse, rat and dog plasma. In human pooled plasma samples, approximately 30 metabolites with each >1% of the total peak area were detected in total. While the parent compound was detected as a minor component (<1%), two major (>10%) metabolites were detected, which were GNX+O+gluc(uronide) at 21% and GNX+2O at 16% of total drug-related exposure in humans. The glucuronide GNX+O+gluc was also found in dog plasma (at 4%), and female mouse plasma (12%)

but not in rat plasma or male mouse plasma. The other major metabolite, GNX+2O was not detected in rat, mouse or dog plasma. In addition, six other human metabolites were found, with each a presence of 4% - 9% of total exposure, of which five were glucuronides (~5% GNX+O+gluc, 7-9% GNX+2O+gluc, 6-8% GNX+H2O+gluc, 4-6% GNX+H2O+gluc, 4-6% GNX+H2O+O+gluc) and one not (4-5% GNX+H2O+H2). Of these latter metabolites, four with a total exposure of 18% - 25% were not present in the pivotal preclinical chronic toxicology species (female) rat and dog.

2.5.3.4. Excretion

After a single oral administration of [^{14}C]ganaxolone, the excretion of radioactivity was predominantly through the faecal (biliary) pathway, and, for the mouse, rat, and dog, it accounted for 93%, 64-82% and ~71% of the radioactivity dose, while the urinary route (including cage wash) contributed 4-8%, 12-29% and 8-17%, respectively. The total recovery of radioactivity, 3 (rodents) or 5 (dog) days after dosing, was high in the mouse (>97%), rat (93%) and human (>90%) and moderate in the dog (77-81%).

In separate ADME studies in male Beagle dogs, given [^{14}C]ganaxolone, and in male SD rats, given [^{3}H]ganaxolone, a similar excretion profile was found when dosed orally or intravenously (IV), with predominantly faecal (po ~70%, IV 65-76%) as compared to renal (po 13-21%, IV 15%-23) excretion. The high faecal excretion upon IV administration may indicate that biliary (and/or intestinal) excretion plays an important part in the excretion of ganaxolone.

In male and female bile duct-cannulated SD rats, following intravenous administration of [^{3}H]-GNX, excretion into the bile, faeces, and urine was 78%, 1% and 7-17% of the radioactivity dose, respectively, indicating a predominant biliary clearance. Following oral administration, also 78% was found in bile, while 6% and 4-9% of the dose was excreted into faeces or in urine, confirming the mainly biliary excretion and that this was found after a high oral absorption.

2.5.3.5. PK studies with Human Metabolite M2

In vitro studies with the human metabolite M2 indicate that it is highly protein bound in the plasma of all species tested (mouse, rat, dog and human). M2 significantly inhibited CYP2C8 activity, with 70% to 82% of CYP2C8 activity inhibited at the highest concentration of M2 (50 µM). M2 also exhibited non-NADPH mediated, time-dependent inhibition of human CYP2B6 with pre-incubation.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

Two single-dose toxicity studies were performed with GNX in mice and rats. A non-GLP exploratory study was conducted in male mice to evaluate different intravenous (IV) and oral (PO) vehicle formulations.

A pivotal GLP-compliant study with IV administration of GNX formulated in 50% HP-β-CD was conducted in rats. Animals dosed with $\geq$ 2 mg/kg showed immediate ataxia, and with doses $\geq$ 10 mg/kg these animals lost the righting reflex. The incidence, duration, and severity of clinical signs were dose-dependent and more severe in female rats than in males. These findings were also observed in the repeat-dose toxicity studies.

2.5.4.2. Repeat dose toxicity

GNX was initially formulated in oral suspensions containing β-CD and evaluated in repeat-dose toxicology studies in mice (1 week, dosing up to 250 mg/kg/day), in rats (up to 6 months, dosing up to 400 mg/kg/day) and in dogs (up to 6 months, dosing up to 20 mg/kg/day). In the second stage of development, a new formulation - not containing β-CD - was evaluated in repeat-dose toxicology studies in mice (2 weeks, dosing up to 1000 mg/kg/day), in rats (4 weeks, dosing up to 80 mg/kg/day) and in dogs (up to 12 months, dosing up to 15 mg/kg/day) using a preformulated oral GNX suspension. This preformulated oral suspension is also used in patients with the intended indication. In the third stage, a more aggressive approach to dosing was taken in rodents to compensate for the reduced exposure and accommodation of the acute sedation effects. The preformulated oral GNX suspension was evaluated in repeat-dose toxicology studies in mice (up to 4 weeks, dosing up to 1000 mg/kg/day) and in rats (13 weeks, dosing up to 1000 mg/kg/day) in a twice-daily dosing (BID) with an incremental dosing strategy. In the fourth stage, an IV formulation of GNX was developed. This included repeat-dose toxicity studies in rats (up to 2-weeks, dosing up to 42 mg/kg/day) and in dogs (up to 4 weeks, dosing up to 7.2 mg/kg/day).

Findings common to all repeat-dose toxicology studies in mice, rats, and dogs were CNS-related clinical signs. The incidence, duration, and severity of sedation signs were dose-dependent, ranging from decreased activity to unresponsive, usually within 1-2 hours after dosing, and were usually resolved before dosing the next day. Several treatment-related mortalities occurred in all species due to severe sedation signs, and the sedation effects were considered dose-limiting. Since these CNS-related clinical signs often occurred at the lowest tested dose, no NOAEL for sedation effects was established.

In rodents, increased liver weights were observed, which was associated with hepatocellular hypertrophy. In more recent studies, liver enzyme activity was assessed and demonstrated increased CYP450 activity (mainly CYP3A and CYP2B) in GNX-treated rodents compared to control animals. This autoinduction of CYP isoenzymes also led to a dose-dependent reduction in GNX exposure with repeated dosing in rodents and higher exposures achieved in females compared to males, which resulted in females being more sensitive than males in the rodent repeat-dose studies. Additionally, increased thyroid gland weights associated with follicular cell hyperplasia were observed in the 13-week pre-carcinogenicity study and 12-week juvenile toxicity studies. In general, the liver and thyroid gland findings are believed to be secondary to the autoinduction of CYP isoenzymes in rodents and are considered non-adverse. No findings in the liver and thyroid gland or clear gender differences were observed in dogs.

In dogs, a dose-dependent increase in heart rate observed in the 12-month repeat-dose toxicology study (at doses of 3 to 15 mg/kg/day) with the preformulated GNX suspension: average heart rates at 15 mg/kg/day in the 12-month study were up to 66% higher than those of the control group, which were accompanied by a physiologically normal shortening of the PR and QT intervals. Some dogs dosed with 10 and at 15 mg/kg/day (4/10 dogs for each dose) had an instance (a single instance in 7/8 dogs) of transient sinus tachycardia (heart rate greater than 190 bpm). There were no changes in QRS duration or QTc interval observed nor any changes in blood pressure parameters.

2.5.4.3. Genotoxicity

GNX was evaluated in a GLP-compliant Ames test, *in vitro* mouse lymphoma cell mutagenesis assay and *in vivo* micronucleus assay in rats dosed with GNX up to 140 mg/kg, and demonstrated no genotoxic potential.

In addition to GNX, the genotoxic potential of the human metabolite M2 was evaluated in GLP-compliant *in vitro* genotoxicity studies. While M2 did not increase the number of revertants in the Ames

Test, it did increase the incidence of aberrant metaphases in the *in vitro* mammalian chromosome aberration assay.

A GLP-compliant *in vivo* genotoxicity assay with doses up to 150 mg/kg/day M2 in female and male rat was conducted. This dose was considered the Maximum Tolerated Dose (MTD) based on an exploratory Dose Range Finding (DRF) study. Cmax levels were ~7.6-fold higher compared to humans.

Micronucleus assessment of the bone marrow and a COMET analysis of the liver revealed no genotoxic potential of M2.

2.5.4.4. Carcinogenicity

A 4-week and 13-week pre-carcinogenicity assays were conducted with GNX in CByB6F1-Tg[HRAS]2Jic: Wild Type mice and SD rats.

In the 4-week study in CByB6F1-Tg[HRAS]2Jic: Wild Type mice, all GNX dose groups received 125 mg/kg BID for the first 3 days of dosing and then the mid- and high-dose groups received 250 and 500 mg/kg BID, respectively, for the remaining 25 days of dosing. Findings were limited to increases in body weight gain and food consumption (males only) and non-adverse dose-dependent increases in liver/gall bladder weights, which correlated with a significant increase in CYP2B and CYP3A activity. See also repeat dose toxicity section above for key findings.

2.5.4.5. Reproductive and developmental toxicity

Reproductive and developmental toxicity studies have been completed in rats and mice with GNX formulations containing β-CD. Juvenile toxicity studies were conducted in rats with both β-CD containing formulations and the preformulated GNX suspension.

One GLP-compliant fertility and early embryonic development study was conducted in rats with doses up to 40 mg/kg/day GNX. The sedation effects observed in this study were consistent with the findings in the repeat-dose toxicity studies. An increased incidence of extended oestrus cycle was observed in females dosed with 40 mg/kg/day, which did not affect other reproductive parameters. No effects on the male reproductive system were observed. No adverse findings in the female and male reproductive organs were observed in the repeat-dose toxicity studies in rodents and dogs. Therefore, the NOAEL for fertility and early embryonic development in rats was considered 40 mg/kg/day.

One exploratory and one definitive GLP-compliant embryofetal development study were conducted in mice with doses up to 300 mg/kg/day GNX. The sedation effects observed in these studies were consistent with the findings in the pre-embryofoetal 1-week repeat-dose toxicity study. Dams dosed with ≥ 200 mg/kg/day showed reduced bodyweight gain, which was accompanied by reduced food intake. GNX elicited no teratogenicity, embryotoxicity or fetotoxicity in mice. Therefore, the maternal (excluding sedation effects) and embryo-fetal development NOAELs were considered 175 mg/kg/day and 300 mg/kg/day in mice, respectively.

A GLP-compliant combined embryofoetal- and pre-and postnatal development study was conducted in rats with doses up to 40 mg/kg/day GNX. The sedation effects observed in this study were consistent with the findings in the repeat-dose toxicity studies. Dams dosed with 40 mg/kg/day showed reduced bodyweight gain at gestation days 6-16, which was accompanied by reduced food intake, while, at the same dose, approximately half of the animals showed intermittent body tremors whilst the animals appeared asleep. A slightly prolonged duration of the pregnancy was observed in these animals. In addition, one 10 mg/kg/day and one 40 mg/kg/day females were prematurely sacrificed due to dystocia. Dams treated with 20 and 40 mg/kg/day showed an increase in the mean litter percentage

incidence of foetuses with an additional lumbar rib (24% and 10% at these 2 dosages, respectively compared to 5 % in the control group), although there was no dosage-relationship. GNX elicited no teratogenicity, embryotoxicity or fetotoxicity in rats. Therefore, the maternal (excluding sedation effects) and embryo-fetal development NOAELs were considered 20 mg/kg/day and 40 mg/kg/day in rats, respectively.

F1 animals of dams treated with 40 mg/kg/day showed an increased bodyweight directly after birth, which was likely related to the prolonged duration of the pregnancy. However, at weaning, these F1 animals showed reduced bodyweight gain compared to control animals. F1 animals showed delayed development (delayed attainment of surface righting reflex and air righting reflex in both sexes and delayed attainment of balano-preputial skinfold cleavage in males) but this was likely secondary to reduced body weight gain. No effects were observed on the reproductive performance of F1 animals, and no abnormalities were found in the F2 generation up to day 20 postpartum. Therefore, in rats, the NOAEL for postnatal development was considered 20 mg/kg/day.

Multiple juvenile toxicity studies have been conducted with oral administration of different GNX formulations in neonatal rats (start dosing on PND 7). Oral GNX formulations containing β-CD were evaluated in one 2-week exploratory and one definitive GLP-compliant 6-week study with dosing up to 60 mg/kg/day. The preformulated oral GNX suspension was evaluated in one 3-week exploratory study and one definitive GLP-compliant 12-week study with BID dosing up to 500 mg/kg/day. In general, the findings with both formulations were consistent and demonstrated similar findings as in adult rats, including sedation effects and increased liver and thyroid weights (with histopathological correlates). In the 6-week juvenile toxicity study with GNX formulation containing β-CD, a delay in sexual maturation (vaginal patency) occurred in females dosed with 50 mg/kg/day.

A GLP-compliant acute neuropathy study was also conducted in neonatal rats (Study 00398515). On PND 7 (the time of the brain growth spurt) the rats were administered the same dose as in the 12-week juvenile toxicity study (20, 45, and 90 mg/kg/day as divided doses). An increase in neurodegeneration occurred in multiple brain regions, consistent with findings from other GABA modulators. The incidence, severity, and distribution of the neurodegeneration increased with the GNX dose administered on PND 7, with findings at the highest dose comparable to that of the positive control, the NMDA antagonist, dizocilpine (MK-801).

2.5.4.6. Toxicokinetic data

Toxicokinetic data of GNX in β-CD containing formulations was collected in repeat-dose toxicity studies in the rat and dog. In rodents, exposure levels increased with a dose less than dose-proportional. Exposure levels decreased over time, suggesting metabolism induction after repeated dosing. In addition, females consistently had higher exposure levels compared to male animals. GNX safety exposure margins reached 2.08-2.87 fold in rats after 6 months at the highest tested dose of 40 mg/kg/day. In dogs, exposure levels generally increased in a dose-proportional manner, and no clear gender difference was observed. GNX safety exposure margins reached 2.71-4.11 fold in rats after 6 months at the highest tested dose of 10 mg/kg/day.

Toxicokinetic data of the preformulated GNX suspension was evaluated in repeat-dose toxicity studies in mice, adult and juvenile rats and dogs. Similar trends were observed in these studies, although exposure levels in rats were generally lower than those achieved with β-CD containing formulations. Therefore, an incremental BID dosing approach was implemented for rodents to offset the anticipated liver enzyme induction and avoid overt toxicity. GNX safety exposure margins reached 0.18-0.63 fold in mice after 4 weeks at the highest tested dose of 1000 mg/kg/day. In rats, exposure margins reached 0.35-1.57 fold in adult rats after 13 weeks at the highest tested dose of 1000 mg/kg/day and

0.35-0.98 fold in juvenile rats after 12 weeks at the highest tested dose of 500 mg/kg/day. In the dogs' 12-month repeat-dose toxicity study, exposure margins reached 6.01-7.85 fold at the highest tested dose of 15 mg/kg/day.

2.5.4.7. Local Tolerance

GNX was not considered a local irritant when administered IV (1 mg/kg) or perivascular (0.25 mg/kg) to the ears of NZW rabbits.

2.5.4.8. Other toxicity studies

No studies for antigenicity, immunogenicity, photosafety have been conducted. In agreement with the argumentation of the applicant, this is accepted.

2.5.5. Ecotoxicity/environmental risk assessment

Table 1: Summary of main study results

Substance (INN/Invented Name): ganaxolone					
CAS-number (if available): 38398-32-2					
PBT screening		Result			**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD123	5.30 (pH 5) 5.35 (pH 7) 5.31 (pH 9)			Potential PBT: Y
PBT-assessment					
Parameter	**Result relevant for conclusion**				**Conclusion**
Bioaccumulation	log K_{ow}	5.30 (pH 5) 5.35 (pH 7) 5.31 (pH 9)			
	BCF	1186, 1272 L/kg_{ww}			not B
Persistence	ready biodegradability	not readily biodegradable			potentially P
	DegT50 (at 12°C)	$DT_{50\ water}$ 2.4, 5.0 d $DT_{50\ system}$ >10 000, 293 d			vP
Toxicity	EC10 fish	5.1 µg/L			T
PBT-statement :	Ganaxolone is considered to be not PBT, nor vPvB				
Phase I					
Calculation	**Value**	**Unit**			**Conclusion**
$PEC_{surface\ water}$, refined	0.045	µg/L			>0.01 threshold: Y
Other concerns (e.g. chemical class)					N
Phase II Physical-chemical properties and fate					
Study type	**Test protocol**	**Results**			**Remarks**
Adsorption-Desorption	OECD 106	$K_{oc\ sludge}$ 4145, 4525 L/kg_{oc} $K_{oc\ soil}$ 4153, 20500, 9699 L/kg_{oc}			
Ready Biodegradability Test	OECD 301B	not readily biodegradable			
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	$DT_{50\ water}$ 2.4 d (1), 5.0 d (2) $DT_{50\ system}$ >10 000 d (1), 138 d (2) (1) = silty clay, (2) = sand Sediment shifting 44.1-68.5%			DT_{50} values at 20°C at day 14
Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**value**	**Unit**	**Remarks**

Algae, Growth Inhibition Test/*Raphidocelis subcapitata*	OECD 201	EC_{10}	>42	µg/L	growth rate
Daphnia sp. Reproduction Test	OECD 211	EC_{10}	43	µg/L	mortality
Fish, Early Life Stage Toxicity Test/*Species*	OECD 210	EC_{10}	5.1	µg/L	dry weight
Activated Sludge, Respiration Inhibition Test	OECD 209	NOEC	≥1000	mg/L	respiration
Phase IIb Studies					
Bioaccumulation/*L. macrochirus*	OECD 305	BCF_{kgL} BCF_{kgL}	1186, 1272	L/kg_{lipid} L/kg_{lipid}	normalised to 5% lipid
Sediment dwelling organism/*C.riparius*	OECD218	NOEC	P.M.	mg/kg	normalised to 10% o.c.

2.5.6. Discussion on non-clinical aspects

Pharmacodynamics

The primary pharmacodynamics *in vitro* and *in vivo* studies suggested that GNX acts as a positive allosteric modulator of the $GABA_A$ receptor complex. GNX demonstrated potent, broad, $GABA_A$-mediated anticonvulsant activity in several types of seizure models tested. The proof of concept that GNX is protective of convulsion is adequately demonstrated.

The evaluation of human major metabolite M2 revealed no agonist, nor antagonistic action on $GABA_A$ receptors, human progesterone or oestrogen receptors that would suggest relevant secondary pharmacology, except for the antagonist activity of M2 at the ERβ receptor with an IC50 of 3.03 µM. Consequently, a potential hormonal effect of M2 at levels reached in human plasma cannot be excluded. Accordingly, the information regarding these *in vitro* findings is correctly reflected in section 5.3 of the SmPC. Long term safety, including sexual maturation and growth, is also listed as safety concern/important potential risk in the RMP and will be monitored.

Overall, the dependence studies suggested that GNX may cause physical and behavioural dependence in humans, as evidenced by its reinforcing properties, the sharing of a subjective interoceptive cue with benzodiazepines, as well as the presence of a mild withdrawal syndrome.

In vivo GNX-related effects mainly included dose-dependent sedation, an expected pharmacological effect of GNX. GNX had no notable effects in cardiovascular and respiratory studies. Thus, apart from the sedation, *in vivo* studies did not reveal reasons for concern, however it should be noted that clinical exposures were not reached in rats.

Pharmacokinetics

Gender differences in the metabolic profiles were observed in the preclinical species, caused by a sex difference in hepatic expression of the metabolising (CYP) enzymes yielding different metabolite levels in female as compared to male rodents. As this large difference in CYP expression is rat specific, the impact on the human patient population, especially the adolescent, may be low, although no data are available to assess this.

The observed *in vivo* metabolism of GNX was complex and showed a 20-keto reduction and/or multiple (1-4) hydroxylations followed by subsequent conjugations. A complete overview of all the metabolites formed in the different species, including human, is not available and it is not clear which metabolites are the most relevant at steady-state. Therefore, the MAH committed to further characterise the GNX metabolite pattern at steady state in human (see also clinical pharmacology sections, category 3 study, as detailed in the RMP).

Toxicology

The most common finding across all GNX toxicology studies was sedation, an exaggerated pharmacologic effect of a $GABA_A$ receptor modulator. Some level of sedation (decreased activity, ataxia, prostration, etc.) was observed in almost all toxicity studies. The sedation effects were reversible and dose-dependent in both incidence and severity. The progression of sedation effects tended to follow a stepwise increase in severity, from decreased activity to ataxia to prostration. The CNS effects tended to be most severe within the first few hours after a daily dose was administered, particularly in the first week of dosing. At lower doses, the CNS effects resolved by the next daily dose, suggesting a C_{max}-driven effect, and abated with the duration of administration, suggesting accommodation to the sedation effects over time.

Besides the sedation effects, (intermittent) tremors were observed in several toxicity studies in dogs and pregnant and juvenile rats. These rare events only occurred at the highest GNX exposures and in animals that showed other severe clinical findings. These observations suggest that these adverse events were secondary to severe toxicity rather than directly related to the pharmacology of GNX, and that the likelihood of their occurrence in clinical subjects receiving therapeutic doses of GNX is low.

The only other findings outside of the CNS effects were increased liver and thyroid gland weights in rodents, at times associated with hepatocellular hypertrophy and follicular cell hyperplasia. There is evidence to support that these findings are a result of autoinduction of CYP isoenzymes (particularly CYP3A and CYPB2) that occurs in rodents but not in dogs or humans. Increases in liver and thyroid weights were greater in females than in males, which is in agreement with a more pronounced induction of CYP isoenzymes in females. It is noted however that females achieved higher GNX exposure over males, and yet it was also the females that tended to have a greater magnitude of reduction in exposure with multiple dosing and higher liver weights. The applicant stated that this inconsistency is likely due to male rodents making more CYP3A, which is considered acceptable.

The results of the studies conducted with preformulated GNX suspensions were consistent with the previous repeat-dose toxicology studies (with formulations containing β-CD), except for the additional finding of a dose-dependent increase in heart rate with incidences of sinus tachycardia in the 12-month repeat-dose toxicity study in dogs. There were no histopathological correlates associated with the change in heart rate, and the single-dose cardiovascular safety study in male dogs showed no abnormal findings. The CHMP agreed that, taking into account that the safety margins based on Cmax were 8-fold fold compared to humans, the human relevance of this finding is likely low.

Regarding carcinogenicity, the applicant will provide a 26-week carcinogenicity study report in transgenic mice (category 3 study with final report expected Q1 2025, as listed in the RMP). The applicant also committed to provide a WoE assessment to evaluate the need of a 2-year carcinogenicity study in rat (expected Q2 2024 and listed as category 3 studies, as detailed in the RMP). This is agreed by the CHMP.

The sedation effects observed in the reproductive and developmental toxicity studies were consistent with the findings observed in the repeat-dose toxicity studies.

In the pre-and post-natal development toxicity study in SD rats, dams treated with 20 and 40 mg/kg/day showed an increase in the mean litter percentage incidence of foetuses with an additional lumbar rib (24% and 10% at these 2 dosages respectively compared to 5% in the control group) although there was no dosage-relationship. This skeletal finding is likely secondary to maternal toxicity, as observed by decreased maternal body weight gain associated with maternal stress.

Juvenile rats dosed with ≥ 45 mg/kg/day showed decreased bodyweight gain compared to control animals. This was also observed in the F1 generation dosed with 40 mg/kg/day in the pre-and postnatal development study but not in adult animals. The reduction in body weight gain was seen primarily at the highest dose group, and the effects on growth would probably be due to poor health

and long periods of sedation rather than a direct effect of GNX treatment, also considering that no effects on femur length were observed in any groups.

In juvenile rats, no effect on femur length and bone density was observed at doses up to 500 mg/kg/day.

In the acute neuropathy study in neonatal rats, the incidence, severity, and distribution of neurodegeneration in multiple brain regions increased dose-dependently, starting at the lowest tested dose of 20 mg/kg. However, in the 12-week pivotal repeat-dose juvenile toxicity study, there were no behavioural changes (motor activity, acoustic startle, learning, or memory) identified at any doses (up to 500 mg/kg/day), either during the dosing period or after cessation of dosing. In addition, there were no histological changes in the brain after repeated GNX administration. A discussion on the neurodegenerative findings with $GABA_A$ modulators and NMDA antagonists during the brain growth spurt was provided: in contrast to the other agents, treatment-related apoptotic neurodegeneration after ganaxolone treatment did not lead to behavioural deficits in juvenile rats. In addition, the timing of the brain growth spurt in rats is significantly different compared to humans, indicating that the intended target population of 2 years and older are less susceptible to apoptotic neurodegeneration. Altogether, the CHMP agreed that the relevance of this finding for humans is low. The findings in juvenile animals are adequately reflected in SmPC section 5.3.

In the 6-week juvenile toxicity study with GNX formulation containing β-CD, a delay in sexual maturation (vaginal patency) occurred in females dosed with 50 mg/kg/day. In the second pivotal 12-week study with the preformulated GNX suspension, a significant delay in sexual maturation was again observed in females dosed with ≥ 45 mg/kg/day and females dosed with 20 mg/kg/day also showed a slight delay in sexual maturation. Although there was a significant delay in attaining sexual maturation, this did not affect oestrous cyclicity or any fertility or reproductive parameters at any doses (up to 500 mg/kg/day). Even though it was noted that patients with CDD are unlikely to give birth at a later stage, the applicant is advised to monitor sexual maturation carefully. The findings in juvenile animals are adequately reflected in SmPC section 5.3.

The metabolite M2 was newly identified in human subjects administered GNX and contributes between 10% and 20% of total drug-related material in human plasma. In view of the genotoxicity investigation results, the CHMP agreed to conclude that M2 is not genotoxic.

In a 28-day PK study with ganaxolone in rats, with a dosing regimen reflecting the previously conducted studies, M2 levels were 5-10 fold higher in female rats compared to male rats, and significantly lower compared to the levels formed in humans.

For further characterization of M2, a GLP-compliant 4-week repeat-dose toxicity study in rats was performed. Reduced bodyweight gain was observed in males dosed with ≥ 100 mg/kg/day, and in females dosed with 150 mg/kg/day, which was accompanied by significant reduced food intake at the highest dose level. A dose-dependent decrease in prostate gland weight was observed in males dosed with ≥ 25 mg/kg/day. This correlated with small prostates which were noted macroscopically in males dosed with ≥ 100 mg/kg/day. In addition, males dosed with ≥ 100 mg/kg/day M2 had small seminal vesicle glands. Related microscopic changes included acinar atrophy and decreased secretion in the prostate glands and seminal vesicle glands, with a dose-dependent increase in incidence and severity. Additionally, decreased epididymis weight was observed in males dosed with ≥ 100 mg/kg/day, without histopathological correlates. The applicant states that the finding in the prostate and seminal vesicles gland could be associated with the decrease in bodyweight gain in animals dosed with ≥ 100 mg/kg/day, however that a treatment-related effect cannot be excluded. Also, microscopic findings in the prostate gland were already observed at the lowest tested dose where no effect on bodyweight was noted. In females dosed with ≥ 50 mg/kg/day, a dose-dependent increase in incidence of hepatocellular hypertrophy was observed, and females dosed with 150 mg/kg/day had increased relative weight of the liver. This was

not observed in male animals and could be due to induction of xenobiotic-metabolizing enzymes, however this was not determined. In view of these findings, no NOAEL could be established. The applicant also provided a preliminary summary of a 13-week repeat-dose toxicity study in rats, in which animals were given 25, 50 or 100 mg/kg/day M2 per oral administration. In general, similar findings as in the 4-week repeat-dose toxicity study were observed. Since the clinical relevance of these findings remains unclear, the applicant commits to provide the final results of the 6-month repeat-dose toxicity in rats with M2 post-approval (category 3 study, as listed in the RMP) and will perform an embryofetal developmental toxicity study (category 3 study, as listed in the RMP). These findings are adequately described in the SmPC section 5.3.

Furthermore, the applicant commits to perform a weight-of-evidence (WoE) assessments for the need for a 2-year carcinogenicity study in rat with GNX and M2, and for juvenile toxicity study with M2 (category 3 studies, as listed in the RMP).

Regarding the environmental risk assessment, no risk to surface water, groundwater, soil and the STP is expected. However, in order to complete the ERA regarding the sediment compartment, the applicant agrees to provide the OECD 218 study report and updated risk assessment post-approval.

2.5.7. Conclusion on the non-clinical aspects

The marketing authorisation application for ganaxolone is considered approvable from a non-clinical point of view.

The relevant non-clinical information is appropriately reflected in the Product Information.

The CHMP considers the following measures necessary to address the non-clinical issues:

- Submission of a 26-week GNX and M2 carcinogenicity study report in transgenic mice

- Submission of the final results of the 6-month repeat-dose toxicity in rats with M2

- Submission of an embryofetal developmental toxicity study with M2

- Submission of a WoE assessment reports regarding the need of a 2-year carcinogenicity study in rat with GNX and M2 (6 and applicant 7)

- Submission of a WoE assessment report for a juvenile toxicity study for M2

In addition, the applicant agrees to complete post-approval the ERA sediment compartment study and update the ERA accordingly.

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 Number	Study Design and Purpose	Formulation(s)*
Biopharmaceutic Studies		
1042-0400	Double-blind, single-dose, randomized, placebo-controlled, crossover study of the safety, tolerability, relative bioavailability, and PK of GNX suspension in fasted and fed subjects	**GNX oral suspension, 50 mg/mL**
1042-0118 (year 1998)	Randomized, open-label, single-dose, study of the safety, tolerability, and PK of various formulations of GNX following a standardized meal	GNX HPMC tablets GNX PVA tablets GNX suspension (50 mg/mL GNX equivalent, termed "nanosized") Batch F-046-002B
1042-0106.01 (year 1997)	Randomized, open-label, single-dose, parallel-group study of the PK, bioavailability, safety, and tolerability of 3 formulations of GNX following a standardized meal	GNX PVA tablets GNX HPMC tablets GNX HPMC suspension, 50 mg/mL Batch #F-003-047
1042-0115	Randomized, open-label, single, rising-dose, study of the safety, tolerability, bioavailability, and PK of 2 formulations of GNX in fasted and fed subjects	GNX HPMC tablets GNX β-CD complex suspension (25 mg/mL)
1042-0116	Open-label, single-dose study of the safety tolerability and PK of 2 formulations of GNX in adult subjects with migraine headache	GNX β-CD suspension (25 mg/mL) GNX HPMC tablets
CA042-9407.01	Double-blind, placebo-controlled, rising, single-dose study of the safety, tolerability, and PK of GNX following a high fat meal	GNX β-CD complex suspension in Ora-Plus®/Ora-Sweet® (30 mg/mL) GNX β-CD complex suspension in Ora-Plus®/Ora-Sweet® (15 mg/mL)
1042-0401	Randomized, double-blind, placebo-controlled, single-dose study to compare 2 formulations of GNX in fed and fasted healthy subjects	GNX immediate-release capsules GNX controlled-release (enteric coated) capsules
1042-0111.01	Randomized, open-label, single-dose, 2-period, crossover study of the relative bioavailability of 2 doses of GNX in fasted and fed subjects	GNX β-CD complex suspension in Ora-Plus®/Ora-Sweet® (25 mg/mL)
CA042-9302.01	Open-label, single-dose, crossover study to compare the PK of 3 formulations of GNX following a high-fat meal	GNX β-CD inclusion complex GNX-micronized + SLS GNX HP-β-CD suspension
CA042-9403.01	Double-blind, placebo-controlled, single- and 14-day multiple-dose study of the safety, tolerability, and PK of GNX administered following a high fat meal	GNX β-CD complex in yogurt
CA042-9404.01	Open-label, single morning dose and single evening dose, and 10-day multiple dose study of the safety, tolerability, and PK of GNX administered following a high-fat meal	GNX β-CD complex in yogurt
CA042-9405.01	Open-label, single-dose crossover study comparing the bioavailability of 2 formulations of GNX following a high-fat meal	GNX β-CD complex in yogurt GNX β-CD complex suspension in Ora-Plus®/Ora-Sweet®
1042-HME-1001	a Phase 1, single-dose, open-label, 3-cohort, 6-period, 3- or 6-sequence, crossover study to compare the bioavailability of 2 oral formulations of GNX in healthy adult participants.(**ongoing**)	GNX formulation with GNX 50 mg/mL oral suspension
Phase 1 Studies		
1042-HAP-1001	A randomized, double-blind, placebo- and active-controlled crossover study to evaluate the abuse potential of oral GNX in recreational CNS depressant users	**GNX oral suspension, 50 mg/mL**
1042-DDI-1001	A study to assess the effects of itraconazole and rifampin on the PK of GNX in healthy adult subjects	**GNX oral suspension, 50 mg/mL**
CA042-9401.01	Open-label, single-dose, comparison of the effect of food on the PK of GNX following a 10-hour fast, a high-carbohydrate meal, or a high-fat meal	Neat drug
CA042-9301.01	Open-label, rising, single-dose study of the safety, tolerance, and PK of GNX following a high-fat meal	GNX HP-β-CD suspension

Study Number	Study Design and Purpose	Formulation(s)*
CA042-9402.01	Open-label, single-dose study of the absorption, metabolism, and elimination of GNX following a high-fat meal	[^{14}C] GNX with HP-β-CD
CA042-9501.01	Open-label, randomized, single-dose crossover study of the safety, tolerability, PK, and effect of gender of GNX following a high-fat meal	GNX β-CD complex suspension in Ora-Plus®/Ora-Sweet® (20 mg/mL)
CA042-9505.01	Double-blind, randomized, placebo-controlled, 14-day multiple-dose study of the safety, tolerability, and PK of GNX administered following a high-fat meal	GNX β-CD suspension in Ora-Plus®/Ora-Sweet®
1042-0402	Open-label, 14-day, multiple-dose study of the safety and PK of GNX and of the effects of GNX on the PK of a single dose of midazolam following a standardized meal	GNX immediate-release capsules Midazolam syrup
1042-0403	Double-blind, placebo-controlled, 15-day study of the safety, tolerability, and PK of single doses (crossover with and without a high-fat meal) and multiple doses of GNX in healthy subjects	GNX capsules
1042-0404	Inpatient evaluation of PK, tolerability, and safety in healthy adult subjects of 1200 to 2000 mg/day GNX capsules after multiple doses administered to steady state	GNX capsules
1042-0405	Double-blind, sequential cohort evaluation of intravenous GNX in healthy subjects	GNX intravenous injection, 3 mg/mL
Study 1042 C14.GNX-lac-1001	An open-label study of the excretion of [^{14}C]-GNX into breast milk following a single oral dose in healthy, lactating female subjects	[^{14}C] GNX HP-β-CD
Study 1042 GNX.AME-1001	An open-label study of the absorption, metabolism, and excretion of [^{14}C]-GNX following a single oral dose in healthy male subjects	[^{14}C] GNX HP-β-CD
1042-IRF-1001	Pharmacokinetic Single Dose Study of Ganaxolone in Adult Subjects with Normal and Impaired Renal Function	
1042-IHF-1001	Pharmacokinetic Single Dose Study of Ganaxolone in Adult Subjects with Normal and Impaired Hepatic function	
1042-TQT-1001	Placebo- and Positive-controlled, Parallel-arm Study with Nested Crossover to Investigate the Effects of Ganaxolone on the QT/QTc Interval in Healthy Subjects	
GNX-ADME-083122	Metabolite identification report	
Phase 2 Studies		
1042-0500	Double-blind, placebo-controlled, add-on, dose-escalation, incomplete crossover study to evaluate the safety, tolerability, and anti-seizure activity of GNX in pediatric subjects with infantile spasms	**GNX oral suspension, 50 mg/mL**
1042-0501	Open-label extension of Study 1042-0500 (up to 2 years treatment)	**GNX oral suspension, 50 mg/mL**
1042-0600	Double-blind, randomized, placebo-controlled, add-on, safety, tolerability, PK, and efficacy study of GNX in adults with uncontrolled partial-onset seizures	**GNX oral suspension, 50 mg/mL**
1042-0601	Open-label extension study to evaluate the long-term safety and efficacy of GNX as add-on therapy and to screen for drug-drug interactions with other Anti-seizure medications in adults with uncontrolled, partial-onset seizures who completed Study 1042-0600 (up to 2 years treatment)	**GNX oral suspension, 50 mg/mL**
1042-0602	Open-label extension for adult subjects with uncontrolled partial onset seizures deriving benefit from Study 1042-0601	**GNX oral suspension, 50 mg/mL**
1042-0800	Proof-of-concept, double-blind crossover trial of GNX and placebo in children with fragile X syndrome	**GNX oral suspension, 50 mg/mL**
1042-0900	Open-label, proof-of-concept study of GNX in children with PCDH 19-related female pediatric epilepsy and other rare genetic epilepsies	**GNX suspension, 50 mg/mL** GNX capsules

Study Number	Study Design and Purpose	Formulation(s)*
1042-0104	Double-blind, placebo-controlled 8-day, multiple dose study of the safety, tolerability, and anti-seizure activity of GNX in subjects undergoing evaluation for surgical treatment of epilepsy	GNX β-CD complex suspension (25 mg/mL)
CA042-9408.01 Stage 1	Open-label, 2-month, flexible escalating-dose, add-on study of the safety, tolerability, dose range, and potential efficacy of GNX in pediatric or adolescent subjects with refractory partial and/or generalized seizures, with open-label extension	GNX β-CD suspension in Ora-Plus®/ Ora-Sweet®
CA042-9408.01 Stage 2	Open-label, 17-week, flexible escalating-dose, add-on study of the safety, tolerability, optimal dose, and potential efficacy of GNX in pediatric or adolescent subjects with refractory partial and/or generalized seizures, with open-label extension	GNX β-CD suspension in Ora-Plus®/ Ora-Sweet®
1042-0101	Open-label, 3-month, add-on, flexible-dose, dose-escalation study of the safety, tolerability, and potential efficacy of GNX in pediatric subjects with refractory seizures and a history of infantile spasms	GNX β-CD suspension (25 mg/mL) in Ora-Plus®/Ora-Sweet®
1042-0112	Double-blind, parallel-group, placebo-controlled, single-dose study of GNX for the treatment of migraine headaches with and without aura in females	GNX β-CD suspension (1, 5, 10, and 25 mg/mL)
1042-SE-2001	Double-blind, randomized, placebo-controlled study to evaluate the safety, tolerability, efficacy, and PK of intravenous GNX as adjunctive therapy to treat in adolescent and adult subjects (≥ 12 years) with status epilepticus	GNX intravenous injection, 1 and 3 mg/mL
1042-0120 #	Open-label, add-on, safety, tolerability, PK, and potential efficacy study of GNX in subjects with catamenial epilepsy	GNX β-CD suspension (25 mg/mL)
1042-0700	Proof-of-concept, 6-week double-blind, randomized study of GNX and placebo, followed by 6-week open-label treatment in adults with posttraumatic stress disorder	GNX capsules
1042-0117	Double-blind, parallel-group, placebo-controlled, single-dose study of GNX for the treatment of adult subjects with migraine headaches with and without aura	GNX tablets
1042-PPD-2002	Double-blind, placebo-controlled, multiple-dose escalation study to evaluate safety, PK, and efficacy of GNX in women with PPD	GNX intravenous injection, 3 mg/mL GNX capsules
1042-PPD-2003	Double-blind, placebo-controlled multicenter study to evaluate safety, tolerability, and efficacy of GNX in women with PPD	GNX capsules
1042-TSC-2001	A Phase 2 Open-label 12-week Trial of Adjunctive Ganaxolone Treatment (Part A) in Tuberous Sclerosis Complex-related Epilepsy followed by Long-term Treatment (Part B).	
1042-CDD-EAP-3005	compassionate use extension in 17 patients who had completed the 1042-CDD-3001 trial	
Phase 3 Studies		
1042-CDD-3001 (**Pivotal trial)**	A double-blind, randomized, placebo-controlled trial of adjunctive GNX treatment in **children and young adults with CDD** which consists of a 6-week prospective baseline period to collect seizure data, followed by a 17-week double-blind treatment phase, which is then followed by a long-term open-label phase	**GNX oral suspension, 50 mg/mL**
1042-0603	Phase 3, double-blind, randomized, placebo-controlled, add-on, safety, tolerability, PK, and efficacy study of GNX in adults with uncontrolled partial-onset seizures followed by long-term open-label treatment	GNX capsules
1042-0604	Second year open-label extension study of GNX in subjects with drug-resistant partial-onset seizures	GNX capsules

Study Number	Study Design and Purpose	Formulation(s)*

CDD = cyclin-dependent kinase-like 5 deficiency disorder; CNS = central nervous system; GNX = ganaxolone; HP = hydroxypropyl; HPMC = hydroxypropylmethylcellulose; PCDH = protocadherin; PK = pharmacokinetics; PPD = postpartum depression; PVA = polyvinyl alcohol; SLS = sodium lauryl sulfate.

Studies with the GNX oral suspension, 50 mg/mL formulation are presented in black; early/different formulations are presented in grey

*For GNX oral suspension formulation, 50 mg/ml different milling systems were used: (to-be marketed)

Study 1042-0120 is mentioned in the clinical overviews but study has been terminated due to enrolment challenges

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Population PK studies

Two population PK analysis studies were conducted, Study 1042-CDD-POPPK-001 and 09-0600-POP-PK-01.

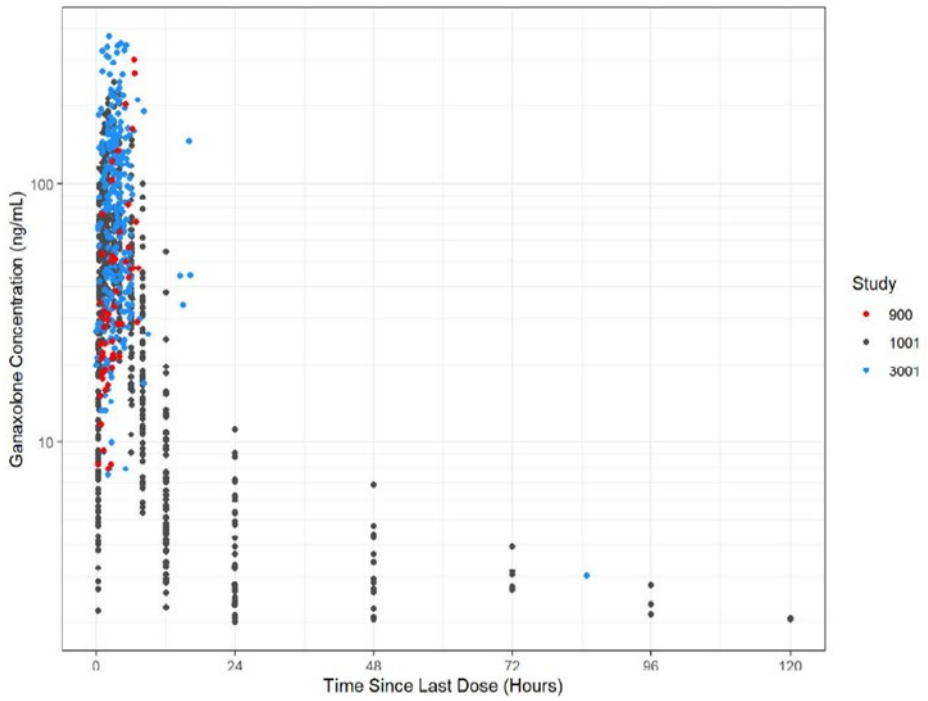
1042-CDD-POPPK-001

The population PK-study 1042-CDD-POPPK-001 was conducted to estimate the exposure in paediatric patients. Data from 3 clinical studies was included in the analysis (Study 1042 0900 (Phase 2a), Study 1042 CDD-3001 (Phase 3) and GNX alone treatment arms of Study 1042 DDI 1001 (Phase 1)). Overall, the population PK analysis included 146 subjects and 694 PK observation records. Covariates tested included age, sex, race, height, body weight, body mass index, body surface area, albumin, ALT, AST, ALP, bilirubin, and creatinine clearance (1042 CDD POPPK 001). The most apparent correlations between covariates were measures of body size: weight, height, BMI, BSA, and age all showed correlations > 0.5.

Figure 2 shows individual measurable plasma drug concentration versus time after dose (semi-log), coloured by study.

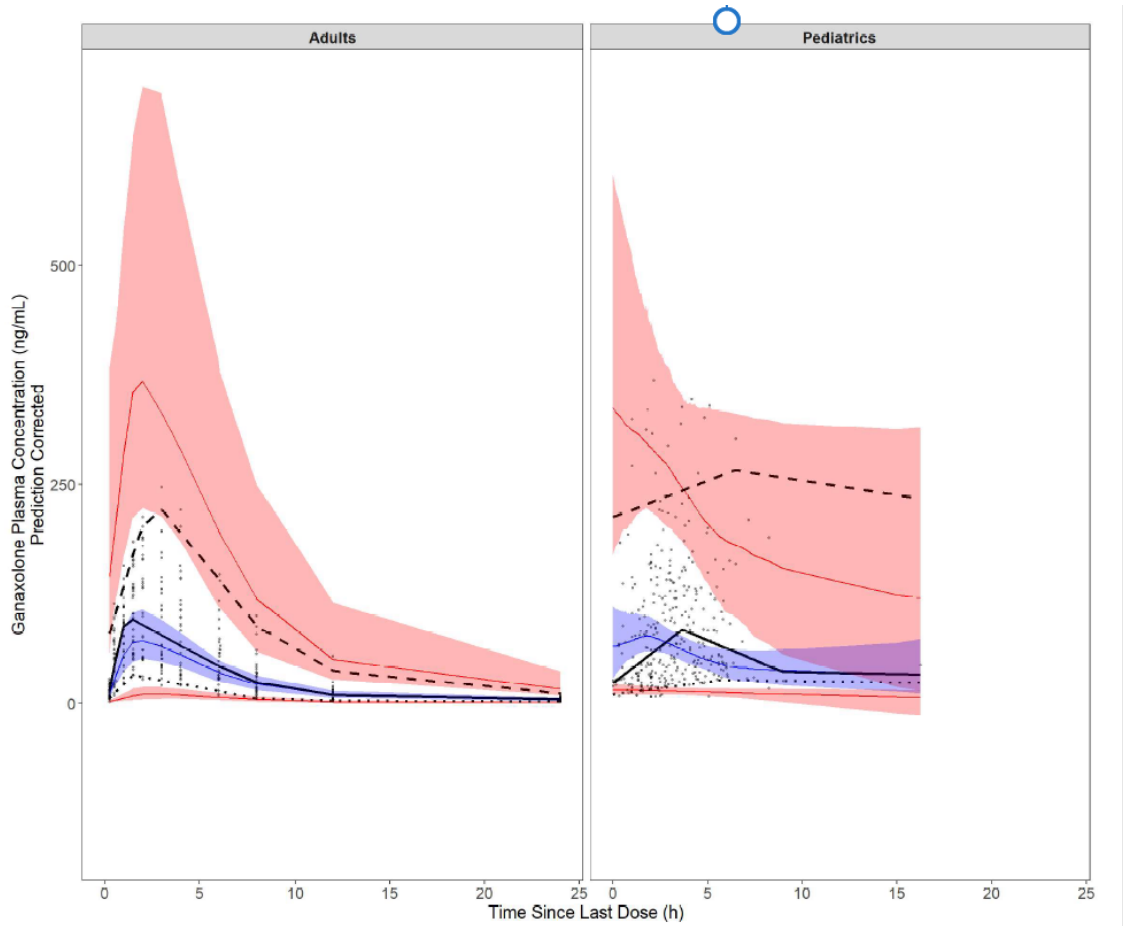
The blue dots concern data in paediatric patients with CDD (Study 1042 CDD-3001). Exploratory analysis showed that GNX appears to exhibit multi-compartment kinetics and that concentrations in paediatric patients were of similar magnitude as observed in healthy volunteers in Study 1042-DDI-1001.

Figure 2: GNX Concentration versus Time Since Last Dose



Although the prediction-corrected VPCs suggest some over-prediction of the GNX concentrations for paediatric subjects from 0 to 4 hours post-dose, likely due to the sparse data in paediatric and a structural trend in the elimination phase, the base population PK model was considered the best model for the data and fit-for-purpose for estimating the exposure in paediatric subjects.

Figure 3: Prediction-Corrected VPC Stratified by Adult and Pediatric Subjects Over The First 24 Hours After Dose Administration



Notes: Black dots are observed data points; black solid line is the observed median; black dashed line is the observed p95 and black dotted line is the observed p5. The blue solid line is the simulated median; red solid lines are simulated p5 and p95. The blue area is the 95% PI of the simulated median, and pink areas are the 95% PI of the simulated p5 and p95.
Abbreviations: p5=5th percentile; p95=95th percentile; PI=prediction interval; VPC=visual predictive check

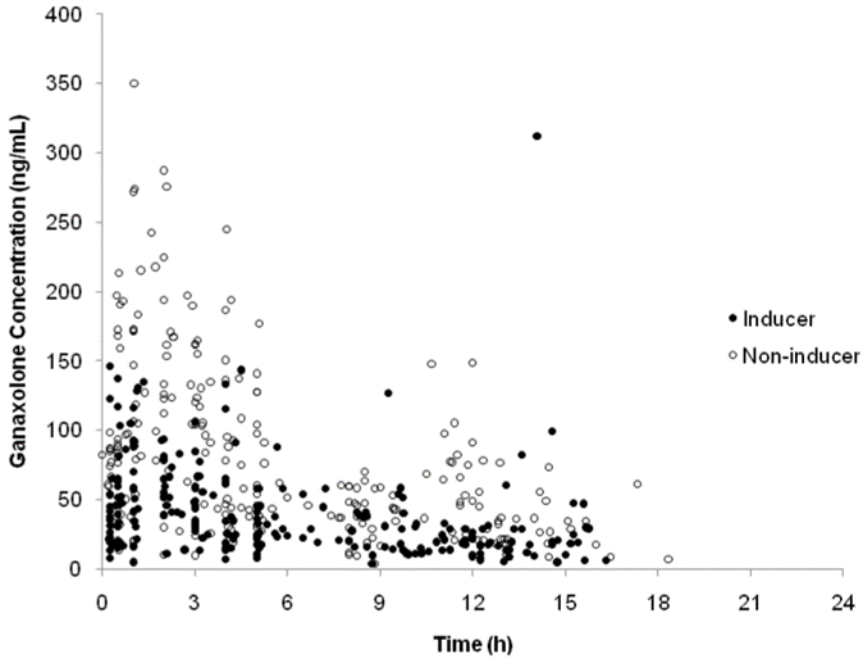
Simulated GNX profiles were generated using the final population PK model for four different age groups: 2 to <6 years, 6 to <12 years, 12 to <18 years, and ≥ 18 years. The median weight for the subjects in each age group category in the analysis dataset was used for the simulations. Doses of 63 mg/kg/day up to 1800 mg were administered until steady-state was achieved. The maximum GNX concentration, minimum GNX concentration, and 24-hour area under the GNX plasma concentration-time curve were calculated at steady-state.

09-0600-POP-PK-01

Population PK-study **09-0600-POP-PK-01** was conducted in 2009 to develop a model to describe the population pharmacokinetics of ganaxolone in adult patients with partial-onset seizures and to identify sources of inter-individual variability in the pharmacokinetics of ganaxolone. Pharmacokinetic data from the phase 2 study **1042-0600** were included in this older version of the pop PK model.

The model included a covariate "inducer category" representing concomitant Anti-seizure medications (ASM) metabolic classification. Concomitant ASMs were classified under the following categories: inducers and non-inducers of CYP. Phenytoin, carbamazepine and phenobarbital were classified as general inducers. All other ASMs were classified as non-inducers.

Figure 4: Steady-state ganaxolone plasma concentrations versus time in adult with refractory epilepsy; patients with concomitant carbamazepine or phenytoin or both medications are designated as inducer while patients with other concomitant anti-epileptic medications are classified as non-inducer



Plasma concentration-time data for this analysis were obtained from 94 adult subjects, with a total of 442 observations used. Approximately six plasma samples were collected from each patient.

Formulation development

As GNX is poorly soluble, several different GNX oral formulations were tested to find a formulation that results in an adequate and predictable absorption of GNX. The final formulation is a GNX oral suspension, 50 mg/mL.

The applicant evaluated the effect of particle size distribution on PK using the data of four different studies (**1042-CDD-3001**, **1042-0900**, **1042-HAP-1001** and **1042-DDI-1001**) and a more detailed analysis of data from study **1042-HAP-1001.**

Several studies were conducted to compare the pharmacokinetics of different formulations. The most relevant comparative studies are study **1042-0118** and **1042-0106.01**. In these studies, the relative bioavailability of a nanosized GNX suspension was compared to other solid GNX formulations when administered following a standardized meal. Based on the biopharmaceutical studies, the applicant concluded that the hydroxypropylmethylcellulose (HPMC) tablet and suspension formulation have a favourable absorption profile with a tmax of approximately 2.5 hours.

Absorption

The PK of GNX in humans have been investigated in both single- and multiple-dose studies. Clinical trials were conducted with several formulations to determine the effects of food, gender, and rising-dose exposures.

Co-administration of the to-be-marketed ganaxolone oral suspension with a high-fat meal increased C_{max} by 2-fold and AUC by 3-fold compared to fasted levels (study **1042-0400**). Following oral administration, GNX is rapidly absorbed without the involvement of active transport proteins. The

maximum concentration is observed at a median tmax of 1.25 hours after single dosing and about 2 -3 hours at a steady state. A steady-state is achieved within 2 to 3 days.

Ganaxolone undergoes extensive first-pass metabolism, the oral bioavailability of ganaxolone following administration of the suspension formulation at a dose level of 600 mg is approximately 13%.

The population PK model (**1042-CDD-POPPK-001**) was used to simulate GNX steady-state exposure data of the GNX suspension, 600mg TID in paediatric and adult CDD Patients; the steady-state exposure (AUC_{0-24}) relevant for safety is approximately 4100 ng*hr/mL, and the maximum concentration ($C_{max,ss}$) is approximately 300 ng/mL. Simulated GNX profiles were generated using the population PK model for 4 different age groups: 2 to < 6 years, 6 to < 12 years, 12 to < 18 years, and ≥ 18 years.

Doses of 63 mg/kg/day up to 1800 mg were administered until steady-state was achieved. At steady-state, the GNX C_{max}, C_{min}, and AUC_{0-24} were calculated (see Table 5).

Table 2: Simulated GNX Steady-state Exposure in Pediatric CDD Patients by weight category(1042-CDD-POPPK-001)

Age Group	Mean Body Weight (kg)	Dose (mg)	AUC_{0-24} (ng*hr/mL)	C_{min} (ng/mL)	C_{max} (ng/mL)
2 to < 6 years	14.8	312	3903	85	247
6 to < 12 years	22.6	475	3998	84	269
12 to < 18 years	36.1	600	4106	84	293
≥ 18 years	35.1	600	4100	84	292

Notes: Dose represents the dose amount in mg administered 3 times daily.
AUC0-24 = 24-hour area under the GNX plasma concentration time curve; Cmax = maximum GNX plasma concentration; Cmin = minimum GNX plasma concentration.

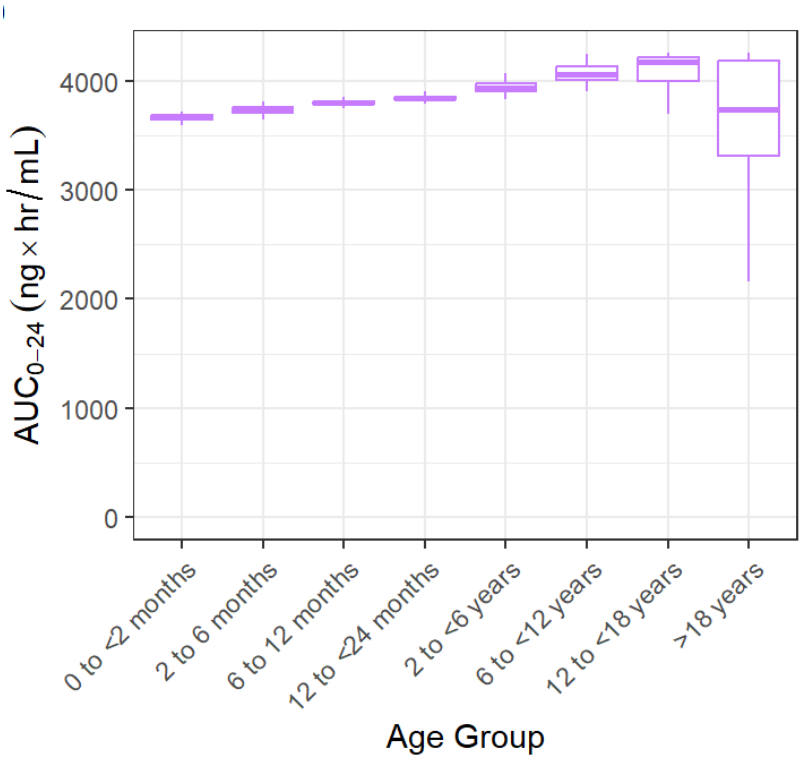
Table 3: Simulated GNX PK Profiles for Paediatric Subjects by Weight Category (1042-CDD-POPPK-001, addendum 2021)

Parameter (Paedeatrics) Mean (SD) (CV%)	**7 mg/kg 2-6 (years) (N=100)**	**14 mg/kg 2-6 (years) (N=100)**	**21 mg/kg 2-6 (years) (N=100)**
AUC_8 (ng*hr/mL)	770 (40.0) (5.2%)	1540 (80.0) (5.2%)	2310 (120) (5.2%)
C_{max} (ng/mL)	144 (6.67) (4.6%)	287 (13.3) (4.6%)	431 (20.0) (4.6%)
T_{max} (hours)	2.00 (0) (0%)	2.00 (0) (0%)	2.00 (0) (0%)
C_{min} (ng/mL)	51.5 (3.05) (5.9%)	103 (6.11) (5.9%)	155 (9.16) (5.9%)
Fluctuation (%)	95.9 (1.26) (1.3%)	95.9 (1.26) (1.3%)	95.9 (1.26) (1.3%)
AUC accumulation ratio	1.63 (0.00810) (0.5%)	1.63 (0.00810) (0.5%)	1.63 (0.00810) (0.5%)
Cmax accumulation ratio	1.40 (0.00713) (0.5%)	1.40 (0.00713) (0.5%)	1.40 (0.00713) (0.5%)
Parameter (Adults) Mean (SD) (CV%)	**200 mg 70 kg Adult (N=100)**	**400 mg 70 kg Adult (N=100)**	**600 mg 70 kg Adult (N=100)**
AUC_8 (ng*hr/mL)	515 (165) (32.0%)	1030 (330) (32.0%)	1550 (495) (32.0%)
C_{max} (ng/mL)	91.3 (31.4) (34.4%)	183 (62.9) (34.4%)	274 (94.3) (34.4%)
T_{max} (hours)	2.00 (0) (0%)	2.00 (0) (0%)	2.00 (0) (0%)
C_{min} (ng/mL)	35.9 (10.7) (29.7%)	71.8 (21.4) (29.7%)	108 (32.0) (29.7%)
Fluctuation (%)	84.9 (3.88) (4.6%)	84.9 (3.88) (4.6%)	84.9 (3.88) (4.6%)
AUC accumulation ratio	1.68 (0.0169) (1.0%)	1.68 (0.0169) (1.0%)	1.68 (0.0169) (1.0%)
Cmax accumulation ratio	1.48 (0.0188) (1.3%)	1.48 (0.0188) (1.3%)	1.48 (0.0188) (1.3%)

CV = coefficient of variation; N = number of subjects; SD = standard deviation

The applicant also provided additional population PK simulations for adult subjects, for the highest dose level of 600 mg three times daily (1800 mg total daily dose) using the popPK model 1042-CDD-POPPK-001.

Figure 5: Exposure (AUC0-24) Versus Age Group Categories



Notes: Exposure versus age group for AUC0-24. Boxplots present the median, lower box is the 25th percentile, the top of the box the 75th percentile and the whiskers maximum observation above the upper fence and the minimum value. Outliers are not presented. Dosage is 21 mg/kg/600 = label dosing scenario where individuals with weights </= 28 kg received 21 mg/kg/dose (63 mg/kg total daily dose) and >28 kg dose of 600 mg (1800 mg total daily dose) for weight values greater than 28 kg.

Distribution

Ganaxolone is extensively distributed throughout the body. Ganaxolone is approximately 99% protein-bound in serum. Ganaxolone demonstrated high binding to plasma proteins in human, rat, and dog plasma after both 4- and 8-hour incubations. Ganaxolone gave mean fu values of <0.001 and 0.01 in the presence of human, plasma respectively, in a 4-hour and 8-hour incubation, respectively.

Study **1042-C14GNX-lac-1001** suggests extensive distribution into the tissues and a slow release of GNX and metabolites from the tissues. The apparent volume of distribution of GNX based on the average of all cohorts from IV study **1042-0405** approximates 800 L (using IV doses in the range of 10-86 mg), which confirms extensive distribution into tissues.

In study **OPT-2016-063**, Ganaxolone did not appear to be a substrate of any of the investigated transporters (BCRP, P-gp, OCT1, OCT2, OATP1B1, or OATP1B3).

Elimination

GNX is rapidly and extensively metabolised with a t1/2 of about 4.5 hours, with negligible excretion of unchanged drug in urine and faeces. A terminal half-life (t½) of 7.8 to 10.1 hours was observed at steady state. The levels of total radioactivity appeared to decline slowly in a multiphasic manner with an arithmetic mean $t_{1/2}$ of 413 hours in both mass balance studies. The excretion of the radioactivity was slow; this could be due to the slow release of GNX and/or metabolites from the tissues. This is supported by the milk excretion study and as the GNX half-life increases with the dose without

apparent increase of exposure. The total amount of radioactivity excreted in faeces and urine was 65.60 – 72.83% and 21.07 – 30.9%, respectively.

A milk excretion study 1042-C14GNX-lac-1001 was conducted in five healthy adult lactating women treated with a 300 mg oral dose of ganaxolone. Concentrations of ganaxolone in breast milk were approximately 4-fold higher than in plasma. The calculated maximum relative infant dose for ganaxolone is approximately 0.157 mg/kg/day based on an average milk intake of 150 mL/kg/day, which is less than 1% of the maternal dose and approximately 0.24% of the labelled paediatric dose of 63 mg/kg/day.

Mass balance and metabolic profiling indicate that GNX is extensively metabolized to multiple steroid metabolites, including metabolites with a very long half-life (up to 230 hours). These metabolites can be expected to accumulate at steady state, especially as GNX is administered three times daily. Due to accumulation, the main metabolites at steady state may differ from those following a single dose.

Different names were used for the metabolites of GNX and metabolite identification data differ between studies.

In vitro studies suggest that Ganaxolone is primarily metabolised by cytochrome P450 (CYP) 3A4 and CYP3A5. CYP2B6, CYP2C19 and CYP2D6 may be involved to a much lesser extent.

In the presence of recombinant enzymes, ganaxolone was a substrate for UGT1A6, UGT1A9, and UGT2B7. Metabolic profiling studies suggest multiple metabolic routes: oxidation, dehydrogenation, hydrogenation, sulphation and glucuronidation have been observed.

Dose proportionality and time dependencies

The single-dose pharmacokinetics of GNX, following the administration of escalation dose levels of GNX Oral Suspension, was investigated under high fat, fed conditions in study **1042-HAP-1001**.

Table 4: Summary of GNX PK Parameters – Dose Selection (Part A) (Dose Selection PK Population) and Main study (part B) (PK Population) (1042-HAP-1001, GNX Oral Suspension [50 mg/mL])

	Part A			Part 2		
Parameter	**GNX 1000 mg (N = 7)**	**GNX 1500 mg (N = 5)**	**GNX 2000 mg (N = 5)**	**GNX 400 mg (N = 46)**	**GNX 800 mg (N = 45)**	**GNX 2000 mg (N = 45)**
Cmax (ng/mL)	115.46 (28.8)	99.24 (32.4)	92.48 (58.1)	59.05 (47.8)	88.37 (57.0)	133.51 (44.8)
tmax (h)	2.03 (1.53, 2.53)	0.53 (0.53, 2.03)	1.53 (1.03, 2.03)	1.03 (0.53, 3.10)	1.53 (0.53, 6.03)	1.53 (1.03, 2.53)
AUC0-t (h*ng/mL)	678.84 (40.3)	580.51 (21.2)	585.03 (39.1)	287.69 (41.9)	435.51 (50.7)	653.61 (36.4)
AUC0-inf (h*ng/mL)	747.28 (41.7)	613.77 (11.0)	687.97 (43.4)	337.98 (44.9)	534.23 (51.3)	762.89 (33.6)

Geometric Mean (%CV)are presented, and for t_{max} Median(min, max)

Several early studies investigated the pharmacokinetics of escalating doses of GNX. These studies include study **1042-0404,** which evaluated the PK of GNX following multiple doses of GNX capsules at a dose level of 600, 800, and 1000 mg BID. Steady state was reached within 3 days of administration. Data suggest that over the dose range of 600 to 1000 mg BID under fed conditions, exposure to GNX increases less than proportionally with increases in dose, with this disproportionality being more pronounced at the high end of the dose range.

Table 5: Summary of Mean (SD) GNX Pharmacokinetic Parameters Following Multiple Dosing of GNX Capsules in Healthy Subjects 1042-0404

Dose	Study Day	$AUC_{0\text{-}tau}$ (ng*h/mL)	C_{max} (ng/mL)	$C_{min,ss}$ (ng/mL)	t_{max} (h)
Study 1042-0404 (BID for 3 days, N = 22) Capsules With a Standard Meal or Snack					
1200 mg/day, BID	Day 6	1160 (461)	224 (100)	38.9 (16.9)	2.00 (1-3)
1600 mg/day, BID	Day 9	1450 (504)	263 (99.2)	52.0 (27.3)	2.00 (2-3)
2000 mg/day, BID	Day 12	1510 (640)	262 (90.8)	56.9 (28.8)	2.00 (1-3)

$AUC_{0\text{-}taut}$ = area under the drug-concentration vs time curve from time zero to the end of dosing interval of 12 hours; BID = twice daily; C_{max} = maximum concentration; $C_{min,ss}$ = minimum concentration at steady state; GNX = ganaxolone; N = number of subjects; t_{max} = time of maximum concentration Median (range).

Less than proportional PK was also observed for early GNX HPMC tablets formulation in the higher doses of 1200-1600mg (study **1042-0115**)

About dose-proportional PK was observed in study **1042-0403**, following single and multiple doses of early GNX capsules in the 200mg-600mg dose range and in **CA042 9301.01**, for early GNX β-CD complex suspension formulation in the 50mg-600mg dose range.

Multiple-dose data from study **1042-0403**, with GNX capsule formulation at doses of 200, 400, and 600 mg BID, showed that steady-state was achieved within 48 hours and accumulation for $AUC_{0\text{-}12}$ was approximately 43 to 81%, yielding an effective t½ with BID dosing of 7 to 10 hours.

Special populations

Renal impairment Study **1042-IRF-1001** assessed the PK and safety and tolerability of adult subjects with normal renal function and severe renal impairment. Renal impairment had no impact on the exposure of ganaxolone; a single dose of ganaxolone 300 mg was well tolerated in adults with normal and severe renal impairment.

Subjects with moderate or severe hepatic impairment were excluded from the Phase 3 clinical trials. Hepatic impairment study **1042-IHF-1001** evaluated the influence of hepatic impairment on the PK of ganaxolone. Patients with severe (Child-Pugh C) hepatic impairment had an approximately 6-fold increase in AUC as compared to those with normal hepatic function. The impact of mild hepatic impairment could be considered negligible ((1.2 fold increase in AUC). In patients with moderate hepatic impairment, a 1.7 fold increase in AUC has been observed.

Studies in healthy subjects have not shown a gender effect for PK with early GNX formulations in studies **CA042-9501.01** and **CA042-9505.01**.

The eldest patient included in the CDD trials was 19 years, but many adults were included in other clinical trials. The pharmacokinetics in the elderly was not discussed.

Table 6: Summary of Older Subjects in Studies with Pharmacokinetic Data, All Treated Subjects (MAA Safety Pool)

Study	Total Number of Subjects	Age 65-74 n (%)	Age 75-84 n (%)	Age 85+ n (%)
1042-SE-2001	17	5 (29.4)	2 (11.8)	1 (5.9)
1042-CDD-3001	101	0	0	0
1042-0900	30	0	0	0
1042-0600	147	1 (0.7)	0	0
1042-HAP-1001	54	0	0	0
1042-C14GNX-lac-1001	5	0	0	0
1042-GNX.AME-1001	8	0	0	0
1042-DDI-1001	32	0	0	0

Percentages are based on the total number of subjects in each study.

Pharmacokinetic interaction studies

Study **1042-DDI-1001** assessed the effects of strong CYP3A4 inhibitor itraconazole (200mg QD for 11 days) and strong inducer rifampin (600mg QD, fasting conditions) on the pharmacokinetics of GNX under fed conditions. Concomitant coadministration with strong CYP3A4 inhibitor itraconazole increased GNX exposure (AUC0-inf) by 17%. Multiple doses of rifampicin, a potent inducer of multiple metabolic pathways, decreased GNX AUCs by approximately 57-68% and C_{max} by approximately 57%. This is in line with the importance of metabolism as an elimination route of GNX and the induction of enzymes by rifampicin.

Study **1042-0402** assessed the effects of GNX (400mg BID) on the pharmacokinetics of probe CYP3A4 substrate midazolam (2mg). There was a minor influence of the pharmacokinetics of midazolam; the $AUC_{0\text{-}inf}$ ratio (90%CI) for GNX+ MDZ/ MDZ was 86.7 (67.7, 111.1).

The drug-drug interaction between GNX and anti-seizures was evaluated using sparse sampling data from study **1042-600**, data were analysed in popPK study **09-0600-POP-PK-01**. Concomitant administration of CYP3A4-inducing anti-seizures (carbamazepine, phenytoin, and phenobarbital) resulted in approximately 40% lower plasma exposures of GNX, as compared to subjects not receiving strong inducers. These results are in line with the results of the DDI study with CYP inducer rifampicin.

Pharmacokinetics using human biomaterials

The applicant conducted 10 *in vitro* drug interaction studies using human biomaterials:

Table 7: Pharmacokinetic Drug Interactions, In vitro

Study Number	Type of Study	In-vitro test system
199N-0501	CYP450 metabolism: metabolism of GNX by the major human liver CYP450 isoenzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5	Human liver isoenzymes
XT154127	CYP reaction phenotyping In vitro : test system expressed with CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4	Human liver microsomes
C1042.361.056	Interaction with valproic acid : effect of various concentrations valproic acid on protein binding of GNX	Human plasma
XT155147	CYP450 inhibition: GNX as direct, time-dependent, or metabolism-dependent inhibitor of CYP1A2, CYP2B6, CYP2C9, CYP2C19, or CYP3A4/5	Human liver microsomes
XT153121	CYP450 induction: GNX as inducer of mRNA expression / CYP1A2, CYP2B6, and CYP3A4/5 activity	Cultured human hepatocytes
11042.HR.IT.95.09	Interaction with CYP3A4 inducer phenobarbital and CYP3A4 inhibitor ketoconazole	Rat and human liver microsomes
11042.HM.VT. 94.08	Interaction with Anti-seizure medications: • The potential for GNX to have an effect on the metabolism of Anti-seizure	Human liver microsomes

	medications carbamazepine, phenytoin, phenobarbitone, valproic acid, and ethosuximide • the potential for these same Anti-seizure medications to affect the metabolism of GNX	
11042.HM.IT.95.04	Interaction with steroids: The potential for GNX to have an effect on the metabolism of steroid hormones used in contraceptives	Human liver microsomes
OPT-2016-063	GNX as a substrate of human drug transporters: GNX, as substrate of human BCRP, P-gp, OCT1, OCT2, OATP1B1, or OATP1B3	Transporters
OPT-2016-020	Inhibition of human drug transporters: The potential for GNX to inhibit thedrug transporters BCRP, P-gp, MATE1, MATE2-K, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, and BSEP	Transporter
CYP2472_R2	• Plasma Protein Binding of Ganaxolone • Inhibition of UGT Enzymes by Ganaxolone UGT Reaction • Phenotyping of Ganaxolone	
B21A34-R	Inhibition potential of GNX for SULT	

Ganaxalone as a substrate

Ganaxolone, at a concentration of 0.12 µM was rapidly metabolised by cytochrome P450 (CYP) 3A4 and CYP3A5 in study 199N-0501. Study XT154127 confirmed that GNX at a concentration range of 1, 10, and 100 µM is primarily metabolised by Cyp 3A4/5 or GNX. This study showed that also CYP2B6, CYP2C19 and CYP2D6 may be involved to a much lesser extent. GNX was not a substrate for CYP1A2, CYP2C8, CYP2C9 and CYP2E1.

Study 11042.HR.IT.95.09 investigated the effects of the CYP3A4 inhibitor ketoconazole on the metabolism of the GNX in human and rat liver microsomes. Ketoconazole inhibited NADPH-dependent oxidation of [^{3}H] GNX in human microsomes by 89.8%.

In studies OPT-2016-063 and OPT-2016-020, ganaxolone did not appear to be a substrate of any of the investigated transporters. Ganaxolone, at concentrations of 1, 3, and 10 µM, was not a substrate of human BCRP, P-gp, OCT1, OCT2, OATP1B1, or OATP1B3.

Ganaxolone is a substrate for UGT1A3, UGT1A6, UGT1A9, and UGT2B15. No formal drug-drug interaction studies have been conducted with ganaxolone in combination with UGT inhibitors such as valproate

The effect of valproic acid, at concentrations of 20-200 µg/mL, on protein binding of ganaxolone, was evaluated in study C1042.361.056. There were no significant differences in the percentages of free and bound GNX in the presence or absence of valproic acid. The average percent of free GNX over all conditions was 0.86%, and the average percent bound was 99.14%.

Ganaxalone as a perpetrator

In studies OPT-2016-063 and OPT-2016-020, ganaxolone did not appear to be an inhibitor of any of the investigated transporters. Ganaxolone, at concentrations ranging from 0.1 to 30 µM, was not an inhibitor or inducer of the transporters BCRP, P-gp, MATE1, MATE2-K, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, and BSEP.

In study XT155147 with ganaxolone, at concentrations ranging from 0.003 - 1 mM, some direct inhibition of CYP2C8 (21%), CYP2D6 (24%), and CYP3A4/5 (as measured by midazolam 1′ - hydroxylation; 27%) was observed; however, the IC50 values were reported as > 30 µM, the highest concentration of GNX tested. No evidence of direct inhibition of CYP1A2, CYP2B6, CYP2C9, CYP2C19, or CYP3A4/5 (as measured by testosterone 6β-hydroxylation) was observed and GNX not appear to cause time dependent or metabolism dependent inhibition of any the CYP450 isoenzymes evaluated.

In study XT153121 with ganaxolone, at concentrations ranging from 0.1 to 30 µM, GNX caused increases in CYP2B6 activity and mRNA levels in all 3 hepatocyte preparations. Activity levels were

increased up to 6.89 fold and were 32.4% of the positive control response. While GNX had little or no effect on CYP3A4/5 activity, it did cause concentration-dependent increases in CYP3A4 mRNA levels in all 3 hepatocyte preparations. CYP3A4 mRNA levels were increased up to 52 fold and were 32.2% of the positive control response. The responses were concentration-dependent in 2 of 3 hepatocyte preparations. GNX had little or no effect (ie, < 2- fold change and/or < 20% of the positive control response) on CYP1A2 activity and mRNA levels. Based on this study it was concluded that GNX is a potential *in vivo* inducer of CYP2B6 and CYP3A4.

Study 11042.HM.VT.94.08 evaluated the potential for GNX (at concentrations of 2 and 20 µM) to have an effect on the metabolism of Anti-seizure medications carbamazepine (50µM or 500 µM), phenytoin (100 µM) , phenobarbitone (340 µM), valproic acid (0.7 mM or 3.5 mM), and ethosuximide (0.5 mM and 1 mM), and conversely, the potential for these same Anti-seizure medications (at concentrations equivalent to their maximum therapeutic plasma range) to affect the metabolism of GNX, in human liver microsomes. At the tested concentrations, none of the 5 anti-seizure agents significantly inhibited or activated the metabolism of [3H]-GNX in human liver microsomes. Carbamazepine metabolism was increased by 20 µM GNX, but not 2 µM GNX. When carbamazepine at concentrations of 50 and 500 µM was incubated with 20 µM GNX the metabolism of carbamazepine was increased by 2 to 4-fold and 1.5-fold, respectively. GNX inhibited the metabolism of phenytoin by up to 55% and 23% in male and female liver microsomes, respectively. GNX had no significant effect on the metabolism of valproic acid. An assessment of the effect of GNX on the metabolism of ethosuximide and phenobarbitone was not possible.

Study 11042.HM.IT.95.04 evaluated the potential for GNX (0.5 µCi of [^{3}H]-GNX) to have an effect on the metabolism of the steroids [^{14}C] progesterone 250 nM, [^{14}C] oestradiol 150 µM, and [^{14}C] norgestrel 100 µM in human liver microsomes. GNX did not inhibit the metabolism of progesterone, but there were minor changes in the metabolite profile that accounted for less than 2% of the overall metabolism. Progesterone was a weak competitive inhibitor of GNX, with an inhibition constant of approximately 30 µM. Since oestradiol and norgestrel were not significantly metabolized by the human liver microsomes, the potential for GNX to inhibit their metabolism could not be assessed. Oestradiol and norgestrel did not significantly affect the metabolism of GNX.

In vitro study CYP2472_R2 showed that GNX did not inhibit UGT1A1, UGT1A6, UGT1A9, and UGT2B7 up to concentrations of 100 µM. Some inhibition of UGT1A3 and UGT1A4 was observed, at a clinically relevant concentration of 40 µM the UGT activity was decreased by approximately 50%. In the presence of recombinant enzymes, ganaxolone was a substrate for UGT1A6, UGT1A9, and UGT2B7.

In *in vitro* study B21A34-R GNX inhibited Estrogen sulfotransferase (SULT1E1) with an IC50 of 2.2 µM. At clinically relevant doses the unbound plasma concentrations of GNX is less than 0.01 µM

2.6.2.2. Pharmacodynamics

Mechanism of action

GNX is a positive allosteric modulator of $GABA_A$ receptors located in the CNS. GNX and benzodiazepines positively modulate synaptic $GABA_A$ receptors comprised of α and γ subunits (Campo-Soria et al., 2006). However, GNX acts via a distinct binding site on the $GABA_A$ receptor and, unlike benzodiazepines, also modulates extrasynaptic $GABA_A$ receptors that are comprised of α and δ subunits (Belelli et al., 2006; Belelli and Lambert, 2005).

GNX potentiates GABAergic transmission at nanomolar concentrations and acts as a direct agonist at micromolar levels (Reddy 2019).

Synaptic GABAA receptors mediate phasic inhibitory neurotransmission, whereas extra-synaptic receptors are involved in maintaining tonic inhibitory currents. Synaptic $GABA_A$ receptors internalize and become unresponsive with seizure and also downregulate and become unresponsive with chronic administration of benzodiazepines. In contrast, extrasynaptic $GABA_A$ receptors maintain their activity after seizure and remain responsive with chronic GNX treatment (Reddy and Rogawski, 2000).

In addition to their anticonvulsant effects, GNX and related neurosteroids may have a neuroprotective activity, particularly relevant to CDD. Research has provided evidence on the role of CDKL5 in the normal growth and structure of neurons and that mutations in CDKL5 responsible for CDD cause neurodevelopmental abnormalities. Moreover, certain neurosteroids may play a role in the repair of neuronal damage caused by CDKL5 mutations (Barbiero et al., 2020; Barbiero et al., 2017). Specifically, normal dynamics of the neuronal cytoskeleton require interaction between CDKL5 and a GTPase--activating protein, IQGAP1, which controls cellular activities dependent on actin and proteins affecting microtubule tracking. A deficiency of CDKL5 displaces IQGAP1 from migrating cells and impairs its ability to interact with the CLIP170. This results in the inability of CLIP170 to guide neuronal microtubules within cells and, thereby, disrupts the normal migration of neurons. CLIP170 is a cellular target of pregnenolone, a neurosteroid precursor to allopregnanolone (the endogenous analogue of GNX). Pregnenolone induces a conformational change in CLIP170, restoring its affinity to microtubules and rescuing neuronal migration defects resulting from CDKL5 deficiency.

Primary and Secondary pharmacology

Primary pharmacology

Study 1042-0405 investigated the safety, PK, and PD of intravenous (IV) ganaxolone administered as an ascending bolus dosing (Stage 1) and as a bolus dosing followed by a continuous infusion (Stage 2) in healthy subjects.

The primary objective of this clinical study was to evaluate the safety and pharmacokinetics (PK) of IV ganaxolone administered as a bolus dose and continuous infusion in healthy subjects. The secondary objectives were to evaluate the pharmacodynamics (PD) effect on EEG parameters, to evaluate the PD effect on Bispectral Index (BIS) parameters, to evaluate the effect on clinical sedation scores and on ventilatory parameters.

A total of 36 healthy volunteers were enrolled in the study, 30 subjects into Stage 1 and 6 subjects into Stage 2.

In Cohort A there was no significant change in BIS values following the 10 mg bolus. Following the 30 mg bolus the BIS showed a significant decrease by 5 minutes (median value 54), which recovered to 80 by 30 minutes postdose and remained at that value by the end of the monitoring period of 60 minutes (median value 82).

In Cohort B with 20 mg bolus over 2 minutes, the median BIS values showed a rapid decline to 81 at 5 minutes, maintained until 45 minutes with a gradual recovery approaching baseline at 60 minutes (BIS value 89).

In Cohort C with 30 mg infusion over 60 minutes, BIS showed an initial change at 15 minutes postdose (median BIS value 84) with a further decline to a nadir of median BIS value 70 by 60 minutes postdose. Once the infusion was discontinued at 60 minutes, the BIS remained low (median BIS value 70 at 75 minutes) with gradual recovery to baseline values at 2 hours post-dose.

In Cohort D with 10 mg infusion over 60 minutes, the BIS values exhibited a similar pattern to those in Cohort C but to a lesser extent. The nadir of the curve at 75 minutes post-dose had a median BIS value of 78, and recovery to baseline value was attained by 90 minutes post-dose.

In Stage 2 Cohort A with an initial bolus of 6 mg over 5 minutes followed by a 4-hour infusion of 20 mg/h, the BIS values showed an initial decline at 5 minutes with a further decrease in median values by 15 minutes. The median BIS values were then maintained between mid-60 to mid-70 values for the duration of the infusion. Following discontinuation of the infusion, the BIS values showed an initial recovery (median BIS value of 82 at 255 minutes) with a return to baseline values by 270 minutes post-dose.

The median MOAA/S scores remained unchanged throughout all dosing cohorts.

Quantitative EEG: Overall, the changes seemed most evident in cohort 1B where a bolus infusion was administered over 2 minutes rather than slower infusions. The mean alpha/delta ratio and spectral edge frequency appeared more informative than alpha power, and these parameters had a temporal inverse relationship with the BIS. Taken together, these 3 qEEG measures suggest that at low doses of ganaxolone, faster EEG frequencies increase (increase in relative alpha power, alpha/delta ratio and spectral edge frequency). With a higher dose (30 mg), delta frequencies increase (relative alpha power and alpha/delta ratio decrease after initial increase). However, no firm conclusions can be made since a control group was not present in Stage 1 Cohort A or Stage 2 Cohort A, and the number of subjects (active and control) were small.

Secondary pharmacology

Study 1042-HAP-1001 was a single-centre, randomized, DB, double-dummy, placebo- and active-controlled, 5-way crossover study to evaluate the abuse potential (primary endpoint) of single oral doses of GNX compared with single oral doses of lorazepam and PBO in healthy, male and female, non-dependent, recreational CNS depressant users. In addition, PK, safety, and tolerability (all secondary endpoints) of single oral doses of GNX were evaluated. Part A of the study was the dose selection, and Part B was the main study. In the dose selection part, sequential escalating doses of GNX were administered, with a wash-out period of 5 days between doses. Three dose levels were tested: 1000mg, 1500mg and 2000mg GNX. The positive control was lorazepam 6 mg.

Each dose of ganaxolone produced responses that showed less abuse potential than lorazepam 6 mg. The lower limits of the 1-sided 95% CIs for comparing lorazepam 6 mg with ganaxolone were 14.3, 12.5, and 8.0 for ganaxolone 400, 800 and 2000 mg, respectively (all $P < 0.0001$). Ganaxolone 400 mg produced responses that showed similar abuse potential compared to placebo, with a difference of less than 11 points (upper CI limit of 8.8; $P = 0.0072$). However, ganaxolone 800 and 2000 mg each produced responses that could not be considered similar to those of placebo as they exceeded the prespecified margin of 11 (upper CI limits of 17.0 [$P = 0.5000$] and 19.0 [$P = 0.6864$], respectively).

Ganaxolone 400 mg was not significantly different from placebo on key secondary endpoints (Overall Drug Liking VAS Emax and Take Drug Again VAS Emax), but did show significantly greater positive, sedative, and other effects compared to placebo. Ganaxolone 800 and 2000 mg showed significantly greater effects than placebo on the key secondary endpoints and measures of positive, sedative, and other drug effects. Ganaxolone was not associated with significant negative effects at any dose level.

2.6.3. Discussion on clinical pharmacology

Methods

Bioanalytical methods: The bioanalytical methods used have been adequately described and appropriately validated.

Pharmacokinetic data analysis and statistical methods: Appropriate single and multiple-dose parameters were determined, and acceptable statistical methods have been used to evaluate the pharmacokinetics of GNX.

Population PK study methodology: The applicant presented two population PK analysis studies.

Population PK-study **1042 CDD POPPK 001** was used to simulate steady-state PK parameters of ganaxolone suspension for the proposed dose regimen in the paediatric CDD population and in healthy paediatric and adult subjects. Although the model may be considered fit-for-purpose for estimating the exposure in paediatric subjects, the PopPK predictions cannot be considered accurate. Simulated PK parameters are indicative and the model could not be used for dosing selection or dosing recommendation in the target population.

Population PK-study **09-0600-POP-PK-01** had several major methodological drawbacks, as only based on one study with sparse sampling and different estimated model parameters than model **1042 CDD POPPK 001**, the model appeared to describe the data in the study reasonably well. Therefore, this model may only be used for estimating the effect of CYP3A*-inducing comedication on GNX PK. Nevertheless, based on this study, it may be concluded that ganaxolone oral clearance was increased by approximately 40% in patients taking either carbamazepine or phenytoin or both drugs.

Formulation development

An oral suspension formulation was selected as the final formulation, this formulation is considered an age and disease appropriate for the intended patient population.

Different formulations have been studied during the development of the ganaloxone suspension. In phase 2 and 3 studies, HPMC GNX tablet and capsule formulations or GNX β-CD complex suspension were used. The bioavailability and the food effect differ between formulations, and no direct comparison of the suspension and any other solid GNX formulations has been made. Comparison of the different formulations is solely based on cross-study comparison as appropriate bridging data are not available.

As the pharmacokinetics of the historical β CD formulations have been characterised extensively, across study comparison can be made. The bioavailability of GNX β-CD complex suspension formulation was higher than the bioavailability of the to-be-marketed HPMC oral suspension under high-fat conditions; however, the variability of the GNX β-CD complex suspension formulation was also higher due to a larger food effect. Because the exposure was generally higher for the historical β CD complex suspension compared to the 50 mg/mL oral suspension formulation, the pooled safety analysis is not expected to underestimate the safety of GNX due to the inclusion of these early trials.

Two comparative bioavailability studies (**1042-0118** and **1042-0106.01**) investigated the PK of nanoparticle-sized GNX suspensions and other solid GNX formulations. However, these studies were not intended to provide performance and bioavailability characteristics of the proposed commercial formulation and are not intended for bridging. There is therefore no formal bridging data.

Despite considerable differences between formulations across studies, the studies with historical formulations can be considered supportive, provided that the interpretation of the clinical results of the studies with different formulations is made with caution. The applicant has simulated the steady state exposure in adults and children based using pop PK model 1042 CDD POPPK 001 which included data of the Phase 1 studies with the final suspension formulation.

The HPMC suspension formulation was selected as the final formulation. The applicant appropriately explained that this formulation was selected as it had the most consistent exposure and least variability with food intake. Further, the applicant explained that the suspension formulation is

appropriate for young children and adults with CDD because motor impairments (including dysphagia) are common in these patients.

Although, data are difficult to compare due to the non-linearity of the higher doses and the different dose regimens used (BID vs TID), the steady-state exposure is expected to be about 25% higher with the GNX oral suspension, based on the comparison between population simulations and the multiple-dose studies conducted with different formulations and doses.

Absorption

For the adults, there are no multiple-dose steady-state PK parameters, for the to-be marketed GNX oral suspension at the 600mg TID dose regimen. The pharmacokinetics of the GNX oral suspension at a dose of 600mg TID (or maximum paediatric TID dose) has been simulated in population PK study **1042-CDD-POPPK-001**. However only two adult patients with a low mean weight of 35 kg and aged 19 years were included in the population dataset. When the model was used to simulate the pharmacokinetics of GNX600mg TID, for a 70kg adult, the exposure was much lower than for the highest dose in paediatric patients. Therefore, PopPK model 1042-CDD-POPPK-001 cannot not be used to simulate PK data for 70kg adults.

No formal absolute bioavailability study was conducted. The bioavailability of the to –be- marketed suspension formulation has been calculated based on the results of DDI Study 1042 DDI-1001, and the results of Study 1042-0405, with IV ganaxolone. The oral bioavailability (F) of the suspension formulation, at a dose level of 600 mg, is approximately 13%.

Studies have shown that the impact of food is formulation dependent. Ganaxolone is a lipophilic steroid drug and its bioavailability of ganaxolone increases under fed conditions. Data appear to suggest that the release of bile salts facilitates the absorption of ganaxolone. Study **1042-0400** showed that administration of the commercial formulation 400 mg GNX suspension 50 mg/mL after a high-fat meal increased plasma concentrations and exposure about 2 to 3-fold as compared with the fasted state. The t_{max} and the elimination half-life of GNX were not significantly affected.

Moreover, study **CA042-9401.01**, conducted with GNX capsules, points out that the type of food may be relevant for the absorption of GNX. It was observed that the peak plasma GNX levels following a high-fat meal were 3 times higher than those observed following a high carbohydrate meal and nearly 12 times higher than those observed following an overnight fast. This study indicates that the type of food may be relevant for the absorption of GNX and may be a source of variability in daily practice. It is unclear if the type of meal has an effect on the PK of the to-be-registered GNX suspension formulation. As the effect of different types of food is not known, a statement that Ztalmy should be administered with similar types of feed if possible is included in the SmPC.

Elimination and metabolism

Mass balance and metabolic profiling data indicate that GNX is extensively metabolized to multiple steroid metabolites, including metabolites with a very long half-life.

Based on single dose mass balance study **1042-GNX.AME-1001** was concluded that metabolites Oxy-didehydro-ganaxolone (M60b = M2) and potentially also oxy-dehydro-ganaxolone sulfate (M43 = M17) are contributing to the long half-life of the radioactivity and, therefore, probably, the most important metabolites at steady state.

The metabolites that were identified as main metabolites in the steady state study **8333043** (ganaxolone+O+gluc and ganaxolone+2O) were apparently not evaluated in long term toxicity studies in animals.

Only metabolite M2 is considered appropriately characterized at steady state. The other steady state metabolites of ganaxolone have not been appropriately characterized. Additional characterisation work is ongoing for M43 = M17.

The applicant commits to provide the results of the additional characterisation of the metabolite M43=M17 (category 3 study, as defined in the RMP). The applicant also commits to characterise the GNX metabolite pattern at steady state (category 3 study, as defined in the RMP).

Dose proportionality and time dependency

The proposed dose of GNX ranges from 150 mg to 600 mg TID (450-1800mg daily) for adults. Dose proportionality of the to-be-marketed 50 mg/ml suspension formulation has been investigated over a range of 400-2000 mg. Less than proportional PK is observed with high doses of ganaxolone suspension (doses of 1000 mg or more). As ganaxolone did not appear to be a substrate for any of the investigated transporters *in vitro* study **OPT-2016-063,** low solubility is the most likely cause.

In studies with early formulations (GNX HPMC tablets, GNX capsules and GNX β-CD complex suspension formulation), dose-proportional PK was observed for doses up to 600mg GNX. Data suggest that over the dose range of 600 to 1000 mg BID under fed conditions, exposure to GNX increases less than proportionally with increases in dose, with this disproportionality being more pronounced at the high end of the dose range. Based on the totality of data, it is expected that the pharmacokinetics of the to-be-marketed 50mg/ml suspension will be approximately dose-proportional in the clinical dose range of 150-600mg for adults.

In the pop PK model, saturable absorption for paediatric patients was included. Saturable absorption was only included in the popPK model for paediatric patients and not for adults, although less than dose proportional PK has been observed in adults in several clinical trials. The highest proposed dose given may be larger than the maximum absorbed dose, even for adults. Bodyweight is apparently an explanation for differences between subjects in the degree of saturable absorption. No changes in the posology are however needed as the GNX dose is titrated based on effect and tolerability.

Based on population PK analysis simulations, the estimated $AUC_{0\text{-}8}$ is approximately 1550 ng*hr/mL for the 600mg TID dose in 70kg adult patients at steady state and 2310 ng*hr/mL for the 21 mg/kg in paediatric subjects of 2-6 years old. The estimated accumulation is about 1.6-fold for the TID dose regimen. The simulated exposure was much higher in paediatric patients when compared to adults.

Pharmacokinetics of Ganaxolone in Adults and Children

The eldest patient included in the CDD trials **1042-CDD-3001** and **1042-09009.00** was 19 years. In other clinical trials, many adults of different ages were included; however, none of these studies evaluated the to-be-marketed formulation and/or the 600mg TID dose regime. The investigated adults were also mosty healthy subjects or a different population. Therefore, data in adults are mainly based on population PK simulations.

The applicant provided population PK simulations for healthy adult subjects using the NHANES database. Following administration of GNX 600 mg three times a day in adults, the expected exposure ($AUC_{0\text{-}24\ hr}$) is about 4000 ng*hr/mL, based on population PK simulations and PK studies in healthy volunteers. Overall, the simulated exposure in adults was reversely correlated with bodyweight and therefore it cannot be excluded that the drug may be less effective in adults with normal body weight.

The applicant also estimated the adult GNX exposure following a 600-mg oral dose of GNX based on single dose study 1042-DDI-1001 (healthy volunteers) and ongoing single dose study 1042-HME-1001. The calculated $AUC_{0\text{-}24hr}$ were 3860 ng*h/mL and 4260 ng*h/mL. This is roughly in line with population PK estimates. The applicant will provide the final report of the single dose study 1042-HME-1001 post-approval.

Special populations

The applicant evaluated the PK of GNX following oral administration to adult and paediatric patients with CDD in pop PK study **1042-CDD-POPPK-001**. Covariate analysis was conducted, and weight was identified as the relevant covariate. None of the other covariates had an additional impact on GNX exposure. However, due to the relatively small and homogenous dataset, no final conclusions can be drawn yet on the potential impact of age, albumin, creatinine clearance and liver parameters.

The impact of Race (white N=83, black N=8, Asian N=1, other N=2) was also evaluated in popPK model 09-0600-POP-PK-01. This analysis indicated that GNX pharmacokinetics are unlikely to be influenced by race.

The applicant evaluated the pharmacokinetics of GNX in patients with impaired renal function in study **1042-IRF-1001**. Based on the results of the study it can be concluded that renal impairment had no impact on the exposure of ganaxolone. There is no experience in patients with end-stage renal disease. The information is correctly reflected in the SmPC.

GNX is extensively metabolised and **study 1042-IHF-1001** showed that hepatic impairment has significant effects on the pharmacokinetics of ganaxolone. The impact of mild hepatic impairment could be considered negligible (1.5 fold increase in Cmax and 1.2 fold increase in AUC0-t). A dose reduction is recommended for patients with severe hepatic impairment and not for patients with mild and moderate hepatic impairment. The information is correctly reflected in the SmPC.

In Studies **CA042-9501.01** and **CA042-9505.01** gender did not affect the PK of GNX. However, both studies evaluated early formulations and study CA042-9505.01 was not designed to evaluate full pharmacokinetic profiles and PK parameters such as C_{max}, t_{max}, AUC, and clearance could not be calculated due to limited sampling. Therefore, the results should be interpreted with caution. Potential gender differences of the GNX metabolic profile have not been evaluated. As GNX is structurally related to progestogen, sex differences cannot be excluded. Like other steroids, GNX is metabolized by multiple pathways, via CYP enzymes but also via sulphation and glucuronidation. It should be noted that the metabolite pattern was different between male and female mice and rats, which is not uncommon for drugs metabolised by CYP enzymes. As studies in healthy subjects have not shown a gender effect on parent GNX and possible sex differences are not expected to affect the clinical efficacy of GNX, the CHMP agreed that this is acceptable.

Interactions

The metabolism of GNX is highly complex. GNX is extensively metabolized by humans to give a variety of Phase 1 and Phase 2 metabolites, and a total of over 50 metabolites have been detected. *In vitro* studies suggest that GNX is primarily metabolised by cytochrome P450 (CYP) 3A4 and CYP3A5. CYP2B6, CYP2C19 and CYP2D6 may be involved to a much lesser extent. Metabolic profiling suggests multiple metabolic routes: oxidation, dehydrogenation, hydrogenation, sulphation and glucuronidation have been observed.

In the presence of recombinant enzymes, GNX was a substrate for UGT1A6, UGT1A9, and UGT2B7. GNX did not inhibit UGT1A1, UGT1A6, UGT1A9, and UGT2B7 up to concentrations of 100 µM. Some inhibition of UGT1A3 and UGT1A4 was observed, at a concentration of 40 µM the UGT activity was decreased by approximately 50% (study **CYP2472_R2**). This information has been added to the SmPC.

In vitro study **XT153121** and non-clinical studies in different species had indicated that GNX could be an inducer of CYP3A4 at concentrations of 0.1 to 30 µM.; however, in clinical DDI study, **1042-0402** GNX only had a minor influence on the pharmacokinetics of midazolam. Hence, GNX is no relevant inducer *in vivo* at 400 mg BID doses. However, it should be noted that the 400 mg BID dose is more

than 2-fold lower than the maximal posology of 600 mg TID, and hence the potential inducing effects are not evaluated under the worst-case situation.

GNX will be frequently co-administered with other Anti-seizure medications. Most Anti-seizure medications are extensively metabolised via CYP and/or UGT-pathways. The applicant has not evaluated the potential effect of GNX on the pharmacokinetics of other Anti-seizure medications *in vivo*. *In vitro* studies suggest that GNX may be an inducer of CYP3A*(study **XT153121**); some direct inhibition of CYP3A was observed (study **XT155147**), and an increase of carbamazepine metabolism and a decrease of valproic acid metabolism was observed in human liver microsomes vitro (**11042.HM.VT. 94.08**). Although these *in vitro* studies are difficult to interpret, drug interaction with other anti-seizures cannot be excluded.

GNX is a substrate for UGT1A3, UGT1A6, UGT1A9, and UGT2B15. In a subgroup analyses (see Clinical efficacy), an effect modification is apparent when GNX is used concomitantly with clobazam, valproate or vigabatrin. The effect appeared most pronounced for valproate, a UGT inhibitor. No formal drug-drug interaction studies have been conducted with ganaxolone in combination with UGT inhibitors such as valproate. Dose reduction of ganaxolone and/or the UGT inhibitor may be necessary when given in combination.

Even though the interaction study with midazolam indicated that there is no induction of CYP3A4, this, however, is considered not sufficient evidence for absence on effect on oral contraceptives especially given the similarities in structure and metabolism between ganaxolone and oral contraceptives. As the CDD population is usually not capable of bearing offspring, a DDI study is currently not warranted. Accordingly, a statement indicating that potential interaction of ganaxolone with oral contraceptives has not been investigated is included in the SmPC Section 4.5.

Pharmacodynamics

The precise mechanism by which GNX exerts its therapeutic effects in the treatment of seizures associated with CDD is unknown, but its anticonvulsant effects are thought to result from the modulation of the $GABA_A$ receptors in the CNS, similar to benzodiazepines (e.g. clobazam) and other anti-seizure medications (ASMs) such as vigabatrin and felbamate. According to the applicant, GNX binds to a distinct site on the GABAA receptor and modulates the extrasynaptic $GABA_A$ receptors.

The potential for abuse of GNX was evaluated in study 1042-HAP-1001. Of the three GNX doses evaluated in the study, the 2000mg dose obtained the highest scores on all endpoints (maximum daily dose for CDD: 1800mg). It is noted however that the abuse potential seems less than for lorazepam 6mg. A limitation of the study is that it only evaluated the abuse potential of single-dose GNX in recreational polydrug users. In clinical practice, GNX will be dosed repeatedly in patients with CDD, most likely in a controlled setting. Therefore the CHMP agreed that GNX has the potential for abuse and a warning is included in section 4.4, with a cross reference to section 5.3.

No pharmacodynamic drug-drug interaction studies were submitted. For this CDD indication, GNX is given as adjunctive to other ASMs. With respect to PK interactions, DDI study 1042-DDI-1001 the strong CYP3A4 inducer rifampin reduced GNX exposure by 57-68% and in pop PK study 09-0600-POP-PK-01 a reduction of about 40% was observed when GNX was concomitantly used with CYP inducing anti-seizures. This information has been reflected in SmPC section 4.5. Subgroup analyses on concomitant use with specific anti-seizure medications indicate that somnolence and sedation occur more frequently when these are used with GNX. Therefore, there is a warning in section 4.4 on somnolence and sedation emphasizes that these effects can be potentiated when anti-seizure medication is used concomitantly.

2.6.4. Conclusions on clinical pharmacology

The CHMP agrees that the pharmacokinetics and pharmacodynamics of ganaxolone have been sufficiently evaluated.

The relevant findings are appropriately reflected in the Product Information.

The CHMP considers the following measures necessary to address the issues related to pharmacology:

- Characterisation of the GNX metabolite pattern at steady state.

- Submission of the results of the additional characterisation of the metabolite M43=M17.

In addition, the applicant agrees to provide post-approval the final report of the single dose study 1042-HME-1001.

2.6.5. Clinical efficacy

Table 8: Overview of clinical studies

Study ID Objective No. of study centres Study period	Design Duration	Study posology Subjects per arm entered/completed Study population	Efficacy endpoint
1042-0900 Proof of concept 10 centres 26 OL phase: May 2015 – January 2018 52 OLE phase: November 2015 – January 2019	OL 26 wks OL + 52 wks OLE	GNX[1] 26 wks OL: 30/15 52 wks OLE: 14/8 CDD + PCDH19 + DS	%Δ from baseline in 28-day total seizure frequency for the sum of individual seizures and clusters in the 26 week OL open-label treatment period
1042-CDD-3001 Efficacy/safety DB phase: June 2018 – July 2020 OLE phase: ongoing	RD DB PC: 4 wks titration + 13 wks maintenance + OLE 4 weeks titration + 3 yrs OLE	101/95 PB: 51/47 GNX: 50/48 CDD	%Δ from baseline in 28-day major motor seizure[2] frequency during the 17 week DB phase 50% Responder rate[3] CGI-I at last visit in the 17 week DB phase

CDD= cyclin-dependent kinase-like 5 deficiency disorder, CGI-I= Clinical Global Impression of Change – Improvement, DB= double-blind, DS= Dravet syndrome, GNX = ganaxolone, OL= open-label, OLE= open-label

extension, PC = placebo-controlled, PB= placebo, PCDH19= protocadherin 19- related epilepsy, RD= randomized, extension.

[1] up to 1800 mg/day

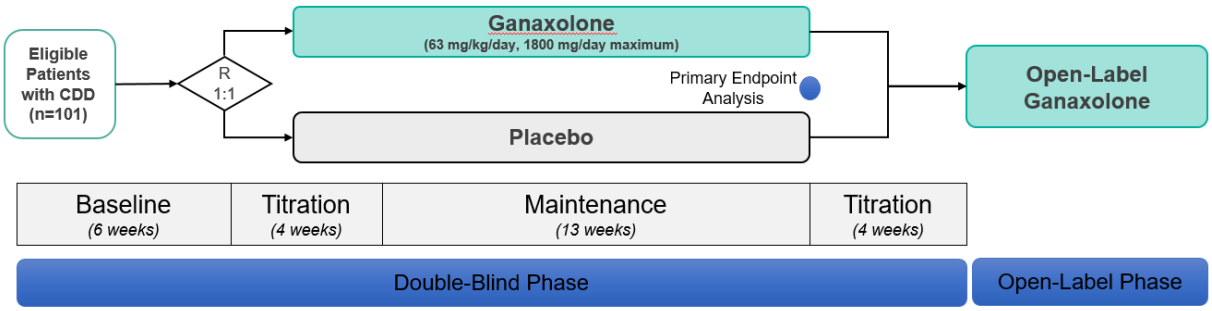
[2] motor seizures defined as bilateral tonic (sustained motor activity ≥ 3 seconds), generalized tonic-clonic, atonic/drop, bilateral clonic, and focal to bilateral tonic-clonic

[3] responder rate defined as proportion of subjects who had at least a 50% reduction in major motor seizures

2.6.5.1. Main study

1042-CDD-3001: A double-blind, randomized, placebo-controlled trial of adjunctive ganaxolone treatment in children and young adults with Cyclin-Dependent Kinase-Like 5 (CDKL5) Deficiency Disorder (CDD) followed by long-term open-label treatment

Figure 6: Study design - study 3001



CDD = CDKL5 deficiency disorder; CDKL5 = cyclin-dependent kinase-like 5; R = randomized

Methods

- **Study Participants**

The main **inclusion** criteria were:

- (a) Molecular confirmation of a pathogenic or likely pathogenic CDKL5 variant, early onset, difficult to control seizures, and neurodevelopmental impairment were required.

The Principal Investigator must have reviewed the results of the genetic analysis and confirmed that gene mutation was likely to be the cause of the epilepsy syndrome. If the subject had a de novo variant of unknown significance in the kinase domain of the CDKL5, parental testing was negative and met all other inclusion criteria; then the subject was included.

- Male or female subjects aged 2 through 21 years, inclusive.
- Failure to control seizures despite appropriate trial of 2 or more anti-seizure medications at therapeutic doses.
- Had at least 16 seizures of major motor seizure types: bilateral tonic (sustained motor activity ≥ 3 seconds), generalized tonic-clonic, bilateral clonic, atonic/drop or focal to bilateral tonic-clonic per 28 days in each 1-month period in the 2-month period prior to screening.
- Subject was approved to participate by sponsor and/or designee (ie, Epilepsy Consortium) after review of medical history, genetic testing, seizure classification, and historical seizure calendars.

- Participants were on a stable regimen of zero to 4 anti-seizure medications (including moderate or strong inducer or inhibitor anti-seizure medications eg, carbamazepine, phenytoin, etc.) for ≥ 1 month prior to the screening visit, without a foreseeable change in dosing for the duration of the double-blind phase. Vagus nerve stimulation (VNS), ketogenic diet, and modified Atkins diet did not count towards this limit but must have been unchanged for 3 months prior to screening.
- Subjects with surgically implanted VNS were allowed to enter the study provided that all of the following conditions were met: VNS had been in place for ≥ 1 year prior to the screening visit, the settings remained constant for 3 months prior to the screening visit and remained constant throughout the double-blind phase, the battery was expected to last for the duration of the double-blind phase.
- Felbamate: The use of felbamate was allowed provided that the subject had been maintained on a stable dose of felbamate for > 6 months and had stable liver function (AST and ALT) and hematology during the course of treatment and was expected to remain constant throughout the double-blind phase.
- Parent/caregiver was able and willing to maintain an accurate and complete daily electronic seizure calendar for the duration of the study.
- Was able and willing to take investigational product with food TID. GNX must have been administered with food.

The key **exclusion** criteria were:

- Previous exposure to GNX.
- Pregnant or breastfeeding.
- West Syndrome with hypsarrhythmia pattern on EEG or seizures predominantly of IS type; if EEG pattern/seizure type was uncertain, study inclusion was reviewed and determined by the sponsor/sponsor delegate.
- Concurrent use of ACTH, prednisone or other glucocorticoid was not permitted, nor use of moderate or strong inducers or inhibitors of CYP3A4/5/7. Moderate or strong inducer or inhibitor ASMs were allowed (eg, carbamazepine, phenytoin, etc.).
- Subjects on ACTH, prednisone, or other systemically (non-inhaled) administered steroids should have been off the product > 28 days prior to screening.
- Subjects with a positive result on THC or CBD test (via urine or plasma drug screen) at the screening visit, and a positive result on THC or CBD test (via plasma) at the baseline visit without prescription for Epidiolex in epilepsy were excluded from the study. Concomitant Epidiolex (CBD) use was allowed in the double-blind phase provided the subject had been on a stable dose for at least 1 month prior to screening and was expected to remain on a stable dose without a foreseeable change for the duration of the double-blind phase. THC and/or CBD were allowed in the open-label phase.
- Use of dietary supplements or herbal preparations were not permitted if subject had been using them consistently for less than 3 months prior to screening or did not plan on remaining on stable doses for the duration of the double-blind phase. Use of St. John's Wort was not permitted.
- Changes in ASMs within the last month prior to screening. All ASMs must have been stable in dose for at least 1 month prior to screening unless otherwise noted.

- Had an active CNS infection, demyelinating disease, degenerative neurological disease, or CNS disease deemed progressive as evaluated by brain imaging (MRI).
- Had any disease or condition (medical or surgical; other than CDKL5) at screening that might have compromised the hematologic, cardiovascular, pulmonary, renal, gastrointestinal, or hepatic systems; or other conditions that might have interfered with the absorption, distribution, metabolism, or excretion of the IP, or would have placed the subject at increased risk.
- An AST (SGOT) or ALT (SGPT) > 3 x ULN at study entry. If AST or ALT increased > 3 x ULN during the study, subject was followed with weekly laboratory repeat testing and continued in study if levels were trending down. Subject was discontinued if levels did not decline to < 3 x ULN.
- Total bilirubin levels greater than ULN at study entry. In cases of documented, stable medical condition (ie, Gilbert's Syndrome) resulting in levels of total bilirubin greater than ULN, the medical monitor determined if a protocol exception could be made. If total bilirubin increased to 1.5 x ULN or more during study, the subject was discontinued.
- Subjects with significant renal insufficiency, eGFR < 30 mL/min (calculated using the Cockcroft-Gault formula, Paediatric GFR calculator or Bedside Schwartz), were excluded from study entry or were discontinued if the criteria was met post-baseline.

- **Treatments**

17-week double-blind treatment phase

Subjects received either GNX or PBO, prescribed in increments of 15 mg/kg/day up to 63 mg/kg/day (max 1800 mg/day) as an oral suspension TID. Subjects weighing ≤ 28 kg were dosed on an mg/kg basis, and subjects weighing > 28 kg were dosed by mg/day. All investigational products prescribed per day was administered in 3 divided doses following a meal or snack. The titration scheme during the initial 28-taper is shown in Table 12.

Table 9: Titration scheme for double-blind treatment period

Oral Suspension (50 mg/mL) Dosing for Subjects Weighing ≤ 28 kg

Dose	Total mg/kg/day	Days
6 mg/kg TID	18	1 - 7
11 mg/kg TID	33	8 - 14
16 mg/kg TID	48	15 - 21
21 mg/kg TID	63	22 - 28

TID = 3 times daily.
Source: Appendix 16.1.1, Protocol Table 4.

Oral Suspension (50 mg/mL) Dosing for Subjects Weighing > 28 kg

Dose	mL per Dose	Total mg/kg/day	Days
150 mg TID	3	450	1 - 7
300 mg TID	6	900	8 - 14
450 mg TID	9	1350	15 - 21
600 mg TID	12	1800	22 - 28

TID = 3 times daily.
Source: Appendix 16.1.1, Protocol Table 5.

Subjects who were not able to tolerate 63 mg/kg/day (or 1,800 mg/day max) were allowed to be maintained on a lower dose. A minimum dose of 33 mg/kg/day or 900 mg/day was generally required during the double-blind phase unless a lower dose was agreed to.

Dose changes, including alternative dosing paradigms (e.g., a lower dose during the daytime and a higher dose in the evening) were discussed with the Sponsor's medical monitor. Subjects who discontinued investigational product underwent a 2-week taper period unless otherwise medically indicated. Subjects who discontinued investigational product treatment before the completion of the double-blind phase were followed per-protocol (PP) and maintained Diary entries until the double-blind phase was completed. These subjects also completed the safety follow-up assessments 2 weeks after the taper.

Subjects or parent/caregiver were to record administration of investigational product and background ASMs in the eDiary/seizure calendar. Compliance with investigational product treatment was assessed by inspecting the subjects' eDiary/seizure calendar and returning supplies with queries as necessary. Subjects that fell below 80% compliance at 2 consecutive visits during the double-blind phase were not included in the per-protocol population.

Open-label extension phase

Following the completion of the 17-week, double-blind phase subjects randomized to PBO will transition to GNX, while subjects randomized to GNX will stay on 63 mg/kg/day suspension (maximum 1800 mg/day) or Maximum Tolerated Dose (MTD). Subjects begun the 4-week blinded dose titration to 63 mg/kg/day or 1800 mg/day (or MTD) after completing Visit 5.

- **Objectives**

The **primary objective** was to assess the efficacy of GNX compared with placebo as adjunctive therapy for the treatment of seizures in children and young adults with genetically confirmed CDD at the end of the 17-week double-blind phase.

The **secondary objectives** were defined as follows:

1) To assess behavioural/neuropsychiatric changes correlated with domains of attention, sleep, and a target behaviour chosen by the parent/caregiver, using objective tests of CNS function for GNX compared with PBO as adjunctive therapy at the end of the 17-week double-blind phase.
2) To assess the safety and tolerability of GNX compared with PBO as adjunctive therapy at the end of the 17-week double-blind phase.
3) To assess PK parameters in subjects receiving GNX doses up to 63 mg/kg/day (1800 mg/day maximum) throughout the study
4) To assess the long-term efficacy, safety, and tolerability of GNX when administered as adjunctive therapy throughout the open-label phase.

The exploratory objectives of this study are the following:

- To assess changes correlated with other behaviour domains as assessed by the Anxiety, Depression, and Mood Scale (ADAMS).
- To assess changes to the quality-of-life scales as assessed by the Parenting Stress Index (PSI) and the Quality-of-Life Inventory-Disability (QI-Disability).
- To assess the potential use of an electroencephalogram (EEG) to study background activity as a biomarker of change in underlying brain function.

- To assess changes to other types of seizures in CDD

- **Outcomes/endpoints**

Primary endpoint:

1. The percentage change from baseline in 28-day major ***motor seizure frequency*** during the 17-week, double-blind treatment phase.

The major motor seizure types included bilateral tonic, generalized tonic-clonic, bilateral clonic, atonic/drop seizures and focal to bilateral tonic-clonic.

Key secondary endpoints:

2. Number (%) of subjects with a ≥ 50% reduction from baseline in major motor seizure frequency.
3. Clinical Global Impression of Improvement (CGI-I) at the last scheduled visit in the 17-week double-blind treatment phase.

Secondary endpoints:

4. Change from baseline in the percentage of seizure-free days during the 17-week double-blind treatment phase, based on major motor seizure types.
5. Caregiver Global Impression of Change in Seizure Intensity/Duration (CGI-CSID) score
6. Caregiver Global Impression of Change (CGI-C) in parent/caregiver identified behavioural target - potential domains include sociability, communication, irritability, and hyperactivity.

Exploratory endpoints:

7. Percent change in 28-day frequency of all seizures.
8. Change from baseline in the percentage of seizure-free days during the 17-week double-blind treatment phase, based on all seizure types.
9. Change from baseline in the longest seizure-free interval during the 17-week double-blind treatment phase, based on major motor seizure types and all seizure types.
10. Percent change from baseline in 28-day seizure frequency through the end of the 4-week titration phase of the double-blind treatment phase (major motor seizure types and all seizure types).
11. Percent change from baseline in 28-day seizure frequency during the maintenance phase of the double-blind treatment phase (ie, from the end of the 4-week titration phase to the end of the 17-week double-blind phase) (major motor seizure types and all seizure types).
12. Percent change from baseline in 28-day seizure frequency during the 17-week double-blind treatment phase of the following countable focal-onset seizures types (secondary seizure types): focal motor with intact awareness or altered awareness, and focal non-motor with altered awareness.
13. Percent change from baseline in 28-day seizure frequency during the 17-week double-blind treatment phase of the following hard to count seizure types (tertiary seizure types): focal non-motor with intact awareness, absence, myoclonic, epileptic spasms.
14. Number (%) of subjects considered treatment responders, defined as those with a ≥ 25%, and ≥ 75% reduction from baseline in seizure frequency.

15. Percent change from baseline in 28-day seizure frequency during the 17-week double-blind treatment phase per seizure subtype.
16. Changes from baseline in Children's Sleep Habit Questionnaire (CSHQ).
17. Changes from baseline in ADAMS.
18. Changes from baseline in neurosteroid levels.
19. Number (%) of subjects taking rescue medication during the 17-week double-blind treatment phase.
20. Number of doses of rescue medication.
21. Changes from baseline in quality-of-life scales (PSI and QI-Disability)

The 28-day **major motor seizure (or primary seizure) frequency** was based on an electronic seizure diary (eDairy) for each subject. Primary seizure types of bilateral tonic (sustained motor activity ≥ 3 seconds), generalized tonic-clonic, bilateral clonic, atonic/drop or focal to bilateral tonic-clonic seizures will be counted towards the primary endpoint.

Parent/caregiver was to record daily seizure frequency denoting seizure type and frequency, ASMs (rescue medications) and compliance with the investigational product treatment in an electronic seizure diary. Seizures were to be noted as occurring as individual seizures, seizures occurring in a cluster with countable seizures or seizures occurring in a cluster with uncountable seizures. In rare cases when an eDiary completion was not feasible, a paper seizure calendar was to be used to log in daily seizure type and frequency (with approval by the Sponsor).

To standardize seizure identification and classification in the study, a Seizure Identification and Diagnostic Review Form (SIF/DRF) were submitted and reviewed by the Epilepsy Study Consortium. Approval of the SIF/DRF was required prior to randomization. In addition to this form, videos of the subject's seizure types were reviewed to confirm proper classification provided that the parent/caregiver. Parents/caregivers had to make every effort to capture one example of each of the primary seizure types that their child experiences.

A **responder analysis** in which subjects with a percent reduction from baseline in 28-day major motor seizure frequency during the double-blind treatment period of at least 50 percentage points were identified and the overall proportion within each treatment group was tabulated.

The **Clinical Global Impression of Improvement (CGI-I)** is a 7-point Likert scale that the parent/caregiver and clinician used to rate the change in overall seizure control, behaviour, safety and tolerability after initiation of investigational product relative to baseline (1- very much improved, 2- much improved, 3-minimally improved, 4- no change, 5- minimally worse, 6- much worse, and 7- very much worse).

The **Caregiver Global Impression of Change in Seizure Intensity/ Duration (CGICSID)** is a 7-point Likert scale (1- very much improved, 2- much improved, 3-minimally improved, 4- no change, 5- minimally worse, 6- much worse, and 7- very much worse). The parent/caregiver assesses change in seizure intensity and/or duration after initiation of the investigational product relative to baseline.

The **Caregiver Global Impression of Change in Attention (CGICA)** is a 7-point Likert scale (1- very much improved, 2- much improved, 3-minimally improved, 4- no change, 5- minimally worse, 6- much worse, and 7- very much worse) in which the parent/caregiver assesses change in attention after initiating investigational product relative to baseline.

The **Caregiver Global Impression of Change (CGI-C– target behavior)** is a 7-point Likert scale (1- very much improved, 2- much improved, 3-minimally improved, 4- no change, 5 - minimally

worse, 6- much worse, and 7- very much worse) in which the caregiver chooses one domain from possible domains of sociability, communication, irritability, and hyperactivity to assess change in target behaviour after the initiation of investigational product relative to baseline.

The **Anxiety, Depression, and Mood Scale (ADAMS)** is a rating scale designed to screen for anxiety and depression in individuals with intellectual disability. The 28-question scale is filled out by the parent/caregiver and is based on the subject's behaviour.

The **Children's Sleep Habits Questionnaire (CSHQ)** is a psychological questionnaire designed to measure sleep behaviours in children and adolescents. The 22-question test was filled out by the parent/caregiver.

The **Parenting Stress Test (PSI)** 4th edition is designed to evaluate the magnitude of stress in the parent-child system. It focuses on three major domains of stress: child characteristics, parent characteristics and situational/demographic life stress. This is an 18-item parent report assessment, with each item rated on a 5-point Likert scale (1- strongly disagree, 2- disagree, 3- undecided, 4- agree, and 5- strongly agree).

The **Quality-of-Life Inventory- Disability (QI-Disability)** is a parent/caregiver reported quality of life scale developed for children with intellectual disabilities. The scale measures six domains over the past month: physical health, positive emotions, negative emotions, social interaction, leisure and the outdoors, and independence. Each question will be rated categorically (never, rarely, sometimes, often, and very often).

To determine the **neurosteroid levels**, blood samples will be drawn at Visit 1 (screening), Visit 5 (Week 17), and at the Final OL Visit.

An additional exploratory endpoint was the measurement of EEG. A routine EEG was to be performed at Visit 2 (baseline), Visit 5 (Week 17) in the double-blind phase, and to be assessed at the Final OL Visit to record seizure activity. All EEG study data collected were to be submitted to a central reviewer for interpretation.

- **Sample size**

Based on data from the 7 patients in Study 1042-0900 evaluating GNX in CDKL5 patients, the standard deviation for the percent change in 28-day seizure frequency was estimated to be 44.5. Therefore, with a difference in 28-day seizure frequency between GNX and PBO, a trial with 100 subjects randomized 1:1 would have 92% power to detect this effect when using an analysis of variance (ANOVA) that preserves a (one-sided) 2.5% false-positive error rate. The actual analysis will use a Wilcoxon rank-sum test, which has approximately the same power as the ANOVA.

- **Randomisation and Blinding (masking)**

Subjects were randomly assigned at a 1:1 ratio (GNX: PBO) based on a randomization schedule at Visit 2 (Week 0). An IWRS centrally randomized subjects and instructed the investigator which numbered bottle to use to dose a subject. The content of each bottle was blinded using labels. Only the investigational product supplier and the sponsor's investigational product manager were unblinded as to the contents of each bottle of investigational product. Study staff as well as subject and caregiver were blinded to treatment

- **Statistical methods**

The Intent to Treat (ITT) **population** comprised all randomized subjects who received at least one dose of the investigational product. Treatment groups were defined according to the treatment assignment at randomisation. The ITT population was the primary population for the efficacy analyses and for the concomitant ASM level and neurosteroid level analyses. The safety population comprised all

randomised subjects who received at least one dose of investigational product. Treatment groups were defined according to the treatment actually received. All safety analyses were performed in the Safety Population. The Per-Protocol (PP) population consisted of ITT subjects who received study drug for at least 6 weeks, provided at least 5 weeks of post-baseline seizure data, and had no major protocol violations (defined prior to database lock). A supportive analysis of the primary and secondary efficacy endpoints was conducted in this population.

Subject demographics, characteristics, and medical history at randomization was summarized using descriptive statistics.

The **estimand** framework for the primary endpoint is as follows:

Population: target study population comprises children and young adults with CDKL5 Deficiency Disorder (CDD) who also meet the inclusion and exclusion criteria as specified in the study protocol. The analysis population is the ITT Population defined as all randomized participants.

Variable: The variable is the primary efficacy endpoint, the percentage change from baseline in 28-day primary seizure frequency during the 17-week DB treatment phase.

Population-Level Summary: The population-level summary will be the difference of the percentage change on primary efficacy endpoint between GNX and PBO groups and its 2-sided 95% CI.

Intercurrent Events (ICEs) and their handling rules are discontinuations due to lack of efficacy and discontinuations due to expected AEs. Both ICEs were addressed by the treatment policy strategy. The use of rescue medication was allowable at any time during the study. A patient may have required rescue medication during the trial but continued with the trial post-rescue medication. Therefore, the efficacy of the trial treatment was be assessed regardless of the need for rescue medication.

A supplemental estimand was defined post-hoc, considering the ICE rescue medication as treatment failure.

The **primary efficacy endpoint** was the percent change in 28-day primary seizure frequency through the end of the double-blind treatment phase relative to the baseline period. Baseline and post-baseline 28-day seizure frequency were calculated as the total number of seizures in the baseline phase or the 17-week DB treatment phase divided by the number of days with seizure data in that phase, multiplied by 28. The calculation for percent change from baseline in 28-day seizure frequency was done as follows for each subject:

$$\left(\frac{[(\text{Post-baseline 28-day seizure frequency}) - (\text{Baseline 28-day seizure frequency})]}{(\text{Baseline 28-day seizure frequency})}\right)\times 100\%$$

The baseline, post-baseline, and arithmetic and percent changes from baseline in 28-day seizure frequency were summarized using descriptive statistics. The difference between the treatment groups in the percent changes was tested for statistical significance at a 2-sided significance level of 0.05, using the Wilcoxon Rank-Sum test (since skewness and/or outliers were anticipated). Moreover, an estimate of the median difference between GNX and PBO, together with the 95% confidence interval, was calculated by the Hodges–Lehmann approach that is used to compare the treatment effect while the data is non-normal distributed.

Intermittent (random/sporadic) **missing data** during the baseline and DB phase were assumed missing completely at random, and the collected data were used to calculate the 28-day seizure frequencies. For early drug termination prior to the end of the 17-week DB phase, caregivers were instructed to continue to provide daily seizure records until the end of the 17-week DB phase.

Three **sensitivity analyses** of the primary efficacy endpoint were performed. Missing data handling for subjects that stop recording was tested using placebo-based imputation and worst case (3rd highest count) imputation. A third sensitivity analysis tested the effect in the subgroup of patients with low Allo-S levels (≤ 2500 pg/mL at baseline).

While the study was ongoing, the applicant was notified that the eDiary could prompt parent/caregivers to re-enter some seizure and medication information, leading to overreporting. In order to mitigate this issue and only map original records for analysis, the eDiary vendor (Signant Health) reviewed all records collected in the eDiary; all confirmed duplicate (second) records were flagged and were not presented in the final datasets.

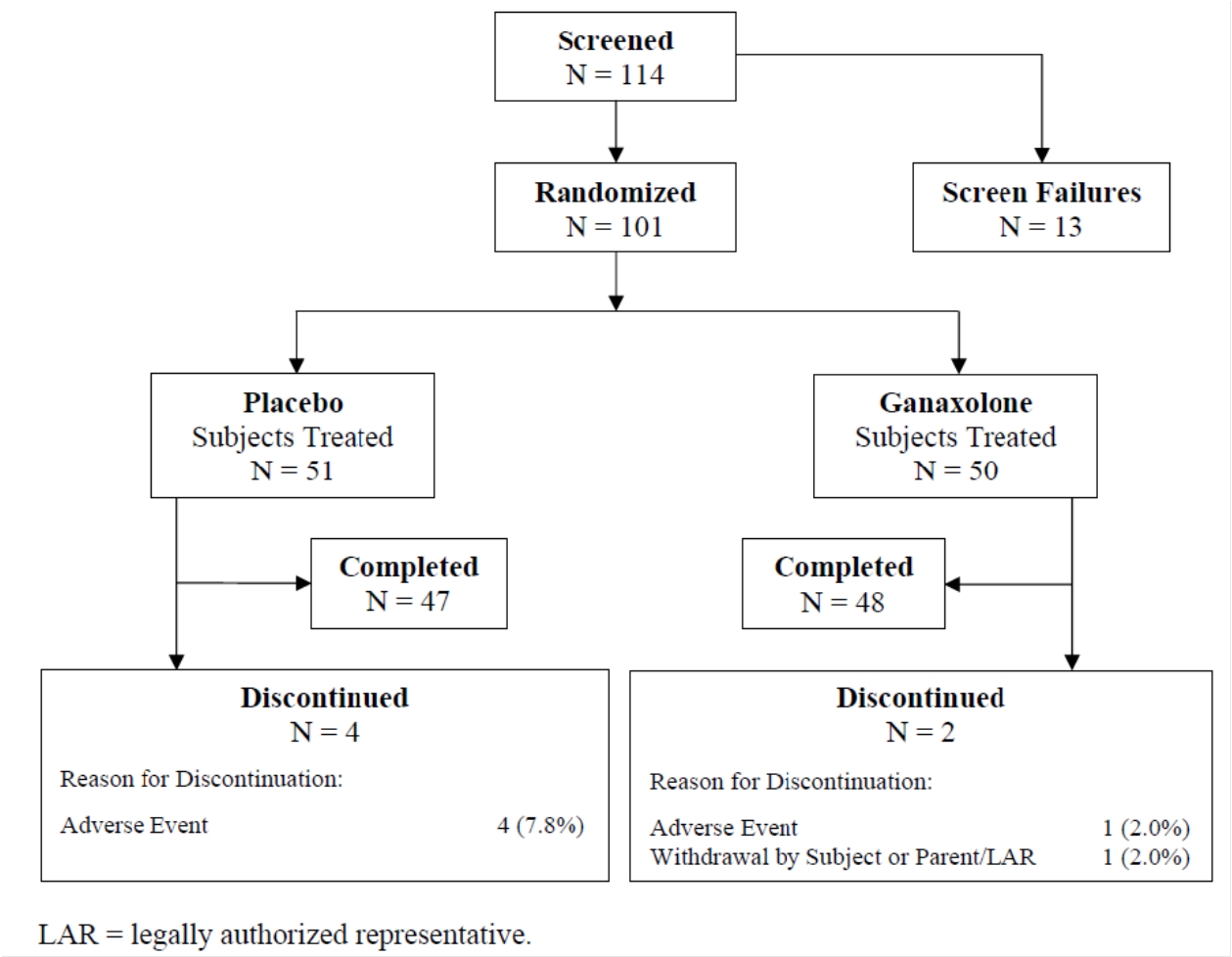
The **key secondary endpoints** were the number (%) of subjects with a ≥50% reduction from baseline in primary seizure frequency and the Clinical Global Impression of Improvement (CGI-I) at the last scheduled visit in the 17- week DB treatment phase. The response rate was analysed using Fisher's Exact test, and the corresponding p-value was provided as well as the difference in proportions along with the 95% CI for the difference. In addition, a cumulative responder curve was provided. The CGI-I score was analysed using ordinal logistic regression. The estimated odds ratios, 95% CI and the p-value was presented.

There was a single primary efficacy endpoint, and formal hypothesis testing was performed for this endpoint first. If the null hypothesis was rejected for the primary efficacy endpoint, statistical hypothesis testing was performed on the 2 key secondary endpoints. First, the responder endpoint was tested, and if statistically significant, the testing process continued with CGI-I.

For other secondary endpoints, the baseline and post-baseline values and the arithmetic changes from baseline were summarized using descriptive statistics for change from baseline endpoints, and the number and percentage of subjects with each score were summarized for score endpoints.

Results

- **Participant flow**



LAR = legally authorized representative.

At the end of the 4-week titration phase, 9 out of 10 of the subjects weighing > 28 kg in the GNX group and 9 out of 11 subjects in the PBO group achieved the optimal dose level of 1800 mg/day, and 31 out of 40 subjects weighing ≤ 28 kg in the GNX group and 33 out of 40 subjects in the PBO group achieved the optimal dose level of 63 mg/kg/day TID.

Overall, 11 subjects in the GNX group and 12 subjects in the PBO group needed a dose reduction after reaching the optimal dose (1800 mg/day for subjects weighing > 28 kg, 63 mg/kg/day for subjects weighing ≤ 28 kg) during titration.

Of 101 subjects randomized, 95 (94.1%) completed the 17-week double-blind phase; 6 (5.9%) subjects discontinued the study. The most common reasons for premature discontinuation are listed in Table 13.

Table 10: Subject Disposition and Reason for Study Discontinuation (17 Week Double blind Phase)

Category	Placebo n (%)	Ganaxolone n (%)	Total n (%)
Subjects screened			114
Not randomized[a]			13 (11.4)
Screen failures[a]			13 (11.4)
Non-screen failures[a]			0
Randomized[a]	51	50	101 (88.6)
Safety/ITT population[b,c]	51 (100)	50 (100)	101 (100)
PP Population[b,d]	48 (94.1)	48 (96.0)	96 (95.0)

Category	Placebo n (%)	Ganaxolone n (%)	Total n (%)
Subjects who completed 17-week DB phase[e]	47 (92.2)	48 (96.0)	95 (94.1)
Subjects who completed 17-week DB phase but stopped taking study drug before the end[e]	0	3 (6.0)	3 (3.0)
Reason for discontinuation[e]			
AE	4 (7.8)	1 (2.0)	5 (5.0)
Lost to follow-up	0	0	0
Lack of efficacy	0	0	0
Physician decision	0	0	0
Withdrawal by subject or parent/LAR	0	1 (2.0)	1 (1.0)
Protocol violation/protocol deviation	0	0	0
Death	0	0	0
Sponsor decision	0	0	0
Other	0	0	0

AE = adverse event; ITT = intent-to-treat; PP = per protocol; DB = double-blind; LAR = legally authorized representative.

a Percentages are based on screened subjects.

b Percentages are based on randomized subjects.

c The safety and intention-to-treat populations include all randomized subjects who received at least 1 dose of study drug.

d The PP population includes ITT subjects who received study drug for at least 6 weeks, provided at least 5 weeks of post-baseline seizure data, and had no major protocol violations.

e Percentages are based on safety population.

- **Baseline data**

The study population was mostly female (79.2%), and the mean age for all subjects was 7.26 years old (range: 2.0 to 19.0 years) (see Table 14).

Table 11: Demographic Characteristics and Physical Measurements at Baseline (Safety Population)

Characteristic	Placebo N = 51	Ganaxolone N = 50	Total N = 101
Age(years)			
n	51	50	101
Mean (SD)	7.73 (4.382)	6.78 (4.705)	7.26 (4.547)
Median	7.00	5.00	6.00
Q1, Q3	4.00, 11.00	3.00, 10.00	3.00, 10.00
Min, Max	2.0, 19.0	2.0, 19.0	2.0, 19.0
Sex, n (%)			
Male	10 (19.6)	11 (22.0)	21 (20.8)
Female	41 (80.4)	39 (78.0)	80 (79.2)
Ethnicity, n (%)			
Hispanic or Latino	6 (11.8)	4 (8.0)	10 (9.9)
Not-Hispanic or Latino	43 (84.3)	44 (88.0)	87 (86.1)
Unknown	1 (2.0)	1 (2.0)	2 (2.0)
Not reported	1 (2.0)	1 (2.0)	2 (2.0)
Race, n (%)			
White	47 (92.2)	46 (92.0)	93 (92.1)
Black or African American	0	0	0
Asian	3 (5.9)	2 (4.0)	5 (5.0)
American Indian or Alaska Native	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Unknown	0	0	0
Not reported	0	0	0
Other	1 (2.0)	2 (4.0)	3 (3.0)
Country			
Australia	2 (3.9)	4 (8.0)	6 (5.9)
France	3 (5.9)	3 (6.0)	6 (5.9)
Israel	1 (2.0)	0	1 (1.0)
Italy	6 (11.8)	9 (18.0)	15 (14.9)
Poland	5 (9.8)	5 (10.0)	10 (9.9)
Russian Federation	7 (13.7)	7 (14.0)	14 (13.9)
United Kingdom	3 (5.9)	4 (8.0)	7 (6.9)
United States	24 (47.1)	18 (36.0)	42 (41.6)

Characteristic	Placebo N = 51	Ganaxolone N = 50	Total N = 101
Height (cm)			
n	51	50	101
Mean (SD)	119.60 (19.554)	112.56 (20.809)	116.11 (20.392)
Median	119.00	108.95	115.00
Q1, Q3	102.00, 133.00	99.30, 129.60	101.00, 130.40
Min, Max	82.5, 160.0	48.5, 160.0	48.5, 160.0
Weight (kg)			
n	51	50	101
Mean (SD)	22.45 (9.054)	19.99 (8.866)	21.23 (9.002)
Median	19.10	16.80	18.30
Q1, Q3	15.80, 27.40	13.50, 23.90	14.60, 26.00
Min, Max	8.7, 46.5	9.7, 42.0	8.7, 46.5
BMI (kg/m^2)			
n	51	50	101
Mean (SD)	15.14 (2.632)	15.94 (8.643)	15.53 (6.343)
Median	14.50	14.64	14.50
Q1, Q3	13.04, 16.51	12.88, 16.30	12.94, 16.30
Min, Max	11.9, 22.7	11.2, 73.1	11.2, 73.1

BMI = body mass index; Max = maximum; Min = minimum; Q1 = first quartile; Q3 = third quartile.

Genetic testing

More than half of the subjects in the GNX (33 [66.0%] subjects) and PBO (26 [51.0%] subjects) groups had confirmed pathogenic CDKL5 variants. For the other subjects, likely pathogenic CDKL5 variants were identified in 11 (22.0%) subjects in the GNX group and 20 (39.2%) subjects in the PBO group and variants of unknown significance were identified in 6 (12.0%) subjects in the GNX group and 5 (9.8%) subjects in the PBO group. Two (4.0%) subjects in the GNX group also had a variant of unknown significance in the kinase domain.

Seizures

All subjects had seizure onset by 1 year of age. The median age at the first seizure for all subjects was 2.0 months (range: 0 to 14 months).

Historical seizure information recorded in the 2 months prior indicates that bilateral tonic seizures were the most frequent seizure types recorded during the 2 months historical control period, with a median number of seizures of 20.0 (range: 0 to 379) for all subjects (median number of seizures of 20.5 [range: 0 to 379] for subjects in the GNX group and 18.0 [range: 0 to 203] for subjects in the PBO group) during Month 1, and median number of seizures of 18.0 (range: 0 to 334) for all subjects (median number of seizures of 21.0 [range: 0 to 334] for subjects in the GNX group and 18.0 [range: 0 to 189] for subjects in the PBO group) during Month 2. Of all seizures captured during the diagnostic review, the most frequently observed were bilateral tonic seizures, observed in 31 (30.7%) subjects (13 [26.0%] subjects in the GNX group and 18 [35.3%] subjects in the PBO group)

Anti-epileptic Drug Medications

Overall, 98 (97.0%) subjects (48 [96.0%] subjects in the GNX group, 50 [98.0%] subjects in the PBO group) used any prior ASM medication. The median number of ASM medications taken and stopped prior to treatment for all subjects was 7 (range: 1 to 16) (7 [range: 2 to 16] for subjects in the GNX group, 7 [range: 1 to 14] for subjects in the PBO group). Subjects enrolled in the study were allowed to be on a stable regimen of up to 4 concomitant antiseizure medications. Concomitant ASM

medications were used by 97 (96.0%) subjects (49 [98.0%] subjects in the GNX group, 48 [94.1%] of subjects in the PBO group).

Table 12: Summary of Prior and Concurrent ASM Medications - Study 1042-CDD-3001

	Placebo (N = 51)	**Ganaxolone (N = 50)**
Number of ASM Medications Taken and Stopped Prior to Treatment		
N	50	48
Mean (SD)	6.82 (3.293)	7.75 (3.528)
Median	7.00	7.00
Q1, Q3	4.00, 9.00	5.00, 10.00
Min, Max	1.0, 14.0	2.0, 16.0
Prior ASM Medications (≥ 20% of Subjects)		
Levetiracetam (Keppra)	32 (62.7)	36 (72.0)
Topiramate (Topamax)	30 (58.8)	31 (62.0)
Vigabtrin (Sabril)	25 (49.0)	28 (56.0)
Phenobarbital	28 (54.9)	23 (46.0)
Valproate/Valproic Acid (Depakote)	23 (45.1)	26 (52.0)
Clobazam	26 (51.0)	21 (42.0)
Lamotrigine (Lamictal)	15 (29.4)	19 (38.0)
Clonazepam	17 (33.3)	14 (28.0)
Rufinamide (Banzel)	17 (33.3)	12 (24.0)
Cannabidiol (Cbd)	9 (17.6)	17 (34.0)
Carbamazepine (Tegretal)	13 (25.5)	10 (20.0)
Oxcarbazepine (Trileptal)	10 (19.6)	13 (26.0)
Zonisamide (Zonegran)	12 (23.5)	11 (22.0)
Number of Concomitant ASM Medications		
N	51	50
Mean (SD)	2.18 (1.144)	2.62 (1.398)
Median	2.00	2.00
Q1, Q3	1.00, 3.00	2.00, 4.00
Min, Max	0.0, 5.0	0.0, 6.0
Concomitant ASM Medications (≥ 20% of Subjects)		
Valproate Semisodium	16 (31.4)	18 (36.0)
Levetiracetam	13 (25.5)	13 (26.0)
Clobazam	13 (25.5)	12 (24.0)
Vigabatrin	12 (23.5)	10 (20.0)

Refer to source tables in 1042-CDD-3001 CSR for full data.
Source: 1042-CDD-3001 CSR, Table 14.1.2.1, Table 14.1.5, Table 14.1.7.3.1, Table 14.1.7.3.

Non anti-seizure Drug Medications

Overall, 21 (20.8%) subjects (7 [14.0%] subjects in the GNX group, 14 [27.5%] subjects in the PBO group) used any prior non-ASM medication.

Concomitant non-ASM medications were used by 89 (88.1%) subjects (42 [84.0%] subjects in the GNX group, 47 [92.2%] subjects in the PBO group). The most frequent (used by ≥ 10 subjects in either treatment group) concomitant non-ASM medications were paracetamol (15 [30.0%] subjects in the GNX group, 15 [29.4%] subjects in the PBO group) and Macrogol 3350 (5 [10.0%] subjects in the GNX group, 11 [21.6%] subjects in the PBO group).

Non-pharmacological Therapies

Overall, 17 (16.8%) subjects (9 [18.0%] subjects in the GNX group, 8 [15.7%] subjects in the PBO group) had prior therapies (used and discontinued by subjects prior to the first dosing day of the study drug). A ketogenic diet was the most frequent prior therapy, administered to 6 (5.9%) subjects (3 [6.0%] subjects in the GNX group, 3 [5.9%] subjects in the PBO group); all other prior therapies were administered to ≤ 2 subjects in either treatment group.

Concomitant therapies were administered to 55 (54.5%) subjects (29 [58.0%] subjects in the GNX group, 26 [51.0%] subjects in the PBO group). The most frequent (used by ≥ 10 subjects in either treatment group) concomitant therapies were physiotherapy (20 [40.0%] subjects in the GNX group, 21 [41.2%] subjects in the PBO group), speech rehabilitation (15 [30.0%] subjects in the GNX group, 18 [35.3%] subjects in the PBO group), and occupational therapy (12 [24.0%] subjects in the GNX group, 15 [29.4%] subjects in the PBO group).

- **Outcomes and estimation**

  Primary endpoint:

  Within the 13-week maintenance portion of the 17-week double-blind phase, subjects in the GNX group had greater improvement from baseline in 28-day seizure frequency for major motor seizure types compared to subjects in the PBO group. There was a statistically significant difference in the median percent change from baseline in 28-day seizure frequency for major motor seizure types between the GNX and PBO groups (-29.39% for subjects in the GNX group, -6.49% for subjects in the PBO group; Wilcoxon Test p = 0.0081, Table 16).

Table 13: Summary of Efficacy Results – 13-Week Maintenance Phase, Intent-to-Treat Population (Study 1042-CDD-3001)

Variable	Placebo	Ganaxolone
28-Day Seizure Frequency for Primary Seizure Types, N	51	49
Baseline		
Mean (SD)	103.94 (172.983)	115.32 (138.380)
Medan (Q1, Q3)	49.17 (18.67, 120.00)	54.00 (31.33, 147.33)
13 Weeks Maintenance, Percent change		
Mean (SD)	70.19 (312.441)	-12.18 (78.345)
Median (Q1, Q3)	-6.49 (-26.77, 38.46)	-29.39 (-65.78, 1.30
Hodges-Lehmann Estimate of Location Shift (95% CI)		-29.08 (-51.17, -8.41)
Wilcoxon Test p-value		0.0097
Response Rate, N	50	49
n (%)	6 (12.0)	15 (30.6)
Difference (95% CI)		18.6 (2.0, 34.9)
p-value[a]		0.0283

[a] Response is defined as at least 50% reduction from baseline in 28-day Seizure Primary Seizures Frequency. P-value is based on Fisher's Exact test.
Source: MAA D90 Response Table 2.1.1
Notes: Seizure counts are based on the sum of the individual seizures, the countable seizures, and the clusters with uncountable seizures (each cluster with uncountable seizures counts as 1 seizure). Within the baseline and post baseline intervals, 28-day seizure frequency was calculated as the total number of seizures in the interval divided by the number of days with available seizure data in the interval, multiplied by 28.

The primary seizure types include bilateral tonic (sustained motor activity ≥3 seconds), generalized tonic-clonic, atonic/drop, bilateral clonic, and focal to bilateral tonic-clonic.
The baseline interval consists of 6 weeks prior to the first dose.

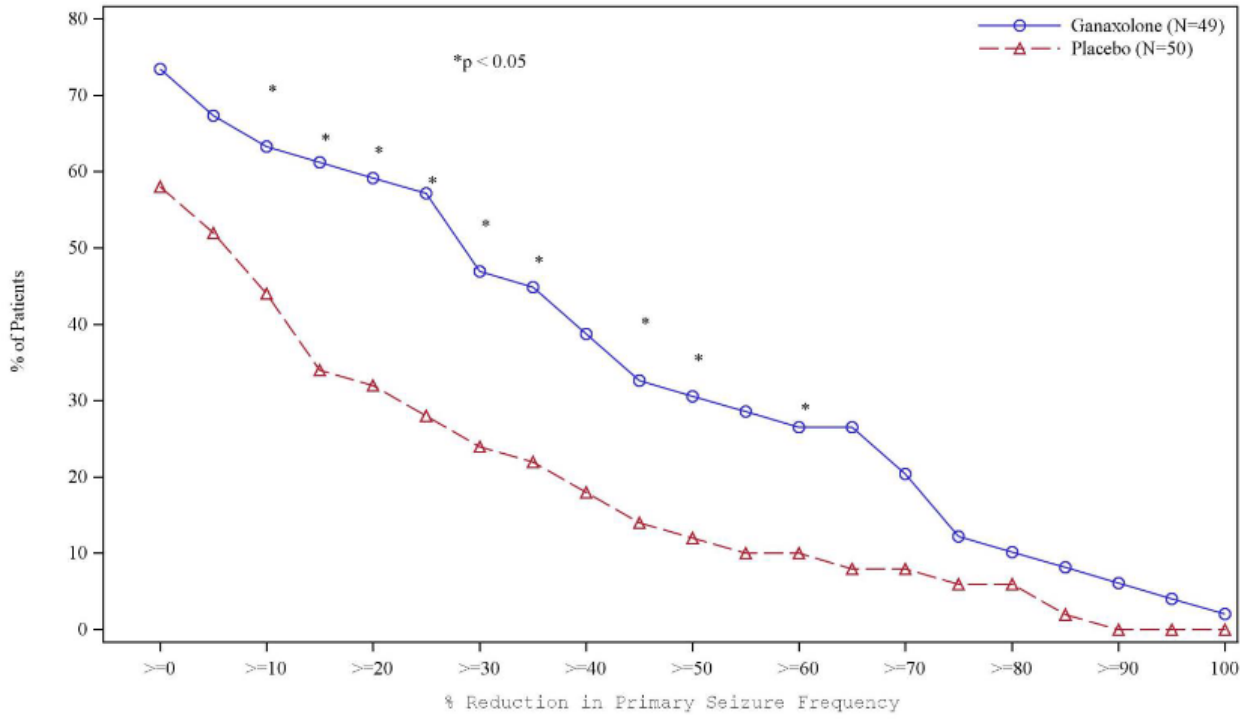
Secondary endpoints

50% responder rate

The number (%) of subjects with a ≥ 50% reduction from baseline in major motor seizure frequency (response rate) numerically favoured the GNX group (12 [24.5%] subjects in the GNX group, 5 [9.8%] subjects in the PBO group; p = 0.0643). In the maintenance phase of the study, the responder rate was nominal statistically significant in favour of GNX (p=0.0283, see Table 16).

A cumulative responder curve of 28-day seizure frequency for major motor seizure types for subjects in the ITT population over the maintenance phase is presented in Figure 7.

Figure 7: Cumulative Responder Curve of 28-Day Seizure Frequency for Major Motor Seizure Types (13 week Maintenance Phase, Intent-to-treat Population)



*p-value is based on Fisher Exact Test

CGI-I

Table 14: Response Rate and CGI-I Scores at End of 17 week Double blind Treatment Phase (ITT Population)

Variable	Placebo	Ganaxolone
Response Rate, N	51	49
n (%)	5 (9.8)	12 (24.5)
Difference (95% CI)		14.7 (-4.7, 33.8)
p-value[a]		0.0643
CGI-I (Parent/Caregiver), N	48	48
Very much improved, n (%)	1 (2.1)	0

Variable	Placebo	Ganaxolone
Much improved, n (%)	7 (14.6)	13 (27.1)
Minimally improved, n (%)	13 (27.1)	17 (35.4)
No change, n (%)	22 (45.8)	14 (29.2)
Minimally worse, n (%)	4 (8.3)	2 (4.2)
Much worse, n (%)	1 (2.1)	2 (4.2)
Very much worse, n (%)	0	0
Odds ratio (95% CI)		1.87 (0.89, 3.91)
Logistic regression p-value[b]	9	0.0971
CGI-I (Clinician), N	48	48
Very much improved, n (%)	0	0
Much improved, n (%)	7 (14.6)	7 (14.6)
Minimally improved, n (%)	13 (27.1)	19 (39.6)
No change, n (%)	19 (39.6)	16 (33.3)
Minimally worse, n (%)	9 (18.8)	2 (4.2)
Much worse, n (%)	0	3 (6.3)
Very much worse, n (%)	0	1 (2.1)
Odds ratio (95% CI)		1.41 (0.68, 2.94)
Logistic regression p-value[b]		0.3518

Seizure counts were based on the sum of the individual seizures, the countable seizures, and the clusters with uncountable seizures (each cluster with uncountable seizures counts as 1 seizure). Within the baseline and post baseline intervals, 28-day seizure frequency was calculated as the total number of seizures in the interval divided by the number of days with available seizure data in the interval, multiplied by 28.
The major motor seizure- types included bilateral tonic (sustained motor activity ≥ 3 seconds), generalized tonic clonic, atonic/drop, bilateral clonic, and focal to bilateral tonic clonic.
The baseline interval consisted of the 6 weeks prior to the first dose.
Duplicate seizure diary entries were excluded from this analysis.
CDD = cyclin-dependent kinase-like 5 deficiency disorder; CGI-I = clinical global impression of improvement; CI = 95% confidence interval; ITT = intent-to-treat.
a Response was defined as at least 50% reduction from baseline in 28-day major motor seizures frequency. P-value was based on Fisher's Exact test.
b CGI-I analysis was based on ordinal logistic regression model adjusted for treatment group as a fixed factor. The analysis is based on the CGI-I values reported at the last scheduled visit in the 17-weeks double-blind treatment phase.

Other secondary endpoints

Major motor seizure free days

The percentage of seizure-free days for major motor seizure types at baseline and at the end of the 17-week double-blind phase are presented in Table 18. The median change from baseline in the percentage of seizure-free days was 4.91% for subjects in the GNX group and 0.17% for subjects in the PBO group, with a median shift from the PBO group to the GNX group of 1.72% (Table 18).

Table 15: Summary of the Percentage of Seizure-free Days for Major Motor Seizure Types (17 Week Double-blind Phase, ITT Population)

Interval	Placebo (N = 51)	Ganaxolone (N = 50)
Baseline		
N	51	49
Mean (SD)	30.32 (27.070)	22.57 (25.761)
Median (95% Distribution-Free CI)	23.81 (10.53, 35.71)	11.90 (0.00, 30.95)
Q1, Q3	2.63, 54.76	0.00, 40.48
Min, Max	0.0, 97.5	0.0, 82.9
Change From Baseline		
N	51	49

Interval	Placebo (N = 51)	Ganaxolone (N = 50)
Mean (SD)	5.86 (15.350)	9.62 (21.364)
Median (95% Distribution-Free CI)	0.17 (0.00, 8.13)	4.91 (0.00, 11.11)
Q1, Q3	-2.97, 15.24	0.00, 15.60
Min, Max	-24.9, 53.9	-29.4, 91.4
Hodges-Lehmann Estimate of Location Shift (95% CI)[a]	1.72 (-2.71, 7.84)	

Summaries are based on the individual seizures, the countable seizures, and the clusters with uncountable seizures.

The major motor seizure types include bilateral tonic (sustained motor activity = 3 seconds), generalized tonic-clonic, atonic/drop, bilateral clonic, and focal to bilateral tonic-clonic.

Within the baseline and post baseline intervals, the percentage of seizure-free days is the number of days in the interval with zero seizures divided by the number of days with seizure data in the interval, multiplied by 100.

The baseline interval consists of the 6 weeks prior to the first dose.

The 17-week postbaseline interval consists of the first day after the first dose up to the day before Visit 5 (Week 17), if available; otherwise up to the last day with seizure data. However, if a subject successfully completes the double-blind phase without a Visit 5, with a Taper Visit, and does not enter the open-label extension, then the interval ends the day before the Taper Visit.

Duplicate seizure diary entries were excluded from this analysis.

CI = 95% confidence interval; Max = maximum; Min = minimum; Q1 = first quartile; Q3 = third quartile; SD = standard deviation.

a An estimate of how far the responses in the GNX group are shifted from the PBO group

Adapted by Assessor to improve readability

Caregiver Global Impression of Change in Seizure Intensity/duration

The majority of GNX subjects showed CGI-CSID scores of very much improved, much improved, or minimally improved (nominal p = 0.015) at their last visit when administered by the parent/caregiver (see Table 19Table 19:).

Table 16: Summary of Caregiver Global Impression of Change in Seizure Intensity/duration (17 week Double blind Phase, ITT Population)

Visit Category	Placebo (N = 51)	Ganaxolone (N = 50)
Visit 5 (End of Week 17)		
Total observed, n	47	45
Very much improved	1 (2.1%)	2 (4.4%)
Much improved	5 (10.6%)	15 (33.3%)
Minimally improved	11 (23.4%)	11 (24.4%)
No change	21 (44.7%)	10 (22.2%)
Minimally worse	5 (10.6%)	3 (6.7%)
Much worse	4 (8.5%)	2 (4.4%)
Very much worse	0	2 (4.4%)

Percentages are based on the number observed.

ITT = intent-to-treat

Table adapted by Assessor to improve readability

Behavioral/Neuropsychiatric Evaluation

For the CGICA and CGI-C, in general higher proportion of caregivers of subjects in the GNX group reported any improvement ("minimally improved, "much improved," or "very much improved") in attention at Visit 3, Visit 4, and Visit 5 compared to caregivers of subjects in the PBO group. However, at the end of Week 17 the CGICA result favouring GNX group was of only "minimally improved" (46.7% vs 29.8% for the PBO group) and the CGI-C result was of only "much improved" (44.4% vs 30.4% for the PBO group).

Exploratory endpoints

Median percent change from baseline in seizure frequency – all types

The median percent change from baseline in seizure frequency for all seizure types was -19.09% for subjects in the GNX group and -8.91% for subjects in the PBO group, with a median shift from the PBO group to the GNX group of -17.38%, indicating improvement in the GNX group compared to the PBO group.

Percent Change from Baseline in 28-day Seizure Frequency per Seizure Subtype

For the following seizure types, which were considered part of major motor seizures, a numerical improvement for GNX over placebo could be observed: bilateral tonic (median percent decrease from baseline -16.75%), generalized tonic-clonic (-47.89%), atonic/drop, (-49.18%), bilateral clonic (-159.05%), and focal to bilateral tonic-clonic (-150.0%) seizures.

With regard to the remaining seizure subtypes measured during the study, a numerical improvement in seizure frequency in the GNX group over placebo was observed with the exception of focal motor with intact awareness or altered awareness seizures and epileptic spams. In the former, a greater reduction in seizure frequency was reported for subjects treated with placebo (-52.56%, n=6) compared to GNX-treated subjects (-16.19%, n=10). For the latter, worsening of epileptic spasms was recorded for subjects treated with GNX (1.55%, n=12), whereas a minor reduction was seen for subjects treated with placebo (-3.66%, n=10).

Use of rescue medication

Twenty-five (50.0%) subjects in the GNX group and 22 (43.1%) subjects in the PBO group took at least 1 rescue medication during the 17-week double-blind phase, and 20 (40.0%) and 17 (33.3%), respectively, in the maintenance phase. See Table 20 for an overview of the most commonly used rescue medication during the maintenance phase. Of those subjects who took at least 1 rescue medication, the median number of doses taken was 8.0 (range: 1 to 112) for subjects in the GNX group and 6.0 (range: 1 to 79) for subjects in the PBO group. The median number of days rescue medication was used was lower in the ganaxolone arm (462; 273 - 560) than in the placebo arm (499; 406 - 740). Sensitivity analysis assessing the influence of handling rescue medication in the analysis is described below.

Table 17: Summary of Rescue Medication Use in Patients Taking the Most Commonly Used ASMs – Maintenance Phase, Intent-to-Treat Population (Study 1042-CDD-3001)

	Placebo (N = 51) n (%)	**Ganaxolone (N = 50) n (%)**
Number of Days on ASM and ASM Rescue Medications During the Phase		
Mean (SD)	2049.3 (1237.89)	2071.5 (1668.84)
Median (95% Distribution-Free CI)	1845.0 (1319.0, 2269.0)	1564.0 (1247.0, 1954.0)
Q1, Q3	1051.5, 2713.0	1148.0, 2585.0
Min, Max	3, 4802	206, 7843
Valproate		
Yes	16 (31.4)	18 (36.0)
No	35 (68.6)	32 (64.0)
Levetiracetam		
Yes	13 (25.5)	13 (26.0)

	Placebo (N = 51) n (%)	Ganaxolone (N = 50) n (%)
No	38 (74.5)	37 (74.0)
Clobazam		
Yes	15 (29.4)	13 (26.0)
No	36 (70.6)	37 (74.0)
Vigabatrin		
Yes	12 (23.5)	10 (20.0)
No	39 (76.5)	40 (80.0)

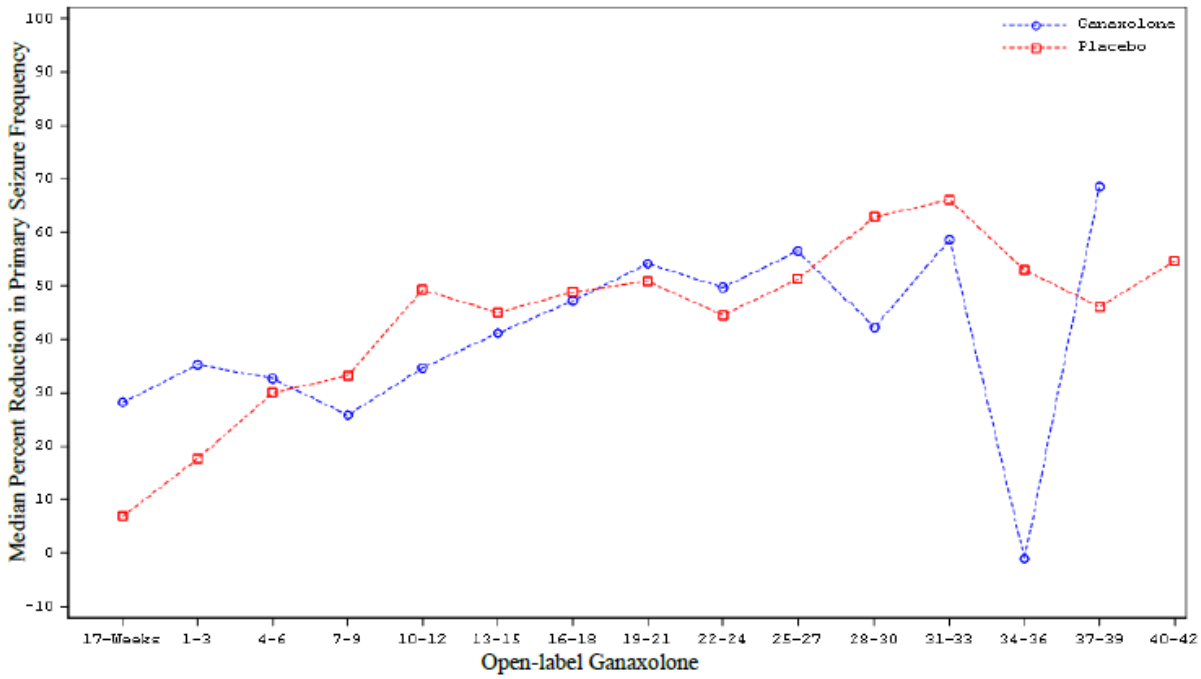
ASM = Anti-seizure medications; CI = confidence interval; Max = Maximum; Min = Minimum
Percentage is based on the number of subjects in each treatment group.
Source: MAA D90 Response Table 1.1.3a

Open-label extension phase

88 subjects continued from the double-blind treatment period into the open-label extension. As of the data cut-off 30 June 2022, 40 subjects are still in the study.

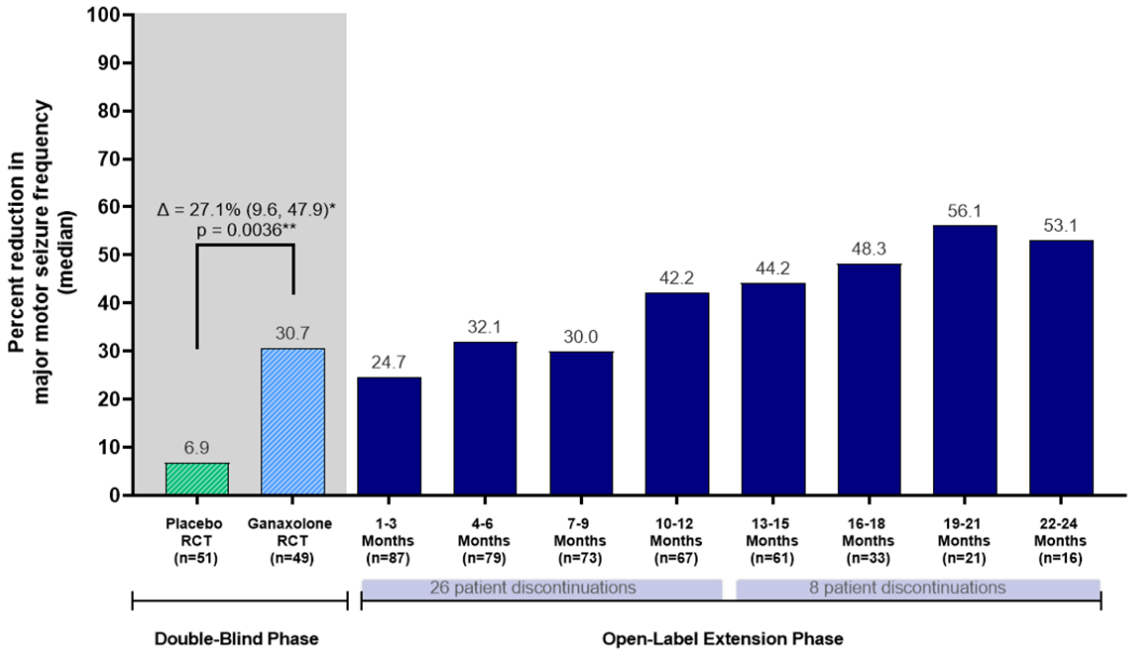
Primary endpoint – change from baseline in median major motor seizure frequency

Figure 8: Percent Reduction in Major Motor Seizure Frequency at the End of the Double-blind Phase and Then at 3-month Intervals Within the Open-label Extension Phase (Intent-to-treat Population, data cut-off:)



Only subjects that completed a 3-month interval were included at that time point. Sample size varies due to subject discontinuations and due to subjects still ongoing within the open-label extension. Subjects are grouped by their treatment assignment during the double-blind phase. All subjects received open-label ganaxolone in the open-label extension independent of their double-blind treatment assignment. Source: 2-year OLE interim report,

Figure 9: Percent reduction in major motor seizure frequency - Study 3001 Open Label Extension phase (data cut-off 22 June 2021)



- **Ancillary analyses**

The three sensitivity analyses and the per-protocol analyses supported the primary analysis. Post-hoc sensitivity analyses were provided where rescue medication was seen as a treatment failure. For the primary endpoint, a tipping point analysis penalising rescue treatment from 0 to 30% in both study arms showed that rescue medication did not affect the treatment estimate. In another analysis penalising the ganaxolone arm only, the treatment difference was no longer significant when rescue treatment would reduce the treatment effect in the ganaxolone arm by 25%. For the 50% responder endpoint, regarding rescue medication as non-response reduced the number of responders. In the primary analysis, 12 (24.5%) subjects in the ganaxolone group and 5 (9.8%) subjects in the placebo group showed a 50% reduction from baseline in major motor seizure frequency (p = 0.064), including rescue medication as non-response lowered the responders to 6 (12.2%) and 3 (6.0%) (p = 0.3178).

Adult population

In the double-blind phase of study 3001, there were two subjects > 18 years of age at study entry. Both subjects were 19 year-old, one was randomized to placebo and the other to GNX.

Additional limited efficacy data on use of GNX for the treatment of seizures associated with CDD in adult patients comes from the OLE phase of study 3001 (see below).

Table 18: Percent Change from Baseline to Last 3-Month Open-Label Period in Subjects Aged 15-19

DB Treatment	Last OL period (Months)	Percent reduction in MMSF	Seizure Types				
			At	T	C	TCS	Focal
PBO	40-42	86.2%	x	x		x	
PBO	37-39	47.4%		x		x	x
PBO	28-30	58.1%	x	x			x
PBO	13-15	32.1%	x	x	x	x	x
GNX	28-30	30.4%		x		x	x
GNX	31-33	89.8%		x	x	x	x
GNX	19-21	10.5%		x		x	
GNX	28-30	-72.7%				x	x
GNX	25-27	-21.6%		x		x	x

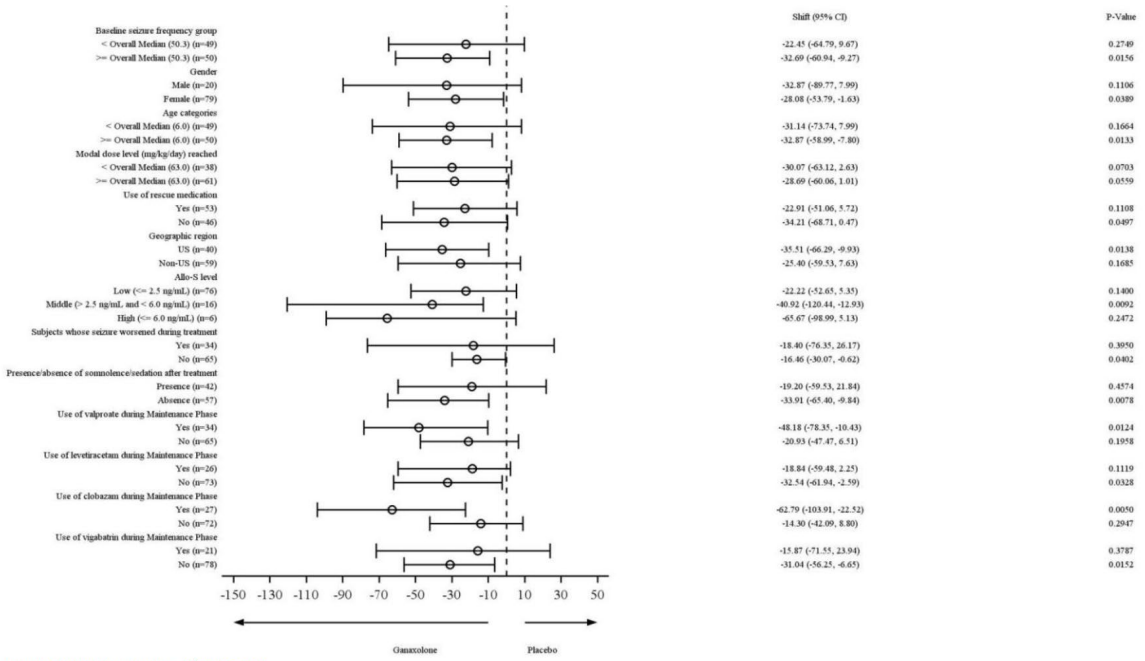
Abbreviations: DB=double-blind; PBO=placebo; GNX=ganaxolone; OL=open-label; MMSF=major motor seizure frequency; At=atonic; T, tonic; C, clonic; TCS, tonic-clonic seizures (focal-to-bilateral or generalized); Focal, all focal onset seizures

Source: MAA D180 Response Listing 23.2.6C and 16.2.3.7.2B ; 1042-CDD-3001 Listing 16.2.3.1

Subgroup analyses

Subgroup analyses are displayed in the forest plot below:

Figure 10 Figure 13 Forest Plot of Percent Change in 28-day Major Motor Seizure Frequency by Subgroup



Source: MAA D90 Responses, Figure 2.4.3

Subgroup analyses – concomitant valproate, clobazam and vigabatrin

Additional subgroup analyses were provided to further evaluate the impact of concomitant use of valproate, clobazam and vigabatrin on seizure frequency. See below.

Figure 11: Cumulative Responder Curves of 28-day Seizure Frequency for Concomitant Valproate (Yes/No) - 13-Week Maintenance Period, Intent-to-Treat Population (Study 1042-CDD-3001)

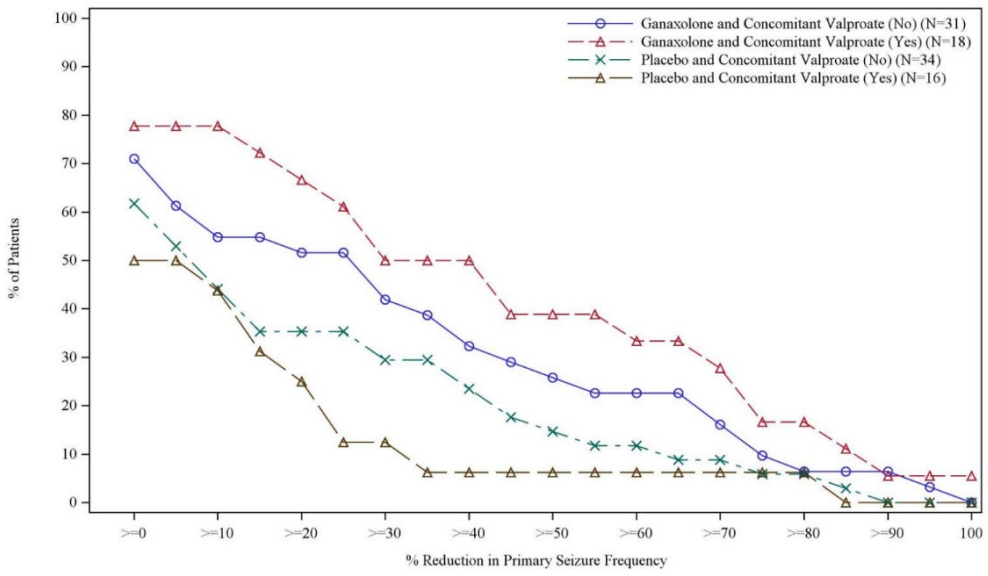


Figure 12: Cumulative Responder Curves of 28-day Seizure Frequency for Concomitant Clobazam (Yes/No) - 13-Week Maintenance Period, Intent-to-Treat Population (Study 1042-CDD-3001)

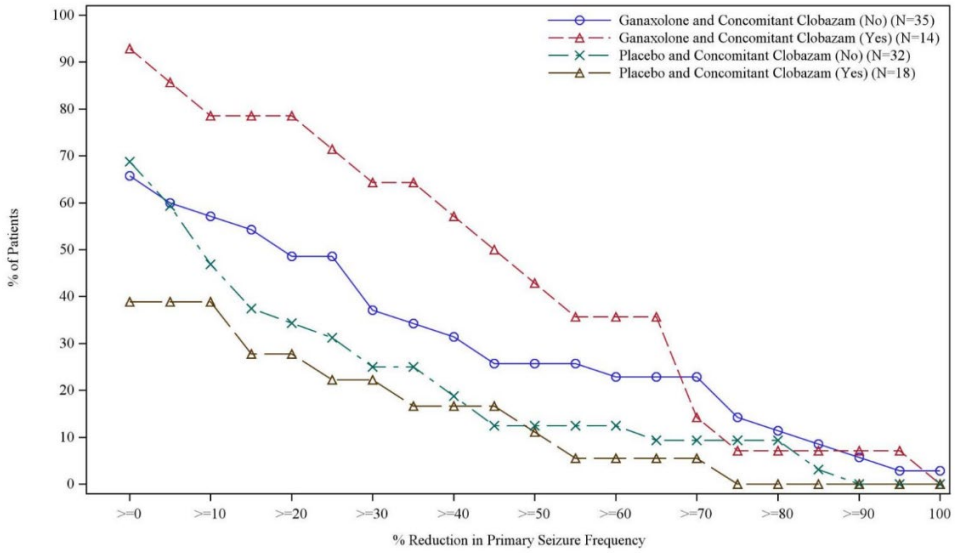
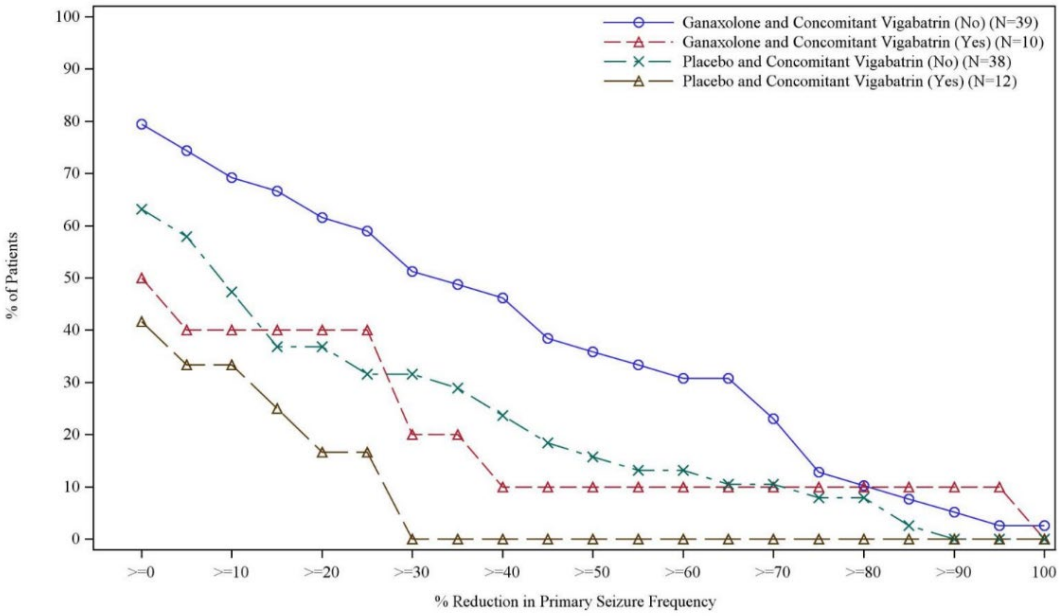


Figure 13: Cumulative Responder Curves of 28-day Seizure Frequency for Concomitant Vigabatrin (Yes/No) - 13-Week Maintenance Period, Intent-to-Treat Population (Study 1042-CDD-3001)



- **Summary of main efficacy results**

The following table summarises the efficacy results from the main study supporting the present application. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 19: Summary of efficacy for study 3001

Title: A double-blind, randomized, placebo-controlled trial of adjunctive ganaxolone treatment in children and young adults with cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD) followed by long-term open-label treatment.			
Study identifier	1042-CDD-3001 NCT03572933 EudraCT NO: 2018-001180-23		
Design	Double-blind, randomized, placebo-controlled		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		17 weeks N/A Ongoing
Hypothesis	Superiority		
Treatments groups	Placebo (PBO)		Placebo oral suspension, 17 weeks, n=51
	Ganaxolone (GNX)		Ganaxolone oral suspension, 17 weeks, n=50 For patients weighing ≤ 28 kg (mg/kg dosing) up to 63 mg/kg/day For patients weighing >28 kg up to 1800 mg/day
Endpoints and definitions	Primary Endpoint	Percent Change from Baseline in major motor seizure Frequency	Percentage change from baseline in 28-day major motor seizure frequency during the 13-week maintenance phase.
	Key Secondary	Responder Rate	Number (%) of subjects with a ≥50% reduction from baseline in major motor seizure frequency.
	Key Secondary	Clinical Global Impression of Improvement – Parent/Caregiver (CGI-C)	CGI-C in parent/caregiver/LAR identified behavioural target (potential domains include sociability,

			communication, irritability, and hyperactivity).
	Key Secondary	Clinical Global Impression of Improvement – Clinician (CGI-I)	CGI-I at the last scheduled visit in the 17-week double-blind treatment phase.
Database lock	01 Sept 2020		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Percent Change from Baseline in 28-Day Seizure Frequency for Major Motor Seizure Types (During the 13-week Maintenance Phase, ITT Population)		
Descriptive statistics and estimate variability	Treatment group	PBO (N=51)	GNX (N=50)
	Number of subjects	51	49
	Percentage change from baseline in 28-day major motor seizure frequency (median)	-6.49%	-29.39%
	95% Confidence Interval (CI)	-11.46, 20.60	-42.12, -10.46
Effect estimates per comparison	Percentage change from baseline in 28-day major motor seizure frequency during the 13-week double-blind treatment phase.	Comparison groups	PBO vs. GNX
		Hodges-Lehmann Estimate of Location Shift[a]	-29.31
		95% CI	-51.45, -8.90
		Z-value (Wilcoxon Test)	-2.6489
		P-value (Wilcoxon Test)	*0.0081*
Notes	[a] An estimate of how far the responses in the GNX group are shifted from the PBO group.		
Analysis description	**50% Responder rate**		
Analysis population and time point description	Intent to treat (ITT) During the 17-week Double-blind Treatment Phase:		
Descriptive statistics and	Treatment group	PBO (N=51)	GNX (N=50)
	Number of subjects	51	49

estimate variability	Number (%) of subjects 50% reduction from baseline in major motor seizure frequency	5 (9.8)	12 (24.5)
Effect estimates per comparison	Number (%) of subjects with a ≥50% reduction from baseline in major motor seizure frequency.	Comparison groups	PBO vs. GNX
		Difference	14.7
		95% CI	-4.7, 33.8
		P-value (Fisher's Exact Test)[a]	0.0643
Notes	Rounded to 4 decimal places and therefore presented as 0.xxxx;p-values smaller than 0.0001 as '< 0.0001'; p-values greater than 0.9999 as '> 0.9999'.		
Analysis description	**Clinical Global Impression of Improvement (Parent/Caregiver)**		
Analysis population and time point description	Intent to treat (ITT) During the 17-week Double-blind Phase:		
Descriptive statistics and estimate variability	Treatment group	PBO (N=51)	GNX (N=50)
	CGI-I (Parent/Caregiver), N	48	48
	Very Much Improved, n (%)	1 (2.1)	0
	Much Improved, n (%)	7 (14.6)	13 (27.1)
	Minimally Improved, n (%)	13 (27.1)	17 (35.4)
	No Change, n (%)	22 (45.8)	14 (29.2)
	Minimally Worse, n (%)	4 (8.3)	2 (4.2)
	Much Worse, n (%)	1 (2.1)	2 (4.2)
	Very Much Worse, n (%)	0	0
	CGI-I (Parent/Caregiver) at the last scheduled visit in the 17-week double-blind treatment phase.	Comparison groups	PBO vs. GNX
		Odds Ratio (95% CI)	1.87 (0.89, 3.91)
		P-value	*0.0971*
Analysis description	**Clinical Global Impression of Improvement (Clinician)**		

Analysis population and time point description	Intent to treat (ITT) During the 17-week Double-blind Phase:		
Descriptive statistics and estimate variability	Treatment group	PBO	GNX
	CGI-I (Clinician), n	48	48
Effect estimates per comparison	Very Much Improved, n (%)	0	0
	Much Improved, n (%)	7 (14.6)	7 (14.6)
	Minimally Improved, n (%)	13 (27.1)	19 (39.6)
	No Change, n (%)	19 (39.6)	16 (33.3)
	Minimally Worse, n (%)	9 (18.8)	2 (4.2)
	Much Worse, n (%)	0	3 (6.3)
	Very Much Worse, n (%)	0	1 (2.1)
	CGI-I (Clinician) at the last scheduled visit in the 17-week double-blind treatment phase.	Comparison groups	PBO vs. GNX
		Odds Ratio (95% CI)	1.41 (0.68, 2.94)
		P-value	*0.3518*

2.6.5.2. Supportive study(ies)

Study 1042-0900

The study was a phase 2a multicenter, 26-week open-label proof-of-concept trial evaluating GNX as adjunctive therapy for uncontrolled seizures in children with PCDH19 Epilepsy, CDD, Dravet Syndrome, and other epileptic syndromes such as LGS and CSWS followed by 52 weeks of additional open-label treatment.

Design

The study consisted of a 26-week open-label treatment period followed by a 52-week open-label extension period. For entry into the 52-week open-label extension, subjects must have completed all scheduled clinical study visits and shown a min of 35% improvement in mean seizure frequency per 28 days vs baseline over the 28-day period preceding study entry or upon sponsor approval after consultation with the principal investigator, Subjects were treated with open-label GNX oral suspension or GNX capsules at doses up to 1800 mg/day.

The **primary efficacy endpoint** was the percent change in total seizure frequency (sum of individual seizures and clusters) per 28 days relative to baseline.

A total of 30 subjects were enrolled (including 7 CDD subjects) in the 26-week open-label treatment period, and 15 subjects (50.0%) completed treatment. 15 subjects (50.0%) discontinued during the 26-week open-

label treatment period, and the main reasons were lack of efficacy (8 subjects [26.7%]) and AE or SAE (4 subjects [13.3%)]). Of the 15 subjects eligible for a 52-week extension period, 14 subjects were enrolled, and 8 (57.1%) completed treatment. 6 subjects (42.9%) discontinued treatment during the extension period and all discontinued due to a lack of efficacy.

26-week open-label treatment period

For the primary endpoint, the median percent change in 28-day total seizure frequency for the sum of individual seizures and clusters in the 26-week open-label treatment period relative to the baseline was -47.34%, -10.22%, and -25.98% at Day 91 for the CDD, LGS, and PCDH19 cohorts, respectively.

At Week 26, the median percent change from baseline was -37.70%, -9.19%, and -24.59% for the CDD, LGS, and PCDH19 cohorts, respectively.

52-week open-label treatment period

The median percentage change in 28-day total seizure frequency for the sum of individual seizures and clusters in the 52-week open-label extension period relative to the baseline was reduced in all 3 cohorts. At Day 181, the median percentage change was -58.94%, -38.74% and -13.48% for the CDD, LGS, and PCDH19 cohorts, respectively.

At Week 52, the median percentage change from baseline was -61.93%, -37.75% and -13.48% for the CDD, LGS, and PCDH19 cohorts, respectively.

2.6.6. Discussion on clinical efficacy

The efficacy of GNX for the treatment of seizures associated with CDD was investigated in a single pivotal phase 3 study 3001 and an exploratory phase 2a proof of concept basket trial study 0900.

Design and conduct of clinical studies

Study 0900

This was a 26-week open-label study evaluating GNX as adjunctive therapy for uncontrolled seizures in children with epileptic syndromes, followed by 52 weeks of additional open-label treatment. The study included subjects with seizures associated with CDD, PCDH19 Epilepsy and the Lennox-Gastaut Syndrome. The primary endpoint was the median percent change in total seizure frequency (sum of individual seizures and clusters) per 28 days relative to baseline.

Study 3001

Design

This was a phase 3 randomized, double-blind, placebo controlled study that evaluated adjunctive GNX on top of other anti-seizure medications (ASMs) in children and young adults with CDD.

The study consisted of a 6-week prospective baseline period, 17-week double-blind treatment phase with a 4 weeks titration and 13 weeks maintenance period. The study has an ongoing open-label extension phase, including an additional 4-week titration phase in order to maintain blinding for treatment received in the double-blind.

This study design is considered conventional for studies in refractory epilepsy and in line with the recommendations of the EMA Epilepsy guideline.

Target population

Overall, the main inclusion/exclusion criteria enabled the selection of a patient population that increased the likelihood of detecting the GNX's effect.

The key inclusion criteria with respect to confirmation of CDD was the requirement of molecular confirmation of a pathogenic or likely pathogenic CDKL5 variant, early-onset difficult-to-control seizures, and neurodevelopmental impairment.

Another key inclusion criterion was age 2 through 21 years (inclusive). The choice of the lower limit of 2 years is considered a reasonable compromise for a clinical trial that has the objective of showing the actual efficacy of GNX, in order to exclude the confounding effect of the presence of a "honeymoon" period.

The remaining in-and exclusion criteria are agreed.

Outcomes

The **primary endpoint** was the (median) percentage change from baseline in 28-day major motor seizure frequency over the 17-week, double-blind treatment phase. Major motor seizures, or primary seizures, are defined as bilateral tonic (sustained motor activity ≥ 3 seconds), generalized tonic-clonic, bilateral clonic, atonic/drop or focal to bilateral tonic-clonic seizures. It was agreed in previous Scientific Advice that these seizure types are considered relevant for CDD and easier to count compared to other types of seizures since they are more clearly observable (EMEA/H/SA/3732/1/2018/PED/SME/III corrigendum). The percentage change is based on median seizure frequency, which is supported given the skewed distribution of seizure frequency.

The **key secondary endpoints** are the 50% responder rate, defined as the number (%) of subjects with a ≥ 50% reduction from baseline in major motor seizure frequency and the Clinical Global Impression of Improvement (CGI-I) at the last scheduled visit in the 17-week double-blind treatment phase. The CGI-I is scored by Parents/Caregivers and Clinicians separately.

For rare epilepsies such as CDD, seizure frequency is considered acceptable as primary endpoint as long as a responder rate, which is usually preferred as the primary endpoint in the EMA Epilepsy guideline, has been included as a key secondary endpoint. This is the case for study 3001.

Additional secondary and exploratory outcome variables deemed relevant for the evaluation of GNX for seizures associated with CDD are: *days of motor seizure freedom, Clinical Global Impression of Change in target domains identified by parent/caregiver (CGI-C), Caregiver Global Impression of Change in Attention (CGICA), Caregiver Global Impression of Change in Seizure Intensity/Duration (CGICSID), and change in seizure frequency for all seizure type*.

EEG measurements were included as an exploratory pharmacodynamic endpoint in study 3001. However, the available number of EEG data available was too limited to perform any analyses.

The applicant clarified that no exit criteria (e.g., nth seizure) were used in the Study 3001, hence failed efficacy was not defined a priori, despite the recommendations of EMA Guideline that consider the treatment retention time as an important variable to allow the assessment of the global clinical effectiveness of an Anti-seizure medication. During the study, subjects could be on a stable regimen of 0-4 other ASMs. In addition, the use of rescue medication was permitted. This was also captured by an exploratory endpoint. Rescue

medication were considered as background therapy and no recommendation was made with respect to dosing.

Randomisation and blinding

The blinding procedure is considered adequate. The randomisation procedure using IWRS, is acceptable.

Statistical methods

The sample size was based on the treatment effect observed in the PoC study and the calculation is correct. It was increased from 70 to 100 in protocol amendment 1.

There are five versions of the SAP, the last two dated after the database lock only related to the OLE extension analyses

The definition for the ITT, safety and per-protocol population are considered standard and acceptable. The estimand definition, using a treatment policy strategy in case of discontinuation or use of rescue medication, together with a supplemental estimand, using a hypothetical/composite strategy in case of rescue medication (treatment failure), are agreed.

The analysis of the primary endpoint was performed with the Wilcoxon Rank-Sum test; given the skewness of seizure frequencies, this is an acceptable method. Moreover, an estimate of the median difference between GNX and PBO, together with the 95% confidence interval, was calculated by the Hodges–Lehmann approach that is used to compare the treatment effect while the data is non-normal distributed.

For the analysis of the primary endpoint, in addition to the non-parametric approach as the Wilcoxon rank-sum test, the applicant should apply a parametric model as supportive analysis, i.e., the percentage reduction from baseline should be estimated using a negative binomial regression model. Moreover, factors known to influence outcome, such as baseline seizure frequency and seizure severity (if available) should be taken into account. The evaluation and discussion of concomitant products that could have impact on the overall efficacy were provided by the applicant after the requested use of a parametric model as supportive analysis, i.e., the percentage reduction from baseline using a Negative binomial regression model. A non-significant treatment effect was shown, although a trend in favor of GNX was found. Of note, the justification provided by the applicant for the correct use of non-parametric model is not supported since the underlined assumptions of the model do not require normal distribution of the data. For the analysis of count data, a Poisson regression or, in case of overdispersion, a negative binomial regression can be actually used to consider covariates, hence the proposed model is deemed appropriate to analyze seizure rates.

Missing seizure data will be considered missing completely at random and collected data will be used to calculate the 28-day seizure frequencies. This can be acceptable as the amount of missing data is sporadic and appears to be at random. Two sensitivity analyses were performed to test the missing data assumption, placebo-based imputation and worst-case imputation, which are considered acceptable.

The key secondary 50% responder endpoint was analysed using Fisher's exact test, which is acceptable. Ordinal regression was used for the key secondary CGI-I endpoint. As it is unlikely that the assumption of proportional odds was met, post-hoc the CGI-I endpoints were reanalysed using Chi-square, which led to the same results. Type I error rate across the primary and key secondary endpoints was appropriately handled by a sequential testing procedure.

The primary and secondary endpoints use data from the titration plus maintenance phase. According to the Guideline on clinical investigation of medicinal products in the treatment of epileptic disorders, efficacy endpoints should be based on the maintenance phase only. The reason is that the effect size observed in this

period is no longer confounded by the dose adaptation of GNX. Upon request, analyses performed over the maintenance period were provided only for the primary, secondary, and seizure-related exploratory endpoints.

Due to a diary error, some seizures were entered twice. This was repaired by flagging duplicate entries. As the first entry will be more reliable, this was used in the analysis.

The applicant planned subgroup analyses on Allo-S level and gender, which is acceptable. However, since this is a single pivotal study, consistency of effect is needed. Additional (post hoc) subgroup analyses have been provided to evaluate consistency: baseline seizure frequency, gender, age categories, dose level, reached, use of rescue medication (yes/no), geographic region, Allo-S level, subjects whose seizure worsened during treatment (yes/no) and most commonly used concomitant ASMs (valproate, levetiracetam, clobazam, vigabatrin: yes/no and/or combinations thereof), presence/absence of somnolence and changes in background concomitant ASM medication.

Efficacy data and additional analyses

Study 0900

The study enrolled subjects with CDD (n=7), protocadherin-19 epilepsy (PCDH19, n=11) and Lennox-Gastaut Syndrome (LGS, n=10). The assessment of this study will be restricted to the CDD cohort.

At the end of the 26-week open-label phase, the median percentage change from baseline in seizure frequency was -37.70% for subjects with CDD. Interpretation of the results from this study is hampered due to the lack of a control arm and the very small sample size. In the end, only 4 subjects with CDD finished the 26-week open-label treatment phase.

The contribution of the results of this study to support the indication is limited.

Study 3001

Conduct of the study

Several changes to the study protocol were made through Amendment 1.0, dated 05 May 2019, and Amendment 2.0, dated 26 May 2020. The most important changes were the addition of subgroup analyses by the Allo-S levels and Gender to the protocol and of a third sensitivity analysis on subjects with low Allo-S levels (Amendment 1.0), and the order of (key) secondary endpoints (the 50% responder rate and the CGI-I became the key secondary endpoints) (Amendment 2.0).”

Of the 114 subjects screened, 13 (11.4%) were considered screen failures in accordance with the exclusion criteria.

At the end of the 4-week titration phase, not every subject reached the target dose of 1800 mg/day (or 63 mg/kg/day). There were also 23 subjects (11 in GNX, 12 in placebo) who required a dose reduction after initially reaching the target dose. The reasoning for not reaching the target dose or requiring a dose reduction at a later stage in the study was mostly related to the occurrence of adverse events. In clinical practice, GNX will be titrated based on tolerance. The mean model dose of GNX administered during both the double-blind and open-label phase are close to the target dose (1500 mg and 1690 mg, respectively), indicating that subjects were able to tolerate high doses of GNX.

Study population

Overall the study population appears to be representative of a CDD population, and most of the baseline characteristics seem to be well-balanced over the treatment arms.

The median (range) number of concomitant ASMs was 2 overall (max 6) in both GNX (max 6) and PBO (max 5) groups. According to the EMA Guidelines on epilepsy "…add-on trial should be conducted optimally in the presence of only one or two pre-existing ASMs, which plasma levels are kept stable within appropriate limits. …". Based on the demographic data, GNX patients tended to take a higher number of concomitant ASMs than PBO patients.

Regarding the concomitant ASM medications, it was shown by the applicant that the vast majority of them were between 2 and 5 at each treatment group. Moreover, around 50% of non-pharmacological treatments like ketogenic diet or VNS were used in the 2 to 3 concomitant AES medications at each group. Although the 28-Day mean (SD) seizure frequency at baseline was 141.1 (200.9) for the PBO group and 223.9 (598.71) for the GNX group, their use did not show striking difference in the 13-week maintenance period and the OL phase that could have differentiated or privileged specific subgroups in each treatment arm.

Efficacy results – double blind treatment period

For the primary endpoint and 50% responder rate, an analysis over the maintenance period was provided. For the remaining endpoints, the analysis provided was performed over the titration+maintenance period (referred to as the 17-week double-blind phase).

The median percent change from baseline in major motor seizure frequency in the maintenance phase was -6.49% for subjects in the placebo group and -29.39% for subjects in the GNX group. The median shift was -29.31% (CI95% -51.45, -8.90), and the difference between the group was statistically significant in favour of GNX treatment ($p < 0.0081$). Sensitivity analyses testing the handling of missing data supported the findings of the primary analysis.

The results described above indicate a possible beneficial effect of GNX on major motor seizures associated with CDD. Initially, it could be excluded that the high use of rescue medication partially explains this effect, i.e., half of the subjects in GNX received rescue medication at some point, more than PBO subjects (50% [n=25] vs. 43.1% [n=22]). In addition, the median number of doses taken was higher for the GNX group compared to the placebo group (8 vs. 6), this is even reflected in a higher maximum dose (max 112 doses vs max 79 doses for the placebo group). The median number of days rescue medication was used was however lower in the GNX arm (462; 273 - 560) than in the placebo arm (499; 406 - 740). Post-hoc sensitivity analysis testing the influence of rescue medication on the primary and seizure-related secondary endpoints where rescue medication was seen as treatment failure. The sensitivity analysis using a tipping point penalising both treatment arms up to 30% did not affect the treatment estimate for the primary endpoint, while when penalising the ganaxolone arm only needed, the primary endpoint was no longer statistically significant when a 25% reduction in treatment effect in case of rescue medication was added to the ganaxolone arm. In the analysis of the 50% responder endpoint, the number of responders dropped when rescue medication was seen as non-response, but it remained numerically in favour of the ganaxolone arm.

Based on the data, the most commonly used rescue medications during the maintenance period were benzodiazepines (e.g. midazolam, diazepam and clonazepam), with an imbalance specifically for midazolam in the GNX arm. The indications of these drugs suggest that rescue medication was most often used in situations wherein rapid cessation of prolonged seizures/status epilepticus was warranted. Additional analyses have been provided showing that the use of rescue medication was not followed by a decrease of major motor seizure frequency (i.e. the primary endpoint).

As dose changes of concomitant ASM therapies were not collected in a structured way it is not possible to gather any detailed summary tabular data by treatment group in the 13-week maintenance period and in the open-label phase.

The number (%) of subjects with a ≥ 50% reduction from baseline in major motor seizure frequency (response rate) at the end of the 17-week double-blind phase numerically favoured the GNX group (12 [24.5%] subjects in the GNX group, 5 [9.8%] subjects in the PBO group, net difference 14.7%). Nevertheless, as this endpoint did not reach statistical significance, the sequential testing procedure stopped here, and none of the secondary endpoints were formally statistically significant. Upon request, the responder rate over the maintenance phase only was provided. Herein 15 subjects (30.6%) in the GNX group and 6 subjects (12%) in the PBO group were considered a responder. The difference compared to placebo was nominal statistically significant (p=0.0283). This reiterates the importance of conducting the main analyses over maintenance period only, as the effect size estimate could be influenced by allowed and planned dose adaptations in the titration period. Moreover, the cumulative response curves show a clear separation between the GNX and PBO arms, further supporting a relevant effect of GNX. Thus, the results from these analyses, support that GNX has a clinically relevant effect on primary seizures.

Higher proportions of parents/caregivers of subjects in the GNX group rated the response to treatment at the end of the 17-week double-blind phase as "minimally improved" or "much improved" compared to parents/caregivers of subjects in the PBO group. Although a modest improvement was observed, there was no difference between the groups on the CGI-I score (nominal p = 0.0971). With respect to clinicians, higher proportions of subjects in the GNX group were rated the response to treatment at the end of the 17-week double-blind phase as "minimally improved" compared to clinicians of subjects in the PBO group. No difference was observed between the groups on the CGI-I (nominal p = 0.3518). Moreover, in both CGI-I scores, more subjects in the GNX group were rated as "much worse" or "very much worse" compared to placebo.

The change from baseline in the percentage of median seizure-free days was 4.91% for subjects in the GNX group and 0.17% for subjects in the PBO group, with a median shift from the PBO group to the GNX group of 1.72%.

At the end of Week 17, the CGICA result favouring GNX group was "minimally improved" (46.7% vs 29.8% for the PBO group), and the CGI-C result was "much improved" (44.4% vs 30.4% for the PBO group).

Taken together, while not all (seizure-related) secondary endpoints are supportive of the primary, the 50% responder rate and response curve performed over the maintenance phase indicate a clinically relevant reduction in seizure frequency for GNX when compared to placebo.

Subgroup analyses

Pre-specified (gender, Allo-S levels) subgroup analyses, as well as specifically requested analyses were provided.

Most of the requested subgroup analyses show a consistent effect in favour of GNX, with the exception of the subgroup analyses on Allo-S levels (low/mid/high) and concomitant anti-seizure medication (valproate, clobazam and vigabatrin).

For the Allo-S subgroup analysis, the forest plots suggest a trend that a higher allo-S level leads to better efficacy of GNX. Allo-S levels were initially included as subgroup analyses based on previous research suggesting better efficacy of GNX with low Allo-S levels. The findings of the subgroup analyses over study

3001 seem to suggest the opposite effect (i.e. higher Allo-S better efficacy); however, given the large confidence intervals that also mostly overlap with each other, no definitive conclusions can be drawn.

Initial subgroup analyses performed over the maintenance period for concomitant use with valproate, clobazam or vigabatrin, suggested effect modification GNX. Note that during the maintenance period, subjects were not permitted to start new anti-seizure medication. The effect modification suggested a larger effect of GNX on major motor seizures when it was taken concomitantly with valproate or clobazam, whereas concomitant vigabatrin resulted in a much smaller effect size for GNX. Cumulative response curves on primary seizure frequency for these specific subgroups have been provided. Although it seems that a larger effect is obtained when GNX is combined with clobazam and valproate, GNX is still more effective than either of the placebo arms when it is not combined with these specific ASMs. For vigabatrin, both the placebo as well as the GNX group perform worse compared to those who did not take concomitant vigabatrin. A post hoc analysis on seizure worsening indicated that a larger group of subjects experienced some kind of seizure worsening in the GNX + vigabatrin group compared to subjects who did not combine GNX with vigabatrin. But the largest worsening is observed for subjects in the placebo group + vigabatrin. Altogether, a specific interaction between vigabatrin and GNX with respect to seizure worsening is not observed.

Overall, the provided response curves do not indicate any effect modification of GNX + respective concomitant anti-seizure medication (ASM) that warrant any changes to the SmPC.

Efficacy results - Ongoing open-label extension phase

An interim report for the OLE phase has been provided (data cut-off June 2022). The interim data suggest that subjects who switched from placebo to GNX in the OLE phase benefitted from treatment, as shown by the increase in median percentage reduction in seizure frequency over time. With respect to the GNX arm, except for months 34-36, the reduction in seizure frequency does not fluctuate that much over time. At month 34-36, the median percentage change from baseline in major motor seizure frequency was 1.04 (-82.62, 110.69) in the GNX group. This seems to be primarily driven by a single patient who experienced a seizure worsening of 101.7%, which is not an uncommon event for severe refractory epilepsies.

Efficacy in special populations

Additional studies in special populations were not performed.

Approval was initially sought for the treatment of seizures associated with CDD in patients from 2 years of age. CDD is relatively recently characterised as a distinctive disorder. Of several other developmental epileptic encephalopathies, it is known that the disease changes over time and this is associated with changes in the seizure profile. However, for CDD, it is unclear how the disease, in particular the seizure characteristics, progresses into adulthood. The controlled data available in adult CDD patients treated with GNX is very limited. There were 2 subjects aged > 18 years, one in placebo and the other in GNX (both 19 years old), and precluded conclusions whether GNX was efficacious. Therefore, the applicant was requested to perform an extrapolation from children with CDD to adults (with reference to the Extrapolation of efficacy and safety in paediatric medicine development (EMA/189724/2018).

Based on the provided response, efficacy obtained with GNX in children with CDD cannot be extrapolated to adults with CDD, as disease similarity has not been shown between these patient subpopulations. The claim that seizure semiology and disease severity are generally stable into adulthood could not be verified through scientific literature. There seems to be no natural history data or longitudinal observational data available that supports this. Although limited efficacy data have been provided on 7 subjects who turned > 18 years old during the OLE phase of the study, the results hamper interpretation whether seizure semiology remains

the same as it focuses only on primary seizure types and lack a comparison to childhood. Moreover, the results from these subjects are mixed, and do not provide convincing data to start treatment with GNX in naïve adults with CDD. In addition, based on the provided PK modelling, lower GNX exposure is expected for adults with normal body weight, which may lead to GNX being less effective at the target dose. Although GNX is titrated based on response, higher doses than the maximum dose of 1800 mg/day have not been investigated. Thus, as efficacy cannot be extrapolated, the current data do not support use of GNX in treatment naïve adults. The CHMP agreed that the indication should therefore be for studied population ie patients 2 to 17 years of age. However, while the data preclude use of GNX in treatment naïve adults with CDD, the limited OLE efficacy data do indicate that there is no reason to withdraw treatment in adulthood if a patient has initiated treatment during adolescence and perceived a clear benefit.

2.6.7. Conclusions on the clinical efficacy

GNX treatment resulted in a statistically significant reduction in monthly number of major motor seizures. Although support from secondary endpoints is limited, the median percentage reduction is supported by the 50% responder rated and cumulative response rate over the maintenance period, indicating a clinically relevant reduction in seizures when compared to placebo.

The CHMP agreed the efficacy obtained in paediatric patients with CDD cannot be extrapolated to adult patients with CDD and starting treatment with GNX in naïve adults with CDD cannot not be recommended. It was however agreed that treatment with GNX may be continued into adulthood if initiated in childhood/adolescence.

The relevant findings are correctly reflected in the SmPC.

2.6.8. Clinical safety

2.6.8.1. Patient exposure

GNX has been investigated in a broad range of CNS conditions (several epileptic syndromes, migraine, post-partum depression and PTSD). A total of 1930 subjects were exposed to GNX (1837 subjects across 42 completed clinical studies to oral GNX, 77 subjects exposed to IV GNX and 16 subjects administered both oral and IV GNX). 379 subjects were exposed to the to-be-marketed oral suspension formulation and dose of GNX in 8 Phase 2/3 studies (238 paediatric subjects and 141 adults).

Three safety pools were defined (data lock point is 24 February 2021):

Pool A = CDD Exposure Set (all patients with CDD exposed to GNX). 102 patients were included in the safety database for GNX in CDD (50 patients on GNX in DB phase Study 3001, 45 patients who switched from placebo to GNX in OL phase of Study 3001 and 7 CDD patients from basket Study 0900 in rare genetic epilepsies).

Pool B = All Exposure Set (all patients exposed to GNX regardless of the indication and formulation used). N=1135.

Pool C = Paediatric Exposure Set (a subset of Pool B with only paediatric safety data <18 years) N=238.

<u>Duration of exposure</u>

As of the 24 Feb 2021 cut-off date, a total of 466 subjects received at least 1 dose of the to-be-marketed formulation of oral GNX in clinical trials. This includes 325 subjects who received oral GNX up to the maximum recommended therapeutic dose for the treatment of CDD up to 1800 mg/day (for subjects weighing > 28 kg).

Table 20: Total Duration of Exposure for the Intended To-be marketed Formulation of Oral Ganaxolone

	Number of subjects exposed to the study drug:			
Dosage	**≥ 1 dose**	**≥ 6 months**	**≥ 12 months**	**≥ 18 months**
Placebo	256	1	0	0
GNX Dose: ≤ 100 mg	0	0	0	0
GNX Dose: > 100 to ≤ 500 mg	48	18	5	1
GNX Dose: > 500 to ≤ 1000 mg	40	15	8	2
GNX Dose > 1000 to ≤ 1800 mg	325	165	78	28
GNX Dose > 1800 mg	53	0	0	0
GNX: All doses combined	466	198	91	31

Notes: The doses cited in the table refer to daily doses.
The Table summarizes dose information for the studies utilizing the to be marketed formulation. This formulation was utilized in Studies: 1042-DDI-1001, 1042-HAP-1001, 1042-0600, 1042-0601, 1042-0602, 1042-0500, 1042-0501, 1042-0800, 1042-0900, 1042-CDD-3001. Study 1042-0602 also used to be marketed formulation of oral GNX. But due to the lack to study level data Populations, the duration of exposure from this study was not included.
GNX = ganaxolone.

All CDD Patients

At the cut-off date of 30 June 2022, the mean duration of exposure was 613.7 days for subjects in the CDD Exposure Set. The subjects in the PBO group received a mean of 533.8 days of GNX treatment in the OLE phase. Subjects spent the majority of their time at the target dose of 1800 mg/day for subjects > 28 kg or 63 mg/kg/day for subjects ≤ 28 kg.

Table 21: Duration of Exposure to Study Treatment (Double-blind and Open-label Extension Phases, Safety Population)

	Treatment Group During the Double-blind Phase		
	Placebo (N = 45)	Ganaxolone (N = 43)	Total (N = 88)
Exposure duration categories, n (%)			
Any exposure	45 (100)	43 (100)	88 (100)
≥ 1 month	43 (95.6)	43 (100)	86 (97.7)
≥ 2 months	39 (86.7)	43 (100)	82 (93.2)
≥ 3 months	38 (84.4)	43 (100)	81 (92.0)
≥ 4 months	38 (84.4)	43 (100)	81 (92.0)
≥ 5 months	35 (77.8)	43 (100)	78 (88.6)
≥ 6 months	35 (77.8)	41 (95.3)	76 (86.4)
≥ 9 months	30 (66.7)	40 (93.0)	70 (79.5)
≥ 12 months	29 (64.4)	33 (76.7)	62 (70.5)
≥ 15 months	23 (51.1)	29 (67.4)	52 (59.1)
≥ 18 months	23 (51.1)	28 (65.1)	51 (58.0)
≥ 21 months	21 (46.7)	26 (60.5)	47 (53.4)
≥ 24 months	18 (40.0)	25 (58.1)	43 (48.9)
≥ 27 months	10 (22.2)	23 (53.5)	33 (37.5)
≥ 30 months	7 (15.6)	11 (25.6)	18 (20.5)
≥ 33 months	5 (11.1)	7 (16.3)	12 (13.6)
≥ 36 months	4 (8.9)	3 (7.0)	7 (8.0)
≥ 39 months	1 (2.2)	1 (2.3)	2 (2.3)
≥ 42 months	1 (2.2)	0	1 (1.1)
Exposure duration (days)			
n	45	43	88
Mean	533.8	697.3	613.7
SD	367.38	313.53	349.96
Min	16	161	16
Median	554.0	838.0	702.5
Max	1317	1210	1317
Sum	24021	29983	54004
Subjects > 28 kg (30 kg): Modal dose (dose of longest exposure), n (%)			
< 1800 mg/day	2 (4.4)	3 (7.0)	5 (5.7)
1800 mg/day	9 (20.0)	8 (18.6)	17 (19.3)
Subjects ≤ 28 kg (30 kg): Modal dose (dose of longest exposure), n (%)			
< 63 mg/kg/day	9 (20.0)	4 (9.3)	13 (14.8)
63 mg/kg/day	25 (55.6)	28 (65.1)	53 (60.2)

Note: Exposure duration is calculated as date of last dose – date of first dose + 1.
The exposure in the ganaxolone group includes both the double-blind and open-label extension phases.
Subjects weighting ≤ 28 kg were dosed on a mg/kg basis, and subjects weighting > 28 kg were dosed by mg/day.
Max = maximum; Min = minimum; SD = standard deviation.
Source: 2-year OLE interim report, Table 4.

2.6.8.2. Adverse events

A similar proportion of patients on GNX (86%) and placebo (88.2%) experienced AEs. For approximately half of the patients in placebo, these were mild and for approximately half of the patients on GNX these were moderate. Serious AEs were reported for 9.8% of patients on placebo and 12% for patients on GNX. Treatment-related AEs were reported for 43.1% of patients on placebo and 70% for patients on GNX. Slightly more patients on placebo stopped because of AEs, while on GNX these leaded to dose reduction or temporary study drug discontinuation.

Table 22: Overview Summary of Adverse Events (Study 1042-CDD-3001 17 week Double blind Phase Safety Population)

	PBO (N = 51)		GNX (N = 50)	
Category	**Subjects n (%)**	**Events n**	**Subjects n (%)**	**Events n**
TEAEs[a]	45 (88.2)	175	43 (86.0)	153
TEAE by Severity[b]				
Mild	27 (52.9)	134	16 (32.0)	102
Moderate	15 (29.4)	37	26 (52.0)	50
Severe	3 (5.9)	4	1 (2.0)	1
Serious TEAEs	5 (9.8)	10	6 (12.0)	6
Treatment-related TEAE	22 (43.1)	60	35 (70.0)	79
TEAE Leading to Study Drug Discontinuation	4 (7.8)	8	2 (4.0)	4
TEAE Leading to Dose Reduction or Temporary Study Drug Discontinuation	8 (15.7)	11	11 (22.0)	17
TEAE of Special Interest[c]	3 (5.9)	3	4 (8.0)	4
TEAE Resulting in Death	0	0	0	0

Note: If a subject experienced more than 1 adverse event in a category, the subject was counted only once in that category.
CSR = clinical study report; PBO = placebo; TEAE = treatment-emergent adverse event.
[a] TEAE, occurred or worsened on the day of or after the first dose of study drug and, for subjects who entered the open-label extension phase, before the first dosing day of that phase.
[b] Highest severity for subjects.
[c] Includes Rash and TEAEs in the reproductive system and breast disorders system organ class.
Source: Study 1042-CDD-3001 DB CSR, Table 14.3.2.2.1 and Listing 16.2.10

In the CDD exposure set, a similar proportion of patients on GNX (91.2) and placebo (88.2%) experienced AEs in the DB phase of study 3001. Severe AEs were reported for 5.9% of patients on placebo and 20.6% for patients on GNX. Treatment-related AEs were reported for 43.1% of patients on placebo and 59.8% for patients on GNX. More patients on GNX stopped because of AEs than patients on placebo; 12.7% vs 7.8%. One death on GNX was reported.

Table 23: Overview of Treatment-Emergent Adverse Events (All Treated CDD Subjects)

	PBO DB (N = 51) n (%)	All GNX CDD (N = 102) n (%)
Number of Subjects with at least 1 TEAE	45 (88.2)	93 (91.2)
Number of Subjects with at least 1 Severe TEAE	3 (5.9)	21 (20.6)
Number of Subjects with at least 1 drug-related TEAE	22 (43.1)	61 (59.8)
Number of Subjects with at least 1 severe drug-related TEAE	1 (2.0)	7 (6.9)
Number of Subjects with at least 1 treatment-emergent SAE	5 (9.8)	26 (25.5)
Leading to death	0	1 (1.0)
Non-fatal	5 (9.8)	26 (25.5)
Number of Subjects with at least 1 drug-related treatment-emergent SAE	1 (2.0)	6 (5.9)
Leading to death	0	0
Non-fatal	1 (2.0)	6 (5.9)
Number of Subjects with at least 1 TEAE leading to study drug discontinuation	4 (7.8)	13 (12.7)
Number of Subjects with at least 1 treatment-emergent SAE leading to study drug discontinuation	1 (2.0)	5 (4.9)
Number of Subjects with at least 1 TEAE leading to study drug interruption	1 (2.0)	3 (2.9)
Number of Subjects with at least 1 TEAE leading to study drug dose changes	7 (13.7)	21 (20.6)
Number of Subjects with at least 1 TEAE leading to dose reduction or temporary study drug discontinuation	8 (15.7)	24 (23.5)

Notes: The "PBO DB" group consists of subjects treated with PBO during DB phase of Study 1042-CDD-3001. The "All GNX CDD" group consists of all subjects exposed to GNX in any phase of Study 1042-CDD-3001 and the CDD subset of subjects from Study 1042-0900
CDD = cyclin-dependent kinase-like 5 deficiency disorder; DB = double-blind; GNX = ganaxolone; PBO = placebo; SAE = serious adverse event; TEAE = treatment-emergent adverse event
Source: ISS, Table 14.3.1.1.1.A

80% of the AEs reported for the GNX group were considered to be treatment-related by the investigator, in contrast to 43.1% for placebo. The most common treatment-related AE was somnolence: 34% for GNX vs 5.9% for placebo. Seizure was the second most common treatment-related AE: 10% for GNX vs 7.8% for placebo. Sedation was reported as treatment-related AE for 6% for patients on GNX and 3.9% for patients on placebo. Constipation was reported as treatment related AE for 6% for patients on GNX and 0% for patients on placebo. Salivary hypersecretion was reported as treatment-related AE for 6% for patients on GNX and 2% for patients on placebo.

Table 24: Summary of Study Drug-related Treatment-Emergent Adverse Events by SOC and Preferred Term (⩾ 3% in Either Treatment Group) (Study 1042 CDD 3001 17 week Double blind Phase, Safety Population)

	PBO (N = 51)		GNX (N = 50)	
System Organ Class Preferred Term	**Subjects n (%)**	**Events n**	**Subjects n (%)**	**Events n**
Any Study Drug-related TEAE[a]	22 (43.1)	60	35 (70.0)	79
Nervous System Disorders				
Somnolence	3 (5.9)	3	17 (34.0)	19
Seizure	4 (7.8)	7	5 (10.0)	4
Sedation	2 (3.9)	2	3 (6.0)	3
Hypersomnia	0	0	2 (4.0)	2
Lethargy	2 (3.9)	2	2 (4.0)	2
Hyperaesthesia	2 (3.9)	2	0	0
Gastrointestinal Disorders				
Constipation	0	0	3 (6.0)	3
Salivary Hypersecretion	1 (2.0)	1	3 (6.0)	3
Vomiting	2 (3.9)	3	2 (4.0)	2
Psychiatric Disorders				
Insomnia	2 (3.9)	3	2 (4.0)	2
Irritability	2 (3.9)	2	2 (4.0)	2
General Disorders and Administration Site Conditions				
Gait Disturbance	1 (2.0)	1	2 (4.0)	2

Note: If a subject experienced more than 1 adverse event in a category, the subject was counted only once in that category

GNX = ganaxolone; PBO = placebo; SOC = system organ class; TEAE = treatment-emergent adverse event.

[a] TEAE occurred or worsened on the day of or after the first dose of study drug and, for subjects who entered the open-label extension phase, before the first dosing day of that phase.

Source: Study 1042-CDD-3001 DB CSR, Table 14.3.2.5

In Pool A (CDD Exposure Set), the most common treatment-related AE was somnolence: 26.5%. Seizure was the second most common treatment-related AE: 13.7%. Weight decrease and decreased appetite were reported for 2.9% and 4.9% of patients, respectively. Rash as a treatment-related AE was reported for 2 (2%) patients.

In the pool with all subjects exposed to GNX (N=1135), the most common drug-related AEs were in CNS effects: somnolence, dizziness, headache, sedation and seizures.

2.6.8.3. Serious adverse event/deaths/other significant events

Serious adverse events

A comparable incidence of serious AE (SAEs) was seen between GNX treatment and placebo: 12% vs. 9.8%. Three subjects in the GNX group and 1 subject in the placebo group had SAEs that led to dose reduction or temporary or permanent withdrawal of the study drug. In the GNX group, two SAEs were considered not to be related to GNX (urinary tract infection and bronchitis). While decreased oxygen saturation was reported

for one patient on GNX, the unresponsiveness to stimuli was considered related to GNX and placebo, respectively.

Table 25: Summary of Serious Treatment-Emergent Adverse Events by SOC and Preferred Term – (Study 1042-CDD-3001 17 week Double-blind Phase, Safety Population)

System Organ Class Preferred Term	PBO (N = 51) Subjects n (%)	PBO (N = 51) Events n	GNX (N = 50) Subjects n (%)	GNX (N = 50) Events n
Any Serious TEAE[a]	5 (9.8)	10	6 (12.0)	6
Infections and Infestations	2 (3.9)	3	3 (6.0)	3
Bronchitis	0	0	1 (2.0)	1
Rhinovirus Infection	0	0	1 (2.0)	1
Urinary Tract Infection	0	0	1 (2.0)	1
Pneumonia Mycoplasmal	1 (2.0)	1	0	0
Pneumonia Viral	1 (2.0)	1	0	0
Respiratory Syncytial Virus Bronchiolitis	1 (2.0)	1	0	0
Investigations	0	0	1 (2.0)	1
Oxygen Saturation Decreased	0	0	1 (2.0)	1
Metabolism and Nutrition Disorders	0	0	1 (2.0)	1
Food Refusal	0	0	1 (2.0)	1
Respiratory, Thoracic and Mediastinal Disorders	1 (2.0)	2	1 (2.0)	1
Pneumonia Aspiration	0	0	1 (2.0)	1
Hypoxia	1 (2.0)	2	0	0
Gastrointestinal Disorders	1 (2.0)	1	0	0
Faecaloma	1 (2.0)	1	0	0
Nervous System Disorders	2 (3.9)	4	0	0
Hypotonia	1 (2.0)	1	0	0
Seizure	1 (2.0)	1	0	0
Unresponsive to Stimuli	1 (2.0)	2	0	0

Note: If a subject experienced more than 1 adverse event in a category, the subject was counted only once in that category

CSR = clinical study report; GNX = ganaxolone; PBO = placebo; SOC = system organ class; TEAE = treatment-emergent adverse event.

[a] TEAE occurred or worsened on the day of or after the first dose of study drug and, for subjects who entered the open-label extension phase, before the first dosing day of that phase.

Source: Study 1042-CDD-3001 DB CSR, Table 14.3.2.6.1

In all CDD patients who were exposed to GNX (Pool A), the most common reported SAE was seizures: 4.9%.

Deaths

As of 30 Jun 2022, thirteen patients died in the total clinical development program of GNX: twelve were during or after GNX treatment and one after placebo treatment.

Three patients in the CDD exposure set died: the most likely causes of death were meningococcal sepsis, experienced SUDEP (sudden unexplained death in epilepsy) and cardiac arrest (possibly SUDEP). The first two cases are considered not related to GNX, while the latter was considered as possible related to GNX dose decrease rather than to the GNX itself.

In study 0900, two patients died: one patient with Lennox-Gastaut syndrome died of hepatic failure and one patient with (most likely) CDD died of cardiac arrest in the OLE phase of the study. Two patients with infantile spasms (IS), three adults with status epilepticus (SE), one patient with drug-resistant partial-onset

seizures and one patient with complex partial seizures randomized to placebo deceased. According to the applicant, all cases are not related to GNX.

Rash, reproductive system AEs and breast disorders

Rash, reproductive system AEs and breast disorders were firstly considered adverse events of special interest (AESI). Rash was included as an AESI in the GNX development program due to its structural and pharmacologic similarity to endogenous allopregnanolone, allopregnanolone being derived from progesterone, which is associated with autoimmune dermatitis and rash.

In the DB phase of study 3001, rash was reported for 2 (4%) of patients on GNX and for 2 (3.9%) on placebo. One subject (2%) on GNX reported dermatitis atopic. In the CDD Exposure Set population, 9 (8.8%) subjects reported a TEAE in the reproductive system and breast disorders SOC and 7 (6.9%) subjects reported rash. In all patients exposed to GNX regardless of indication (Pool B, N=1135), rash was reported in 39 (3.4%) of patients. 38 Of these patients received a daily dose of between 1000 and 1800mg.

In all CDD patients treated with GNX, the following AESIs in the reproductive system and breast disorders were reported: dysmenorrhoea, menorrhagia and polymenorrhoea were reported for 2 (2.0%), other AE's in this SOC were each reported once (1%).

In all patients exposed to GNX regardless of indication, 48 (4.2%) of patients reported AESIs of reproductive system and breast disorders. 47 of these patients received a daily dose between 1000 and 1800mg. Dysmenorrhoea and menorrhagia was reported for 6 (0.5%) of patients, menstruation irregular and polymenorrhoea was reported for 5 (0.4%) of patients, and vaginal haemorrhage was reported for 4 (0.4%) of patients. Breast tenderness was reported for 7 (0.6%) of patients. Other AESIs were reported once (0.1%).

Table 26: Treatment-Emergent Adverse Events of Special Interest (All Treated CDD Subjects)

System Organ Class (SOC)/ Preferred Term (PT)	PBO DB (N = 51) n (%)	All Ganaxolone CDD (N = 102) n (%)
Reproductive System and Breast Disorders	0	9 (8.8)
Dysmenorrhoea	0	2 (2.0)
Menorrhagia	0	2 (2.0)
Polymenorrhoea	0	2 (2.0)
Breast Enlargement	0	1 (1.0)
Menstrual Discomfort	0	1 (1.0)
Menstruation Irregular	0	1 (1.0)
Oligomenorrhoea	0	1 (1.0)
Spontaneous Penile Erection	0	1 (1.0)
Skin and Subcutaneous Tissue Disorders	7 (13.7)	14 (13.7)
Rash	4 (7.8)	7 (6.9)

Notes: A subject with multiple adverse events within a SOC or PT is counted only once.
- MedDRA Version 23.0 was used for reporting adverse events
- The "Placebo DB" group consists of subjects treated with placebo during DB phase of Study 1042-3001. The "All Ganaxolone.
- CDD" group consists of all subjects exposed to ganaxolone in any phase of Study 1042-3001 and the CDD subset of subjects from Study 1042-0900.

CDD = cyclin-dependent kinase-like 5 deficiency disorder; DB = double-blind; PBO = placebo.
Source: ISS, Table 14.3.1.2.1.A

Sexual maturation and bone density

Table 30 provides the Tanner stage of patients who continued in the OLE phase of study 3001 at baseline and at week 52.

Table 27: Tanner stage at baseline and week 52 (study 1042-CDD-3001 OLE)

	Treatment Group during Double-Blind Phase			
	Placebo (N=45) N (%)	GNX (N=43) N (%)	Placebo (N=18) N (%)	GNX (N=21) N (%)
Tanner stage	Baseline		Week 52	
Stage 1	26 (65.0)	25 (71.4)	11 (57.9)	13 (61.9)
Stage 2	5 (12.5)	5 (14.3)	5 (26.3)	3 (14.3)
Stage 3	7 (17.5)	1 (2.9)	1 (5.3)	2 (9.5)
Stage 4	2 (5.0)	1 (2.9)	2 (10.5)	0
Stage 5	0	3 (8.6)	0	3 (14.3)

Based on Table 14.3.10A Summary of Tanner Staging (Open-Label Extension Phase, Safety Population), cut-off date: 23 Feb 2022.

2.6.8.4. Laboratory findings

The laboratory evaluations and vital signs were determined in the DB and OL phase of study 3001, study 0900 and in the 3 safety pools. No clinically relevant changes in laboratory values regarding haematology, chemistry, urinalysis or vital signs were observed.

ECGs and Potential Effects on Cardiac Safety:

A cardiac safety report assessed cardiac data from non-clinical trials, 4 clinical trials (studies 1042-SE-2001, 1042-PPD-2002, 1042-PPD-2003) and available results from the ongoing study 1042-CDD-3001 DB phase. In addition, a Phase 1 thorough QT/QTc study 1042-TQT-1001 provided data related to the effects of maximal steady-state GNX exposures following oral dosing and is intended to provide information that will further inform the understanding of the effects of GNX on QTc and other ECG parameters. The study showed that adverse events associated with GNX in this study were: dizziness (10 [17.5%] of 57 subjects), somnolence (9 [15.8%] of 57 subjects), fatigue (4 [7.0%] of 57 subjects each), headache and nausea (3 [5.3%] of 57 subjects each), and dyspepsia and gastritis (2 [3.5%] of 57 subjects each). No clinically meaningful signals of QTc prolongation or other cardiovascular toxicities have been observed in clinical trials to date.

2.6.8.5. Safety in special populations

Intrinsic Factors

The results from the subgroup analyses for intrinsic factors (age [<12 and ≥12 years old], race, ethnicity, and weight suggest that the safety profile of GNX is similar in several subgroups. Regarding gender, drug-related somnolence was reported for 35% of males (n=7) and 28% of females (n=23); for drug-related seizures this was 25% for males and 24.4% for females, and for drug-related sedation this was 10% for males and 2.4% for females.

Extrinsic Factors

The results from the subgroup analyses for geographic region did not reveal differences. The GNX oral suspension (50 mg/mL) demonstrated 2-fold and 3-fold higher exposure (by Cmax and AUC0-inf, respectively) when administered under high-fat, fed conditions compared to fasted levels. To ensure sufficient bioavailability of GNX, it should be taken with food. In clinical trials with CDD subjects, doses were administered following a meal or snack. Therefore, no additional safety concerns are expected from food effects.

Pregnancy and Lactation

Given the nature of CDD and the age of the patients included, no pregnancies were reported for the CDD population.

GNX is excreted in human milk. Concentrations of ganaxolone in breast milk were approximately 4-fold higher than in plasma.

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

Concomitant ASMs

Concomitant medication use was evaluated for the CDD Exposures Set. Most subjects in the CDD Exposure Set received at least 1 concomitant ASM (97.1%). The median duration of concomitant ASM use during the study in the CDD Exposure Set was 1446.0 days (range 1 to 6977 days).

The most common concomitant ASMs were valproate sodium (33.3%), clobazam (31.4%), levetiracetam (25.5%), and vigabatrin (19.6%).

Additional analyses showed that for concomitant valproate use, somnolence was the most reported AE when valproate and GNX were used concomitantly: 55.6% (n=10). This was lower for patients on GNX, who did not use valproate concomitantly: 21.9% (n=7). For the other ASMs the incidence of somnolence was comparable or lower when concomitantly used with GNX.

For concomitant clobazam use, the overall incidence of AEs was higher when clobazam was not used concomitantly for both patients on GNX and placebo.

The incidence of somnolence and seizure is comparable in the patients on GNX regardless of concomitant levetiracetam use. Sedation is more frequent when levetiracetam is not concomitantly used: 8.1 vs 0%.

For concomitant vigabatrin use, sedation was the most common AE: 30% (n=3) when concomitantly used with GNX. When vigabatrin was not concomitantly used, somnolence was most common: 40% (n=16), with an incidence higher than when vigabatrin was concomitantly used with GNX (40 vs 10%). The incidence of seizures was lower when vigabatrin was concomitantly used with GNX (0 vs 12.5%).

Other CNS depressants

GNX is associated with CNS-effects. Concomitant use of GNX with CNS depressants may increase the risk of sedation, somnolence, and gait disturbances which have been reported as drug-related TEAEs for GNX.

Alcohol

In animal models, GNX has been shown to potentiate the effects of alcohol in a similar manner to that of benzodiazepines and valproic acid. No reports of adverse effects with GNX and adult epileptic subjects have been reported. However, subjects should be prohibited from drinking alcohol during treatment with GNX.

2.6.8.7. Discontinuation due to adverse events

In the DB phase of study 3001, seizures were the most reported AE leading to discontinuation for both the placebo group (2 (3.9%)) and the GNX group (2 (4.0%)). In the OL phase, seizure was the most reported AE leading to discontinuation for patients who switched to GNX: 3 (6.7%) vs 0 in the patients who remained on GNX treatment. In the pool of CDD patients exposed to GNX, 13 (12.7%) of patients discontinued because of AEs. Most reported AEs were seizures, 5 (4.9%) and somnolence 3 (2.9%).

In Pool B, containing all exposed subjects to GNX, 128 (11.3%) subjects have discontinued the study drug due to AEs. This is comparable to the pool of CDD patients exposed to GNX. The most common AEs leading to discontinuation in Pool B were fatigue (21 [1.9%] subjects), dizziness (19 [1.7%] subjects), somnolence (19 [1.7%] subjects), and seizure (12 [1.1%] subjects.

Table 28: Summary of Treatment-Emergent Adverse Events Leading to Study Drug Discontinuation by SOC and Preferred Term (Study 1042-CDD-3001 17 week Double-blind Phase, Safety Population)

System Organ Class Preferred Term	PBO Subjects n (%)	PBO Events n	GNX Subjects n (%)	GNX Events n
Any TEAE[a] Leading to Study Drug Discontinuation	4 (7.8)	8	2 (4.0)	4
Nervous System Disorders	4 (7.8)	5	2 (4.0)	4
Seizure	2 (3.9)	3	2 (4.0)	2
Somnolence	0	0	1 (2.0)	2
Sedation	1 (2.0)	1	0	0
Unresponsive to Stimuli	1 (2.0)	1	0	0
Gastrointestinal Disorders	1 (2.0)	1	0	0
Abdominal Pain	1 (2.0)	1	0	0
Respiratory, Thoracic and Mediastinal Disorders	2 (3.9)	2	0	0
Hypoxia	1 (2.0)	1	0	0
Respiratory Failure	1 (2.0)	1	0	0

Note: If a subject experienced more than 1 adverse event in a category, the subject was counted only once in that category

GNX = ganaxolone; PBO = placebo; SOC = system organ class; TEAE = treatment-emergent adverse event.

[a] TEAE occurred or worsened on the day of or after the first dose of study drug and, for subjects who entered the open-label extension phase, before the first dosing day of that phase.

Source: Study 1042-CDD-3001 DB CSR, Table 14.3.2.7

2.6.8.8. Supportive safety data

Open-label extension of Study 3001

Of the 88 patients who continued from the double-blind phase into the OLE phase, 40 (45.5%) patients were ongoing in the OLE phase as of the data cut-off point of 30 Jun 2022 (19 [44.2%] patients in the GNX group, 21 [46.7%] patients in the PBO group). Sixty-one patients (69.3%) were treated in the OLE for at least 1 year, 50 (56.8%) were treated in the OLE for at least 2 years, and 11 (12.5%) were treated in the OLE for at least 3 years. The patient with the longest treatment time in the OLE was treated for at least 3.5 years.

A total of 41 patients (46.6%) in the OLE phase reported a total of 101 AEs that were considered related to study drug. Treatment-related AEs that were reported in > 2 subjects were: somnolence (15 patients, 17.0%), seizure (10 patients, 11.4%), decreased appetite (5 patients, 5.7%), weight decreased (4 patients, 4.5%), attention-seeking behaviour and gait disturbance (3 patients each, 3.4%).

Severe CNS AEs were seizure (3 patients, 3.4%; 5 severe AEs) and somnolence and status epilepticus (2 patients each, 2.3%; 2 severe AEs each).

Serious AEs were: seizure (6 patients, 6.8%), pneumonia (5 patients 5.7%), acute respiratory failure, dehydration, and pneumonia aspiration (3 patients each, 3.4%), Covid-19, influenza, pneumonia viral, sepsis, status epilepticus, and vomiting.

A total of 11 patients (12.5%) discontinued study drug due to an AE during the OLE phase.

Two patients were 19 years old at time of study 3001 enrolment and seven patients turned 18 years of age during the OLE phase. The reported AEs seems to be in line with the observed safety data in the overall study

population. Of the 44 TEAE's, four were SAEs: three were considered unrelated to study treatment (sepsis, urinary tract infection, seizure) and one was classified as related (unresponsive to stimuli). In the SOC of Infections and Infestations, AEs were also reported in the 9 adults: 11 TEAEs, generally mild or moderate, with the exception of two SAEs considered not related to GNX (sepsis and urinary tract infection).

Withdrawal

Based on non-clinical data, GNX was tapered off in clinical practice to minimize withdrawal effects or symptoms. 13.7% of patients on GNX compared to 2% of patients on placebo reported AEs after discontinuation. For the patients who were randomised to GNX, vomiting and pyrexia (3.9% each) were most reported AEs. AEs like diarrhoea and seizures were reported in 1.0% and 2.0% of patients, respectively.

2.6.8.9. Post marketing experience

GNX has not been previously authorised and marketed in EU.

GNX was approved by the FDA in the US on 18 March 2022. No new safety information has been reported since the date of commercialization.

2.6.9. Discussion on clinical safety

Safety database

The main evidence for the safety profile of GNX in CDD comes from the completed double-blind (DB), randomized, placebo-controlled study 3001. Supportive evidence comes from 7 paediatric patients with CDD who are included in a 26-week open-label, proof-of-concept basket study 0900 in children with rare genetic epilepsies.

At the cut-off date of 24 February 2021, 101 patients are included in DB phase of study 3001. In addition, Pool A (CDD Exposure Set) provides safety data of 102 CDD patients exposed to GNX in study 3001 (DB and OLE) and 0900. These patients received GNX via the proposed route of administration (oral) and according to the proposed posology.

Other safety pools include an All Exposure Set (n= 1135, including all subjects exposed to GNX regardless of the indication and formulation used) and the Paediatric Exposure Set (n= 238, including only paediatric safety data [<18 years]).

At the data cut-off of 30 June 2022, the mean exposure duration for all CDD patients treated with GNX is 613.7 days; the median is 702.5 days. 70.5% Of patients have an exposure for ≥12 months, 58% of patients have an exposure for ≥18 months, 48.9% for ≥24 months and 8% for ≥36 months.

Adverse events

Most reported adverse events (AEs) were in the domains of Nervous System Disorders, Infections and Infestations, Gastrointestinal disorders, General Disorders and Administration Site, Respiratory, Thoracic and Mediastinal Disorders, Psychiatric Disorders and Skin and Subcutaneous Tissue Disorders.

Compared to placebo, the incidence of the following AEs was higher in GNX: somnolence, sedation, pyrexia and upper respiratory tract infection. The most common reported AE for patients on placebo were seizure and vomiting. Some of the observed AE's may comply with the normal background incidence in paediatrics

subjects, i.e. diarrhoea, constipation, gastroesophageal reflux, vomiting and ear infection. Others may be disease-related, like gastrointestinal disorders and salivary hypersecretion.

In the DB phase of study 3001, 70% of the AEs reported for the GNX group were considered to be treatment-related by the investigator versus 43.1% for placebo. The most common treatment-related AEs for GNX were somnolence, seizure, sedation, constipation and salivary hypersecretion.

In the OLE phase of study 3001 (data cut-off 30 Jun 2022) and in the Pool containing all CDD patients exposed to GNX, the observed AEs were in line with the DB phase of the study. For the all exposure set (Pool B, N=1135) the most common drug-related AEs were CNS effects: somnolence, dizziness, headache, sedation and seizures. The incidence of somnolence and headache was dose-dependent. For dizziness, sedation and seizures, there was a suggestion of dose-dependency. This seems in line with non-clinical findings of dose-dependent incidence, duration, and severity of CNS-effects.

The incidence of drug-related somnolence (34% vs. 5.9%) and sedation (6.0% vs. 3.9%) in the DB phase of Study 3001 was higher in the GNX group than in placebo. For lethargy (4.0% vs 3.9%) the incidence was comparable. All three are considered to fall within the spectrum of sedation. The applicant provided additional informaiton showing that most events of somnolence, sedation and lethargy occurred relatively quick after the initiation of GNX treatment and most cases resolved during treatment. It appears that over time patients develop tolerance for GNX.

Decreased appetite was reported for 2.2% of patients who switched from placebo to GNX vs. 7% for patients who remained on GNX in the OLE phase of study 3001. Also, weight decrease was reported for 2.2% of patients who switched from placebo to GNX vs. 4.7% for patients who remained on GNX. For one patient, the drug-related weight decrease was a serious AE. It is acknowledged that CDD is associated with gastrointestinal problems. The 3 out of 56 patients who had a week 132 visit had a median change from baseline of -1.3kg; this implies that 2 out of 3 patients lost weight. Moreover, these two patients received doses between 1000-1800mg, which is in line with the proposed posology. Additional information is provided indicating that for both patients the events of weight decrease were considered related to GNX and led to dose reductions. For both patients the events resolved. It should be noted that the 1 patient on a dose >1800mg is also losing weight until week 52 visit, and at week 68 his change from baseline is 0.9 kg which is considered comparable to baseline.

The relevant adverse drugs reactions are appropriately reflected in the Product Information.

Serious adverse events

In the DB phase of study 3001, three subjects in the GNX group and 1 subject in the placebo group had serious AEs (SAEs) that led to dose reduction or temporary or permanent withdrawal of the study drug. The incidence of SAEs was comparable between the GNX and the placebo groups (12% vs 9.8%, respectively). The most frequently reported SAEs were under the SOC of infections and infestations (6% in the GNX group; 3.9% in the placebo group). In the OLE phase, SAEs were more frequent for both the placebo and the GNX groups (24.4% and 25.6%, respectively). Events in the Nervous System Disorders SOC were the most common (11.1% in the PBO group; 11.6% in the GNX group), as well as events in the Infections and Infestations SOC (8.9% in the PBO group; 14% in the GNX group). Based on the available data, it is not possible to rule out that the occurrence of these SAEs under the SOC of infections and infestations was not related to the assumption of GNX. Therefore, they should be monitored through routine pharmacovigilance and with the development of GNX in other indications, especially in a paediatric setting.

In the OLE phase of study 3001, the most common SAE was seizure: 6.8% of patients.

In all CDD patients exposed to GNX, the most commonly reported SAE was seizures 4.9%, dehydration, aspiration pneumonia and vomiting (2.9%). Of note, SAEs were reported more frequently in the GNX group (25.5%) than in the placebo group (9.8%), but the greater duration of exposure may explain this. Of the SAEs considered treatment-related, no clear profile can be disentangled because of the low number of events and the variety of events.

Adverse events of special interest

Rash

Rash was included as an adverse event of special interest (AESI) in the GNX development program due to its structural and pharmacologic similarity to endogenous allopregnanolone. Allopregnanolone is derived from progesterone, associated with autoimmune dermatitis and rash. Rash was reported in with incidences of 4% of patients on GNX and for 3.9% on placebo in the DB phase of study 3001, 4.4% in the OL phase of study 3001, 6.9% of all CDD patients and 3.4% in all patients exposed to GNX (N=1135). Two cases of non-serious rash as a drug-related AE for GNX were reported in DB phase Study 3001. One case was mild; the other was considered moderate. Throughout the GNX clinical development program, there have been no cases of Stevens-Johnson syndrome, toxic epidermal necrolysis or any other clinically important rashes reported in the clinical development program. The additional analyses show that a history of allergic reactions does not predispose subjects to later cutaneous adverse reactions when taking GNX, nor is there currently evidence that GNX use is associated with an increased risk of rash. It is agreed that rash can be removed from the listed AESI.

During the CHMP scientific advice, it appeared that the 13-w juvenile toxicity study in rats to assess potential effects on bone density and sexual maturation/reproductive performance had monitored neonates up to 49 days of life, equivalent to 12 years of age, just before adolescence. Thus, effects on sexual maturation were not explored non-clinically. Although it was acknowledged that CDD patients are unlikely to give birth, it was strongly advised to monitor bone density and also sexual maturation during phase 3.

Reproductive system and breast disorders

The reported incidences of AEs like menorrhagia, menstruation irregular, oligomenorrhoea and polymenorrhoea fall within the incidences in a normal population. Therefore no solid conclusions can be drawn. These events should be monitored in adolescents through routine pharmacovigilance and reported in upcoming PSUR.

Sexual maturation

Tanner stages for patients in study 3001 are provided. Clinically, at baseline, most patients were in Tanner Stage 1, which is in line with the mean age of the cohort of 7.3 years. A Tanner staging at week 52 is available for about half of the patients. With this limited number of patients and a short duration of exposure, which is clearly before the age at the start of sexual maturation, no proper assessment can be made. Delays in sexual maturation and growth were observed non-clinically. It seems reasonable that the non-clinically observed delay in sexual maturation is caused by the delay in growth. Effects on sexual maturation and growth need to be followed up for a longer period, considering that these adverse events have a longer latency. In the RMP, sexual maturation and growth are included as an important potential risk under 'long term safety' in the safety specifications. The applicant will participate to the CDKL5 Registry, with questions dedicated to physical, neurological, and reproductive development asked at baseline and annually thereafter. In addition, assessments of height and weight will be monitored.

Deaths

As of 30 Jun 2022, thirteen patients deceased in the total clinical development program of GNX; twelve during or after GNX treatment and one after placebo treatment. The relation between GNX and the cause of death for most patients is unlikely.

Laboratory findings

No clinically relevant changes in laboratory values regarding haematology, chemistry, urinalysis or vital signs were observed.

ECGs and Potential Effects on Cardiac Safety

In a 1-year dog toxicity study, transient sinus tachycardia (>190 beats per minute [bpm]) was observed after 3 months of dosing in 4 animals and was accompanied by decreased PR and QT interval but no treatment effect on QRS duration or QTc. Because of these non-clinical findings, the CHMP advised sufficient ECG monitoring during the clinical trial.

TQT Study (1042-TQT-1001) showed that adverse events associated with GNX in this study were: dizziness (10 [17.5%] of 57 subjects), somnolence (9 [15.8%] of 57 subjects), fatigue (4 [7.0%] of 57 subjects each), headache and nausea (3 [5.3%] of 57 subjects each), and dyspepsia and gastritis (2 [3.5%] of 57 subjects each). This is in line with the known safety profile of GNX, which mainly consists of CNS-related adverse events. This study investigated higher doses than what will be used in clinical practice in CDD. With regard to short-term safety, these results are reassuring.

For all CDD patients, tachycardia paroxysmal was reported for one patient on placebo and for one patient on GNX, tachycardia was reported. In the large pool of all treated subjects with GNX, bradycardia and tachycardia were reported for two patients each. Other cardiac preferred terms were reported for one patient each. These low incidences are considered to fall within the incidences of a normal population.

An analysis of the available clinical safety data does not reveal an apparent increased risk of cardiac arrest in study participants exposed to GNX at this time, however the risk cannot be excluded and these events (in particular SAE of cardiac arrest and/or SUDEP) should be promptly reported and trigger additional evaluations.

Special populations

Two patients were 19 years old at time of study enrolment and seven patients turned 18 years of age during the OL phase of the study. For these patients (n= 9) separate safety data has been provided. The reported AEs seems to be in line with the observed safety data in the overall study population. Of the 44 TEAE's, four were SAEs: three were considered unrelated to study treatment (sepsis, urinary tract infection, seizure) and one was classified as related (unresponsive to stimuli). In the SOC of Infections and Infestations, AEs were also reported in the 9 adults: 11 TEAEs, generally mild or moderate, with the exception of two SAEs considered not related to GNX (sepsis and urinary tract infection). The reported AEs like menorrhagia, menstruation irregular, oligomenorrhoea and polymenorrhoea fall within the incidence in a normal population, but at the moment no definitive conclusion can be drawn. Therefore, these events should be monitored in adolescents through routine pharmacovigilance and reported in upcoming PSUR.

The Study 1042-IRF-1001 showed that renal impairment has no impact on the exposure of ganaxolone, a single dose of ganaxolone 300 mg was well tolerated in adults with normal and severe renal impairment. With regard to the use of GNX in patients with pre-existing hepatic failure (Study 1042-IHF-1001), adverse events seem in line with the safety profile of GNX. Patients with moderate hepatic impairment reported more

treatment related AEs. The proposed dosing recommendations and warning for hepatic impairment are agreed.

From the subgroup analyses provided, no apparent differences for several subgroups seemed present. Pyrexia was more commonly reported in subjects <12 years old compared with subjects > 12 years old in the CDD Exposure Set, which is expected in a paediatric population. TEAEs of seizure were more frequent in subjects who worsened during the treatment than in subjects who did not worsen.

Pregnancy and lactation

As expected, given the nature of CDD and the age of the patients included, no pregnancies were reported for the CDD population and there is limited data on the use of GNX in pregnant women

Animal studies did not indicate (in)direct harmful effects. However, the exposure in animals was lower than in humans.

GNX is excreted in human milk. Concentrations of ganaxolone in breast milk were approximately 4-fold higher than in plasma. It should therefore be evaluated if breastfeeding should be discontinued or that GNX should be discontinued taking into account the benefits of both for the child as well as for the mother.

The information is appropriately reflected in section 4.6 of the SmPC.

Drug-drug interactions

No formal DDI studies were conducted. Additional subgroup analyses have been provided. Based on these analyses, a warning that other CNS depressants, including other anti-seizure medications, could potentiate the effect on somnolence and sedation has been included in section 4.4 of the SmPC.

Non-clinical data indicate that patients should not be drinking alcohol while being treated with GNX. In the SmPC, a warning not to use alcohol during GNX treatment and interaction with alcohol is included.

Supportive safety data

Withdrawal

Based on non-clinical data, GNX is tapered off in clinical practice to minimize withdrawal effects or symptoms. 13.7% of patients on GNX compared to 2% of patients on placebo reported AEs after discontinuation. The patient randomised to placebo reported hypoxia after discontinuation. For the patients who were randomised to GNX, vomiting and pyrexia (3.9% each) were most reported AEs. AEs like diarrhoea and seizures were reported in 1.0% and 2.0% of patients, respectively. Down titration at discontinuation is included in section 4.2 of the SmPC.

Abuse

As discussed in the clinical pharmacology sections, the potential for abuse of GNX was evaluated in study 1042-HAP-1001. Of the three GNX doses evaluated in the study, the 2000mg dose obtained the highest scores on all endpoints (maximum daily dose for CDD: 1800mg). It is noted however that the abuse potential seems less than for lorazepam 6mg. A limitation of the study is that it only evaluated the abuse potential of single-dose GNX in recreational polydrug users. In clinical practice, GNX will be dosed repeatedly in patients with CDD, most likely in a controlled setting. Therefore the CHMP agreed that GNX has the potential for abuse and a warning is included in section 4.4, with a cross reference to section 5.3.

Overdose

No intentional overdose or misuse of GNX in clinical studies has been reported to date. Accidental overdose has been reported in a total of 4 subjects. All cases of unintentional overdose were resolved.

Suicidality

For the CDD population, no information is available. A general warning regarding suicidal behaviour and ideation in patients treated with ASMs is included in the SmPC.

Long term safety

The long-term safety data for GNX in CDD is limited. 70.5% of patients have an exposure for ≥12 months, 58% of patients have an exposure for ≥18 months, 48.9% for ≥24 months and 8% for ≥36 months. The long-term safety of GNX in CDD will be monitored post-authorisation.

From the safety database all the relevant adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.6.10. Conclusions on clinical safety

In conclusion, the safety data presented is sufficient to allow a benefit-risk assessment in the paediatric population in the CDD indication.

2.7. Risk Management Plan

2.7.1. Safety concerns

Table 29: Summary of safety concerns

Summary of safety concerns	
Important identified risks	• None
Important potential risks	• Long term safety (including sexual maturation and growth) • Suicidal behaviour and ideation
Missing information	• Use in pregnancy and during breastfeeding

2.7.2. Pharmacovigilance plan

Table 30: 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				

Study **Status**	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
None				
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				
None				
Category 3 - Required additional pharmacovigilance activities				
Study 1042-CDD-3001 A Double-blind, Randomised, Placebo-controlled Trial of Adjunctive GNX Treatment in Children and Young Adults with CDD Followed by Long-term Open-label Treatment Ongoing	To assess the long-term safety and tolerability of GNX when administered as adjunctive therapy throughout the open-label phase	Important potential risk of suicidal behaviour and ideation	Open-label trial completion	Q2 2023
			CSR filing	2023
Participation in EURAP (European Registry of Antiepileptic Drugs and Pregnancy) Observational Study Planned	To evaluate the risk of GNX during pregnancy	Missing information on use in pregnancy and during breast-feeding	Regular updates	Data will be reviewed on an on-going basis as a part of signal detection and reported within PSURs, when available.

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Study LLF001 (CANDID observational study) Endpoint Enabling Study in Cyclin-dependent kinase-like 5 Deficiency Disorder Planned	To assess the clinical characteristics of CDD across multiple domains and to understand the longitudinal trajectories of CDD patients across the various clinical outcomes (eg, related to seizures, sleep, behaviour, cognition, global development, and quality of life) To conduct regular safety assessments, including adverse events.	Long term safety	Annual updates	Milestone reports after 50 and 100 participants have completed the first-year visit
CDD-IPR-CDD-01 CDKL5 Deficiency Disorder International Patient Registry Planned	The primary objective of the registry is to collect and enable sharing of real-world demographic, clinical, PRO, and treatment data collected from patients with CDD or their legal guardians and ultimately, their clinicians. Secondarily, the CDKL5 Registry aims to aid in establishing best practices in standard of care for CDD, to inform clinical study design by collecting real-world data and providing access to the scientific and research community, to enable research through data sharing, to understand patient needs and quality of life factors, and to enable rapid recruitment by connecting patients to clinical trials.	Long term safety (including delayed sexual maturation and growth)	Six monthly updates	Q2 2024

Study Status	Summary of objectives		Safety concerns addressed	Milestones	Due dates
GNX Steady-State Metabolite Study Planned		To characterise the GNX metabolite pattern at steady state	Long term safety	Re-analysis of 1042-TQT-1001 study (if feasible) PK study (if relevant)	Q3 2023 Q1 2025
GNX and M2 Transgenic Mouse Carcinogenicity Study A 26-week Oral Gavage Carcinogenicity Study of Ganaxolone (GNX) and M2 (Ganaxolone Metabolite) in Hemizygous CByB6F1-Tg(HRAS)2Jic Mice Planned		To further evaluate mortality and morbidity, terminal histopathology, and tumour statistics for GNX and its metabolites	Long term safety (including delayed sexual maturation and growth)	Study start Final report	Q4 2023 Q1 2025
M2 Chronic ToxicityStudy A 26-Week Oral Gavage Toxicity Study of M2 (Ganaxolone metabolite) in the Albino Rat Followed by a 14-Day Recovery Ongoing	To further evaluate treatment-related clinical signs, M2 exposure and accumulation, and gender differences		Long term safety (including delayed sexual maturation and growth)	Study start Final report	March 2023 Q2 2024
M2 Embryo-foetal Development Study Planned	To detect potential adverse effects of the M2 metabolite of GNX on pregnant female rats and the development of embryos and foetuses after receiving M2 during organogenesis.		Use in pregnancy	Study start Final report	Q1 2024 Q3 2024

Study Status	Summary of objectives		Safety concerns addressed	Milestones	Due dates
WoE assessment	To evaluate the need for a 2-year carcinogenicity study in rats with GNX	Long term safety	Final report	Q2 2024	
WoE assessment	To evaluate the need for a 2-year carcinogenicity study in rats with M2	Long term safety	Final report	Q2 2024	
WoE assessment	To evaluate the need for a juvenile toxicity study with M2	Long term safety	Final report	Q2 2024	
M17 in vitro DDI Studies	To determine if the M17 sulphated metabolite has the potential to cause DDIs.	Long term safety	Final report	Q4 2023	
M17 in vivo PK Study with Brain Penetrance	To determine if M17 is capable of penetrating into the brain and, if so, the levels of M17 in the brain.	Long term safety	Final report	Q4 2023	

2.7.3. Risk minimisation measures

Table 31: Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Long term safety (including delay in sexual maturation and growth)	Routine risk minimisation measures: • *Information in SmPC section 5.3* • *Prescription only medicine* • *Prescribed by physicians experienced in the treatment of epilepsy* Additional risk minimisation measures: *None*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *None* Additional pharmacovigilance activities: • *Study LLF001 (CANDID)* • *CDKL5 Deficiency Disorder International Patient Registry* • *GNX steady-state metabolite study* • *M2 chronic toxicity study* • *GNX and M2 Transgenic Mouse Carcinogenicity Study* • *WoE assessments* • *M17 in vitro DDI Studies* • *M17 in vivo PK Study with Brain Penetrance*
Suicidal behaviour and ideation	Routine risk minimisation measures: • *Warning in SmPC section 4.4* • *Warning in PL section 2 in lay language* • *Prescription only medicine* • *Prescribed by physicians experienced in the treatment of epilepsy* Additional risk minimisation measures: • *None*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *None* Additional pharmacovigilance activities: • *Study 1042-CDD-3001 (open label extension phase)*
Use in pregnancy and during breastfeeding	Routine risk minimisation measures: • *Warning/information in SmPC section 4.6* • *Warning in PL section 2 in lay language* • *Prescription only medicine* • *Prescribed by physicians experienced in the treatment of epilepsy* Additional risk minimisation measures: • *None*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *Pregnancy Notification and Outcome follow-up forms* Additional pharmacovigilance activities: • *EURAP Observational Study* • *M2 Embryo-foetal development study*

2.7.4. Conclusion

The CHMP considers that the risk management plan version 1.0 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 submission

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 18.03.2022. 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, Ztalmy (ganaxolone) is included in the additional monitoring list as ganaxolone is a new active substance and authorised after 1 January 2011.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

The CDKL5 gene is located on the X chromosome. Mutations in certain regions of the CDKL5 gene result in a rare X-linked disorder known as CDKL5 deficiency disorder. This disorder manifests as early-onset with severe neuro-developmental impairment, behavioural abnormalities and difficult-to-control seizures.

Neurologic manifestations include deficits in speech or language, limited or complete loss of functional hand use, stereotypies, hypotonia, cortical blindness, autistic features, and sleep disturbances. Patients may also have dysautonomia, impaired swallowing and constipation.

3.1.2. Available therapies and unmet medical need

There is currently no treatment approved specifically for seizures associated with CDD. Seizures in CDD are treated with anti-seizure medications, corticosteroids, ketogenic diet, vagal nerve stimulation and neurosurgery.

Despite these treatments, patients with CDD remain highly refractory. Thus, there is a need for more effective therapies of seizures associated with CDD.

3.1.3. Main clinical studies

Study 3001 was a randomized, double-blind, placebo-controlled study that evaluated the efficacy and safety of ganaxolone as adjunctive to concomitant anti-seizure medications for the treatment of seizures associated with CDD. The study consisted of a 4-week baseline phase, a 4-week titration phase, and a 13-week maintenance phase. Upon completion of the maintenance phase, subjects could enter the open-label extension phase, which is ongoing. Subjects on placebo switched to GNX.

Subjects between 2 – 21 years of age were eligible to enrol into the study if they had a molecular confirmation of a pathogenic or likely pathogenic CDKL5 variant, early-onset difficult-to-control seizures and neurodevelopmental impairment. In addition, subjects had to have at least 16 major motor seizures per month prior to screening and a history of failure to control seizures despite an appropriate trial of 2 or more anti-seizure medications.

A total of 101 subjects were randomized to GNX (n=50) or placebo treatment (n=51). The target dose of GNX oral suspension was 63 mg/kg/day for subjects weighing ≤ 28 kg or 1800 mg/day for subjects weighing > 28 kg.

The primary endpoint was the percentage change from baseline in 28-day (median) major motor seizure frequency during the 17-week, double-blind treatment phase. Major motor seizures, or primary seizures, are defined as bilateral tonic (sustained motor activity ≥ 3 seconds), generalized tonic-clonic, bilateral clonic, atonic/drop or focal to bilateral tonic-clonic seizures.

The key secondary endpoints are the 50% responder rate, defined as the number (%) of subjects with a ≥ 50% reduction from baseline in major motor seizure frequency and the Clinical Global Impression of Improvement (CGI-I) by caregivers at the last scheduled visit in the 17-week double-blind treatment phase. The CGI-I is scored by Parents/Caregivers and Clinicians separately.

Additional secondary endpoints are the days of motor seizure freedom, the Clinical Global Impression of Change in Seizure Intensity/Duration (CGICSID), the Clinical Global Impression of Change in target domains identified by parent/caregiver (CGI-C) and the Caregiver Global Impression of Change in Attention (CGICA).

3.2. Favourable effects

The percentage change from baseline in median major motor seizure frequency in the maintenance phase was -6.49% for subjects in the placebo group and -29.39% for subjects in the GNX group. The median shift between groups was -29.31% (95% CI: -51.45; -8.90), indicating a statistically significant improvement in the GNX group over placebo (p= 0.0081).

The number (%) of subjects with a ≥ 50% reduction from baseline in major motor seizure frequency (response rate) numerically favoured the GNX group (12 [24.5%] subjects in the GNX group, 5 [9.8%] subjects in the PBO group; 95% CI: -4.7;33.8, p > 0.0643). Over the maintenance phase, there were 15 subjects in the GNX group (30.6%) and 12 subjects in the PBO group (12.0%) who were considered a responder and the difference compared to placebo was nominal statistically significant (p=0.0283)

The Clinical Global Impression of Change – Improvement (CGI-I) as rated by the Parents/Caregivers, reported numerically higher proportions in the GNX treatment group that subjects "minimally improved" (27.1% vs. 14.6%) or "much improved" (35.4% vs. 27.1%) compared to the placebo group. A higher percentage of subjects were rated as having "no change" by their parents/caregivers in the placebo group (45.8%) compared to the GNX group (29.2%).

In the CGI-I as rated by clinicians, subjects were reported "much improved" equally across the treatment groups (14.6% for both treatment groups). The clinicians scored more subjects in the GNX group as "minimally improved" than the placebo group (39.6% vs. 27.1% for the GNX and placebo groups, respectively). A similar percentage of subjects was reported for "no change" for both treatment arms (33.3% vs 39.6% for GNX and placebo, respectively).

The change from baseline in the percentage of median seizure-free days was 4.91% for subjects in the GNX group and 0.17% for subjects in the PBO group, with a median shift from the PBO group to the GNX group of 1.72% (95% CI: -2.71, 7.84).

The majority of GNX subjects showed Caregiver Global Impression of Change in Seizure Intensity/Duration (CGI-CSID) scores of very much improved (4.4% for GNX vs 2.1% for placebo), much improved (33.3% for GNX vs 10.6% for placebo), or minimally improved (24.4% for GNX vs 23.4% for placebo). The nominal p-value was 0.015 (last visit).

For the CGICA and CGI-C, at the end of Week 17 the CGICA result favouring GNX group was of only "minimally improved" (46.7% vs 29.8% for the PBO group) and the CGI-C result was of only "much improved" (44.4% vs 30.4% for the PBO group).

3.3. Uncertainties and limitations about favourable effects

The efficacy of GNX as an adjunctive treatment for seizures associated with CDD in patients from 2 years of age is based on a single pivotal study 3001. Thus, the study should be particularly compelling concerning internal and external validity, clinical relevance, statistical significance, data quality and internal consistency.

While a statistically significant reduction in favour of GNX treatment was observed for major motor seizures, it was questioned whether the high use of rescue medication could partially explain this effect, as half of the subjects in the GNX group received rescue medication at some point. However, the median number of days rescue medication used was lower in the GNX arm (462) than in the PBO arm (499). Post-hoc sensitivity analysis using a tipping point penalising both treatment arms up to 30% did not affect the treatment estimate for the primary endpoint. In the analysis of the 50% responder endpoint, the number of responders dropped when rescue medication was seen as non-response, but it remained numerically in favour of the GNX arm (24.5% for GNX and 9.8% for PBO, respectively). Thus, rescue medication may have affected the efficacy measurement, but this was to a similar degree in both treatment arms, and the treatment difference found was not affected.

Although there is inconsistent support from secondary endpoints, the seizure reduction observed under GNX treatment can be considered clinically relevant through the 50% responder rate and cumulative response curve analysed over the maintenance period.

The majority of the study population consisted of children and young adolescents (mean age was 7 years). There were 2 "adult" subjects, which were both aged 19 years (one in placebo and one in GNX). In light of the limited data available, the available efficacy/safety data in adult patients with CDD, as well as an extrapolation exercise, taking into account the seizure characteristics, were discussed. The efficacy obtained with GNX in children with CDD cannot be extrapolated to adults with CDD, as disease similarity has not been shown between these patient subpopulations. The claim that seizure semiology and disease severity are generally stable into adulthood could not be verified through scientific literature. There seems to be no natural history data or longitudinal observational data available that supports this. Limited efficacy data have been provided on 7 subjects who were 15 years of age at study entry and became > 18 years during the OLE phase of the study. The results from these subjects were mixed and did not provide convincing data to start treatment with GNX in naïve adults with CDD. Moreover, the results hamper interpretation whether seizure semiology remains the same as it only focuses on primary seizure types and lack a comparison to childhood. In addition, based on the provided PK modelling simulations, the simulated exposure in adults was reversely correlated with bodyweight and therefore it cannot be excluded that the drug may be less effective in adults with normal body weight. Although GNX is titrated based on response, higher doses than the maximum dose of 1800 mg/day have not been investigated and thus are not recommended. Thus, as efficacy cannot be extrapolated, the current data do not support use of GNX in treatment naïve adults. While the data preclude use of GNX in treatment naïve adults with CDD, the limited OLE efficacy data do indicate that there is no reason to withdraw treatment in adulthood if a patient has initiated treatment during adolescence and perceived a clear benefit. These recommendations are included in the agreed restricted indication of Ztalmy.

3.4. Unfavourable effects

The safety profile of GNX was studied in 1930 subjects with different indications: several epileptic syndromes, migraine, post-partum depression, and post-traumatic stress disorder (PTSD).

The main evidence of the oral use of GNX in patients with CDD comes from the completed study 1042-CDD-3001. Supportive evidence comes from seven paediatric patients with CDD who are included in a 26-week open-label, proof-of-concept basket study 0900 in children with rare genetic epilepsies.

Safety Pool A provides data of 102 CDD patients exposed to GNX. These patients received GNX via the proposed route of administration (oral) and according to the proposed posology.

In the DB phase of study 3001, 70% of the AEs reported for the GNX group were considered to be treatment-related by the investigator versus 43.1% for placebo. The most common treatment-related AEs for GNX were somnolence (34%), seizure (10%), sedation, constipation and salivary hypersecretion (all 6%). This profile was also observed in the OLE phase of study 3001 and in Pool A.

The incidence of drug-related somnolence (34% vs 5.9%) and sedation (6.0% vs 3.9%) was higher in the GNX group than in the placebo group. For lethargy (4.0% vs 3.9%), the incidence was comparable. All three are considered to fall within the spectrum of sedation. 50% Of the patients on GNX and 43.1% of the patients on placebo used rescue mediation. All rescue medications were CNS depressants. Additional analyses show that the incidence of somnolence-related AEs was not associated with the use of rescue medication.

Decreased appetite was reported for 2.2% of patients who switched from placebo to GNX vs 7% for patients who remained on GNX in the OLE phase of study 3001. Also, weight decrease was reported for 2.2% of patients who switched from placebo to GNX vs. 4.7% for patients who remained on GNX. The drug-related weight decrease was a serious AE for one patient.

In the DB phase of study 3001, three subjects in the GNX group and 1 subject in the placebo group had serious SAEs that led to dose reduction or temporary or permanent withdrawal of the study drug. In the GNX group, two SAEs were considered not being related to GNX (urinary tract infection and bronchitis). No causality for decreased oxygen saturation in one patient on GNX could be concluded.

In the OLE phase of study 3001, the most common SAE in both groups was seizure: 4.7% for patients continuing GNX treatment and 6.7% for patients who switched from placebo to GNX. In all CDD patients who were exposed to GNX, the most commonly reported SAE was seizures: 4.9%. The imbalance between placebo and GNX seems to be explained by a longer exposure to GNX in the OLE.

In the DB phase of study 3001, two patients on GNX and four on placebo discontinued because of AEs. Seizures were the most reported AE leading to discontinuation for both the placebo group; 2 (3.9%), as the GNX group 2 (4.0%). In the OLE phase, ten patients who switched from placebo to GNX discontinued because of AEs versus one patient in the group who remained on GNX. Seizures were the most reported AE leading to discontinuation for both patients who switched to GNX. In the pool of CDD patients exposed to GNX, 13 (12.7%) of patients discontinued because of AEs; the most reported AEs were seizures and somnolence.

Rash was included as an adverse event of special interest (AESI) in the GNX development program due to its structural and pharmacologic similarity to endogenous allopregnanolone. However, since the accumulated evidence suggests that the reports of treatment-emergent skin rashes reported do not differ from non-serious rashes that are relatively commonly encountered, and there has not been evidence to date of significant autoimmune skin reactions to GNX, the applicant decided to remove rash as an AESI. One mild and one moderate case of rash as a drug-related AE for GNX were reported in DB phase Study 3001. Both were considered non-serious. Throughout the GNX clinical development program, there have been no cases of Stevens-Johnson syndrome, toxic epidermal necrolysis or any other clinically important rashes reported in the clinical development program. The additional analyses show that a history of allergic reactions is not associated

with rash, nor is there currently evidence that GNX use is associated with an increased risk of rash. It is agreed that rash can be removed from the listed AESI.

As of 30 Jun 2022, thirteen patients deceased in the total clinical development program of GNX: twelve during or after GNX treatment and one after placebo treatment. The relation between GNX and the cause of death for most patients is unlikely. Three patients in the CDD exposure set deceased; the most likely cause of death is meningococcal sepsis, experienced SUDEP (sudden unexplained death in epilepsy) and cardiac arrest (possibly SUDEP). The first two cases are considered not related to GNX, while the latter was considered as possible related to GNX dose decrease rather than to the GNX itself.

3.5. Uncertainties and limitations about unfavourable effects

Non-clinical and PK uncertainties still exist concerning the metabolites formed in humans.

GNX is rapidly and extensively metabolized. In the mass balance studies, the excretion of the radioactivity was slow, with a half-life of 413 hours. This could be due to metabolites with a long half-life. Due to accumulation, the main metabolites at steady-state may differ from those following a single dose. The steady-state metabolite pattern of GNX has been evaluated in Study 8333043. In this study ganaxolone+O+gluc and Ganaxolone+2O were identified as major human steady-state metabolites. However, due to misinterpretation of results, possible stability issues and the lack of appropriate reference metabolite compounds, the identity of the main steady-state metabolites could not be confirmed and the study cannot be used as supportive evidence.

Based on single dose data, metabolites oxy-didehydro-ganaxolone (M60b = M2) and potentially also oxy-dehydro-ganaxolone sulfate (M43 = M17) appear to be contributing to the long half-life of the radioactivity and are, therefore, possibly the most important metabolites at steady-state. Additional characterization work is ongoing for M43= M17 and the applicant commits to submit the results of this characterisation once available (category 3 study, as listed in the RMP).

As the GNX metabolite pattern at steady state remains unclear, the applicant commits to characterise the GNX metabolite pattern at steady state (category 3 study, as listed in the RMP).

The putative major human metabolite M2 demonstrated no functional activity at the GABAA receptor. However, a potential hormonal effect of metabolite M2 at clinical exposures cannot be excluded. M2 was considered not genotoxic in a standard battery of genotoxicity assays. However, in a short-term repeat-dose toxicity study in rats, findings in the seminal vesicles and prostate glands of males were observed. These findings could potentially be treatment-related and clinically relevant. A stepwise approach for the non-clinical characterization of the safety profile of M2 is still ongoing and will be conducted post-approval (category 3 studies, as listed in the RMP).

The clinical safety database in CDD is relatively small (101 patients are included in DB phase of study 3001 at the cut-off date of 24 February 2021). The number of adults who were treated with GNX is limited: two patients were 19 years old at time of study enrolment and seven patients turned 18 years of age during the OLE phase of the study. For these patients (n= 9) separate safety data has been provided. The reported AEs seems to be in line with the observed safety data in the overall study population. The safety provided in adults with CDD is not considered comprehensive, nor useful to justify the extrapolation of the indication to adults with CDD. However, the applicant accepted the restricted indication to use in children/adolescents.

Long-term safety data for GNX in CDD is limited, and there are some concerns concerning the long-term safety of GNX, especially sexual maturation and growth. A delay in sexual maturation was observed in non-clinical studies. Tanner stages for study 3001 are provided. At baseline, most patients included were in Tanner Stage 1, this is compliant with the mean age of the cohort of 7.3 years. A Tanner staging at week 52 is available for about half of the patients. Due to the limited number of patients with Tanner stages, no proper assessment can be made.

Limited discussion on growth is available. Instead, descriptive data are provided. It is acknowledged that CDD is associated with gastrointestinal problems. The data implies that 2 out of 3 patients lost weight at week 132 while receiving a dose in line with the proposed posology. Additional information indicated that for both patients the events of weight decrease were considered related to GNX and led to dose reductions. For both patients the events resolved. It is agreed that based on these data no amendments are made to the SmPC. It seems reasonable that the non-clinically observed delay in sexual maturation is caused by the delay in growth. Effects on sexual maturation and growth need to be followed up for a longer period, considering that these adverse events have a longer latency. The applicant will participate in the CDKL5 Registry and questions dedicated to physical, neurological, and reproductive development will be asked at baseline and annually thereafter. In addition, assessments of height and weight will be monitored.

An analysis of the available clinical safety data does not reveal an apparent increased risk of cardiac arrest in study participants exposed to GNX at this time. Though, the risk cannot be excluded and these events (in particular SAE of cardiac arrest and/or SUDEP) should be closely monitored, be promptly reported and trigger additional evaluations.

3.6. Effects Table

Table 32: Effects Table for GNX in CDD (data cut-off: 24 February 2021)

Effect	Short Description	Unit	GNX	Placebo	Uncertainties/ Strength of evidence	References
Favourable Effects						
Change in major motor seizure frequency	Median percentage change from baseline in 28-day seizure frequency for major motor seizure types	% (95% CI)	-29.39 (-42.12, -10.46)	-6.49 (-11.46, 20.60)	**SoE**: Hodges-Lehmann Estimate of Location shift: -29.31%, vs. placebo. 95%CI -51.45, -8.90. $p < 0.0081$. Proportion of subjects with a $\geqslant$ 50% reduction in major motor seizure frequency, 15 (30.6%) versus 6 (12%); nominal $p > 0.0283$ **Unc**: Effect not consistently supported by secondary endpoints, 50% responder rate analysed over maintenance period not formally tested. Extrapolation of efficacy from children to adults not possible.	Study 3001 Maintenance phase
CGI-I	Clinical Global Impression of improvement scores at end of week 17 DB rated by Parent/Caregiver or Clinician	N (%) Very much improved Much improved Very much improved Much improved	Parent/Caregiver: 0 13 (27.1%) Clinician: 0 7 (14.6%)	Parent/Caregiver: 1 (2.1%) 7 (14.6%) Clinician: 0 7 (14.6%)	**Unc**: Vs. placebo: Nominal $p > 0.0971$ (Parent/Caregiver), nominal $p > 0.3518$ (Clinician) not formally tested due to sequential testing procedure.	Study 3001 DB phase

Effect	Short Description	Unit	GNX	Placebo	Uncertainties/ Strength of evidence	References
Major motor seizure free days	Median percentage of seizure-free days for major motor seizures at end of 17-week DB	Median (95% CI)	4.91 (0.00, 11.11)	0.17 (0.00, 8.13)	**Unc**: Hodges-Lehmann Estimate of Location Shift: 1.72 (95%CI -2.71, 7.84) Disbalance in baseline median seizure-free days impacts interpretation	Study 3001 DB phase
CGI-CSID	Caregiver Global Impression of Change in Seizure Intensity/ Duration	N (%) Very much improved Much improved	2 (4.4%) 15 (33.3%)	1 (2.1%) 5 (10.6%)	**SoE**: Vs. placebo nominal $p < 0.015$ **Unc**: Not formally tested, support for primary endpoint limited	Study 3001 DB phase
Unfavourable Effects						
Somnolence	Drug-related adverse event	%	34.0	5.9	**SoE:** Incidence almost 6 times higher than for placebo	Study 3001 DB phase
Sedation	Drug-related adverse event	%	6.0	3.9	**SoE:** Incidence higher than for placebo	Study 3001 DB phase
Seizure	Drug-related adverse event	%	10.0	7.8	**SoE:** Incidence higher than for placebo	Study 3001 DB phase

Abbreviations: CDD= cyclin-dependent kinase-like 5 deficiency disorder, CGI-I = Caregiver Global Impression of Change, CGI-CSID = Caregiver Global Impression of Change in Seizure Intensity/Duration, CI = Confidence Interval, DB= Double-Blind, GNX= Ganaxolone, N= Number of patients, SoE = Strength of Evidence, Unc = Uncertainty

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Treatment with GNX resulted in a clinically relevant reduction in major motor seizures associated with CDD as reflected in the median seizure frequency, 50% responder rate and cumulative response curve.

GNX treatment is associated with somnolence and sedation. This is consistent with the safety profiles of other anti-seizure medications. Somnolence and sedation occurred relatively quick after GNX initiation, and most cases resolved during treatment.

The long-term safety data in patients is limited. A delay in sexual maturation and growth was observed after exposure to GNX in non-clinical studies, and preliminary studies with putative major human metabolite M2 did not exclude a potential hormonal effect of M2. Since GNX is a chronic treatment in growing children, evaluation of the possible impact of GNX on sexual maturation and growth is important. Effects on sexual maturation and

growth have a long latency, therefore a long follow-up period is needed. Sexual maturation and growth are included as an important potential risk under 'long term safety' in the safety specifications. In the CDKL5 Registry, in which the applicant will participate, questions dedicated to sexual maturation and the assessment of growth will be investigated at baseline and annually thereafter.

Extrapolation of efficacy from children to adults with CDD in not possible and in view of the limited efficacy data from subjects who became >18 years during the OLE phase of the study, the indication of GNX should be restricted to use in children and adolescents. However, for patients, who initiated treatment in childhood/adolescence, for whom a clear benefit is observed treatment with GNX can be continued when they reach adulthood.

3.7.2. Balance of benefits and risks

Based on the provided data and argumentation, treatment with GNX is approvable for use in children and adolescents. GNX treatment may be continued into adulthood in patients who initiated in childhood/adolescence if a clear benefit has been observed.

3.7.3. Additional considerations on the benefit-risk balance

As part of a pilot program 'CHMP early contact with patient organizations', relevant patient organizations for CDD were contacted by the EMA. The aim is to enable patients to share their experiences, concerns and needs related to their condition with the Rapporteurs/CHMP so that this can be considered in a timely manner during the assessment process, if appropriate. The feedback received indicated that additional medication for CDD is welcomed.

3.8. Conclusions

The overall benefit risk balance of Ztalmy (ganaxolone) for the adjunctive treatment of epileptic seizures associated with cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD) in patients 2 to 17 years of age is positive. Ztalmy may be continued in patients 18 years of age and older.

4. Recommendations

Outcome

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

Ztalmy is indicated for the adjunctive treatment of epileptic seizures associated with cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD) in patients 2 to 17 years of age. Ztalmy may be continued in patients 18 years of age and older.

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 restricted 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.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that ganaxolone is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

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/0171/2021 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.

Appendix

1. CHMP AR on New Active Substance (NAS) as adopted on 25 May 2023