29 January 2026
EMA/37828/2026
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Kygevvi

International non-proprietary name: doxecitine / doxribtimine

Procedure No. EMEA/H/C/005119/0000

Note

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

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Table of contents

List of abbreviations

ADL	activities of daily living
ADME	absorption, distribution, metabolism and excretion
ADR	adverse drug reaction
AE	adverse event
AoO	Age of onset
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
BMI	body mass index
Bw	bodyweight
CHOP INTEND	Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders
CI	confidence interval
CK	creatine kinase
CL	clearance
CO_2	carbon dioxide
CPP	critical process parameter
CSR	clinical study report
dC	doxecitine
dCK	deoxycytidine kinase
dCMP	deoxycytidine monophosphate
dCTP	deoxycytidine triphosphate
dNTP	deoxyribonucleoside triphosphate
dNS	deuterated nucleosides
DSC	differential scanning calorimetry
dT	doxribtimine
dTMP	deoxythymidine monophosphate
dTTP	deoxythymidine triphosphate
EAP	extended access program
eCRF	electronic case report form
EFD	embryofoetal development
EK	Egen Klassifikation
FEED	fertility and early embryonic development
FGF-21	fibroblast growth factor 21
FVC	forced vital capacity
GDF-15	growth differentiation factor 15
GC	gas chromatography
GCP	Good Clinical Practice
GGT	gammaglutamyltransferase
GI	gastro-intestinal
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HDPE	High Density Polyethylene
HFMSE	Hammersmith Functional Motor Scale Expanded
HPLC	High performance liquid chromatography
HR	Hazard ratio
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	Identification

IPC	In-process control
ISE	integrated summary of efficacy
ISS	integrated summary of safety
IV	intravenous
KM	Kaplan-Meier
LDPE	Low density polyethylene
LDPE	Low Density Polyethylene
MA	marketing authorisation
MAA	marketing authorisation application
MDDS	mitochondrial DNA depletion syndrome
MOA	mechanism of action
MOE	margin of exposure
MRI	magnetic resonance imaging
mtCOX1	mitochondrial cyclooxygenase subunit 1
mtDNA	mitochondrial deoxyribonucleic acid
(M)UPD	(modified) untreated patient database
MWP	methodological working party
NA	not applicable
NAS	New Active Substance
NfG	Note for Guidance
nGAPDH	nuclear glyceraldehyde-3-phosphate dehydrogenase
NMT	Not more than
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NSAA	North Star Ambulatory Assessment
PAR	Proven Acceptable Range
PASS	post-authorisation safety study
PDILI	potential drug-induced liver injury
PGNBE	paediatric growth, neurodevelopment, behaviour, and endocrine-related events
Ph. Eur.	European Pharmacopoeia
PK	pharmacokinetic
PND	post-natal day
PPND	pre- and postnatal development
PSD	Particle Size Distribution
PYE	Patient-years of exposure
QTPP	Quality target product profile
RCE	respiratory chain enzyme
RCT	randomised controlled trial
REC	recommendation
RED	rapid equilibrium dialysis
RH	Relative Humidity
RMST	restricted mean survival time
ROW	rest of the world
RULM	Revised Upper Limb Module
SAE	serious adverse events
SAG	scientific advisory group
SAP	statistical analysis plan
SAT	single-arm trial
SD	standard deviation
SmPC	summary of product characteristics

SMQ	standardised MedDRA queries
6MWT	six-minute walking test
SmPC	Summary of Product Characteristics
TAMC	Total Aerobic Microbial Count
TG	transgenic
TK1	thymidine kinase 1
TK2	thymidine kinase 2
TK2d	thymidine kinase 2 deficiency
TK2KI	thymidine kinase 2 knock-in
TK2KO	thymidine kinase 2 knock-out
TYMC	Total Combined Yeasts/Moulds Count
USP	United States Pharmacopoeia
UV	Ultraviolet
Vd	volume of distribution
WHO	World Health Organization
WT	wild-type
XR(P)D	X-Ray (Powder) Diffraction

1. Background information on the procedure

1.1. Submission of the dossier

The applicant UCB Pharma submitted on 6 November 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Kygevvi, through the centralised procedure falling within the Article 3(1) and point 4 of Annex I of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 28 June 2018.

Kygevvi, was designated as an orphan medicinal product EU/3/17/1870 on 20 April 2017 in the following condition: "Treatment of mitochondrial DNA depletion syndrome, myopathic form".

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

The applicant applied for the following indication:

Kygevvi is indicated for the treatment of paediatric and adult patients with thymidine kinase 2 deficiency (TK2d) with an age of symptom onset on or before 12 years.

The final indication granted by CHMP is the following:

Kygevvi is indicated for the treatment of paediatric and adult patients with genetically confirmed thymidine kinase 2 deficiency (TK2d) with an age of symptom onset on or before 12 years.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - full and stand-alone 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/0287/2022 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0287/2022 was completed.

The PDCO issued an opinion on compliance for the PIP P/0287/2022.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

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

847/2000, the applicant did 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. Marketing authorisation under exceptional circumstances

The applicant requested consideration of its application for a marketing authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation.

1.5.2. New active Substance status

The applicant requested the active substance Doxecitine / Doxribtimine 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.

The new active substance claim for Doxribtimine has been withdrawn.

1.6. PRIME

Kygevvi was granted eligibility to PRIME in June 2018 in the following indication: Treatment of Thymidine Kinase 2 Deficiency.

Eligibility to PRIME was granted at the time in view of the following:

- Thymidine Kinase 2 Deficiency is a progressive and devastating disease with high mortality rates especially in patients with infantile-onset and persistent and significant morbidities for all affected patients; treatment is currently limited to supportive care and symptomatic treatment of disease complications. The unmet medical need is recognised.
- Non-clinical data in two relevant animal models provide evidence of proof of principle with significant improvement in survival and parameters evaluating locomotion.
- Clinical data appear to have an effect on survival in a small number of patients with infantile-onset; there is also evidence of clinically significant reductions on need for ventilation and enteric feeding in some patients. Improvements were also reported in patients with childhood onset or the milder phenotype of adult onset.

Upon granting of eligibility to PRIME, Hans Hillege was appointed by the CHMP as rapporteur.

A PRIME kick-off meeting was held on 19 October 2018. The objective of the meeting was to discuss the development programme and regulatory strategy for the product. The applicant was recommended to address the following key issues through relevant regulatory procedures:

The design and statistical analysis plan for a retrospective, medical chart review study to contextualise the efficacy of doxecitine / doxribtimine.

A PRIME submission readiness meeting was held on 3 October 2023. The objective of the meeting was to discuss the status of development programme, implementation of scientific advice, and the planned dossier to support the MAA. The applicant was recommended to address the following key issues through relevant regulatory procedures:

1.7. 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
15 November 2018	EMEA/H/SA/3933/1/2018/PA/PED/SME/PR/III	*André Elferink and Kolbeinn Gudmundsson*
25 June 2020	EMEA/H/SA/3933/1/FU/1/2020/PA/II	*André Elferink and Karl-Heinz Huemer*
16 December 2021	EMA/SA/0000070763	*Ewa Balkowiec Iskra and André Elferink*
23 June 2022	EMA/SA/0000087473	*Johannes Hendrikus Ovelgonne and Dieter Deforce*
20 July 2023	EMA/SA/0000138751	*Rosalía Ruano Camps, Andreas Kirisits*

The Protocol assistance pertained to the following quality, non-clinical, and clinical aspects:

Quality:

- Proposal to supply MT1621 as a dual component medicinal product, filled in separate sachets.
- Strategy to demonstrate equivalency between different sources of the drug substance.
- Comparability data package for the drug substance process change.
- Suitability of starting materials.
- Starting materials and their specifications and proposed intermediate specifications.
- Proposed drug substance specifications, in-process controls, and control of chiral and potential genotoxic impurities.
- Control strategy for the commercial manufacture of drug product.
- Need for device usability studies.
- Demonstration of equivalence between 1st generation and 2nd generation devices.

Non-clinical:

- Extent of the proposed NC data package needed for MA (and general strategy regarding genotox- and carcinogenicity testing).
- Agreement that a two-year carcinogenicity study can be waived.

Clinical:

- Characterization of the pharmacokinetics.
- Contribution of primary evidence to the MAA evaluation.
- Projected safety data for the MAA.

- Acceptability of studies MT-1621-101 and MT-1621-102 for the MAA.
- Justifications to support the planned MAA based on exceptional circumstances.
- Dose selection and dose regimen.
- Adequacy of the PK characterization for the MAA.
- Retrospective chart review study and natural history study.
- Acceptability of the MT-1621-102 study design and the planned safety database for the MAA.
- Proposed overall clinical development plan to support the initial MAA based on exceptional circumstances.
- Acceptability of the studies MT-1621-101 and MT-1621-102 compared to untreated natural history data to support the proposed indication 'Treatment of paediatric and adult patients with TK2d with onset of symptoms on or before 12 years'.
- Proposed final program for clinical pharmacology.
- Body weight -based dose banding approach to replace individual dosing (and preparation).

1.8. Steps taken for the assessment of the product

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

Rapporteur: Peter Mol Co-Rapporteur: Ewa Balkowiec Iskra

The application was received by the EMA on	6 November 2024
The procedure started on	28 November 2024
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	17 February 2025
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	3 March 2025
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	27 March 2025
The applicant submitted the responses to the CHMP consolidated List of Questions on	11 July 2025
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	25 August 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	04 September 2025
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	18 September 2025
The applicant submitted the responses to the CHMP List of Outstanding	07 November 2025

Issues on	
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	26 November 2025
SAG was convened to address questions raised by the CHMP on The CHMP considered the views of the SAG as presented in the minutes of this meeting.	02 December 2025
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	09 December 2025
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	11 December 2025
The applicant submitted the responses to the CHMP List of Outstanding Issues on	19 December 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	14 January 2026
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 Kygevvi on	29 January 2026
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)	18 September 2025

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Kygevvi is intended to be used for the treatment of paediatric and adult patients with genetically confirmed thymidine kinase 2 deficiency (TK2d) with an age of symptom onset on or before 12 years.

2.1.2. Epidemiology

The estimated prevalence of Mitochondrial DNA depletion syndrome (MDDS), myopathic form, is <1 in 1,000,000 (ORPHAcode: 254875; www.orpha.net). In a literature search conducted in 2022, data suggested a prevalence of TK2d of 1.64 per 1,000,000 population (Ma et al, 2023). Published data indicate that the majority of patients with TK2d are children (84% were 0 to 4 years old at onset in Wang et al, 2018; 84.4% were 0 to 12 years old at onset in Garone et al, 2018).

2.1.3. Aetiology and pathogenesis

Thymidine kinase 2 is a mitochondrial matrix enzyme that phosphorylates dC and dT to generate deoxycytidine monophosphate (dCMP) and deoxythymidine monophosphate (dTMP), which in turn, are converted to deoxyribonucleoside triphosphates (dNTPs) required for mtDNA synthesis in replicating cells (Berardo et al, 2022; Hirano et al, 2001). Mutations in the human thymidine kinase 2 gene (TK2) reduce the phosphorylation of dT, and in some tissues dC, needed for synthesis of dNTPs and mtDNA replication, maintenance and repair, with the downstream effect of reduced adenosine triphosphate synthesis (Berardo et al, 2022; Gorman et al, 2016; Hirano et al, 2001).

Thymidine kinase 2 deficiency (TK2d), first identified in 2001, is one of several mitochondrial autosomal recessive disorders that are collectively referred to as mitochondrial deoxyribonucleic acid (mtDNA) depletion and deletion syndrome (MDDS) which encompass clinically and genetically heterogeneous disorders associated with reduction of mtDNA copy number in tissues, leading to insufficient synthesis of mitochondrial respiratory chain enzyme (RCE) complexes (Berardo et al, 2022; Hirano et al, 2001; Saada et al, 2001). The impairment is only partially compensated by the cytosolic production of dNTPs through the thymidine kinase 1 (TK1) and deoxycytidine kinase (dCK) cytosolic pathway. Mitochondrial deoxyribonucleic acid depletion is defined as a residual mtDNA copy number of <30% compared with age-matched controls (Rahman and Poulton, 2009). In TK2d, skeletal muscle is particularly impacted, likely due to reduced thymidine kinase 2 activity in muscle tissue and the high energy demand of skeletal muscle tissue (Alberio et al, 2007). More than 50 distinct TK2 mutations have been linked to TK2d. In general, published data suggest a poor correlation between genotype and phenotype.

2.1.4. Clinical presentation, diagnosis and prognosis

The clinical presentation of TK2d varies, depending on the age of TK2d symptom onset:

- In patients with a symptom onset **<2 years**, the typical clinical picture is hypotonia, rapidly progressive muscle weakness, loss of previously acquired motor skills, respiratory impairment, intestinal dysmotility, failure to thrive; up to 30% of patients have neurologic symptoms such

as encephalopathy, seizures, cognitive impairment and hearing loss and some patients experience cardiomyopathy (Berardo et al, 2022; Garone et al, 2018; Wang et al, 2012).

- In patients that have symptom onset **after 1-2 years and <12 years**, the prominent clinical feature is progressive proximal muscle weakness which typically impacts gross motor function such as ambulation; dysphagia, facial muscle weakness including ptosis, restrictive lung disease; neurologic symptoms can be seen but are generally less common and other non-myopathic symptoms can include cardiac arrhythmias and multiple bone fractures (Berardo et al, 2022; Garone et al, 2018; Wang et al, 2012).

Thymidine kinase 2 deficiency is diagnosed based on a detailed patient history, clinical examination, and laboratory and genetic tests (Berardo et al, 2022). Supportive laboratory tests include elevated serum creatine phosphokinase, elevated lactate and elevated transaminases, or skeletal muscle biopsy or electromyography (Berardo et al, 2022; de Barcelos et al, 2019; Wang et al, 2012). The gold standard for diagnosing TK2d is genetic testing which may reveal various pathologic variants in the nuclear TK2 gene (de Barcelos et al, 2019).

There are no prospective natural history studies or disease registries that are specific to TK2d (Balcells et al, 2023). From case series, medical literature, and chart reviews of untreated patients from the clinical studies in the current application, it appears that TK2d is a relentlessly progressive disease which does not spontaneously improve. TK2d mainly impacts motor function, with progressive proximal muscle weakness that results in impairment of ambulation and activities of daily living, respiratory muscle changes leading to restrictive lung disease, and bulbar muscle changes leading to dysphagia (Berardo et al, 2022; Garone et al, 2018; Wang et al, 2018). Eventually, a substantial percentage of patients die, especially those with an age at symptom onset ≤12 years.

The disease has a large impact on quality of life for patients, their caregivers and families (Amtmann et al, 2023; UMDF, 2022); the unmet medical need herewith is substantial.

2.1.5. Management

There are no medicinal products currently approved for the treatment of TK2d. Disease management is limited to supportive measures, such as ventilatory and feeding support, and devices to assist with mobility (Berardo et al, 2022; Garone et al, 2018; El Hattab and Scaglia, 2013). This involves a coordinated multidisciplinary care team approach, often led by a neurologist and can include other specialists, such as pulmonologists to manage pulmonary infections and the need for ventilatory support, surgeons to manage the need for feeding tubes, and physiotherapists (Ahmed et al, 2018). The supportive care options for patients with TK2d have remained largely unchanged since the disease was first described in 2001 (Berardo et al, 2022; Garone et al, 2018; Saada et al, 2001).

2.2. About the product

2.3. Type of Application and aspects on development

The CHMP did not agree to the applicant's request for an accelerated assessment. The CHMP considered that the data package was atypical and characterized by methodological shortcomings in itself, and this was further complicated by the fact that the presented patient, efficacy, and safety data were preliminary and incomplete. In light of these uncertainties and limitations, the assessment was considered not compatible with the timelines as established in an accelerated assessment.

The applicant requested consideration of its application for a Marketing Authorisation under

exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation based on the following:

Due to the currently estimated prevalence of TK2d (<1 in 1,000,000, Orphanet 2023), it is not feasible to provide comprehensive data for a robust confirmation on morbidity and mortality. In a literature search conducted by the applicant in 2022, a prevalence of 1.64 per 1,000,000 population was found (Ma et al, 2023). Given the high unmet medical need, with currently no European Union (EU) approved treatments to treat the life-threatening or chronically debilitating TK2d disease and anticipating that the level of evidence is considerably less than what would normally be required for a standard marketing authorisation (with expected robust confirmatory data), a different regulatory approach is foreseen and is expected to be followed.

The applicant has data of 17 of an estimated 736 patients in the EU (from Study MT-1621-101 and Study TK0102). Comprehensive clinical data on the product are not available. Due to the indication for which the product is intended is encountered so rarely, the applicant cannot reasonably be expected to provide comprehensive data, and it is unlikely that the applicant will be able to provide such data in a short timeframe; a randomised controlled trial is not feasible. Therefore, a marketing authorisation under exceptional circumstances is proposed by the applicant based on the following ground: *"Inability to provide comprehensive efficacy and safety data due to rarity of the indication"* (see EMEA/357981/2005).

Consequently, specific procedures/obligations are proposed to form the basis for annual reassessment in the context of marketing authorisation under exceptional circumstances. A PASS is proposed as a non-interventional study with 8 years of follow-up, that will describe the safety and clinical outcomes of doxecitine and doxribtimine treatment in paediatric and adult patients with TK2d with an age of symptom onset on or before 12 years; the primary objective is to describe safety. In order to ensure adequate monitoring of safety and efficacy of Kygevvi in the treatment of patients with thymidine kinase 2 deficiency (TK2d), the MAH shall also provide yearly updates on any new information concerning the safety and efficacy of Kygevvi.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as powder for oral solution containing 2 g of doxecitine and 2 g of doxribtimine as active substances.

Other ingredients are: silica colloidal anhydrous (E551) and magnesium stearate (E470b).
The product is available in a foil sachet, made of laminated polyethylene terephthalate (PET), aluminium and low-density polyethylene (LDPE), as described in section 6.5 of the SmPC.

The oral solution is prepared by reconstituting the powder in water. For the reconstitution of the finished product and the administration of the reconstituted product, an administration device kit is cross-referenced in the SmPC. This cross-referenced kit consists of a mixing bottle with dosing cup and two oral syringes.

2.4.2. Active Substance

This medicinal product contains two active substances: doxecitine and doxribtimine, both novel small molecules for which the applicant applied for new active substance (NAS) status. Full information on both active substances has been provided.

Doxecitine (dC)

2.4.2.1. General information

The chemical name of doxecitine is 4-amino-1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1*H*)-one corresponding to the molecular formula $C_9H_{13}N_3O_4$. It has a relative molecular mass of 227.22 and the following structure:

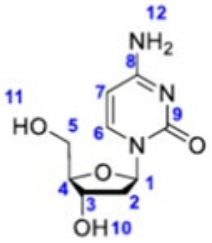


Figure 1. active substance structure

The chemical structure of doxecitine was confirmed by elemental analysis, UV, IR, ^{1}H, ^{13}C NMR, MS, specific optical rotation, X-ray diffraction (XRD), DSC, and DVS. The absolute configuration of the structure containing three chiral centres has been established with a single crystal structure determination.

The active substance is a white to off-white powder, freely soluble in water, very slightly soluble in acetone, soluble in methanol, slightly soluble in dichloromethane, sparingly soluble in ethanol. Doxecitine is non-hygroscopic and photostable.

The active substance is consistently manufactured having the same polymorphic form, , which is the most thermodynamically stable anhydrous form. It was demonstrated as part of the active substance stability studies that the polymorphic form does not change during storage for up to 60 months at long-term conditions, 12 months at intermediate conditions and 6 months at accelerated conditions. The polymorphic form of the active substance is controlled as part of the active substance specification.

The particle size distribution is considered not relevant for the active substance control/clinical performance.

The doxecitine molecules is a β-D-ribose compound with three chiral centres in 2R, 4S, 5R configuration stereoisomer by IUPAC numbering*. The chiral purity is controlled by the specification of the regulatory starting material specification () as well as release of the CII8 intermediate and doxecitine active substance by chiral HPLC.

** Please note that in traditional sugar chemistry numbering the stereochemistry would be (1R, 3S, 4R)*

Polymorphism has been observed for doxecitine. A comprehensive polymorphism screening study of the doxecitine active substance was performed to identify potential polymorphs/solvates and to understand the relationship between these forms. The polymorph screenings have shown the existence of 7 solid forms of doxecitine; anhydrous Forms A, B and G, hydrate Form C, dioxane solvate Form E and two undefined Forms D and F which were not fully characterized.

As indicated above, the applicant claimed new active substance (NAS) status for doxecitine. Based on the review of available data, the CHMP considers that doxecitine is to be qualified as NAS in itself as it is not a constituent of a medicinal product previously authorised within the European Union, nor a salt, ester, ether, isomer, mixture of isomers, complex or derivative of an active substance authorised in the

European Union. Hence, the administration of doxecitine does not expose the patient to the same therapeutic moiety as any already authorised active substance.

2.4.2.2. Manufacture, characterisation and process controls

Doxecitine (dC) is manufactured . from the two starting materials and commercially available chemicals through a series of organic synthesis reactions, purification processes, and a powder-conditioning step.

The process consists of three processing stages: step 1-Synthesis of YII7 ; step 2-synthesis of CII8 (YII7 to CII8), and step 3-synthesis of doxecitine (CII8 to doxecitine), which comprise chemical transformation steps, extractive work-ups, and crystallizations prior to the active substance. After introduction of , there are chemical transformation steps, extractive work-ups, and crystallizations prior to the active substance. There are two isolatedintermediates.

A chemical reaction scheme and a detailed procedural narrative including points for in-process controls (IPCs) are provided. Sufficient details on quantities, temperatures, times, pH and endpoints are given. The critical steps of the manufacturing process and the related critical process parameters and IPCs have been described and are adequate. The batch size of the final active substance has been clearly stated. For some process parameter settings proven acceptable ranges (PARs) have been defined. Since no Design Space is claimed, the applicant clearly stated in S.2.2 that the setting of a parameter can only be varied within a PAR, while keeping other parameters constant.

A justification for selecting the proposed starting materials has been provided and is considered acceptable in view of ICH Q11 and ICH Q11 Q&A. The starting materials are incorporated as a significant structural fragment into the structure of the active substance and have defined chemical properties and structure. Their introduction into the synthesis is followed by sufficient chemical transformation steps with two and one isolated intermediate, respectively. The control strategy of potential impurities from the proposed starting materials is acceptable.

The specifications and control methods for intermediate products, starting materials, the two intermediates and reagents have been presented and are acceptable.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised. A list of specified and observed organic impurities for the doxecitine active substance, summarizing structure, origin, content limits, observed levels and controls has been presented. Also, impurities found in the starting materials or intermediates of the doxecitine active substance, summarizing structure, origin, content limits, observed levels and controls have been summarised. These impurities have not been detected in batches of the doxecitine active substance.

All potential compounds were evaluated with an ICH M7 compliant toxicological analysis for bacterial mutagenicity, based on literature review and/or (Q)SAR evaluation employing a combination of expert rule-based and statistical model-based methodologies. A summary of the outcome of this evaluation with the ICH M7 classification results for class 1, 2, and 3 structures was provided. Two Class 1 impurities (), three Class 2 impurity (), and one Class 3 impurity () were identified. The remaining impurities were identified as ICH M7 Class 5. The control strategy of potential mutagenic Class 1, 2, or 3 impurities has been evaluated based on ICH M7

The applicant has included in 3.2.S.3.2 a summary of the elemental impurities risk assessment and the nitrosamine risk assessment related to the manufacturing process of doxecitine. The results are reported in the finished product section.

Residual solvents used in the manufacturing process have been evaluated. The control of residual solvents has been adequately justified.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. The applicant is proposing a second-generation process (G2) for commercial use. The optimisation of the synthesis of the active substance including the impact of the changes between the G1 and G2 process, the scale up, (critical) process parameters, have been adequately discussed. A statistical comparison of active substance produced by either the G1 (21 batches) or the G2 (3 batches) process has been provided to demonstrate that there is no difference in quality between the G1-process material used for manufacturing of the clinical product batch and later G2-process material proposed for commercialization. Changes introduced have been presented in sufficient detail and have been justified.

As indicated above, PARs beyond the normal operating ranges (NOR) are investigated and defined for multiple process parameters in each step. The available development data, the proposed control strategy and batch analysis data from commercial scale batches fully support the proposed PARs. A risk assessment evaluating the critical material attributes of starting materials and intermediates is provided.

Overall, sufficient information on the manufacturing process development has been provided.

The active substance is packaged in double colourless transparent LDPE bags, each closed with a cable tie. The secondary packaging materials are a LDPE bag with silica gel packets, closed with a cable tie inside a high-density polyethylene (HDPE) drum. Compliance of the LDPE bag with Ph. Eur. 3.1.3 and EU 10/2011 is confirmed.

2.4.2.3. Specification

The active substance specification includes tests for appearance, identification (IR, XRPD, HPLC), assay (HPLC), related substances (HPLC), water content (Ph. Eur.), optical rotation (Ph. Eur.), residual solvents (GC), residue on ignition/sulfated ash (Ph. Eur.), microbial enumeration tests (TAMC, TYMC) (Ph. Eur.), and E.coli (Ph. Eur.).

The proposed active substance specification is acceptable.

The limit of one impurity is above qualification level for maximum daily doses above 2g/day. A toxicology review was conducted to conclude that this impurity would be safe. The limit of this impurity in the active substance is considered acceptableThe proposed limit for individual unspecified impurities is above the qualification and identification threshold for maximum daily doses above 2g/day. However, the limit for individual unspecified impurities in the finished product is in line with ICH Q3B. Considering the levels of the highest unspecified impurity observed, the limit for unspecified impurities was deemed acceptable.

The limit for total impurities was tightened based on batch analytical and stability data as requested by CHMP.The control of class 1 solvent in both active substances doxecitine and doxribtimine in the original submission was not acceptable (Major Objection 1). This solvent may be present as impurity in the solventused in the process, but no control of at any stage of the manufacturing process was proposed. Therefore, the applicant was requested to control this solvent with the ICH in the specification of either the originator solvent, or the active substance as per requirements of the Annex to ICH Q3C. In response, the applicant updated the specifications to include an acceptable limit for the Class 1 solvent. Omission of routine testing for class 1 solvents has been sufficiently justified.

Omission of a test for particle size distribution (PSD) has been adequately justified in line with ICH Q6A decision tree #3. Omission of a test for elemental impurities has been justified based on the fact that they were less than 30% of PDE for the batches manufactured using the commercial G2 process. The risk assessment of potential genotoxic impurities has been performed according to ICH M7, and no related substances need to be controlled according to ICH M7 class 1, 2, or 3.

Sufficient description is provided for all analytical methods included in the specification of doxecitine. Validation of the in-house methods has been adequately performed in accordance with the ICH guidelines. The methods for microbial enumeration and specified microorganisms have been validated to demonstrate the suitability of the method. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data on three commercial scale batches manufactured according to the current and G2 process proposed for commercial use each have been provided, demonstrating that the site is capable of manufacturing doxecitine to a consistent and acceptable standard.

2.4.2.4. Stability

Stability data from six commercial scale batches of active substance from the proposed manufacturer manufactured using the G1 and G2 process stored in a container closure system representative of that intended for the market for up to 36 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided.

The following parameters were tested: appearance, identification by IR, HPLC and XRPD, related substances (specified, unspecified and total impurities), assay, water content, specific optical rotation, specified microorganisms, and microbial enumeration. The analytical methods used were the same as for release (with the exception of water content testing which was tested as per Ph. Eur. 2.5.12.) and were stability indicating.

During the long term and accelerated stability studies the tested parameters remained stable and within the proposed limits. No significant upward or downward trends were observed. Furthermore, no notable difference was observed between the data generated for the G1 and the G2 studies.

Additionally, a photostability study, freeze-thaw cycling study, and a forced degradation study have been performed.

Photostability testing following the ICH guideline Q1B was performed on one commercial scale batch. Following irradiation, the samples were tested for appearance, identification, assay, related substances and water content. The data demonstrate that the active substance doxecitine is photostable. It is agreed that no special storage conditions to protect from light are required.

The effect of temperature cycling was evaluated in a study wherein doxecitine was cycled back and forth between -20°C±5°C, 25°C±2°C and 60°C±2°C, being held at each condition for 48 hrs and repeated two additional times for a total of three times; thus resulting in a total of 6 days at -20°C, 12 days at 25°C and 6 days at 60°C. Samples were tested for appearance, identification, assay, related substances, purity and water content. No notable changes were observed during the study, demonstrating that temperature cycling did not affect doxecitine either chemically or physically.

Forced degradation was evaluated with respect to acid, base, oxidation, heat, humidity, and light. The samples were tested for assay and related substances. The results demonstrated the stability indicating capacity of the in-house assay and related substances 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 with no special storage conditions in the proposed container.

Doxribtimine (dT)

2.4.2.5. General information

The chemical name of doxribtimine is 1-((2*R*,4*S*,5*R*)-4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione corresponding to the molecular formula $C_{10}H_{14}N_2O_5$. It has a relative molecular mass of 242.23 and the following structure:

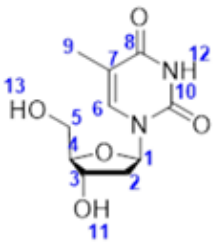


Figure 2. active substance structure

The structure of doxribtimine is confirmed by elemental analysis, UV, IR, ^{1}H, ^{13}C NMR, MS, specific optical rotation, XRD, DSC, and DVS. The absolute configuration of the structure containing three chiral centers has been established with a single crystal x-ray structure determination.

The active substance is a white to off-white powder, soluble in water, slightly soluble in acetone and sparingly soluble in methanol. Doxribtimine is non-hygroscopic and photostable. It is manufactured as polymorphic form A, which is the only form observed. The particle size distribution is considered not relevant for the active substance.

The doxribtimine molecule is a β-D-ribose compound with three chiral centres and is the (2R, 4S, 5R) stereoisomer by IUPAC numbering*. The chiral purity is controlled by the specification of the regulatory starting material specification () as well as release of the CII8 intermediate and doxribtimine active substance by chiral HPLC.

* Please note that in traditional sugar chemistry numbering the stereochemistry would be (1R, 3S, 4R).

Anhydrous Form A is the only observed crystalline form of doxribtimine and is the one produced from the commercial synthesis process.

In the original submission, the applicant claimed NAS status for doxribtimine. Based on the review of available data, the CHMP concluded that doxribtimine is not a constituent of a medicinal product previously authorised within the European Union, nor a salt, ester, ether, complex or derivative of an active substance authorised in the European Union. However, doxribtimine is the stereoisomer of the already authorised active substance telbivudine. Hence, from a quality point of view doxribtimine cannot be considered a new active substance in itself.

2.4.2.6. Manufacture, characterisation and process controls

Doxribtimine (dT) is manufactured by from the two starting materials and commercially available chemicals through a series of organic synthesis reactions, purification processes, and a powder-conditioning step.

The process consists of three processing stages, which comprise chemical transformation steps, extractive work-ups, and crystallizations prior to the active substance. There are two isolated intermediates. A chemical reaction scheme and a detailed procedural narrative including points for IPCs are provided.

Sufficient details on quantities, temperatures, times, pH and endpoints are given. The critical steps of the manufacturing process and the related critical process parameters and IPCs are described. The applicant clearly defined the batch size of the final active substance. For some process parameter settings PARs are defined. Since no Design Space is claimed, the applicant has clearly stated in S.2.2 that the setting of a parameter can only be varied within a PAR, while keeping other parameters constant.

A justification for selecting the proposed starting materials has been provided and is considered acceptable in view of ICH Q11 and ICH Q11 Q&A. Both starting materials are incorporated as a significant structural fragment into the structure of the active substance and have defined chemical properties and structure. Their introduction into the synthesis is followed by sufficient chemical transformation steps with two, respectively one isolated intermediate. The specifications of the starting materials, and of the two isolated intermediates are acceptable.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.
The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities were well discussed with regards to their origin and characterised. A list of specified and observed organic impurities for the doxribtimine active substance, summarizing structure, origin, content limits, observed levels and controls has been presented. Also impurities found in the starting materials or intermediates of the doxribtimine active substance, summarizing structure, origin, content limits, observed levels and controls have been summarised. These impurities have not been detected in batches of the doxribtimine active substance.

An ICH M7 compliant toxicological analysis for bacterial mutagenicity, based on literature review and/or (Q)SAR evaluation employing a combination of expert rule-based (and statistical model-based methodologies coupled with expert judgement, was conducted for doxribtimine starting materials, reagents and impurities that are both known to exist in the process as well as ones that are theoretical based on process knowledge. After an overall evaluation of all potential compounds, two structures were classified as Class 1 , three structures were classified as Class 2 , one structure was classified as Class 3 , 2 were out of scope of ICH M7 and the rest of the structures were classified as Class 5.The results of purge assessments indicate that the purge factors satisfactorily manage all evaluated potential mutagenic impurities and that they are therefore controlled by the overall process, aligning with an ICH M7 .

A summary of the elemental impurities risk assessment and the nitrosamine risk assessment related to the manufacturing process of doxribtimine have been included.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. The applicant is proposing a second-generation process (G2) for commercial use. The optimisation of the synthesis of doxribtimine including the impact of the changes

between the G1 and G2 process, the scale up, (critical) process parameters, are adequately discussed. A statistical comparison of active substance produced by either the G1 (21 batches) or the G2 (3 batches) process is provided to demonstrate that there is no difference in quality between the G1-process material used for manufacturing of the clinical finished product batch and later G2-process material.

PARs beyond the NORs are investigated and defined for multiple process parameters in each step. A risk assessment evaluating the critical material attributes of starting materials and intermediates is provided. Process parameters and in-process controls have been evaluated and the critical process parameters are defined. A control strategy concerning all CQAs of the active substance is discussed.

Overall, sufficient information on the manufacturing process development has been provided.

Doxribtimine is packaged in double colourless transparent LDPE bags, each closed with a cable tie. The secondary packaging materials are a LDPE bag with silica gel packets, closed with a cable tie inside a HDPE drum. Compliance of the LDPE bag with Ph. Eur. 3.1.3 and EU 10/2011 is confirmed.

2.4.2.7. Specification

The active substance specification includes tests for appearance (visual), identification (IR, HPLC), assay (HPLC), related substances (HPLC), water content (Ph. Eur.),residual solvents (GC), residue on ignition/sulphated ash (Ph. Eur.) and microbial enumeration (TAMC, TYMC) and specified microorganisms (*E.coli*).

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set.

The proposed active substance specification is acceptable.

As indicated under doxecitine section above, the control of a class 1 solvent in both active substances doxecitine and doxribtimine in the original submission was not acceptable (Major Objection 1). This solvent may be present as impurity in a solvent used in the process, but no control at any stage of the manufacturing process was proposed. Therefore, the applicant was requested to control this solvent with the ICH in the specification of either the originator solvent, or the active substance as per requirements of the Annex to ICH Q3C. In response, the applicant updated the specifications in doxecitine and doxribtimine to include an acceptable limit . Omission of routine testing for class 1 solvents has been sufficiently justified.

The omission of a test for PSD has been justified in line with ICH Q6A decision tree #3. The omission of a test for polymorphism has been justified as it has been demonstrated that doxribtimine exists in only one polymorphic form.

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with the ICH guidelines. The methods for microbial enumeration and specified microorganisms have been validated to demonstrate the suitability of the method in the presence of the active substance. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data on three commercial scale batches of the active substance manufactured according to the current G2 process have been provided. The results are within the specifications and consistent from batch to batch.

2.4.2.8. Stability

Stability data from three commercial scale (batches of active substance from the proposed manufacturer manufactured using the proposed G2 process and stored in a container closure system representative of that intended for the marketfor up to 36 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided.

The following parameters were tested*:* appearance, ID by IR and HPLC, related substances (specified, unspecified and total impurities), assay, water content, specific optical rotation, specified microorganisms, and microbial enumeration. The analytical methods used were the same as for release and were stability indicating.

During the long term and accelerated stability studies the tested parameters remained stable and within the proposed specification limits. No significant upward or downward trends observed.

Additionally, a photostability study, freeze-thaw cycling study, and a forced degradation study have been performed.

Photostability testing following the ICH guideline Q1B was performed on one commercial scale batch. Following irradiation, the samples were tested for appearance, identification, assay, related substances and water content. The data demonstrate that the active substance doxribtimine is photostable. It is agreed that no special storage conditions to protect from light are required.

The effect of temperature cycling was evaluated in a study wherein doxribtimine was cycled back and forth between -20°C±5°C, 25°C±2°C and 60°C±2°C, being held at each condition for 48 hrs and repeated two additional times for a total of three times; thus resulting in a total of 6 days at -20 °C, 12 days at 25 °C and 6 days at 60 °C. Samples were tested for appearance, identification, assay, related substances, purity and water content. No notable changes were observed during the study, demonstrating that temperature cycling did not affect doxribtimine either chemically or physically.

Forced degradation was evaluated with respect to acid, base, oxidation, heat, humidity, and light. The samples were tested for assay and related substances. The results demonstrate the stability indicating capacity of the in-house assay and related substances 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 with no special storage conditions in the proposed container.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Doxecitine and doxribtimine powder for oral solution 4 g is a white to off-white powder containing 1:1 mixture of doxecitine (2 g) and doxribtimine (2 g). The oral solution is prepared by reconstituting the powder in water. Excipients are silica, colloidal anhydrous and magnesium stearate.

The finished product is packed in a foil sachet, made of laminated polyethylene terephthalate (PET), aluminium and low-density polyethylene (LDPE). The secondary packaging consists of a folding carton and two tamper evident seals.

The active substance doxecitine is a new active substance. Neither of the active substances are described in the Ph. Eur., the USP, or any pharmacopeia of a third member state. Both active substances are white to almost white powders that are freely soluble in water of different pH.

The applicant has justified that polymorphism of doxecitine has no influence on dissolution of the finished product, as all possible polymorphs) exhibit a high solubility above the nominal concentration in the reconstituted oral solution. Doxribtimine only exits in one polymorphic form, which is sufficiently soluble.

The two selected excipients, silica colloidal anhydrous and magnesium stearate are accepted for use in pharmaceuticals and are standard ingredients in oral dosage formulations. The excipients are of Ph. Eur. quality and are used to improve powder flow properties for filling based on their glidant and lubricant actions, respectively. The applicant has justified the absence of a test for the functionality-related characteristic PSD in the specification of magnesium stearate. The compatibility between both active substances and the two excipients has been sufficiently investigated, and reference is also made to the stability studies, which demonstrated stability for up to 18 months.

The influence of doxecitine and doxribtimine PSD of the final blend and flowability on reconstitution time and uniformity of dosage units has been evaluated. Final blends with different PSD were manufactured for this purpose. It has been sufficiently demonstrated that the PSD has no significant impact on critical quality attributes reconstitution time and uniformity of dosage units.

A powder for oral solution of doxecitine and doxribtimine was developed according to the defined Quality target product profile (QTPP).

QTPP elements	Target	Justification
	doxecitine and 50mg/mL of doxribtimine)	doxecitine, the solubility of doxribtimine increases to approx. 70mg/mL, at room temperature) and to maintain acceptable intake volumes, considering the dose range and the target population.
Route of administration	Oral Alternative: feeding tube	To facilitate administration of the drug product in target population.
Method of administration (use of device)	Powder has to be reconstituted, once per day, with water in a mixing bottle. Prescribed dose is to be taken with dosing cup and/or oral syringe	Convenient for body weight-based dosing across a wide range of body weights (pediatric to adult). To ease the administration, even by untrained caregivers and to avoid medication error. Water is selected based on solubility of doxecitine and doxribtimine.
Dosing regimen	3 times per day (6 hours apart) with food	Pharmacokinetic requirement
Pharmacokinetics	PK profile similar to MT-1621-103 and MT-1621-105	Immediate release formulation; doxecitine and doxribtimine are in solution.
Stability	At least 24 months shelf-life at room temperature, for climatic zone II	Sufficient stability to meet the shelf-life.
	At least 16 hours in-use shelf-life after reconstitution in the mixing bottle, at room temperature or in a refrigerator	Sufficient in-use stability to allow once a day reconstitution at room temperature of the required dose and storage at room temperature or in a refrigerator. To ease the administration in out-patient setting.

The formulation development studies have been described in detail.

Early clinical formulations, used in early investigator-initiated studies, consisted of non-GMP grade active substances = provided to patients as per their dosing needs. Nucleotide monophosphates (deoxycytidine monophosphate(dCMP) and deoxythymidine monophosphate (dTMP) were initially used as active substances which were later changed to nucleosides i.e., doxecitine and doxribtimine. No excipients were used in this case.

Then a two-sachet formulation, one for doxecitine and one for doxribtimine were developed. The formulations consisted of GMP-grade active substances (doxecitine or doxribtimine) and excipients packed in respective sachets. This formulation was developed in two strengths – 500mg and 2000mg (of each active ingredient).

The major development entailed the change from the two-sachet formulation to a one-sachet formulation, designed for ease of use and minimizing dosing errors. In the one-sachet formulation, the two active substances are mixed in a 1:1 ratio with the excipients before filling into one sachet. The excipients silica colloidal anhydrous as a glidant and magnesium stearate as a lubricant are the same in both formulations. This formulation was used in the clinical program (clinical studies TK0102, MT-1621-105, MT-1621-106) and it is the proposed formulation for commercialisation.

The differences in qualitative composition between the two-sachet formulation and the one-sachet formulation are minimal. Pharmacokinetic studies have been performed to bridge the two-sachet and one-sachet formulation (see clinical assessment).

Palatability of the doxecitine and doxribtimine reconstituted oral solution in water in adult and paediatric subjects was assessed in clinical studies and it was observed that the palatability of the reconstituted oral solution in water is not optimal. However, there were no reports of patient non-compliance in clinical studies which could be attributed to the severe nature of the disease and the relatively high rate of enteral feeding tube use in the patient population.

The minimum volume of the reconstituted powder for oral solution that patients may take for one dose is 2.5 mL (corresponding to a 3kg bodyweight and 260mg/kg/day dosage), which can be conveniently measured using the administration device i.e., 10mL syringe. The maximum volume for one dose that a patient may take is 300 mL (corresponding to a 110-120 kg bodyweight) which is less or similar to a glass of water and therefore considered as acceptable volume to be taken in one administration.

The dosage form, reconstitution of the finished product and method of administration have been sufficiently justified for the paediatric population in line with EMA Guideline on pharmaceutical development of medicines for paediatric use (EMA/CHMP/QWP/805880/2012 Rev. 2).

As per EMA Q&A on administration of oral immediate release medicinal products through enteral feeding tubes, the applicant performed studies on dose recovery and flush volumes, physicochemical compatibility, and tube blocking. Worst case scenarios are adequately chosen for each of the studies.

Flush volumes to achieve an acceptable dose recovery have been determined (a volume of tap water equivalent to the tube's priming volume, i.e. 1.5 – 6.2 mL). No clogging has been observed. Physicochemical stability has been demonstrated, since no significant changes are observed in clarity of solution, assay and related substances.

The doxecitine and doxribtimine powder for oral solution patient labelling describes the preparation and administration of doxecitine and doxribtimine oral solution, and the use of administration devices that are delivered separately from the finished product and manufactured by Europlaz Ltd. The selection of the devices has been adequately described by the applicant. The pharmaceutical development of the dosing device has been adequately described as per requirements of the Guideline on quality documentation for medicinal products when used with a medical device (EMA/CHMP/QWP/BWP/259165/2019).

The administration devices are provided as a kit (Europlaz Reference ZX2000) and consist of:

- One (1) multiple-use ZX1000 dosing system (manufactured by Europlaz) packed in one box and comprised of:
  - 1 mixing bottle.
  - 1 dosing cup (with a seal) that serves also as lid for the mixing bottle.
  - 2 spare seals as replacement for the dosing cup seal.
- Two (2) 10mL oral syringes manufactured by Rovipharm (a member of SGH).
- One (1) Kit Instruction for Use.

The mixing bottle has volume markings that aid the user in measuring the required amount of water as well as markings that aid the user in selecting the right number of finished product sachets required for their dose. The dosing cup is also the lid of the mixing bottle and is used to administer higher doses at or greater than 50mL. The dosing cup incorporates a replaceable seal, and includes volume markings every 5mL.

Doses below 50mL are to be administered using the 10mL oral syringes.

Adequate documentation on the ZX2000 administration device, including descriptions, specifications, manufacturers and pictures has been provided.

Compliance with the European Medical Devices Requirements has been confirmed.

For the ZX1000 dosing system a copy of the Notified Body CE Certificate for Europlaz and a copy of the Europlaz CE Declaration of Conformity have been provided.
For the 10mL oral syringes a copy of the Notified Body CE Certificate for Rovipharm, and a copy of the Rovipharm CE Declaration of Conformity have been provided.

For the ZX2000 Procedure Pack a statement from Europlaz pursuant to paragraph 1 of Article 22 of the MDR has been provided.

A description of the intended use and a rationale for the choice of device has been provided. Quality attributes of the dosing system (e.g. dose uniformity, durability of marking, tightness, compatibility, in-use stability, functionality of the syringe after repeated use) have been adequately described. The applicant performed a verification study to demonstrate the suitability of the ZX1000 dosing system to reconstitute doxecitine and doxribtimine powder for oral administration

The graduated scale for the mixing bottle and the dosing cup is acceptable in accordance with the EMA Q&A: Part 2" on Graduation of measuring devices for liquid dosage forms. However, a MO2 was raised during the review on the choice of the 10 mL syringe for administration of doses between 10 mL and 50 mL in a single handling. For patient or caretaker convenience and for minimizing the risk for dosing errors, the applicant was requested to refer to an alternative syringe and to perform all relevant studies with the alternative device.

The applicant extensively evaluated the use of other suitable dosing devices. In his view, the benefit of avoiding dosing errors during administration of 10-50 mL of the reconstituted product solution in a single administration with an alternative device instead of multiple doses administered with a 10 mL syringe, does not outweigh the drawbacks that may be encountered

As a conclusion, despite the recommendation by EMA Q&A: Part 2 on Graduation of measuring devices for liquid dosage forms to use a dosing device that enables dosing the prescribed amount in a single administration, the use of the referenced 10 mL syringe is considered acceptable for administration of 10-50 mL doses of the reconstituted product solution in this particular case. The potential dosing errors related to multiple dosing are outweighed by the drawbacks of using an alternative device,.

Furthermore, in line with the above-mentioned guideline and Q&A, the applicant adequately demonstrated dose uniformity both during and at the end of the lifecycle of the bottle. No leakage was detected after the in-use period of the mixing bottle/cup. Functionality of the syringe after repeated use has been demonstrated for manual functionality, tightness and dose uniformity. It has been demonstrated that the markings on the bottle and syringe remain readable during common daily use and cleaning. Suitability and compatibility of the reconstituted product with the devices have been demonstrated by means of extractables/leachables studies and in-use stability studies.

The ZX1000 dosing system (mixing bottle and dosing cup) is to be used as container to store the daily volume of reconstituted oral solution to permit the three daily administrations over the course of the day.

The applicant performed a compatibility and in-use stability study on the administration devices especially considering that the ZX1000 dosing system is also intended to store the daily reconstituted doxecitine and doxribtimine oral solution for 16 hours. The results obtained demonstrated stability and compatibility between the administration devices and the reconstituted solution of doxecitine and doxribtimine, for 16 hours in line with the daily practice (see stability section).

The ZX1000 dosing system has an in-use shelf-life of 6 months (183 cycles). A cycle is defined as a one-day usage of the ZX1000 dosing system. One cycle consists of:

- Preparation/mixing of the oral solution.
- A total of three individual doses by means of the dosing cup, and
- The intermediate and final cleaning.

This corresponds to a total of 4 opening and closing (unscrewing/screwing) actions per cycle/one-day usage.

A reconstitution study was adequately performed and demonstrated that the mixing bottle is suitable for dissolution of the finished product in tap water at room temperature over the entire dosage range.

Interaction studies (extractables/leachables) were also performed.

The performed studies are in line with the requirements of the EMA guideline on quality documentation for medicinal products when used with a medical device. Material compatibility, reconstitution compatibility, and extractables/leachables are sufficiently demonstrated.

During the manufacturing process development, the applicant has adequately investigated the parameter settings of the pre-blending, sieving and final blending steps. The influence of blending time and speed, , on the bulk product properties, such as flowability, PSD, and density were evaluated, and parameter settings were adequately defined for the control of the bulk product properties. Parameter settings of the sachet filling are adequately evaluated and defined for the manufacturing process. The applicant has discussed the changes in manufacturing process during the development of the proposed product from two-sachet to one-sachet formulation. The differences can be attributed to equipment-specific parameters, process development and optimization, and an increase in batch size.

Based on the QTPP, critical quality attributes (CQAs) of the finished product have been identified. Following the manufacturing process development, the control strategy for each CQA has been defined by means of control of material attributes (control of active substances and excipients), in-process controls, critical process parameter controls, release and stability testing.

The primary container closure system for the finished product consists of a laminated foil sachet that has three layers with the inner layer made of low-density polyethylene (LDPE), the middle layer made of aluminium (Alu), and the outer layer made of polyethylene terephthalate (PET). Compliance of the laminated foil with Commission Regulation (EU) No 10/2011 has been confirmed. Information on the printing/labelling of the sachets has been provided. It has been confirmed that the finished product

powder filled and sealed in the sachet does not come in contact with the printing ink or the over lacquer on the exterior. The secondary packaging is a folding carton with a tamper evident seal. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

2.4.3.2. Manufacture of the product and process controls

There is one single manufacturing site for the manufacturing of the finished product: Catalent Germany Schorndorf GmbH (Steinbeisstrasse 1-2, Schorndorf, Baden-Wuerttemberg, 73614, Germany). The site is also responsible for primary and secondary packaging, quality control testing, stability testing (except microbiological tests and 'Clarity of Solution' test), and batch release. TECHPharm GmbH (Draisstraße 14, Bruchsal, Baden-Württemberg, 76646, Germany) is responsible for the 'Clarity of Solution' test. BAV Institut fuer Hygiene und Qualitaetssicherung GmbH (Hanns-Martin-Schleyer-Strasse 25, Waltersweier, Offenburg, Baden-Wuerttemberg, 77656 Germany) is responsible for microbiological tests.

The manufacturing of the finished product consists of pre-blending the active substances with silica colloidal anhydride, followed by sieving and deagglomeration, final blending with magnesium stearate and automated forming-filling-sealing of sachets including weight check.

A hold time of up to 30 days at 20±5°C and ≤65% RH is proposed for the bulk powder (final blend) stored in stainless steel barrels. Packaging of the bulk powder into sachet shall be completed within the 30 day period. The hold time of 30 days is confirmed by a hold time study and it is considered acceptable.

The manufacturing process has been adequately described in sufficient detail. A list of equipment, narrative description with process parameters, flow diagram of the manufacturing process (see below), and in-process controls with acceptance criteria have been presented. No bulk holding time is claimed in P.3.3, but the applicant indicated in P.3.4 a holding time of the final blend before sachet filling of 30 days. This is acceptable for solid oral dosage forms in line with the EMA Guideline on manufacture of the finished dosage form. Compliance with the EMA Note for Guidance (NfG) on start of shelf-life of the finished dosage form has been stated in P.3.3.

All the steps in the finished product manufacturing process are deemed critical and are controlled by critical process parameters. Process parameters such as mixing times and speeds, and acceptance criteria of the in-process controls have been adequately justified during the manufacturing development. The in-process controls are adequate for this type of manufacturing process.

There are no intermediates in the manufacturing process of doxecitine and doxribtimine powder for oral solution.
The applicant provided validation results of three commercial scale batches (4728425, 4728426, 4728427). All IPCs and CPPs, and additional parameters are included in the validation scheme.

The validation results demonstrated a sufficient degree of assurance that the commercial manufacturing process is well controlled with reproducible performance and can consistently produce finished product that meets the proposed commercial specifications.

2.4.3.3. Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance (visual), clarify of solution (Ph. Eur.), pH of solution (Ph. Eur.), identification of doxecitine and doxribtimine (HPLC), identification of doxecitine and doxribtimine (HPLC-retention time and HPLC-UV spectrum), assay of doxecitine and doxribtimine (HPLC), related substances of doxecitine and

doxribtimine (HPLC), reconstitution time for doxecitine and doxribtimine (HPLC), content uniformity (Ph. Eur.), microbial enumeration tests (TAMC, TYMC) (Ph. Eur.) and specified microorganisms (*E. coli*).

Two degradation impurities are specified for the finished product,which are also specified in the active substances Based on the long-term, accelerated, photostability, and thermal cycling stability studies (see stability section), no other degradation impurities are specified. This is acceptable.
Omission of a test for polymorphism has been sufficiently justified. Doxribtimine does not exhibit polymorphism. The potential polymorphs of doxecitine have similar solubilities, hence there is no impact on the reconstitution of the product prior to oral use. In line with decision tree #4 of ICH Q6A, no test for polymorphism in the specification of the finished product is required.

No test for chirality is required in the finished product specification, since the applicant has sufficiently justified that the active substances are stable with respect to chirality and chirality is tested as per active substances specifications.

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

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product has been performed (as requested) 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).

. A risk assessment based on theoretical purge and kinetic modelling was conducted to evaluate the potential for nitrosamine formation in the finished product and the reconstituted preparation. As the modelling approach alone was considered insufficient to exclude the potential formation of a nitrosamine impurity, the Sponsor supplemented the theoretical model with analytical testing from representative batches. Based on the information provided, it is accepted that there is no risk of nitrosamine impurities in the active substance, the related finished product or the reconstituted product. Therefore, no specific control measures are deemed necessary.

Compendial methods for appearance, clarity of solution, pH, water content are according to Ph. Eur. and are considered validated. The suitability of the methods for microbiological quality (microbial enumeration and specified microorganisms) has been confirmed as per requirements of the relevant Ph. Eur. monographs. The validation of the in-house analytical methods for identification and assay of the active substances and related substances by HPLC, and for reconstitution time and uniformity of dosage units by HPLC has been performed in line with the requirements of ICH Q2 for all relevant performance characteristics and documented by providing reports of the validation results.

No other reference standards are used than for the active substance testing. Reference is made to section 3.2.S.5 for the reference standards used in the analytical methods for testing the finished product, which is acceptable.

Batch analysis results are provided on thirteen commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

2.4.3.4. Stability of the product

Stability data from three commercial scale PPQ batches of finished product stored for up to 18 months under long term conditions (25 ºC / 60% RH) and intermediate conditions (30°C/65% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. Additional stability data from two commercial scale supportive stability batches stored for 36 months at 25 ºC / 60% RH and 6 months at 40 ºC / 75% RH was also provided The batches of finished product are representative to those proposed for marketing and were packed in the primary packaging proposed for marketing. The PPQ/Registration batches were manufactured with active substances produced by the second generation (G2) process whereas the supportive stability batches were manufactured with active substances produced by the first generation (G1) process.

Samples were tested for appearance, clarity and pH of solution, assay of both drug substances, related substances, reconstitution time, water content and microbial control. The analytical procedures used are stability indicating.

All results were found to be within the specification limits and no trend was observed under long term or accelerated conditions.

The applicant commits to continue the ongoing stability studies on the PPQ/Registration batches stability study, using three production scale batches through the proposed shelf life. In accordance with EU GMP guidelines[1], any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

One commercial-scaled batch of the finished product was subjected to photostability studies performed under conditions that are in line with the ICH Q1B guideline (option 2). The results show that the finished product is not sensitive to light even when exposed to light outside the primary packaging. Therefore, no further control against light is required.

Stress studies and temperature cycling study were also conducted and further support the inherent stability of the product, both from a chemical and physical standpoint.

All results from temperature cycling studies are within the commercial specifications and no trends were observed. The results support the inherent stability of the product.

The forced degradation (heat, humidity, heat/humidity, light) study confirms that the method for determination of assay and related substances is stability indicating.

In-use stability testing was performed on two commercial scale batches of the finished product. The test date of the aged batch confirms testing at the end of shelf-life, in line with EMA NfG in-use stability testing of human medicinal products. The oral solution was prepared by reconstituting the finished product powder in tap water in the ZX1000 dosing system according to the patient labelling and then stored under defined conditions, namely 5°C and 25°C. At each testing timepoint, a suitable volume of reconstituted solution was poured into the dosing cup and additionally stored to allow 1 hour contact time in the 10mL oral syringe. The parameters tested were clarity of solution, assay and related substances and microbial quality, which is acceptable. The results demonstrate that the reconstituted product is stable at both 5 and 25 °C in the dosing device under conditions of the use in practice.

Based on available stability data, the proposed shelf-life of 30 months with no special storage conditions and the in-use shelf life of 16 h after reconstitution stored in refrigerated conditions

[1] *6.32 of Vol. 4 Part I of the Rules Governing Medicinal products in the European Union*

(5°C±3°C) or at room temperature (15°C-25°C); do not store above 25°C; do not freeze 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.

Active substance and finished product bovine spongiform encephalopathy (BSE) / transmissible spongiform encephalopathies (TSE) statements are provided. Active substance statements were generated based on review of BSE/TSE statements from all raw materials used in the active substance manufacturing process.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

The finished product is presented as a fixed dose combination of doxecitine and doxribtimine formulated as a powder for oral solution. Information on development, manufacture and control of both active substances and finished product has been presented in a satisfactory manner. During the review three MOs were raised pertaining to: MO1. the control of a class 1 solvent in both active substances; MO2. the choice of the 10 mL syringe for administration of doses up to 50 mL; and MO.3 the nitrosamine risk assessment with regard to the potential presence of a nitrosamine in the reconstituted product. These were satisfactorily addressed by the applicant. 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.

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

Not applicable

2.5. Non-clinical aspects

2.5.1. Introduction

Doxecitine (deoxycytidine [dC]) and doxribtimine (deoxythymidine [dT]) are chemically synthesized unmodified pyrimidine nucleosides. The product is being developed for the treatment of paediatric and adult patients with thymidine kinase 2 deficiency (TK2d) with an age of symptom onset on or before 12 years.

TK2 deficiency (TK2d) is a rare mitochondrial DNA depletion syndrome caused by autosomal recessive mutations in the TK2 gene, leading to impaired mitochondrial DNA replication and reduced ATP production, primarily affecting skeletal muscle. The condition has a prevalence of approximately 1.64 per million and typically presents in childhood with progressive muscle weakness, motor regression, respiratory impairment, dysphagia, and neurologic symptoms, with high mortality rates, especially in

early-onset cases. Diagnosis relies on clinical features, lab findings, muscle biopsy, and genetic confirmation. Management is currently limited to supportive care, including ventilatory support and mobility aids, as there are no approved treatments targeting the root cause. This leaves patients with significant morbidity and high caregiver burden. The unmet medical need is driving the development of therapies such as doxecitine and doxribtimine, which target mitochondrial dysfunction.

The nonclinical program was designed to support the chronic oral administration of doxecitine and doxribtimine at a maintenance dose of 800 mg/kg/day (400 mg/kg/day each) for paediatric and adult TK2d patients. The studies, conducted in alignment with ICH guidelines, utilized mouse, rat, rabbit, and dog models, leveraging shared mitochondrial TK2 nucleoside phosphorylation and cytosolic nucleoside pathways, as well as conserved mitochondrial genomes and incorporation pathways analogous to those in humans. The CHMP deemed the program sufficient for the marketing authorisation application (MAA) during multiple Scientific Advice meetings.

The mechanism of action of doxecitine and doxribtimine involves the incorporation of nucleosides deoxycytidine (dC) and deoxythymidine (dT) into skeletal muscle mtDNA, aiming to restore mtDNA copy number and improve skeletal muscle function in TK2d patients. The hypothesis is that doxecitine and doxribtimine utilize residual TK2 activity as well as the cytosolic phosphorylation pathway via thymidine kinase 1 [TK1] and deoxycytidine kinase [dCK] to increase mtDNA precursors (deoxycytidine triphosphate (dCTP) and deoxythymidine triphosphate (dTTP)) in the mitochondria.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Mechanism of action:

The provided mechanism of action (MOA) study aimed to evaluate the incorporation of stable isotope-labelled nucleosides, [^{15}N]dC and [^{15}N]dT, into mitochondrial DNA (mtDNA) of skeletal muscle in both TK2 knockout (TK2KO) and wild-type (WT) mice.

WT (N=3–6/group) and TK2KO (N=2–4/group) mice were orally administered vehicle (PBS) or 800 mg/kg/day [^{15}N]dC and [^{15}N]dT (400 mg/kg each) from post-natal day (PND) 4 to PND 13. At the end of dosing (PND 13), skeletal muscle samples from the left and right hindlimb muscles and diaphragm were collected, processed, and analyzed for mtDNA content using LC-MS to detect isotopically labelled nucleosides. The heavy/light isotope ratios of dC and dT were quantified and compared between treatment groups.

In PBS controls, heavy/light ratios were similar in WT and TK2KO mice, reflecting baseline levels. In treated groups, the ratios were significantly elevated in both WT and TK2KO mice, indicating incorporation of exogenous dC and dT into mtDNA.

These findings confirmed that substrate enhancement therapy with dC and dT can augment mtDNA replication even in the absence of TK2, supporting mtDNA incorporation via a TK2-independent phosphorylation pathway as the MOA for doxecitine and doxribtimine nucleoside therapy in TK2d mice.

Primary pharmacodynamics

A total of five non-GLP primary pharmacodynamic studies, including three literature studies, were conducted using wild-type (WT), Tk2KO, and Tk2KI mouse models to evaluate the effects of nucleoside and nucleotide therapies on TK2 deficiency (TK2d).

<u>Study design</u>

In a mechanism of action study (MT1621-19-014), isotopically labelled [15N]dC and [15N]dT (800 mg/kg/day) were administered to WT and Tk2KO mice from postnatal day (PND) 4 to PND 13 to assess mtDNA incorporation in skeletal muscle. A related study (MT1621-21-015) measured mtDNA copy number in the diaphragm under the same conditions. A survival, growth, and motor function study (MT1621-21-016) tested doxecitine and doxribtimine (1:1 mixture at 660 or 1040 mg/kg/day) in WT and Tk2KI mice from PND 3 to PND 53. Lopez-Gomez et al. (2017) examined dC/dT (520 or 1040 mg/kg/day) and dCMP/dTMP (400 mg/kg/day) effects on survival and mtDNA copy number in WT and TK2KI mice (PND 4 to 70). Garone et al. (2014) tested dCMP/dTMP (400 or 800 mg/kg/day) in WT and TK2KI mice (PND 4 to 54). Finally, Blázquez-Bermejo et al. (2019) assessed dC/dT (800 mg/kg/day) and dCMP/dTMP (1240 mg/kg/day) effects on metabolic and mitochondrial function in WT and TK2KO mice (PND 4 to PND 41–51). These studies collectively demonstrated the therapeutic potential of nucleoside and nucleotide therapies in addressing TK2 deficiency

Mouse models

Two TK2d mouse models were used in PD studies. The TK2KI model carries a H126N mutation, leading to growth deceleration, progressive weakness, and survival limited to 2–3 weeks, with reduced mtDNA levels and unbalanced dNTP pools. The Tk2KO model involves complete deletion of Tk2, resulting in growth retardation, hypothermia, with an 80% reduction in skeletal muscle mtDNA in 14-day-old animals. The Tk2KO mice exhibited a high rate of early mortality by the second week of life with no animal surviving more than 30 days. Both models exhibit mitochondrial dysfunction and respiratory chain defects, mimicking the severe mitochondrial DNA (mtDNA) depletion caused by thymidine kinase 2 deficiency, which closely resembles human infantile encephalomyopathy.

Survival

Four studies (MT1621-21-016, article 2014, 2017 and 2019) evaluated the impact of nucleoside and nucleotide therapies on survival in TK2-deficient mouse models. WT mice, whether treated or untreated, exhibited normal survival (up to PND70) without adverse effects, confirming treatment safety. In untreated Tk2KI mice, survival was severely compromised, with females having a median lifespan of ~5 days and males ~17 days. Similarly, previous studies reported mean lifespans of 13–16 days in untreated Tk2KI and Tk2KO mice. Treatment with nucleoside and nucleotide therapies significantly extended survival across four studies: In Tk2KI mice, doxecitine and doxribtimine prolonged median survival to 30–36.5 days, while dCMP/dTMP and dC/dT therapies extended lifespan by 2–3 times. In Tk2KO mice, treatment with dC/dT or dCMP/dTMP increased survival to ~41–51 days, approximately 2.5–3 times longer than untreated controls.

Survival is the key clinical outcome as TK2d is marked by premature death in most patients with the disease. Findings from all four studies demonstrated that nucleoside and nucleotide therapies significantly extended survival (by 2–5 times) in TK2d mice, highlighting their therapeutic potential in reducing TK2d-associated mortality. However, it should be noted that all treated mice still succumbed prematurely, with lifespans remaining below postnatal day 40.

Body weight growth maintenance

In patients with TK2d, feeding support may be necessary due to feeding muscle dysfunction caused by bulbar muscle impairment and/or nutritional deficiencies. Body weight and weight gain serve as nonclinical surrogate markers for muscle-related feeding symptoms in TK2d patients. In three out of five in vivo studies on TK2-deficient mouse models, body weight was evaluated as an indicator of overall health and muscle-related feeding symptoms.

Wild-type groups treated or untreated gained weight over the course of study, indicating lack of detrimental effect of nucleoside or nucleotide in these animals. Across three studies (MT1621-21-016, article 2014 and 2019) in TK2d mouse models, untreated mice exhibited progressive weight loss

starting around postnatal day 10–14, correlating with disease progression and preceding mortality. In contrast, treatment with nucleoside and nucleotide therapies helped maintain body weight and growth patterns comparable to wild-type controls until approximately PND 20–30.

These findings highlight the therapeutic potential of nucleoside and nucleotide therapies in mitigating weight loss and feeding dysfunction in TK2d. However, despite this temporary benefit, weight gain did not extend into adulthood, and all treated TK2d mice ultimately experienced premature mortality.

Rescued mtDNA depletion:

In three in vivo studies (article 2014, 2017 and 2019) mtDNA level was assessed as an indicator for potential restoration of mitochondrial function. The quantitative PCR (qPCR) method was used to measure mtDNA content in isolated mitochondria from various tissues (brain, liver, muscle, kidney, intestine) and was normalized to nuclear DNA.

Study 1 (Garone et al., 2014) investigated the effects of dCMP+dTMP (400 or 800 mg/kg/day) in Tk2KI mice at PND 13 and PND 29. The results showed significant mtDNA depletion in untreated Tk2KI mice, with the most severe depletion observed in the brain cerebrum (~21% remaining compared to WT), muscle (~47%), and liver (~32%), but in brain cerebellum stable depletion was seen (~59% remaining). At PND 13, treatment with 200 mg/kg/day and 400 mg/kg/day dCMP/dTMP restored mtDNA to near-normal levels in most tissues, except for a mild depletion in the cerebrum (~66% remaining). However, by PND 29, mtDNA depletion reverted to baseline levels, with the brain exhibiting the most significant deterioration. In Study 2 (Lopez-Gomez et al., 2017), treatment with 260 mg/kg/day dC+dT and 520 mg/kg/day dC+dT in Tk2KI mice showed rescued mtDNA depletion in heart, liver, intestine, and muscle but only partially in brain at PND13. Effects were largely lost by PND 29 with the exception of intestine. Finally, in Study 3 (Blázquez-Bermejo et al., 2019), mtDNA depletion was found in brain, skeletal muscle but not in small intestine and liver at D12 in TK2KO vehicle mice when compared to WT vehicle mice. Administration of dC/dT (800 mg/kg/day) in Tk2KO mice demonstrated mtDNA rescue in skeletal muscle and liver. The mtDNA level significantly increased in TK2KO treated mice compared to untreated mice. However, these effects failed to provide long-term benefits, as mtDNA level in TK2KO treated mice was significantly lower than that of WT treated or vehicle mice in brain, liver and skeletal muscle by PND29.

The three studies consistently demonstrated the ability of nucleoside and nucleotide therapies to restore mtDNA levels in various tissues of TK2-deficient mice, with the strongest effects observed in skeletal muscle and minimal efficacy in the brain. However, these effects were transient, with mtDNA levels reverting to baseline or near-baseline levels by PND29 in most tissues.

Motor function:

Two studies, MT1621-21-016 and Blázquez-Bermejo et al., 2019, assessed the efficacy of nucleoside and nucleotide therapies on motor function in Tk2d and wild-type mice.

Study 1 evaluated the effects of doxecitine and doxribtimine (1:1 mixture) on motor and behavioural performance in Tk2KI and WT mice using multiple tests, including neuroscore evaluation, righting reflex, rotarod, treadmill, and open-field tests. The animals in the Tk2KI control group were lost due to early death from their phenotype by 5 days for females and by 17 days for males, whereas treated Tk2KI mice demonstrated motor and behavioural performance comparable to WT mice. In the neuroscore evaluation assessing motor and behavioral deficits, treated Tk2KI mice exhibited scores comparable to both treated and vehicle-treated WT mice until 8 weeks. In the righting reflex test, TK2KI mice treated with 660 mg/kg performed similarly to WT mice through 8 weeks, however, TK2KI mice treated with 1040 mg/kg showed transiently worse performance at 4-5 weeks (not statistically significant) before recovering by 6-8 weeks when compared to WT mice. In the Rotarod tests, treated Tk2KI mice performed similarly to WT mice, with latencies to fall being comparable at 4 weeks but

lower in surviving females at 8 weeks. In the treadmill test, treated Tk2KI mice travelled similar distances and reached exhaustion at similar times as WT mice at 4 weeks. In the open field assay, treated Tk2KI mice showed comparable or greater locomotion than WT controls (travelled the same or longer distance), exhibited similar vertical activity, and yielded inconclusive results in the margin time test due to data variability. Study 2 evaluated the effects of dCMP/dTMP (200 mg/kg/day or 400 mg/kg/day) in TK2KO and control mice using the open-field test with an Opto-Varimetrix-3 sensor system. Results showed no significant differences in average distance travelled, ambulatory and resting times, or horizontal and vertical movements between treated TK2KO and WT mice at 29 days of age. However, the untreated WT and TK2KI mice were not included in the comparison.

In summary, TK2d mice treated with doxecitine and doxribtimine or dCMP/dTMP demonstrated similar scores /results compared to WT mice in motor function, as assessed by neuroscore, righting reflex, Rotarod, open field, and treadmill tests, over a period of at least 4 weeks. Since motor skills were likely impaired in untreated TK2d mice, the results may suggest a potential restoration or stabilization of motor function in treated TK2d animals. However, due to the absence of an untreated TK2d control group, it remains unclear whether this improvement is a direct effect of treatment or reflects variability in disease progression. Besides, there was a gradual decline in body condition over time after 4 weeks.

Respiratory function:

The WT and Tk2KO mice were orally given 800mg/kg/day isotopically labelled [^{15}N]dC and [^{15}N]dT (400mg/kg/day [^{15}N]dC and 400mg/kg/day [^{15}N]dT) once daily from postnatal day 4 to PND 13. Diaphragm samples were collected and analyzed for mitochondrial DNA content using qPCR, with mtDNA quantified through the mitochondrial cyclooxygenase subunit 1 (mtCOX1) gene expression normalized against nuclear glyceraldehyde-3-phosphate dehydrogenase (nGAPDH).

In untreated animals, Tk2KO mice showed significantly reduced mtDNA copy numbers, evidenced by a marked decrease in mtCOX1 expression compared to WT mice. Treatment with deuterated nucleosides (dNS) led to partial restoration of mtDNA levels in the diaphragm of Tk2KO mice; however, this improvement was not statistically significant due to the small sample size (n=2). Interestingly, in the WT+dNS group, the mtDNA:nDNA ratio was slightly lower compared to WT+PBS controls, an unexpected finding since the incorporation of dC and dT into mtDNA would theoretically increase mtDNA content in treated WT mice.

Respiratory failure is the leading cause of death in patients with TK2 deficiency, underscoring the importance of evaluating pulmonary function in therapeutic studies. While direct pulmonary function testing in murine models is challenging due to the small size and early mortality of untreated animals, mtDNA copy number in the diaphragm serves as a surrogate for respiratory function. This study suggests that administering external nucleosides may offer a potential therapeutic benefit by partially restoring mtDNA content in the diaphragm of TK2KO mice, which could correlate with improved respiratory function.

2.5.2.2. Secondary pharmacodynamic studies

The waiving of secondary pharmacodynamic studies is agreed. dC and dT are naturally occurring nucleosides. The MOA of doxecitine and doxribtimine involves improving mtDNA copy number through nucleoside incorporation, which aligns with their physiological roles. The lack of off-target effects in repeat-dose and juvenile toxicity studies at high doses in mice, rats, and dogs suggests a low risk of unintended secondary pharmacology. Furthermore, the confirmation by the PRIME readiness meeting (October 3, 2023) that the nonclinical package seems to be sufficient for the marketing authorization application (MAA) supports this conclusion.

2.5.2.3. Safety pharmacology programme

No dedicated in vitro and in vivo safety pharmacology studies were conducted due to the endogenous nature of doxecitine and doxribtimine.

In the 13-week toxicity study in dogs, cardiovascular endpoints were evaluated. No abnormal findings on ECGs, cardiovascular-related macroscopic, organ weight, or microscopic finding in the heart were noted. In the low dose group, a decrease in heart rate up to 22.6% was observed in males. This was considered treatment unrelated and the no observed effect level (NOEL) was determined at 100 mg/kg/day, with an exposure margin of >2000-fold for dC and >200-fold for dT based on Cmax. This is agreed.

Neurobehavioral and locomotor activities were assessed in Tk2KI mice, however, these pharmacodynamic studies are not designed to serve as stand-alone safety assessments. Since these models were used to evaluate pharmacodynamic effects rather than toxicology, no dedicated toxicity studies were conducted, no definitive conclusions on treatment-related toxicity can be drawn. Respiratory function was not directly measured in the Tk2KO mouse model due to the young age of the animals (PND4 at study start), instead, mtDNA copy number in the diaphragm was assessed as a surrogate marker. Based on the pharmacodynamic models and the mechanism of action, improvements in these organ systems were anticipated. No safety concerns were identified during neurological, respiratory, and GI tract function assessment in the 10-week juvenile animal study in rats (PND7 at the study start), 26-week toxicity study in rats (PND21 at the study start), and 13-week toxicity study in dogs. The exposure margin of these toxicology studies were at least 25-fold higher than human exposure based on baseline-adjusted AUC_{0-24}. The safety pharmacology is considered sufficiently addressed.

2.5.2.4. Pharmacodynamic drug interactions

Due to the endogenous nature of doxecitine and doxribtimine, as well as the clinical experience available to date, no pharmacodynamic drug interaction studies were conducted, which is acceptable.

2.5.3. Pharmacokinetics

Methods

The bioanalytical methods were developed to support TK analyses of deoxycytidine (dC) and deoxythymidine (dT) in mouse, rat, rabbit, and dog plasma in the repeat-dose toxicity studies, developmental and reproductive toxicity studies and juvenile studies. Due to the varying presence of endogenous dC and dT in the different species, the analytical range varied. The provided validation reports demonstrate that the assays were sensitive, selective and suitable to assess dC and dT concentrations in mouse, rat, rabbit, and dog plasma. Incurred sample reproducibility (ISR) for nonclinical sample analysis was conducted for all methods used in pivotal studies. The validation seems to be fit for purpose and the absence of GLP validation is not expected to affect the conclusions of the toxicity studies.

Absorption

Single dose pharmacokinetic studies (non-GLP) with doxecitine and doxribtimine were performed in mice, rats and dogs, with oral doses of 10- 1000 mg/kg bw. The PK analyses were performed on baseline-adjusted plasma concentrations.

After a single oral dose of doxecitine and doxribtimine (266.6-800 mg/kg) to male mice, absorption was fast yielding a Tmax at 0.5-1 h, after which concentrations decreased again to baseline in 6-8h (T1/2 0.891 to 1.37 hours). Overall, exposure to dC and dT increased in a dose-dependent manner. dT plasma exposures (AUC and Cmax) were 10-19 fold higher than dC plasma exposures (baseline as well as after dosing). In a second study in male and female CBYB6f-Tg[HRAS]2jic homozygous (Wt/Wt) and hemizygous (transgenic (Tg)/Wt) mice with lower single oral doses (10-300 mg/kg) Cmax was reached at 0.25-0.75 h and T1/2 was approximately 1 h. Overall, exposure to dC and dT increased in a less than dose proportional to dose-dependent manner. No relevant sex differences were observed. 100 mg/kg administered as a single dose resulted in the highest Cmax, whereas 100 mg administered as 3 oral doses, 4 hours apart resulted in the highest AUC. Exposure in Tg/Wt mice was slightly higher when compared to Wt/Wt mice.

In juvenile rats, after a single oral dose of doxecitine and doxribtimine (66.6-1000 mg/kg), Cmax was reached at 2-4h h and 0.5-3h for dC and dT, respectively. T1/2 ranged from 0.861-2.47 h for dC and from 0.132- 2.34 for dT. Overall, exposure increased in a less than dose proportional (dC) or greater than dose-proportional (dT) manner. In contrast to what was observed in mice, dC plasma exposures in rats tended to be higher than dT plasma exposures. For dC, clearance (CL) and volume of distribution (Vd) after IV bolus administration was observed at 882 mL/hr/kg and 1100 mL/kg, respectively, for the males and at 714 mL/hr/kg and 1500 mL/kg, respectively, for the females. For dT, Cl and Vd was observed at 2920 mL/hr/kg and 556 mL/kg, respectively, for the females (not reported for males). Bioavailability (compared to an IV dose of 66.6 mg/kg) was low: 23.6-28.5 % (males-females) for dC and 6.7-15.7 % (females-males) for dT. No consequent or relevant gender differences were observed.

Two oral single-dose escalation PK studies with doxecitine and doxribtimine (dose levels of 10-200mg/kg and 35-100 mg/k) in male and female dogs were performed. Maximum plasma concentrations were observed after 0.5-2 h. T1/2 for dC and dT was short (0.549-1.36h for dC and 0.165-2.57h for dT). AUC and Cmax generally increased dose dependently. Plasma concentration of dC was greater than of dT (baseline as well as following dosing). No sex differences were observed for dC, but exposure of dT tended to be slightly higher in females than in males in the first, but not in the second study.

Multiple dose pharmaco- and toxicokinetic studies were performed in mice, juvenile or pregnant rats, pregnant rabbits and dogs, at dose levels of 35-2000 mg/kg bw. Whereas mice and pregnant rabbits had higher dT plasma exposures than dC plasma exposures, rats and dogs had higher dC plasma exposures than dT plasma exposures (except in the 5-day study in dogs, where exposure to dC and dC was relatively similar). The TK parameters following repeat oral administration of doxecitine and doxribtimine were generally similar in both sexes in rats and dogs. In mice, rats and dogs, systemic exposure to dC or dT generally increased with increasing doses, although the increases were not necessarily dose proportional. Accumulation of dC or dT was generally not observed following repeat dosing, except in the 26-week juvenile toxicity study in rats (RAUC 2.38 to 5.81 for dC in females, 2.99 for dT in females, and 2.83 for dT in males). In contrast, in the 10-week juvenile study, RAUCs for dT and dC were clearly below 1. The mean Tmax values were low, between 0.5 and 4 hours postdose, and mean t½ values were short, within 1 to 4 hours postdose in all species.

Distribution

No pharmacokinetic studies on organ distribution were performed with exogenous doxecitine and doxribtimine, since sufficient data was available from literature. Exogenous deoxycytidine and deoxythymidine are distributed extensively in the body, with the highest levels in spleen, liver, kidney, and intestine. Lower levels are found in CNS, brain and muscle.

In vitro protein binding of carbon 14 isotope (14C) dC and 14C dT (1-100 µM) was studied in male mouse, rat, dog, monkey, and human plasma using the rapid equilibrium dialysis (RED) method. Low binding of deoxycytidine was observed in rats (7.4-27%), but plasma protein binding of dT and dC across all other species tested ranged between 0 and 8.1% and between 0 and 16.1% for dC and dT, respectively, and was overall independent of concentration over the range evaluated.

No data was available with regard to distribution to milk or placental transport.

Metabolism

Since pyrimidine nucleosides are metabolized by well-characterized pathways, no specific in vitro or in vivo studies were conducted by the applicant to characterize the metabolism of doxecitine and doxribtimine. Instead, studies investigating the metabolic fate of doxecitine and doxribtimine components dC or dT from the literature are discussed. This is acceptable.

Metabolism of endogenous pyrimidine nucleosides is similar across mammalian species, although there are some differences in the relative activities and distribution of the enzymes involved. The degradation of pyrimidine nucleosides does not involve CYP enzymes. Deoxycytidine and dT are initially broken into nucleobases and deoxyribose sugar moieties by (deoxy)cytidine deaminase and thymidine phosphorylase respectively, with further degradation ultimately leading to the formation of water and carbon dioxide (CO_2) via the citric acid cycle. In mammals, deoxycytidine is deaminated to deoxyuridine and further catabolized to dihydropyrimidines.

Excretion

Since the excretion of deoxynucleosides is well known, no specific in vitro or in vivo studies were conducted by the applicant to characterize the excretion of doxecitine and doxribtimine. Instead, a limited overview based on literature is provided. This is acceptable. In general, absorbed nucleosides are extensively degraded, and their end products excreted in the urine and as expired CO_2. Based on studies in rats and hamsters, there appear to be species differences in the amount of dC and dT excreted via urine. In healthy humans, renal excretion of unchanged dC and dT was a minor pathway at doses in the range relevant for this indication.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

No single-dose toxicity studies were conducted.

2.5.4.2. Repeat dose toxicity

In mice dosed up to 400 mg/kg/day (doxecitine 200 mg/kg/day and doxribtimine 200 mg/kg/day) for 4 weeks, no treatment-related findings were observed, and the NOEL was determined at 400 mg/kg/day, with an exposure margin of higher than 10-fold, based on baseline-adjusted AUC_{0-24} as well as non-baseline adjusted AUC_{0-24}.

In juvenile rats at the age of PND21 at the study start were dosed up to 2000 mg/kg/day (doxecitine 1000 mg/kg/day and doxribtimine 1000 mg/kg/day) for 7 days and 26 weeks. In the 7-day study,

decrease in body weights (~-8%) and body weight gain (~-16%) in males were observed at the high dose. The observed decreases in body weights and body weight gains were considered non-adverse and the no observed adverse effect level NOAEL was determined at 2000 mg/kg/day. In the 26-week study, dose-dependent decrease in triglyceride in females were noted in the mid and high dose group. The observed decreased triglyceride levels were not considered adverse and the NOAEL was determined at 2000 mg/kg/day, with exposure margins higher than 1000-fold, based on baseline-adjusted AUC_{0-24} as well as non-baseline adjusted AUC_{0-24}.

In dogs, doxecitine and doxribtimine up to 100 mg/kg/day (doxecitine 50 mg/kg/day and doxribtimine 50 mg/kg/day) for 13 weeks. Decreased heart rate (up to 22.6%) in males. This was not dose dependent and considered treatment unrelated. The NOEL was determined at 100 mg/kg/day, with a dC exposure margin of higher than 500-fold, and a dT exposure margin of higher than 25-fold based on modelled baseline-adjusted AUC_{0-24} as well as non-baseline adjusted AUC_{0-24}.

2.5.4.3. Genotoxicity

Genotoxicity was tested in an Ames test, in vitro and in vivo micronucleus test. The Ames test was negative. The in vitro micronucleus test using human lymphocytes was conducted to examine the potential of doxecitine and doxribtimine to cause chromosomal damage. Human lymphocytes stimulated using phytohemagglutinin to achieve cell division were treated with doxecitine and doxribtimine at concentrations up to 500 µg/mL for 4 hours with or without metabolic activation (S9 fraction), or for 21 hours without metabolic activation. No statistically significant increase in aberrant metaphases was observed in the 4-hour treatments regardless of metabolic activation. However, 21-hour treatment of doxecitine and doxribtimine resulted in a dose-dependent increase in proportion of chromatid breaks (2.7%, 4.7%, and 5.0%) that was outside the 95% control limit of the laboratory negative historical control range (upper 95% limit: 2.4%). The in vivo micronucleus test in male rats treated with a total dose of 2000 mg/kg/day doxecitine and doxribtimine for two days revealed negative results.

2.5.4.4. Carcinogenicity

No studies on carcinogenicity were conducted.

2.5.4.5. Reproductive and developmental toxicity

Fertility and early embryonic development were evaluated in rats at total doses up to 2000 mg/kg/day. No treatment related findings were observed. The NOAEL was determined at 2000 mg/kg/day.

Embryo-foetal developmental toxicity of doxecitine and doxribtimine was assessed in rats and rabbits at up to a total dose of 2000 mg/kg/day.

In rats, no doxecitine and doxribtimine-related maternal toxicity and embryo-foetal toxicity was shown. The maternal and foetal NOAEL were determined at 2000 mg/kg/day, with an exposure margin of 1223 (1867) fold for dC and 425 (491) fold for dT based on modelled baseline-adjusted AUC_{0-24} (non-baseline adjusted AUC_{0-24}).

In rabbits, the dose range-finding embryofoetal development (EFD) study showed a decreased mean body weight, reduced mean food consumption at 2000 mg/kg/day in both non-pregnant and pregnant rabbits. No foetal abnormalities were observed in the dosing groups. The definitive EFD study revealed a decrease in mean maternal body weight (-35% vs. controls for the interval of GD7 to GD20), reduced mean maternal food consumption (-31% vs. controls) in the dosing period at 2000

mg/kg/day. These rebounded (+28% in body weight vs. controls and +22% food consumption vs. controls) during postdose period GD20 to GD29. Furthermore, increased incidence of distended aorta and narrow pulmonary trunk in 3 foetuses from 3 litters were observed in this definitive study. Other increased incidences of skeletal variations included misshapen sternebrae, incompletely ossified sternebrae, and incompletely ossified cervical centra. The litter incidences of these malformations and variations exceeded the historical control range of the Test Facility. As a result, these malformations were considered related to doxecitine and doxribtimine administration. Furthermore, significantly increased incidence of supernumerary thoracic ribs was observed at the high dose. The maternal and foetal NOAEL were determined at 600 mg/kg/day, with an exposure margin of 729 (824) fold for dC and 126 (141) fold for dT based on modelled baseline-adjusted AUC_{0-24} (non-baseline adjusted AUC_{0-24}).

No pre- and postnatal development study (PPND) studies were conducted due to the lack of findings in the fertility and early embryonic development (FEED) and EFD study in rats.

2.5.4.6. Toxicokinetic data

A substantial amount of toxicokinetic data has been collected by the applicant in the pivotal animal species rat and dog as well as pregnant rat and rabbit. Exposure multiples were calculated based on baseline-adjusted and on request also on non-baseline adjusted AUC_{0-24} values.

2.5.4.7. Local Tolerance

No dedicated studies were conducted.

2.5.4.8. Other toxicity studies

Juvenile toxicity

Juvenile toxicity of doxecitine and doxribtimine was evaluated in rats at the age of PND 7 to PND 70 at total doses up to 1000 mg/kg/day, with a 20-day recovery period. Significant increase in absolute body weight and food consumption during the dosing period, which were reversible in the recovery and considered non-adverse. No doxecitine and doxribtimine-related effects on developmental landmarks, reproductive parameters, neurobehaviour, ophthalmology, clinical pathology, and macro- and microscopic pathology. The NOAEL was determined at 1000 mg/kg/day, with an exposure margin of 856 (2925) fold in males and 1527 (3220) fold in females for dC, and 81 (110) fold in males and 152 (187) fold in females for dT, based on the modelled baseline-adjusted AUC_{0-24} ((non-baseline adjusted AUC_{0-24}).

Impurities

A limit of 152 ppm was proposed for the impurity alpha-hydroxythymidine. No dedicated qualification studies were conducted for the proposed limit.

Phototoxicity

Phototoxicity potential tests of dC and dT were performed. The molar extinction coefficient values greater than 1000L/mol*cm were observed over the range of 290 to 294 nm (MEC=2925 to 1283L /mol*cm, based on the methanol data) for dC and 290 to 293 nm (MECs=1913 to 1040 L/mol*cm, based on water data) for dT. Furthermore, dC and dT are endogenous molecules.

Excipients

No dedicated studies were conducted for the excipients.

2.5.5. Ecotoxicity/environmental risk assessment

It can be agreed that 2'-Deoxycytidine and 2'-deoxythymidine are naturally occurring nitrogenous bases of DNA that exist in all living organisms and are not expected to pose any risk to the environment. Therefore, Doxecitine (deoxycytidine [dC]) and doxribtimine (deoxythymidine [dT]) is not expected to pose a risk to the environment.

2.5.6. Discussion on non-clinical aspects

Pharmacology

In MOA studies, mtDNA incorporation was observed in TK2KO mice following dC and dT therapy. The applicant discussed the relevance of TK2 mutant mouse models to human TK2 deficiency (TK2d), including the role of alternative phosphorylation pathways, and explained the transient nature of treatment effects in mice while justifying their relevance to humans.

TK2 mutant mice are validated models for mitochondrial disease, reproducing key features of TK2d—particularly the infantile-onset form—and demonstrating that the mechanism of action of deoxynucleoside therapy is comparable in mice and humans, as the key enzymes involved in its metabolic activation (TK2, TK1, dCK) are highly conserved across species in both structure and function. These models replicate the mitochondrial dysfunction, muscle pathology, and mtDNA depletion seen in patients, with alternative pathways (TK1, dCK, residual TK2) able to partially compensate for TK2 loss. Treatment improves survival, mtDNA content, and motor, respiratory, and weight outcomes, aligning with key clinical endpoints in humans, although no patient data confirm mtDNA restoration. The waning efficacy in mice is likely due to a species-specific age-related decline in TK1 activity, less pronounced in humans, supporting sustained clinical benefit. However, most non-clinical data derive from infantile models, limiting applicability to later-onset disease, and the contribution of residual TK2 or dCK activity to long-term efficacy has not been addressed. Additionally, no human data confirm increased muscular mtDNA following treatment. Nevertheless, given the ultra-rare nature of TK2 deficiency, the limited scientific knowledge, and absence of alternative disease-modifying therapies, together with a well-substantiated MoA supported by robust non-clinical data and regulatory precedents in similar contexts, these uncertainties may be acceptable and can further be mitigated by demonstrating a robust treatment effect in patients with TK2d.

The MT1621 product is a 1:1 mixture of doxecitine and doxribtimine wherein dT is the active component responsible for restoration of mtDNA copy number in TK2d mice and dC is provided in a balanced mass to restore the dCTP pools and prevent an inhibitory effect on of DNA synthesis by excess dT. No inhibitory effect on DNA synthesis was detected for the 1:1 mixture of doxecitine and doxribtimine in in vitro or in vivo toxicology studies, as clinical or pathological signs typically linked to such inhibition (e.g., alopecia, gastrointestinal inflammation, impaired fertility, embryo–foetal effects) were absent. Besides, the mixture was non-genotoxic in the Ames test and rat micronucleus assay. In 26-week juvenile rat and 13-week dog studies, no evidence of tumorigenicity, hyperplasia, preneoplastic lesions, neoplasia, or hormonal/immune modulation was observed.

Pharmacokinetics

Deoxycytidine and dT are endogenous nucleosides present in all tissues. They are distributed extensively in the body, facilitated by the family of SLC28 and SLC29 nucleoside transporters expressed broadly in cell types and organelles including mitochondria. A study quantified the in vitro protein binding of 14C dC and 14C dT in male mouse, rat, dog, monkey, and human plasma. Plasma

protein binding of dC or dT was relatively weak across all species tested under the in vitro test conditions and was independent of concentration over the range evaluated.

Toxicology

The decrease in body weight and body weight gains observed in the 7-day juvenile rat study were considered non-adverse as 1) the extent of reduction is limited, 2) no abnormalities were observed during clinical observations and necropsy examination, and 3) these were observed in males only and not observed in the 26-week study. Given no clinical and histopathological correlates, fully reversible characteristic, and the extent of the effect (0.48 fold and 0.4 fold of the control group mean value), the observed decreased triglyceride levels in the 26-week juvenile rat study were not considered adverse.

The in vitro micronucleus test showed positive. According to the applicant, excess of dTTP can inhibit the enzymatic conversion of dCTP by inhibiting TK2 and ribonucleotide reductase (RNR), leading to dCTP pool depletion. The limited availability of dCTP impairs DNA synthesis, and excess dTTP-induced genotoxicity can thus be observed (Bjurdell and Reichard, 1973; Gonzalez-Vioque et al., 2011). However, it was indicated that the presence of dC in the cell culture medium should circumvent the dT- or dTTP-induced impaired DNA synthesis, since the added dC can be phosphorylated via a dCK-mediated salvage pathway (Yang et al, 1966). The cells in the in vitro micronucleus test were treated with the drug product containing dT and dC at the ratio of 1:1, and genotoxicity should not be expected. Nevertheless, the applicant argued that the regulation of cytosolic nucleotide concentrations under in vitro conditions may not reflect that in vivo, and that an in vivo micronucleus test is more representative of human situations than an in vitro study. While the result of the conducted in vitro micronucleus test was not in line with literature in vitro data where cells cultured with dC and dT mixture were negative for genotoxicity, it is agreed that the negative result of in vivo micronucleus test can overrule the positive in vitro micronucleus test. Furthermore, excess dTTP is not expected to occur in TK2d patients. Should an excess of dTTP occur, there is limited concern for inhibition of TK2, the subsequent dCTP pool depletion and impaired DNA synthesis, as the TK2 activity in TK2d patients is already low. Overall, the concern for genotoxicity is limited in the clinical settings considering the totality of evidence.

Provided the endogenous nature of the compounds, up to 10 years clinical experience of the use of the compounds, and that no evidence of tumorigenicity observed in the 26-week toxicity study in juvenile rats and in the 13-week toxicity study in dogs, it is agreed that the potential for doxecitine and doxribtimine to cause carcinogenicity is low and a two-year carcinogenicity study can be waived.

While developmental toxicities were observed in the conducted EFD study in rabbits, these malformations and variations were observed in foetuses born from dams with maternal toxicity. In addition, the exposure margin of the high dose (2000 mg/kg/day) was more than 100-fold for dC and dT. The human relevance of these visceral malformations and skeletal variations was considered limited.

The applicant has calculated exposure multiples based on baseline-adjusted $AUC_{0\text{-}24}$ values. Since the occurrence of toxicological effects will depend on the total amount of endogenous and administered dC and dT, on request, also non-baseline adjusted margins of exposure (MOEs) were provided.

The impurity alpha-hydroxythymidine is a dT analogue. Also, this is an oxidation product of dT that can be endogenously created. As a dT analogue, misincorporation of alpha-hydroxythymidine into DNA or mispairing can lead to DNA damages and the subsequent cellular death and organ toxicity.

In vitro, administration of alpha-hydroxythymidine alone to bacteria in Ames tests was demonstrated to be mutagenic (Bilimoria et al., 1986; Shirnamé-Moré et al, 1987). However, when administration of alpha-hydroxythymidine together with dT and dC, it was demonstrated that the presence of dT in as low as the amount of 0.1-fold alpha-hydroxythymidine and dC can protect cells from alpha-

hydroxythymidine induced-genotoxicity and cytotoxicity. This was likely due to competition between alpha-hydroxythymidine and dT for uptake, phosphorylation, and DNA incorporation (Kaufman, 1986). Furthermore, in silico predictions did not reveal alerting structures, and the compound was considered not associated with DNA-reactive mutagenic potential.

In the applicant's opinion, the concern for genotoxicity and cytotoxicity induced by impurity alpha-hydroxythymidine present in the drug product was low given that the compound can be endogenously created; misincorporated nucleotides were expected to be removed by natural DNA repair mechanism; and that the drug product contains dC and dT in the amount that is much higher than the amount of this impurity, which further reduces misincorporation of alpha-hydroxythymidine into DNA due to competitive effect. Therefore, for this impurity, a PDE of 420 µg/day (which means 15 ppm of alpha-OH-thymidine in the dT-batches) was calculated according to the ICH Q3C guideline rather than following the TTC value recommended in the ICH M7 guideline. Nevertheless, the applicant indicated that all the doxribtimine batches contain alpha-hydroxythymidine ranging most of the time from 52 ppm up to 152 ppm. Furthermore, the batch containing 128 ppm alpha-hydroxythymidine was tested in the in vivo micronucleus test and in the 26-week study in rats. No toxicity was observed in these studies. In addition, TK2d is a lethal rare disease without other therapeutics alternatives. Considering the above-mentioned weight of evidence and benefit-risk profile, a limit of 152 ppm was proposed.

In the CHMP's opinion, the calculated PDE value of 420 µg/day (15 ppm) is not relevant as the calculation was based on a study in mice receiving only alpha-hydroxythymidine rather than a mixture of alpha-hydroxythymidine, dT, and dC. The applicant proposed a limit of 152 ppm. When a patient is dosed with 400 mg/kg/day doxribtimine containing 152 ppm alpha-hydroxythymidine, the patient is expected to receive 60.8 µg/kg/day alpha-hydroxythymidine. Comparing the amount of alpha-hydroxythymidine received by the rats, dosed with 1000 mg/kg/day doxribtimine containing 128 ppm alpha-hydroxythymidine (128 µg/kg/day), with the amount that the patient is going to receive (60.8 µg/kg/day), the dose of alpha-hydroxythymidine was 2-fold higher in rats than in the patient, and the animal studies did not show toxicities. Considering the protective effect of dT and dC in the drug product and their amount that is much higher than alpha-hydroxythymidine, the natural DNA repair mechanism, a twofold higher dose in the animal studies than in humans, and that TK2d is an ultra-rare disease without other therapeutic alternatives, it is agreed that this impurity has been sufficiently qualified for this case, and the limit of 152 ppm in the doxribtimine batches is acceptable.

Deoxycytidine and deoxythymidine are endogenous molecules. It is agreed that phototoxicity potential of doxecitine and doxribtimine is low. Both doxecitine and doxribtimine were considered photostable and there is no safety concern with the excipients.

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, doxecitine (deoxycytidine [dC]) and doxribtimine (deoxythymidine [dT]) is not expected to pose a risk to the environment.

2.5.7. Conclusion on the non-clinical aspects

Pharmacology

The non-clinical pharmacology studies in Tk2d mice demonstrated the mode of action by showing the incorporation of radiolabelled dT and dC into skeletal muscle mtDNA. Additionally, these studies highlighted the therapeutic potential of nucleoside and nucleotide therapies in addressing the key clinical manifestations of TK2 deficiency, including survival, weight maintenance, motor function, respiratory function and mitochondrial DNA depletion. While these therapies provided notable early benefits, their effects were transient likely due to a species-specific age-related decline of TK1 activity, which is less pronounced in humans. The similarity in enzymatic pathways, mitochondrial dysfunction,

and treatment outcomes between mice and humans support the clinical relevance of the mice model and the translatability of the treatment effects.

Pharmacokinetics

The pharmacokinetics of dC and dT was studied in non-GLP single dose PK studies in mice, rats, and dogs and GLP repeat-dose TK studies in pregnant rats and rabbits, juvenile rats, and dogs. Species differences in dC/dT plasma ratios were observed, with higher dC exposure in rats and dogs, while mice and pregnant rabbits showed higher dT exposure. No significant species differences were observed in Tmax (0.5 and 4h) or t½ (1-4h). Sex differences were minimal. dC and dT are distributed extensively in the body, metabolised by anabolic biotransformation as well as catabolic degradation and their end products excreted in the urine and as expired CO_2. The non-clinical data and discussions provided by the applicant are considered sufficient.

Toxicology

No safety concerns for repeated-dose toxicity, genotoxicity, toxicity on reproduction and development, juvenile toxicity, impurities, phototoxicity, and excipients were identified. The non-clinical data and discussions provided by the applicant are considered sufficient.

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 Union were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Table 1. Tabular overview of clinical studies and other sources evaluating efficacy and safety of doxecitine and doxribtimine in TK2d

Study ID/ location(s) / Study dates/ Status	Population	Number of participants	Design	Drug product	Key efficacy assessments
MT-1621-101 North America, Europe, RoW/ 30 Oct 2018 to 28 Mar 2019/ Completed	Participants with TK2d treated with non-GMP pyrimidine nucleos(t)ides	38 treated (unique)	Phase 2, multicenter, retrospective, noninterventional chart review	Non-GMP dC/dT or non-GMP dCMP/dTMP through compassionate use	• Survival (time to death) • Developmental motor milestones • Ventilatory support • Feeding support
TK0102 North America, Europe, RoW/ 10 Jul 2019/ Ongoing	Participants with TK2d who previously participated in MT-1621-101 and participants treated with non-GMP dC/dT, dCMP/dTMP, or doxecitine and doxribtimine who did not participate in MT-1621-101	47 (12 unique, 35 from MT-1621-101) as of the 15 Mar 2024 data cutoff date	Phase 2, prospective, open-label, single-arm, continuation treatment study of the efficacy and safety of doxecitine and doxribtimine in participants with TK2d	Doxecitine and doxribtimine	• Survival (time to death) • Developmental motor milestones • Ventilatory support • Feeding support

Study ID/ location(s) / Study dates/ Status	Population	Number of participants	Design	Drug product	Key efficacy assessments
MT-1621-107 North America, Europe, RoW/ 22 Sep 2021 to 21 Jan 2022/ Completed	Participants with TK2d treated with non-GMP dC/dT, dCMP/dTMP, or doxecitine and doxribtimine outside of a Company-sponsored study	18 treated (17 unique, 1 from MT-1621-101) 43 untreated	Phase 2, noninterventional, multicenter chart review study to collect survival (time to death) data and related information on treated and untreated participants with TK2d outside of a Company-sponsored study	Non-GMP dC/dT or non-GMP dCMP/dTMP, or doxecitine and doxribtimine outside of a Company-sponsored study	• Survival (time to death) • Developmental motor milestones • Ventilatory support • Feeding support
TK0110 Completed	Treated participants and untreated participants included in MT-1621-101, TK0102, and MT-1621-107 who indicated they had a family member affected by TK2d	43	Noninterventional study to collect additional data from participants with TK2d in the doxecitine and doxribtimine clinical studies	NA	NA
TK0112 Completed	Participants identified from the TK2d literature who were included in the ISE-MUPD and did not participate in MT-1621-101, TK0102, MT-1621-107, or the company-supported EAP	49 participants with data available	Noninterventional study to collect additional data on patients contributing to the Untreated Patient Database for the ISE (ISE-MUPD)	NA	NA
TK0114 North America, Europe, RoW/ Completed	Pediatric and adult patients with TK2d with rapid disease progression and who are the most severely affected in accordance with eligibility criteria	43 (34 unique, 9 from MT-1621-107 as of the data cutoff date of 01 Mar 2024).	Noninterventional to collect data from patients in the company supported EAPs	Doxecitine and doxribtimine (provided as compassionate use)	Survival available for all participants

CSR=clinical study report; dC=deoxycytidine; dCMP=deoxycytidine monophosphate; dT=deoxythymidine; dTMP=deoxythymidine monophosphate; EAP=Expanded Access Program; GMP=Good Manufacturing Practice; ISE=Integrated Summary of Efficacy; MUPD=Modified Untreated Patient Database; NA=not applicable; RoW=rest of the world; TK2d=thymidine kinase 2 deficiency

The **pharmacokinetic** profile of doxecitine/doxribtimine after a single-ascending dose in healthy volunteers was evaluated in 2 clinical trials (**MT-1621-103**, **MT-1621-105**), 1 clinical trial (**MT-1621-106**) evaluated the influence of impaired kidney function on the pharmacokinetic profile of dC and dT, and 2 clinical trials (I-IND 136653, MT-1621-102) evaluated the pharmacokinetic profile of dC and dT in paediatric and adult patients with TK2d.

The applicant proposes to perform a **post-authorisation safety study** (TK0109) as an additional pharmacovigilance activity. The primary objective is to describe safety and clinical outcomes through a non-interventional post-authorisation safety study.

2.6.2. Clinical pharmacology

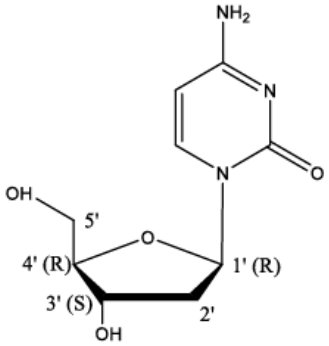
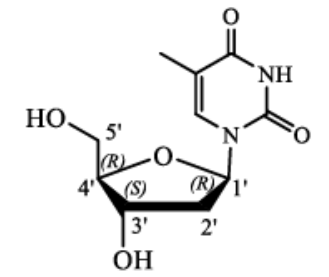
2.6.2.1. Pharmacokinetics

Doxecitine (deoxycytidine [dC]) and doxribtimine (deoxythymidine [dT]), previously known as MT1621, powder for oral solution in a single sachet (containing 2g doxecitine and 2g doxribtimine) is being developed as a treatment for thymidine kinase 2 (TK2) deficiency (TK2d) in patients with age of symptom onset on or before 12 years. The proposed dosing regimen consists of an initial dose of 260 mg/kg/day (1:1 ratio, consisting of 130 mg/kg/day doxecitine and 130 mg/kg/day doxribtimine),

which is uptitrated to 520 mg/kg/day (1:1 ratio) and finally 800 mg/kg/day (1:1 ratio) after 14 and 28 days, respectively. The reconstituted oral solution should be taken with food 3 times a day in equally divided doses (approximately 6±2 hours apart).

Deoxycytidine and deoxythymidine (Table 2) are endogenous pyrimidine nucleosides that are expected to be integral to multiple biochemical and physiological processes, including synthesis of mitochondrial deoxyribonucleic acid (mtDNA), amino acids, lipids, uridine diphosphate-sugars, ribonucleic acid (RNA), and nuclear deoxyribonucleic acid (DNA). Thymidine kinase 2 is a mitochondrial-specific enzyme that phosphorylates dC and dT to deoxycytidine monophosphate (dCMP) and deoxythymidine monophosphate (dTMP), which are subsequently phosphorylated to deoxycytidine triphosphate (dCTP) and deoxythymidine triphosphate (dTTP) for incorporation into replicating mtDNA. Thymidine kinase 2 phosphorylates dT with greater efficiency than dC.TK2d (orphan indication) is characterized by progressive muscle weakness, dysphagia, and respiratory involvement with a broad range of severity and age of onset. Increasing the levels of dC and dT in TK2d is hypothesized to help restore deoxyribonucleoside triphosphates (dNTPs) through cytosolic pathways that phosphorylate dC and dT, which then cross the mitochondrial membrane to support mtDNA replication, along with maximizing any residual TK2 activity within the mitochondria.

Table 2. Key general physicochemical properties of dC and dT

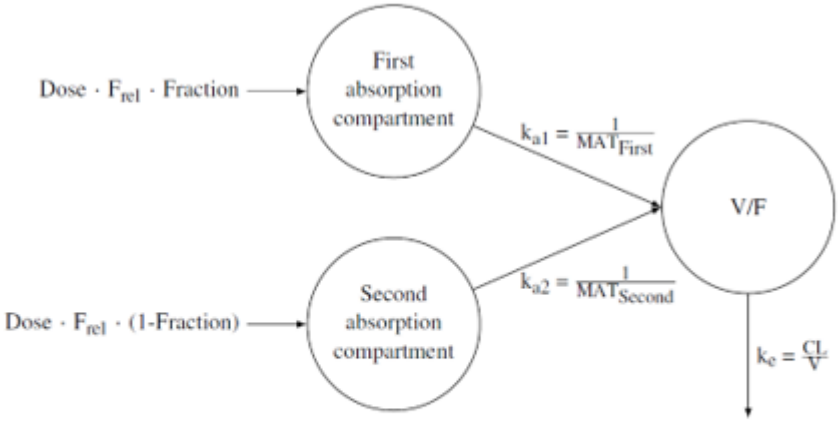
	**Doxecitine** Molecular formula: $C_9H_{13}N_3O_4$ Molecular weight: 227.2 g/mol Partition Coefficient (n-octanol/water): <0.01 Solubility • Water: 632 mg/mL • Buffer (pH 1.2 to 7.5): 619 to 488 mg/mL Stereochemistry: Doxecitine has three chiral centers at the C-1', C-3' and C-4' carbons. The configuration of doxecitine is R, S, R, respectively.
	**Doxribitimine** Molecular formula: $C_{10}H_{14}N_2O_5$ Molecular weight: 242.2 g/mol Partition Coefficient (n-octanol/water): <0.01 Solubility • Water: 47.6 mg/mL • Buffer (pH 1.2 to 7.5): 48 to 44 mg/mL Stereochemistry: Doxribtimine has three chiral centers at the C-1', C-3' and C-4' carbons. The configuration of doxribtimine is R, S, R, respectively.

The clinical development programme (Table 1) consisted of two clinical trials (**MT-1621-103**, **MT-1621-105**) that evaluated the pharmacokinetic profile of dC and dT after a single-ascending dose in healthy volunteers. Additionally, one clinical trial (**MT-1621-106**) evaluated the influence of impaired kidney function on the pharmacokinetic profile of dC and dT and two clinical trials (**I-IND 136653**, **MT-1621-102**) evaluated the pharmacokinetic profile of dC and dT in paediatric and adult patients with TK2d. Furthermore, several *in vitro* studies were conducted that evaluated plasma protein binding (**MT1621-20-003**), permeability (**MT1621-19-013**), metabolic stability in human liver microsomes (**MT1621-20-001**) and whether dC or dT were substrates of CYP enzymes and transporters (**MT1621-20-001, MT1621-19-013, NCD3991**). Furthermore, *in vitro* studies were performed to

investigate whether dC or dT were inhibitors (**MT1621-19-012**) or inducers (**MT1621-19-011**) of CYP enzymes or transporters.

Methods

Population pharmacokinetic model: For dC, the model consisted of a one-compartment model with first-order absorption and first-order elimination with an estimated baseline parameter. Absorption was best described in the fed state by separation of the first-order absorption process and an additional first-order absorption process for the fed state. Between-subject variability was generally assumed to be log-normally distributed and was larger in patients with TK2d for the relative bioavailability parameter. Residual error was modelled as proportional error with an exponential between-subject variability term. Also, residual variability was larger in patients with TK2d compared to healthy volunteers. Bodyweight, ALT and renal impairment were included as covariates on apparent clearance (CL/F), bodyweight on apparent volume of distribution (Vd/F), dose and food status on relative bioavailability and food status and age on the estimated baseline.



$$\text{BASE (ng/mL)} = 3.65 \cdot e^{-0.00389 \cdot (\text{Age} - 32.2 \text{ years})}$$

$$\text{CL/F (L/h)} = 4.54 \cdot 10^{5} \text{ (L/h)} \cdot \left(\frac{\text{WT}}{60 \text{ (kg)}}\right)^{0.741} \cdot e^{0.350 \cdot (\log(\text{ALT}) - \log(18.76 \text{ U/L}))} \cdot \begin{cases} 1 & \text{if normal renal function} \\ 1 - 0.410 & \text{if moderate/severe renal impairment} \end{cases}$$

$$\text{V/F (L)} = 9.16 \text{ (L)} \cdot \left(\frac{\text{WT}}{60 \text{ (kg)}}\right)^{1}$$

$$F_{rel} = 1 \cdot \left(\frac{\text{Dose}}{133.3 \text{ (mg/kg)}}\right)^{-0.422} \cdot \begin{cases} 1 & \text{if fasted} \\ 1 + 1.77 & \text{if fed} \end{cases}$$

$$\text{MAT}_{\text{First}} \text{ (h)} = 0.624 \text{ (h)} \cdot \begin{cases} 1 & \text{if fasted} \\ 1 - 0.521 & \text{if fed} \end{cases}$$

$$\text{MAT}_{\text{Second}} \text{ (h)} = 2.06 \text{ (h)} \cdot \begin{cases} 1 & \text{if TSLD<=4.5 h} \\ 1 - 0.872 & \text{if TSLD>4.5 h} \end{cases}$$

CL=clearance; Frel=relative bioavailability; ka1=first-order absorption rate constant associated with the first absorption compartment; ka2=first-order absorption rate constant associated with the second absorption compartment; ke=first-order elimination rate constant from central compartment; MATFirst=mean absorption time associated with the first absorption compartment; MATSecond=mean absorption time associated with the second absorption compartment; K=pharmacokinetic(s); V=volume of distribution; V/F=apparent volume of distribution
Note: Fraction corresponds to the fraction of the bioavailable dose absorbed through the first absorption compartment (fixed to 1 in fasted).

Figure 3. Schematic for the final deoxycytidine population pharmacokinetic model

Table 3. Parameter estimates of the final dC population pharmacokinetic model

Parameter	Unit	Value	RSE (%)	SHR (%)
BASE	ng/mL	3.65	3.01	NA
WT on CL[a]	NA	0.741	29.4	NA
WT on V[a]	NA	1.00	(FIX)	NA
CL/F	(10^5 L/h)	4.54	4.63	NA
V/F	(10^5 L)	9.16	19.7	NA
MAT_{First}	(h)	0.624	36.1	NA
MAT_{Second}	(h)	2.06	20.6	NA
Fraction[b]	NA	0.130	32.1	NA
Time effect on MAT_{Second}[c]	NA	-0.872	4.19	NA
Scaling factor on IIV F_{rel}[d]	NA	1.58	25.1	NA
Scaling factor on RUV[d]	NA	1.90	19.5	NA
Dose on F_{rel}[a]	NA	-0.422	10.3	NA
Fed on F_{rel} relative to fasted	NA	1.77	16.3	NA
Fed on MAT_{First} relative to fasted	NA	-0.521	48.7	NA
Age on BASE	NA	-0.00389	41.2	NA
ALT on CL	NA	0.350	43.5	NA
Moderate/severe renal impairment on CL relative to normal	NA	-0.410	34.7	NA
IIV RUV	(CV)	0.229	12.3	20.3
IIV BASE	(CV)	0.185	10.3	22.7
IIV F_{rel}	(CV)	0.512	12.2	9.05
IIV V	(CV)	0.420	16.3	31.5
IIV MAT_{First}	(CV)	0.882	14.7	35.9
IIV MAT_{Second}	(CV)	0.450	25.3	41.0
IIV Fraction[e]	(CV)	1.14	25.3	53.6
RUV	(CV)	0.146	5.39	2.10

Table 3. Parameter estimates of the final dC population pharmacokinetic model

ALT=alanine aminotransferase; BASE=estimated baseline plasma concentration; CL=clearance; CL/F=apparent clearance; CV=coefficient of variation; dC=deoxycytidine; F_{rel}=relative bioavailability; IIV=interindividual variability; MAT_{First}=mean absorption time associated with the first absorption compartment; MAT_{Second}=mean absorption time associated with the second absorption compartment; NA=not applicable; RSE=relative standard error; RUV=residual unexplained variability; SD=standard deviation; SHR=shrinkage; TK2d=thymidine kinase 2 deficiency; V=volume of distribution; V/F=apparent volume of distribution; WT=body weight

Note: The typical participant has a weight of 60kg, is dosed with 133.3 doxecitine mg/kg, is in fasted condition, is 32.2 years old, has an ALT of 18.76U/L, and no renal impairment.

The RSE for IIV and RUV parameters are reported as the RSE on the SD (ie, ω and σ) scale.

[a] Power relationship.

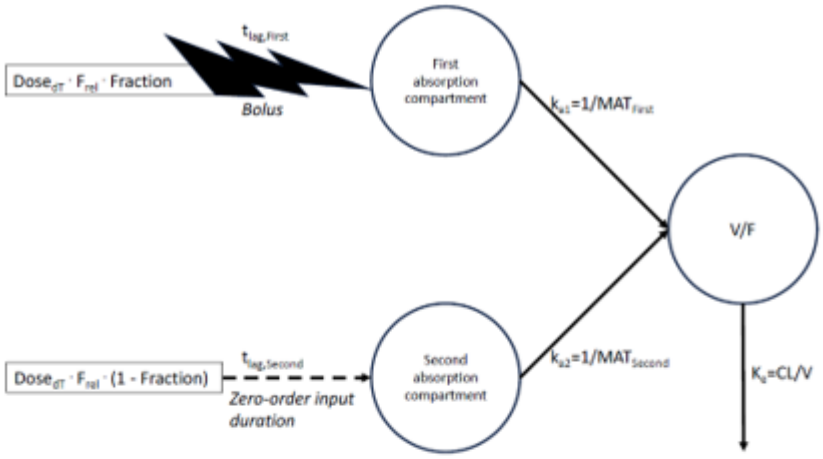
[b] Fraction of the bioavailable dose absorbed through the first absorption compartment, fixed to 1 in fasted.

[c] 4.5 hours after last dose, relative to before 4.5 hours after last dose.

[d] Scaling for participants with TK2d.

[e] The IIV is reported as the SD on the logit scale.

For dT, a similar model structure was used, but the parallel absorption in the fed state was described by first-order absorption with a lag time and a serial zero-order process followed by first-order absorption. The covariate model included allometric relationships between body weight and apparent clearance (estimated exponent) and volume of distribution (fixed exponent of 1), dose and ethnicity on the relative bioavailability, food status on absorption parameters including relative bioavailability (all except zero-order input duration), and age on baseline dT.



$$BASE\ (ng/mL) = 0.488 \cdot e^{-0.0125 \cdot (Age - 32.05\ years)}$$

$$CL/F\ (L/h) = 3.55 \cdot 10^{5}\ (L/h) \cdot \left(\frac{WT}{60\ (kg)}\right)^{0.777}$$

$$V/F\ (L) = 2.92\ (L) \cdot \left(\frac{WT}{60\ (kg)}\right)^{1}$$

$$F_{rel,fasted} = 1.00 \cdot \left(\frac{Dose}{133.3\ (mg/kg)}\right)^{0.709} \cdot \begin{cases} 1 & \text{if hispanic/latino} \\ 1 + 1.41 & \text{if NOT hispanic/latino} \end{cases}$$

$$F_{rel,fed} = 2.13 \cdot \left(\frac{Dose}{133.3\ (mg/kg)}\right)^{0.709} \cdot \begin{cases} 1 & \text{if hispanic/latino} \\ 1 + 1.41 & \text{if NOT hispanic/latino} \end{cases}$$

$$MAT_{First}\ (h) = \begin{cases} 0.459\ (h) & \text{if fasted} \\ 9.41\ (h) & \text{if fed} \end{cases}$$

$$MAT_{Second}\ (h) = \begin{cases} 3.66\ (h) & \text{if fasted} \\ 0.425\ (h) & \text{if fed} \end{cases}$$

$$t_{lag,First}\ (h) = \begin{cases} 0.225\ (h) & \text{if fasted} \\ 0\ (h) & \text{if fed} \end{cases}$$

$$t_{lag,Second}\ (h) = \begin{cases} 3.38\ (h) & \text{if fasted} \\ 0\ (h) & \text{if fed} \end{cases}$$

CL=clearance; Frel=relative bioavailability; ka1=first-order absorption rate constant associated with the first absorption compartment; k_{a2}=first-order absorption rate constant associated with the second absorption compartment; K_e=first-order elimination rate constant from central compartment; MAT_{First}=mean absorption time associated with the first absorption compartment; MAT_{Second}=mean absorption time associated with the second absorption compartment; $t_{lag,First}$=lag time associated with the first absorption compartment; $t_{lag,Second}$=lag time associated with the second absorption compartment; V=volume of distribution; V/F=apparent volume of distribution
Note: Fraction corresponds to the fraction of the bioavailable dose absorbed through the first absorption compartment (fixed to 1 in fasted).

Figure 4. Schematic of the final deoxythymidine population pharmacokinetic model

Table 4. Parameter estimates of the final deoxythymidine population pharmacokinetic model

Parameters	Unit	Value	RSE (%)	SHR (%)
BASE	ng/mL	0.488	6.06	NA
WT on CL[a]	NA	0.777	23.1	NA
WT on V[a]	NA	1.00	(FIX)	NA
CL/F	(10^5 L/h)	3.55	23.5	NA
V/F	(10^5 L)	2.92	21.4	NA
$t_{lag,First,fasted}$	(h)	0.225	1.44	NA
$t_{lag,Second,fasted}$	(h)	3.38	4.41	NA
$t_{lag,First,fed}$	(h)	0	(FIX)	NA
$t_{lag,Second,fed}$	(h)	0	(FIX)	NA
Zero-order input duration	(h)	3.31	6.80	NA
$MAT_{First,fasted}$	(h)	0.459	15.9	NA
$MAT_{Second,fasted}$	(h)	3.66	20.4	NA
$MAT_{First,fed}$	(h)	9.41	19.4	NA
$MAT_{Second,fed}$	(h)	0.425	28.4	NA
$Fraction_{fasted}$[a]	NA	0.854	3.45	NA
$Fraction_{fed}$[a]	NA	0.114	24.0	NA
$F_{rel,\ fed}$ (ratio of fasted)	NA	2.13	3.24	NA
Dose on F_{rel}[b]	NA	0.709	5.77	NA
Scaling factor on IIV F_{rel}[c]	NA	0.761	42.1	NA
Age on BASE	NA	-0.0125	21.1	NA
Not Hispanic/Latino on F_{rel}	NA	1.41	66.6	NA
IIV RUV	(CV)	0.374	5.58	0
IIV BASE	(CV)	0.381	16.1	21.1
IIV CL	(CV)	0.611	11.0	29.2
IIV F_{rel}	(CV)	1.27	13.0	6.01
IIV MAT_{First}	(CV)	0.771	18.3	26.2
Cor(F_{rel},MAT_{First})	(cor)	0.549	20.8	NA
IIV MAT_{Second}	(CV)	1.17	17.5	29.4
Cor(MAT_{First},MAT_{Second})	(cor)	-0.734	16.3	NA
IIV $Fraction_{fast\ abs}$[d]	(SD)	1.64	13.2	22.1
RUV	(CV)	0.484	4.59	2.19

ALT=alanine aminotransferase; BASE=estimated baseline plasma concentration; CL=clearance; CL/F=apparent clearance; CV=coefficient of variation; dT=deoxythymidine; F_{rel}=relative bioavailability; IIV=interindividual variability; MAT=mean absorption time; NA=not applicable; RSE=relative standard error; RUV=residual unexplained variability; SD=standard deviation; SHR=shrinkage; V=volume of distribution; V/F=apparent volume of distribution; WT=body weight

Note: The typical participant has a weight of 60kg, is dosed with 133.3 doxecitine mg/kg, is in fasted condition, is 32.05 years and not Hispanic or Latino.

The RSE for IIV and RUV parameters are reported as the RSE on the SD (ie, ω and σ) scale.

The RSE for the IIV correlation is reported for the square-root of the η covariance.

[a] Fraction of bioavailable dose into first absorption compartment.

[b] Power relationship.

[c] Scaling for participants with TK2d.

[d] The fast absorption compartment is different for fasted and fed state. The IIV is reported as the SD on the logit scale.

Absorption

Pharmacokinetics in healthy volunteers

The pharmacokinetics of dC and dT are studied in healthy volunteers over a dose range of 43.3 to 133.3 mg/kg (clinical trials **MT-1621-103, MT-1621-105** and **MT-1621-106** [two groups of matched healthy volunteers]). The mean baseline (endogenous) concentration of dC and dT ranged between 3.0 and 4.1 ng/mL and 0.10 and 0.72 ng/mL, respectively. After oral administration of doxecitine and doxribtimine under fasted conditions, the mean plasma peak concentrations (C_{max}) of dC and dT are achieved within approximately 0.5 to 1.5 hours (t_{max}). The geometric mean C_{max} of dC and dT ranged between 4.6 and 7.2 ng/mL and 3.95 to 31.5 ng/mL, respectively, after a single dose of 133.3 mg/kg.

Solubility

dC and dT are soluble in the specified administration volume at dose levels ranging from 260mg/kg/day (130mg/kg/day dC and 130mg/kg/day dT) to 800mg/kg/day (400mg/kg/day dC and 400mg/kg/day dT) given in 3 divided doses and independent of pH (pH 1.2 to 7.5).

Permeability

The absorption of doxecitine and doxribtimine is mediated by a complement of nucleoside transporters that are expressed in various epithelia including enterocytes. In Caco-2 cells, the apparent permeability (P_{app}) of dC in the absorptive direction (apical to basolateral) and the secretory direction (basolateral to apical) is approximately 0.4×10^{-6} and $0.5x10^{-6}$ cm/sec, respectively (**NCD3991**) and efflux ratios ranged between 0.9 to 1.2. For dT, the P_{app} in the absorptive direction is approximately $0.8\ x10^{-6}$ cm/sec at 1 µM, $0.7\ x10^{-6}$ cm/sec at 10 µM, $0.5\ x10^{-6}$ cm/sec at 50 µM and $0.4\ x10^{-6}$ cm/sec at 100 µM, while P_{app} in the secretory direction is approximately $1.4\ x10^{-6}$ cm/sec at 1 µM, $1.4\ x10^{-6}$ cm/sec at 10 µM, $1.1\ x10^{-6}$ cm/sec at 50 µM and $1.0\ x10^{-6}$ cm/sec at 100 µM (**NCD3991**) and efflux ratios ranged between 1.7 to 2.9.

Absolute bioavailability

Not determined in the clinical relevant dose range. Based on literature data (Bhalla *et al*., 1988, Kufe *et al*. (1980), Woodcock *et al*. (1980), Ensminger and Frei (1977), and Howell *et al*. (1978), the absolute bioavailability is expected to be low ranging from 0.12 to 0.36%, for dC, and 0.001 to 8.2%, for dT.

Food effect

Administration of 266.6 mg/kg doxecitine and doxribtimine (133.3 mg/kg doxecitine and 133.3 mg/kg doxribtimine, respectively) with a high-fat, high-calorie meal in the morning increased baseline-adjusted C_{max} and AUC_{0-t} by 79% (1.

79 fold) and 137% (2.37 fold), respectively, for plasma dC, and by 27% (1.27 fold) and 74% (1.74 fold), respectively, for plasma dT compared with fasted conditions, confirming a significant food effect (Table 5). The high-fat and high-calorie breakfast tended to prolong dC and dT tmax. The median tmax for dC was 1.25 to 1.51 hours under fasted conditions and 2.02 hours under fed conditions. The median t_{max} for dT was reported as 0.52 to 1.26 hours under fasted conditions and 4.0 hours under fed conditions.

Table 5. Statistical analysis of baseline-adjusted plasma deoxycytidine and deoxythymidine pharmacokinetic parameters following 266.6mg/kg doxecitine and doxribtimine in fed versus fasted state (**MT-1621-105**).

Parameter	Comparison	GM LSM	GMR (%)	90% CI	Intra-individual CV%
Deoxycytidine					
C_{max} (ng/mL) (n=14)	fasted (R)	7.168	178.88	142.21, 224.99	37.22
	fed (T)	12.82			
AUC_{0-t} (h*ng/mL) (n=14)	fasted (R)	30.79	237.18	182.93, 307.51	42.54
	fed (T)	73.03			
Deoxythymidine					
C_{max} (ng/mL) (n=14)	fasted (R)	31.50	127.39	99.16, 163.65	40.88
	fed (T)	91.15			
AUC_{0-t} (h*ng/mL) (n=14)	fasted (R)	40.13	174.38	135.73, 224.03	40.88
	fed (T)	158.9			

AUC_{0-t}=area under the concentration-time curve from time 0 to the last observed concentration; CI=confidence interval; C_{max}=maximum observed concentration; CV=coefficient of variation; GM=geometric mean; GMR=geometric mean ratio; LSM=least squares mean; T=Test, R=Reference

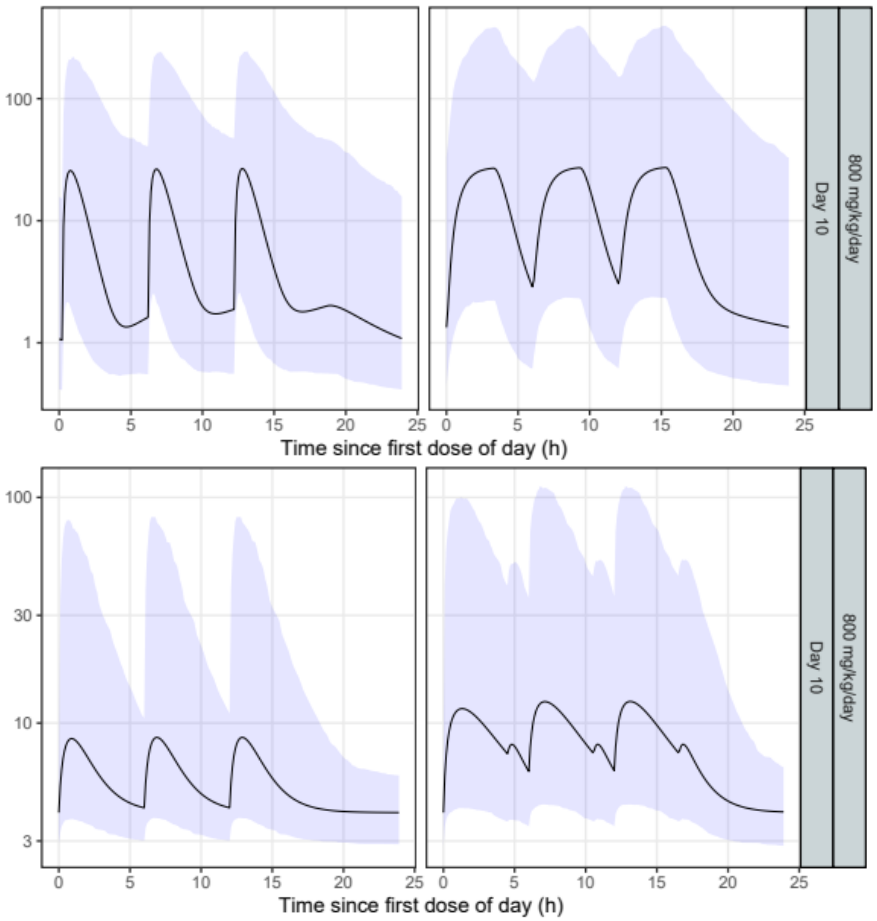


Figure 5. Simulated dC (top) and dT (bottom) plasma concentrations versus time after first dose of day, after administration of 800 mg/kg/day fed (right) or fasted (left), presented at day 10 (taken as steady state), representing a patient of interest (an

8 year old non-Hispanic patient with normal renal function, a weight of 25 kg and an ALT of 29.5 U/L) with associated between-subject variability. The simulated data is presented on semi-logarithmic scale as typical prediction (black line), and with 95% prediction interval (shaded area).

Distribution

In vitro plasma protein binding of dC at final concentrations of 0.5, 5, and 50 µM (113.5, 1136, and 11360 ng/mL) did not reveal any binding to human plasma after 24 hours of incubation. Mean in vitro plasma protein binding of dT at final concentrations of 0.5, 5, and 50 µM (121, 1211, and 12110 ng/mL) was 5.3%, 8.1%, and 0%, respectively, in human plasma.

No results have been provided on the blood-to-plasma ratio.

Both dC and dT are endogenous nucleosides present in all tissues. They are distributed extensively in the body, likely facilitated by the family of SLC28 (concentrative nucleoside transporters [CNT]) and SLC29 (equilibrative nucleoside transporters [ENT]) nucleoside transporters expressed broadly in cell types and organelles including mitochondria (Young, 2016). Following multiple dose administrations of dC and dT 800 mg/kg/day (1:1 ratio) in study participants with TK2d, geometric mean (%CV) V/F of dC and dT is 485,589 (57.5%) L and 150,560 (50.4%) L, respectively.

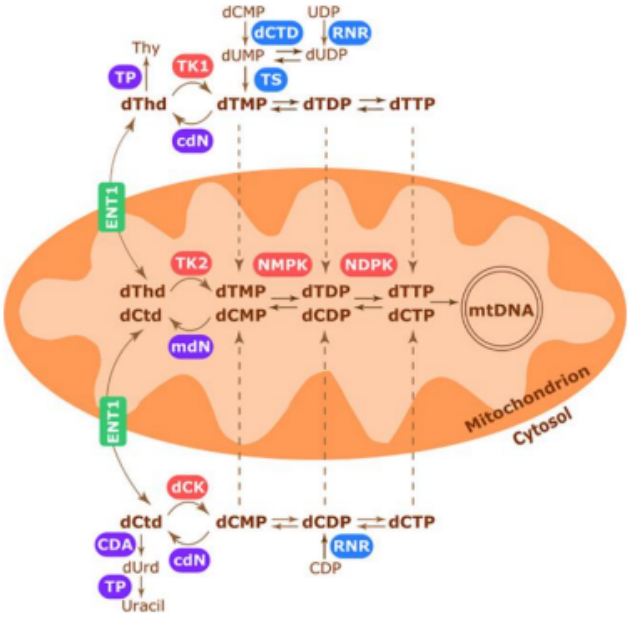
Elimination

Excretion

The mass balance and in vivo metabolic fate of oral doxecitine and doxribtimine has not been determined in humans. The majority of absorbed nucleosides are expected to be extensively metabolised by catabolic pathways, and their end products excreted in the urine and as expired CO_2 (Hess and Greenberg, 2012; Rudolph, 1994). In adult healthy study participants, urinary excretion of intact dC following single oral doses of doxecitine and doxribtimine ranging from 43.3 to 133.3 mg/kg is very low (CL_R approximately 0.027 to 0.069L/h; Fe <1%; **MT-1621-105**). Urinary excretion of intact dT in adult healthy study participants following single oral doses of doxecitine and doxribtimine (43.3 to 133.3 mg/kg) is also very low or negligible (CL_R approximately 0.061 to 0.096 L/h; Fe <1%; **MT-1621-105**).

Metabolism

Pyrimidine nucleosides are metabolized by processes comprising initial catabolism by cytidine deaminase (dC) and thymidine phosphorylase (dT) to their nucleobases and anabolic phosphorylation to nucleotides by various kinases including thymidine kinase 1 and thymidine kinase 2 (Figure 6).



CDA=cytidine deaminase; cdN=cytosolic deoxyribonucleotidase; dCTD=dCMP deaminase; dCK=deoxycytidine kinase; dNTP=deoxyribonucleoside triphosphate; ENT1=equilibrative nucleoside transporter 1; mdN=mitochondrial deoxyribonucleotidase; NDPK=nucleotide diphosphate kinase; NMPK=nucleotide monophosphate kinase; TK1=thymidine kinase 1; TK2=thymidine kinase 2; TP=thymidine phosphorylase; TS=thymidylate synthase; RNR=ribonucleotide reductase

Note: Proteins involved in the de novo synthesis pathway are depicted in blue. Kinases involved in the deoxynucleoside salvage pathway are depicted in red. Enzymes participating in catabolism of dNs are depicted in purple. Nucleoside transporters from cytosol to mitochondria are represented in green.

Figure 6. Schematic representation of the main enzyme pathways involved in mitochondrial pyrimidine dNTP metabolism

The body has evolved mechanisms to limit exposure to endogenous dC and dT metabolites involving the primary and secondary catabolic enzymes such as dihydropyrimidinase, β-ureidopropionase 1, thymidine phosphorylase, uridine phosphorylase 1, and phosphoglucomutase (Garavito et al, 2015). In pooled human liver microsomes, dC and dT were degraded (based on the extent of substrate loss) in a time- and protein concentration-dependent manner, but independent of NADPH as a cofactor, indicating that CYP enzymes are not involved in the metabolism of dC or dT.

The initial catabolic products of dC and dT are the naturally occurring pyrimidine nucleobases thymine, deoxyuridine, and uracil, along with the natural deoxyribose constituents. The primary and secondary catabolic enzymes dihydropyrimidine dehydrogenase, dihydropyrimidinase, β-ureidopropionase 1, uridine phosphorylase 1, and phosphoglucomutase help limit exposures to metabolites of dC and dT. The intermediate metabolites of dC catabolism are naturally existing nucleobase uracil, and dihydrouracil with end products β alanine, ammonia, and CO_2. Thymine, the endogenous nucleobase of dT, is metabolised to dihydrothymine and ultimately to γ amino isobutyric acid and CO_2 (Hill et al, 1975). Plasma uracil is rapidly metabolised to dihydrouracil and ultimately to CO_2 (Ito et al, 2005). The efficient salvage of dC and dT combined with extensive catabolism and rapid elimination suggest that non-physiological accumulation of intact dC and dT or their metabolites is unlikely following the administration of doxecitine and doxribtimine. An *in vitro* study was submitted in which K_m values for the metabolic depletion of dC and dT in human liver S9 and human liver cytosol were determined. K_m values for the human liver S9 and human liver cytosol are 50.8 µM (dC) and 29.3 (dT), and 45.8 µM (dC) and 191 µM (dT), respectively, which is well below the C_{max} at steady state (i.e. 12 ng/mL is approximately 0.05 µM) in plasma.

A literature study was conducted to assess potential influence of polymorphisms in the metabolic pathways of dC and dT. Mutations in the TYMP gene could impact thymidine phosphorylase. Mutations in the DPYD gene result in reduced DPD activity. Errors in DPYS cause dihydropyrimidinase deficiency. Loss of function mutations in the β ureidopropionase gene (UPB1) have been identified. TYMS gene polymorphisms may impact thymidylate synthetase. Several polymorphisms were identified for ENT1, ENT2, ENT3, CNT1, CNT2 and CNT4 transporters.

Doxecitine and doxribtimine are comprised of naturally occurring pure enantiomers β-D-deoxycytidine and β-D-deoxythymidine. Inter-conversion of the enantiomers of pyrimidine nucleosides is not known to occur *in vivo* under physiological conditions.

Dose proportionality and time dependencies

Based on the population pharmacokinetic analysis of the pooled dataset including adult healthy study participants and TK2d participants, the relative bioavailability of dC increased less than dose proportionally whereas that of dT increased in a more than dose proportional manner, consistent with the results from **MT-1621-103** and **MT-1621-105**. The relative bioavailability was estimated to be 61% higher (1.61 fold) and 55% lower (0.45 fold) after administration of 43.3 mg/kg compared to 133.3 mg/kg for dC and dT, respectively.

Geometric mean half-life values of dC and dT were estimated to be approximately 1 hour or under 1 hour in the population pharmacokinetic analysis. No time dependency is therefore to be expected.

Plasma exposures of dC and dT were characterized by moderate to high inter-individual variability in adult healthy study participants and TK2D patients following single ascending oral doses of doxecitine and doxribtimine. Inter-individual variability ranged from 38.1% to 164% and 18.5% to 88.2% on population pharmacokinetic model parameters for dT and dC, respectively. Furthermore, inter-individual variability was also needed in the residual error of dT (37.4%) and dC (22.9%).

Pharmacokinetics in target population

Steady state C_{min}, C_{max}, and AUC_{0-24} values of dC and dT in fed state derived from the population pharmacokinetic models after administration of 400 mg/kg/day doxecitine and 400 mg/kg/day doxribtimine (133.3 mg/kg TID) in participants with TK2d from **TK0102** are presented in Table 6.

Table 6. Steady-state exposures of dC and dT in participants with TK2d (TK0102) at 400mg/kg/day dC and 400 mg/kg/day dT (TID) in fed state and stratified by age group

Analyte	Variable	Age group (years)			
		1.9 to ≤6	>6 to ≤12	>12 to ≤18	>18
dC	N	11	8	5	5
	C_{min} (ng/mL)	4.42 (6.71%)	4.47 (18.43%)	3.74 (8.75%)	5.58 (35.15%)
	C_{max} (ng/mL)	9.05 (29.24%)	9.54 (49.12%)	6.31 (9.59%)	14.45 (92.68%)
	AUC_{0-24} (ng*h/mL)	36.05 (70.43%)	46.5 (100.35%)	20.67 (39.46%)	104.52 (217.2%)
dT	N	11	8	5	5
	C_{min} (ng/mL)	0.78 (47.59%)	1.2 (94.36%)	0.75 (79.97%)	6 (3293.1%)

Analyte	Variable	Age group (years)			
		1.9 to ≤6	>6 to ≤12	>12 to ≤18	>18
	C_{max} (ng/mL)	12.45 (308.73%)	10.85 (180.83%)	7.58 (1097.76%)	561.17 (624.2%)
	AUC_{0-24} (ng*h/mL)	49.18 (491.4%)	56.55 (233.65%)	32.48 (10219.32%)	5124.26 (1011.9%)

AUC_{0-24}=area under the concentration-time curve from time 0 to 24 hours; C_{max}=maximum concentration; C_{min}=minimum concentration; CV%=coefficient of variation; dC=deoxycytidine; dT=deoxythymidine; TID=3 times a day; TK2d=thymidine kinase 2 deficiency.

No therapeutic window has been established.

Special populations

Renal impairment

In the dedicated trial **MT-1621-106**, moderate and severe renal impairment was associated with a substantial increase in systemic exposure to dC and dT following a single oral administration of 266.6 mg/kg (133.3 mg/kg of doxecitine and 133.3 mg/kg of doxribtimine). The plasma exposure of dC and dT in the fasted state was increased in participants with moderate and severe renal insufficiency relative to matched healthy study participants. Baseline adjusted plasma dC C_{max} was 77% (1.77 fold) and 48% (1.48 fold) higher and baseline adjusted plasma dC AUC_{0-t} was 122% (2.22 fold) and 66% (1.66 fold) higher in participants with moderate and severe renal impairment, respectively, compared with matched healthy study participants. Baseline-adjusted plasma dT C_{max} was 210% (3.10 fold) and 146% (2.46 fold) higher and baseline-adjusted dT AUC_{0-t} was 447% (5.47 fold) and 148% (2.48 fold) higher in participants with moderate and severe renal impairment, respectively, compared with matched heathy participants.

Hepatic impairment

No dedicated trial has been conducted.

Gender

No influence of gender has been established in the population pharmacokinetic analysis.

Ethnicity

No influence of ethnicity is to be expected, despite the inclusion of ethnicity in the population pharmacokinetic analysis.

Bodyweight

The pharmacokinetics of dC and dT were described in the population pharmacokinetic analysis using allometric exponents (estimated to be around 0.75 for clearance parameters and 1.0 for volume of distribution parameters).

Age

The median (min, max) age of patients in the **TK0102** PK analysis data set was 9.80 (0.80, 75.5) years. No influence of age was identified in the population pharmacokinetic analysis (other than on the endogenous baseline concentration).

Pharmacokinetic interaction studies

No evidence of direct/reversible inhibition of CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP2D6) and transporters (P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2, BSEP) by dC and dT is expected based on in vitro data. Some potential for induction of

CYP1A2, CYP2B6, and CYP3A4 was observed, but this could be attributed to cytotoxicity at high concentrations.

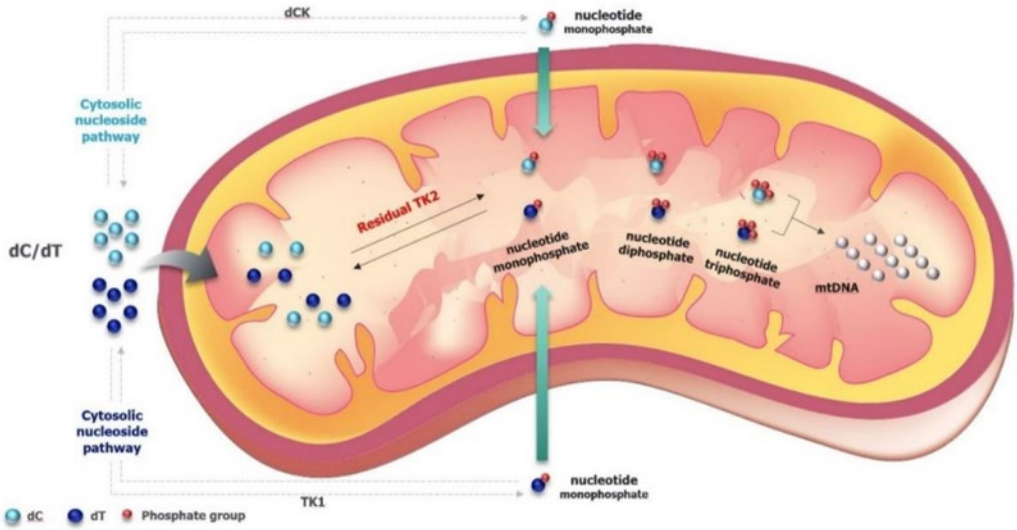
The applicant conducted a literature search to identify drugs that, when administered concomitantly, may influence the pharmacokinetics of dC and dT. In summary, tipiracil was identified as an inhibitor of thymidine phosphorylase. Gimeracil and fluoropyrimidines (e.g. 5-fluorouracil) are substrates of dihydropyrimidine dehydrogenase (DPD). Brivudine is an inhibitor of DP (not EMA approved, but approved in some EU countries). Dihydropyrimidinase is not inhibited by EU-approved drugs. For β-ureidopropionase and Uridine phosphorylase 1, no inhibitors or inducers were identified by the applicant. Finally, lithium may inhibit and induce phosphoglucomutases.

2.6.2.2. Pharmacodynamics

Thymidine kinase (TK) is an enzyme that consists of two forms: A cytosolic form (TK1) and a mitochondrial form (TK2). Generally, TK catalyses (deoxy)thymidine and ATP into (deoxy)thymidine monophosphate and ADP. More specifically, TK2 phosphorylates dC and dT to generate dCMP and dTMP, which are subsequently phosphorylated to dCTP and dTMP required for mtDNA synthesis in replicating cells. The expression of TK2 is cell-cycle independent so its activity becomes critical for the generation of physiological nucleotides (dNTPs) after cell-cycle arrest.

Mechanism of action

The primary mode-of-action of doxecitine and doxribtimine is the anticipated incorporation of the nucleosides dC and dT into mtDNA, to increase the mtDNA copy number and hence the ATP production (**Figure 7**). This was studied non-clinically by the incorporation of isotope-labelled nucleosides dC and dT into skeletal muscle mtDNA of WT or homozygous TK2KO mice during a period of rapid muscle growth (Study MT1621-19-014). The primary PD properties of doxecitine and doxribtimine have been characterized in 2 murine genetic models of TK2d (see Non-clinical discussion). No clinical studies in humans on this issue have been performed.



dC=deoxycytidine; dCK=deoxycytidine kinase; dT=deoxythymidine; mtDNA=mitochondrial deoxyribonucleic acid; TK1=thymidine kinase 1; TK2=thymidine kinase 2
Adapted from: Blázquez-Bermejo et al, 2019

Figure 7. Mechanism of action of doxecitine/doxribtimine

Primary and Secondary pharmacology

The primary pharmacological effects of doxecitine and doxribtimine based on in vitro and in vivo studies are described above (refer to the non-clinical section).

In the clinical development program, potential disease-related laboratory biomarkers were creatine kinase (CK) and lactate, FGF-21, and GDF-15, assessed in study 102, an observational, prospective study in patients with TK2d treated with doxecitine and doxribtimine (see also efficacy section for design and efficacy results of this study). Descriptive results for creatine kinase showed a subtle increase over time (n = 38; median 199.5 (U/L), range 28 – 1718) to month 60 (n = 36; median 206, range 20 – 4081)) and a decrease in lactate (n = 47; 1.43, sd 0.31 versus n = 41; 1.36, sd 0.23 at month 48). Mean FGF-21 (ng/ml) at first assessment (i.e. after treatment initiation) was 0.164 (sd 0.3326) compared to 0.066 (0.0718) at month 36, while for GDF-15 (ng/mL) these values were 0.915 (1.6767) and 0.254 (0.2356).

The effects of doxecitine and doxribtimine on muscle morphology (histology) were not available; muscle biopsies were not repeated.

Concerning secondary pharmacology, potential off-target effects of doxecitine and doxribtimine are not expected or likely due to the endogenous and ubiquitous nature of dC and dT, and considering the specific mode-of-action of doxecitine and doxribtimine: Incorporation of nucleosides dC and dT into mtDNA resulting in improved mtDNA copy number. Clinical studies with specific secondary pharmacology endpoints have not been performed. General safety and tolerability was studied in three 'phase 1' studies: as single-ascending dose studies with healthy participants (MT-1621-103 and MT-1621-105) and as single dose study in participants without TK2d but with moderate or severe renal impairment (MT-1621-106). All treatment-emergent AEs in healthy participants were Grade 1 (mild) except for 1 event of headache that was Grade 2 (moderate). There were no deaths or serious adverse events (SAEs) in these studies. It is referred to the Safety section for information regarding participants with TK2d.

2.6.3. Discussion on clinical pharmacology

Overall discussion on clinical pharmacokinetics

Analytical methods: Bioanalytical methods were developed to determine dC and dT in plasma and urine using LC-MS/MS and generally appropriately validated. QC samples were prepared in human matrix containing significant endogenous levels of dC and dT, necessitating use of the additive approach to QC spiking. Reported accuracy measures are therefore not sensitive to evaluate the bioanalytical method. However, calibration results are deemed reasonable. For dT, it should be noted that a large proportion of the samples showed concentration values below the lower limit of quantification. This indicates that the bioanalysis is not sensitive enough to adequately characterise dT pharmacokinetics (especially baseline) and this should be taken into account when interpreting the results throughout this assessment report.

Population pharmacokinetic modelling: The population pharmacokinetic analyses for dC and dT were mainly conducted to describe the variability in the dC and dT pharmacokinetic profile and to quantify the influences of intrinsic and extrinsic factors on the pharmacokinetic profile of dC and dT. In general, the population pharmacokinetic analysis is adequately conducted and assumptions are reasonable (e.g. assumption that the pharmacokinetic profile of dC and dT are similar between healthy volunteers and patients with TK2d). It should however be noted that additional information could have been obtained in the limited amount of data that was collected in this procedure by modelling dT and dC simultaneously as both compounds likely share some mechanistic pathways.

Absorption: After oral administration of doxecitine and doxribtimine under fasting conditions, the mean plasma peak concentrations (C_{max}) of dC and dT are achieved within approximately 0.5 to 1.5 hours (t_{max}). The geometric mean C_{max} of dC and dT ranged between 4.6 and 7.2 ng/mL and 3.95 to 31.5 ng/mL, respectively, after a single dose of 133.3 mg/kg (proposed clinical dose). The mean baseline concentration of dC and dT ranged between 3.0 and 4.1 ng/mL and 0.10 and 0.72 ng/mL, respectively.

With the commercial formulation (MT-1621-105), administration of 133.3 mg/kg doxecitine and 133.3 mg/kg doxribtimine with a high-fat, high-calorie meal in the morning increased baseline-adjusted C_{max} and AUC_{0-t} by 79% and 137%, respectively, for plasma dC, and by 27% and 74%, respectively, for plasma dT compared with fasted conditions. The proposed intake conditions in the Summary of Product Characteristics are to administer dC and dT with food. The exact mechanism of the increased absorption of dC and dT with food is unknown and it is difficult to extrapolate these findings to normal dietary intake conditions.

The applicant assumes that the bioavailability is low based on the bioavailability of a pyrimidine nucleoside (uridine). There is some data on high extraction ratios for dC and dT based on clinical and non-clinical studies. Based on literature data (Bhalla *et al*., 1988, Kufe *et al*. (1980), Woodcock *et al*. (1980), Ensminger and Frei (1977), and Howell *et al*. (1978), the absolute bioavailability is expected to be low (ranging from 0.12 to 0.36% for dC and 0.001 to 8.2% for dT). However, this comparison may be biased by differences in study population, study design, small patient numbers, potential non-linear pharmacokinetics in the higher dose range, high-variability in pharmacokinetics and potential relevant differences in bioanalytical method and should therefore be interpreted with caution. The low bioavailability during fasted conditions is further supported by the high food effect observed in healthy individuals.

Solubility: Both dC and dT are in solution at time of administration. The solution over the relevant pH range (1.2 to 7.5) indicates that no influence of solubility (or pH modifying agents) is to be expected on the pharmacokinetics of dC and dT.

Cell permeability: The apparent permeability (**NCD3991**) was investigated in Caco-2 cells at 1, 10, 50 and 100 µM, which is a relatively low concentration range to obtain reliable estimates for the apparent permeability (even after dilution in the stomach). The efflux ratios at these concentrations suggest that dC is passively transported, but, for dT, efflux ratios were higher than 2. Additionally, there appears to be a concentration dependency in the efflux ratio for dT (both in $P_{app,a>b}$ and $P_{app,a<b}$). Therefore, with the clinical dose range, the apparent permeability of dT will be even lower. No effect of the BCRP inhibitor could be observed in the Caco-2 cells for both dT and dC, which seems to suggest that the observed concentration dependency could be dependent on processes related to osmosis (and perhaps induce the observed side effects of diarrhoea).

Distribution: Based on the population pharmacokinetic analysis, the apparent volumes of distributions were estimated to be high. This indicates that both dC and dT will be extensively distributed throughout the body.

Plasma protein binding for dC and dT can be expected to be low. The concentration range evaluated (113.5 to 11360 ng/mL for dC and 121 to 12110 ng/mL for dT) is however relatively high (C_{min} and C_{max} was estimated to be 6 ng/mL and 11.5 ng/mL for dC and 1.3 ng/mL and 12.6 for dT at steady state at 400 (dC)+400 (dT) mg/kg daily dose). For dT, no protein binding was observed in this high concentration range. For dC, protein binding varied from 5.3%, 8.1% and 0% at concentrations of 1, 10 and 100 µM. However, at these concentration levels, protein binding may have already been saturated ($C_{max,ss}$ of 0.05 µM).

The applicant did not provide any data on the blood-to-plasma ratio. Based on literature data, both dC and dT can be taken up into human erythrocytes via equilibrative nucleoside transporters (bi-

directional). This transport is not expected to be saturated. Furthermore, no metabolising capacity is expected of erythrocytes nor trapping of dC or dT via concentrative nucleoside transporters. Nonetheless, a dedicated *in vitro* study is being conducted by the applicant for which a commitment is requested to submit the results and provide the expected timeline. ***(REC)***

Metabolism: It is anticipated that dC and dT are prone to catabolism. In the cytosol, the major enzymes involved in the metabolism are anticipated to be cytidine deaminase (for dC) and thymidine kinase 1 (for dT), followed by ribonucleotide reductase and thymidylate synthase. Within the mitochondria, dT and dC are metabolised by TK2 to dTMP and dCMP, which are further processed before incorporation in mtDNA. In the absence of functional TK2, ribonucleotide reductase and thymidylate syntase are expected to play a more prominent role in patients with TK2d as these are responsible for formation of dCMP and dTMP in the cytosol. dCMP and dTMP are expected to have the ability to enter the mitochondria from the cytosol. In an *in vitro* study with human liver cytosol and S9 fraction, it was considered demonstrated that saturation in the drug metabolism is considered unlikely at plasma concentrations in the clinical relevant dose range of dC and dT. However, this is conditional on the assumption that the plasma exposure reflects tissue exposure, which can be expected to be significantly higher due to extensive distribution of dC and dT. Potential saturation of metabolites can therefore not be completely excluded. Metabolites are expected to be endogenous in nature.

Similar to the potential drug-drug interactions, the clinical relevance of the identified polymorphisms in the treatment of TK2d with dC and dT remains uncertain.

Excretion: The majority of absorbed nucleosides are expected to be extensively metabolised by catabolic pathways, and their end products excreted in the urine and as expired CO_2. Due to the lack of an ADME study, the exact contribution of the liver, lung and kidney to the total body clearance is unknown. The applicant indicates that renal clearance is expected to be a minor pathway of elimination for dC and dTGiven the impact of kidney impairment on the overall exposure as well as the anticipated low absolute bioavailability, kidney function is expected to play an important role in the elimination of dC and particularly dT from the body. It has been stated that at micromolar concentrations in plasma following intravenous administration of dT, urinary clearance of dT exceeds creatinine clearance by a factor of 4, while at millimolar concentrations this factor is reduced to 1.65, suggesting saturation of tubular secretion and decreased tubular reabsorption of dT at millimolar concentrations (Zaharko et al, 1979). This may partially explain the non-linearity observed in pharmacokinetics observed with high dosages of dT.

Dose proportionality: The pharmacokinetics of both dC and dT display non-linearities with respect to dose. The baseline-adjusted plasma exposures (C_{max} and AUC_{0-t}) increased in a less than dose proportional manner for dC and in a more than dose proportional manner for dT. The latter could potentially be influenced by the concentration-dependency observed in permeability experiments for dT in Caco-2 cells. Alternatively, as mentioned above, it is suggested that there could be non-linearities in excretion of dT through the kidneys. For dC, the mechanism is unknown.

Time dependency: The potential for accumulation of dC and dT (half-life < 1 hour) was considered to be low. However, in the raw data presented in the population pharmacokinetic analysis, some very high observations can be observed in some individuals which could not be explained by any of the identified covariate effects. The high-variability in the pharmacokinetics of dC and dT, after correcting for differences in body size and kidney function, are expected to be mainly driven by high variability in absorption processes. This provides further evidence that the absolute bioavailability is likely low but variable. This however also questions whether alternative routes of administration would not be more beneficial in terms of efficacy and safety.

Intra- and inter-individual variability: The pharmacokinetics of dC and dT are moderately to highly variable, which can be attributed to the expected low bioavailability and anticipated complex

absorption pathways, baseline variability and excessive metabolism. Furthermore, there are severe limitations in the pharmacokinetic sampling (food intake was not recorded in patients and also sampling times were not recorded), which translate in high estimates of the variability in patients with TK2d.

Pharmacokinetics in patients with TK2d: In **TK0102**, doxecitine and doxribtimine could be administered irrespective of food prior to Protocol Amendment 3.0. Study participants were instructed to administer doxecitine and doxribtimine with food after Protocol Amendment 3.0. Only a few pharmacokinetic samples were collected after the protocol amendment and therefore the impact of this amendment on efficacy and safety cannot be estimated based on the pharmacokinetics (and the lack of a quantified exposure-response relationship).

An age covariate was implemented on the baseline of dC and dT in the population pharmacokinetic model, which could be explained by allometric principles in the production rate of dC and dT as age and bodyweight are correlated. The applicant indicates that baseline dC and dT are not expected to be different between patients with TK2d and healthy volunteers. Furthermore, the relationship between plasma dC/dT concentrations and dC/dT concentrations within mitochondria is not evident. Therefore, it is unclear whether plasma dC/dT concentrations can be used for dosing recommendations.

Patients have reported respiratory problems that can result in a need for mechanical ventilation and feeding problems that can result in the use of nasogastric tubes. No impact of administration of dC and dT via nasogastric tubes is to be expected based on the population pharmacokinetic analysis (n = 7 subjects).

Therapeutic window: No therapeutic window could be defined due to the limited number of patients, limited number of dose levels and uncertainties around pretreatment of these patients.

Special populations: For dC, bodyweight, alanine aminotransferase, moderate/severe kidney impairment, dose and fed status influenced the overall plasma exposure. For dT, bodyweight, dose, fed status and ethnicity influenced the pharmacokinetics.

- Renal function: Both for dC and dT, impaired kidney function patients will have a significantly higher plasma exposure. To avoid high exposures with unknown consequences for safety, a slower titration schedule was proposed by the applicant for patients with moderate to severe kidney impairment. This will not prevent the high exposures at steady state. As no therapeutic window has been defined, the potential higher safety risk may not outweigh the risk of inadequate efficacy. Therefore, a warning to clinical practitioners on the additional safety risk with the current posology has been included in section 4.2 of the SmPC with reference to section 5.2.
- Hepatic function: The pharmacokinetics of dC and dT have not been studied in a dedicated hepatic impairment study. No dedicated hepatic impairment study is planned to be conducted by the applicant, although TK2d frequently leads to mild to moderate increase in liver enzymes. No data is available to estimate the risks of treating patients with hepatic impairment with dC and dT. Insufficient knowledge on the pharmacokinetic profile is available to make any dosing recommendations for patients with impaired hepatic function.
- Bodyweight: Pharmacokinetic parameters were scaled based on allometric theory, which is acceptable. Consequently, bodyweight-based dosing can be anticipated to reduce pharmacokinetic variability by scaling the dose for the influence of body size on the pharmacokinetics of dC and dT.

- Age: No relevant effect of age was identified in the population pharmacokinetic analysis after accounting for body size (with the bodyweight-based dosing regimen). Maturation processes cannot be excluded in the ADME pathways of dC and dT.

Pharmacokinetic interaction studies: No evidence of direct/reversible inhibition/induction of CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP2D6) and inhibition transporters (P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2, BSEP) by dC and dT can be expected based on in vitro data. Although such medicines may not be commonly used in patients with TK2 deficiency, occasional use cannot be excluded.

Similar to the potential drug-drug interactions, the clinical relevance of the identified polymorphisms in metabolic pathways of dC and dT remains uncertain in the treatment of TK2d with dC and dT.

Exposure relevant for safety: Exposure in patients with TK2D is most reliably estimated by the population pharmacokinetic model, but is prone to large variability in this small patient population (simulations for n = 18 in fed state). Overall, for dC, a geometric mean C_{max}, C_{min} and $AUC_{0,24,ss}$ of 12.2 ng/mL (min to max: 6.3 to 29.9, 54% CV), 6.0 (4.0 – 9.8, 26% CV) and 108.7 ng*h/mL (25.4 to 381.9 , 97.5% CV) was estimated by the population pharmacokinetic model. For dT, a geometric mean C_{max}, C_{min} and $AUC_{0,24,ss}$ of 12.6 ng/mL (min to max: 0.5 to 8966.8, 1848% CV), 1.3 (0.3 – 908.5, 489% CV) and 191.2 ng*h/mL (1.6 to 109268, 2851.7% CV) was estimated by the population pharmacokinetic model.

Overall conclusions on pharmacodynamics and PK/PD

Data from study 102 (cut-off date 14 March 2025) indicate an increase in creatine kinase when comparing baseline (i.e. first assessment, predominantly after treatment initiation) with month 60; 95% confidence intervals (CI) are largely overlapping. Lactate levels slightly decreased when comparing month 48 with baseline; no data at month 60 are available. Although informative, lactate is a rather non-specific marker. Both FGF-21 and GDF-15 values decreased between baseline and month 36. Studies have shown a correlation between GDF-15 levels and TK2d disease severity and a decline (to normal) after treatment initiation (*Bermejo-Guerrero et al., Mitochondrion, 2024; Dominguez-Gonzalez et al., Nature research, 2020*). Unfortunately, there are no untreated baseline data in the current dossier; comparison of follow-up data with data from healthy controls (from *Davis et al., 2016; Fernandez et al., 2025; Socha-Banasiak et al., 2020*) indicates that treated patients in study 102 had median GDF-15 values that approached normal values. Altogether, the GDF-15 data in this application might add (indirect) support for the efficacy of dC/dT. Correlations with clinical data are not provided, which is understood due to small numbers of observations for especially the clinical endpoints (motor function). FGF-21 and venous lactate levels, although less specific biomarkers, were aligned with this.

Muscle biopsies were performed in studies 101 and 102 but these were not repeated within patients; as such the impact of treatment on muscle histology, to support the MoA, remains unclear.

Off-target effects of doxecitine and doxribtimine are not expected or likely. This is due to the endogenous and ubiquitous nature of dC and dT, and considering the specific MoA of doxecitine and doxribtimine with mitochondria as the site of action. In line with that, no specific secondary pharmacology studies have been performed.

The phase 1 studies in healthy volunteers and in volunteers with moderate or severe renal failure, were important for drug development but are not very informative concerning the safety profile as relevant for the indication or posology. The main reason is that the doses were usually relatively low as compared to the target dose and these doses were only applied once. Doses above the target dose were not tested. Consequently, for informing the safety profile of doxecitine and doxribtimine, it is relied on the safety information from the clinical studies in patients with TK2d.

2.6.4. Conclusions on clinical pharmacology

The pharmacokinetic profiles of dC and dT have been investigated in a limited number of clinical trials and rely heavily on expected similarities between healthy volunteers and patients with TK2d. No other clinical studies are planned that will investigate the pharmacokinetic profile of dC and dT in patients with or without TK2d. The applicant intends to study the pharmacokinetics of dC and dT in future trials but, at this stage, a limited understanding of the pharmacokinetic profile has been established due to the lack of an ADME study as well as high-variability in the pharmacokinetic profiles of dC and dT. This poses uncertainties with respect to dosing and dosing recommendations for intrinsic and extrinsic factors that influence the pharmacokinetic profiles of dC and dT.

Data on primary pharmacology for doxecitine and doxribtimine were scarcely reported and are expected to be discussed in the final CSR of study 102. There are no data on muscle histology over time as biopsies were only taken once, in a subgroup of patients. The impact of treatment with dC and dT on muscle histology thus remains uncertain. Due to the endogenous and ubiquitous nature of dC and dT, and considering the specific mode-of-action of doxecitine and doxribtimine with mitochondria as the site of action, off-target effects of doxecitine and doxribtimine are considered unlikely.

2.6.5. Clinical efficacy

The clinical development program evaluating **efficacy** of doxecitine and doxribtimine consisted of two 'pivotal' phase 2 studies in patients with TK2d (see also Table 1):

- Study MT-1621-**101** (also called study 101 in this AR) is a completed, retrospective noninterventional chart review in which treated patients (n = 38) received non-GMP dC/dT or non-GMP dCMP/dTMP.
- Study MT-1621-**102** (also called study 102 in this AR) is an ongoing, prospective, open label 'follow-up', including 47 patients who were already treated with non-GMP dC/dT or non-GMP dCMP/dTMP; 35 patients originating from study 101 and 12 patients not included in study 101, with a follow-up of up to 5 years (date of data cut-off 15 March 2024).

The study population is broader than the **target population**. Of the treated patients in studies 101 + 102, there were 39 patients who were paediatric or adult TK2d patients with an age of TK2d symptom onset ≤12 years, aligned with the target population.

Untreated patient data were generated from published case series and reports to serve as an external control group to compare with treated patients; the (modified) Untreated Patient Database (**(M-)UPD**).

Supportive data on treated and untreated patients were gathered in studies MT-1621-107 (also called study 107 in this AR), TK0110, TK0112, and TK0114 (also called study 114 in this AR).

2.6.5.1. Dose response study(ies)

No dose-response study was performed.

2.6.5.2. Main studies

The studies **101** and **102**, supported by the comparison with data from the untreated patient database (**UPD**) compose the principal data supporting this application, and are subsequently presented in this section.

Study MT-1621-101: A Retrospective Study of the Combination of Pyrimidine Nucleos(t)ides in Patients with Thymidine Kinase 2 deficiency (TK2d)

Objectives

The *primary* objective of this study was to retrospectively describe the safety, tolerability, and efficacy of treatment with pyrimidine nucleos(t)ides in patients with TK2d. The *secondary* objective of this study was to describe the disease course of TK2d from time of onset of symptoms until initiation of treatment.

Design

Study 101 is a completed, retrospective medical chart review of patients with TK2d, treated with non-Good Manufacturing Practice (non-GMP) pyrimidine nucleosides (dC/dT) and/or nucleotides (dCMP/dTMP). Data from these patients as from the time of disease presentation were stored in medical charts by their treating physician, and reviewed retrospectively. The data collection process was anticipated to last approximately 1 year.

Participants

Treated patients were recruited based on chart reviews in centres in 3 countries: The USA, Spain, and Israel. The inclusion criteria were:

- A signed informed consent by patient or caregivers, unless the institutional review board or ethics committee provided an appropriate consent waiver prior to data collection,
- Confirmed genetic mutation of the TK2 gene,
- Availability of medical records from the time of onset of TK2d symptoms,
- Use of pyrimidine nucleos(t)ises (dC/dT and / or dCMP/dTMP), and
- Most recent study visit between 1st June and 15th December, 2018.

Patients were excluded if they had been diagnosed with another genetic disease and/or had polygenic disease.

Treatments

The treating physicians had established the dosing regimens of pyrimidine nucleos(t)ides for their patients individually. Patients were treated with nucleos(t)ides up to 800mg/kg/day.

Outcomes/endpoints

Main efficacy endpoints extracted from the charts were 1) survival, 2) motor function including motor milestones, 3) respiratory function, and 4) feeding ability.

1) **Survival** was defined as the vital status at time of the last observation; alive or deceased.
2) **Motor milestones** as defined by the WHO Multicentre Growth Reference Study were extracted, i.e. holding head upright, sitting upright unassisted, standing with or without assistance, walking with or without assistance, climbing stairs with or without assistance, and running. Gain, loss, and / or regain of each motor milestone was documented, which are relevant changes as there are clear reference data including windows of achievement from the WHO Multicentre Growth Reference Study (WHO 2006) based on an international cohort.

Additional motor function endpoints when assessed by the physician, included: The 6-Minute Walk Test (6MWT), the Hammersmith Functional Motor Scale Expanded (HFMSE), the North Star Ambulatory Assessment (NSAA), the Egen Klassifikation (EK), the Revised Upper Limb Module (RULM), and the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND). The endpoints varied by study centre / physician and assessments were not standardised.

3) Respiratory function was assessed by extraction of data on initiation / discontinuation of **ventilatory support** (invasive or non-invasive), transition from one mode of ventilatory support to another (e.g., from tracheostomy/full mechanical ventilation to non-invasive support), and a change in the number of hours of support by at least 4 hours/day.

   Forced Vital Capacity (FVC) was extracted from pulmonary function tests. There was no standardization for pulmonary function testing and no information about the conduct was collected.

4) Data on **feeding support**, including insertion / removal of a feeding tube and management of enteral feeding, was extracted. The requirement of feeding support was at the clinicians' discretion and not standardized.

Sample size

No formal sample size or power calculations were performed.

The Untreated Patient Database (UPD)/ external control group

Objectives

The general objective was to obtain information of the clinical course in untreated patients with genetically confirmed TK2d, for comparison with treated patients in study 101.

Design

To establish the untreated patient database (101-**UPD**), characteristics and clinical outcomes of untreated patients with genetically confirmed TK2d were obtained from published case series and case reports. Clinical outcomes included survival (time to death), ventilatory support (if available), and ambulation (if available). Information on 103 untreated patients with TK2d was collected, with a data cut-off date of 17 June 2019. An updated search was performed with a cut-off date on 31 October 2021 to identify additional untreated participants. The **updated-UPD** includes the publications originally outlined in the 101-UPD, as well as any additional publications reporting untreated individuals with TK2d; a similar methodology to that used for the 101-UPD was used to identify new untreated participants.

The 101-*modified* untreated patient database (101-**MUPD**) was derived from the 101-UPD by excluding all untreated participants in the 101-UPD who died or were censored before the earliest age at which the youngest study participant commenced treatment in MT 1621-101 (which was 1.3 years) and excluding untreated participants who did not have available data on either 1) the age of TK2d symptom onset, or 2) the age of death or age last known alive (post-onset of symptoms).

The **updated-UPD** is pooled with untreated participants from study MT-1621-**107** (see below) to generate the **ISE-UPD**; the **ISE-MUPD** is a subset of the ISE-UPD generated by following the predefined censoring rules.

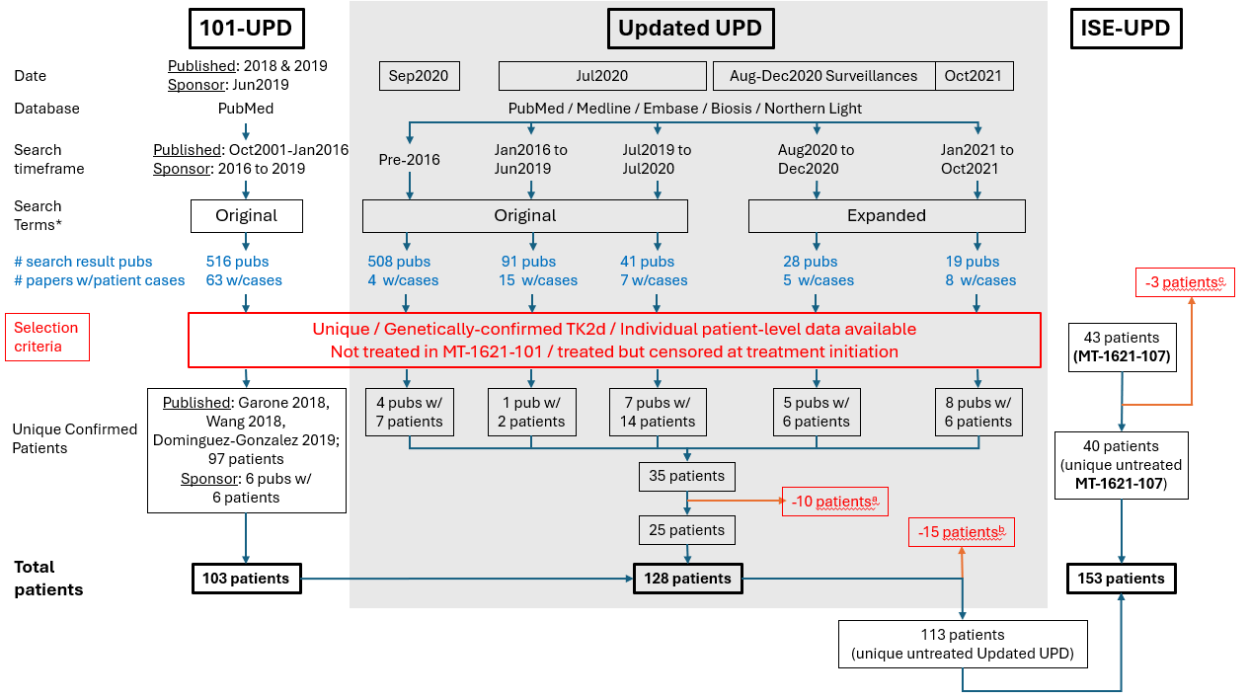
Participants

Study participants who were *treated* in study **101** were excluded from the UPD. In addition, any participants included in the UPD who had received pyrimidine nucleos(t)ides but were not enrolled in study 101 were censored at the time of initiating treatment.

Search strategy and search results

Key terms for the literature searches were 'thymidine kinase 2 deficiency', 'TK2', 'thymidine kinase 2', and 'TK2 mitochondrial DNA maintenance defects', including their variants in MeSH Terms, All Fields, Subheadings or Supplementary Concepts. Eligibility criteria for inclusion in the UPD were availability of individual-level patient data, genetic mutations in the TK2 gene, and availability of source documents in the public domain.

Three key publications were identified in the first search composing the 101-UPD: Two natural history studies of patients with TK2d from Garone et al. (2018) and Wang et al. (2018), and a case series of Dominguez-Gonzalez et al. (2019) (n = 18). Search results and the following selection process for the 101-UPD, Updated UPD, and ISE-UPD are graphically presented in the flow chart below. The selection process was performed by two clinical reviewers to ensure that the patient's data had not previously been reported as part of any of the three key publications and that the patient was not treated / included in any of the (treatment) studies in this Application.



[a] Removed because their data are collected in MT-1621-107
[b] Removed because
- they were treated in TK0102 (n=3)
- they were treated in MT-1621-107 (n=4)
- they were treated in TK0114 (n=1)
- their data were collected in a different clinical study (untreated in MT-1621-107, n=6)
- patient identified as not having confirmed TK2d diagnosis in TK0112 (n=1)

[c] Removed because they were subsequently treated in TK0114

Data extraction

Key variables (sex, mortality status, age at death, age last known alive, ambulation, previous ambulation, age at loss of ambulation, ventilatory support, age at loss of independent ventilation, age

at treatment initiation, feeding support, TK2 alleles, narrative of clinical history) were extracted independently by 2 investigators; this process was verified by a clinical reviewer to resolve discrepancies between those investigators.

If a patient appeared in multiple publications, the most recent was used as main data source for that particular patient.

Outcomes/endpoints

The primary endpoint was survival (time to death).

Study MT-1621-102: A phase 2, prospective, open label, single-arm, continuation treatment study of the efficacy and safety of doxecitine and doxribtimine in participants with TK2d

Objectives

The *primary* objective of study 102 was to characterize the safety and tolerability of doxecitine and doxribtimine in patients with TK2d previously treated with dC/dT, deoxycytidine monophosphate (dCMP)/deoxythymidine monophosphate (dTMP), or doxecitine and doxribtimine.

Secondary objectives were to demonstrate the continued efficacy of doxecitine and doxribtimine, and to evaluate the PK of doxecitine and doxribtimine at steady state.

Design

Study 102 is a prospective, open label interventional study in study participants with TK2d who participated in study 101. Patients who were treated with non-GMP nucleos(t)ides or doxecitine and doxribtimine who did not participate in study 101, were also eligible for enrolment: Those on non-GMP nucleos(t)ide switched to doxecitine and doxribtimine. The study is ongoing; the date of the first data cut-off was 15 March 2024.

Assessments were performed upon enrolment, at 1 month, then every 3 months through 18 months, every 6 months through 36 months, and annually thereafter.

Participants

See section on in- and exclusion for the study population for study 101 above.

Additional *inclusion* criteria compared to study 101 were:

- A negative pregnancy test, not breast feeding, no intention to become pregnant during the study, and a commitment for females to use of birth control methods up to 30 days after end of the study. Male patients had to use condoms for up to 30 days after the end of the study.
- Willingness to maintain the current treatment and exercise regimen during the study, and to comply with the study protocol.

Additional *exclusion* criteria compared to study 101 were:

- History of liver disease including ALT / AST / total bilirubin increases up to 2x ULN, and other significant medical conditions that might interfere with the clinical course of TK2d.

Treatments

The study drug is a 1:1 mixture of doxecitine and doxribtimine. It was supplied as powder in sachets containing 2g of either doxecitine or doxribtimine, or in a single sachet containing 2g of each. The drug had to be mixed with water or apple juice for consumption < 24 hours.

Patients who were on a stable maintenance dose of total 800mg/kg/day non-GMP nucleosides or doxecitine and doxribtimine at baseline, continued with the same dose of doxecitine and doxribtimine administered as 3 equal daily doses.

Patients who were on a total dose <800mg/kg/day were transitioned to 1 of the following regimens administered as 3 equal daily doses, depending on which was closest to the participant's previous dose:

- 260mg/kg/day (130mg/kg/day of doxecitine and 130mg/kg/day of doxribtimine)
- 520mg/kg/day (260mg/kg/day of doxecitine and 260mg/kg/day of doxribtimine)
- 800mg/kg/day (400mg/kg/day of doxecitine and 400mg/kg/day of doxribtimine)

This treatment was continued and titrated according to the schedule in the protocol. The dose could be reduced for tolerability reasons.

Compliance

Treatment compliance was calculated by monitoring the number of drug packets used and the diary of the patient. Compliance had to be at least 80%.

Concomitant treatments

Concomitant treatments were defined as treatments that started on or after baseline and were tabulated by ATC class. All concomitant medications, including mitochondrial cocktails, were recorded and had to be kept at a stable dose and regimen during the study. The use of any other investigational agents for TK2d or any other indication was not allowed. Rescue therapy was not applicable.

Outcomes/endpoints

Efficacy assessments largely overlap with study 101 (see above). The main endpoints were **survival** and the three key functional domains (motor function including **motor milestones**, **ventilatory status, feeding status**), and quality of life. Consecutive assessments were preferably performed by the same personnel, and investigators were instructed to continue using the same protocols / procedures as used in study 101.

Additional assessments in study 102, compared to study 101, were the 10 m, the 100 m walk/run tests, overall global impression from the clinician and the patient (CGI-I and PGI-I), and the Patient-Reported Outcomes Measurement Information System (PROMIS); suitability of tests was aligned to the patients' age (Table 7).

- For the 10 m walk/run test, actual velocity average, preferred/comfortable actual velocity average, and any assistance device and/or bracing utilized were recorded.
- For the 100 m walk/run test, any assistance device and/or bracing utilized, total distance (if not completed), and any interruptions were listed.
- Overall global impression variables included the Clinical Global Impression of Improvement (CGI-I) and Patient Global Impression of Improvement (PGI-I), both scored on a 7-point scale ranging from 1 (very much improved) to 7 (very much worse).
- PROMIS total scores (Physical Function - Upper Extremity total score, Physical Function - Mobility total score, and Physical Function - Total Score), and the response to individual assessment questions were scored on the T-score metric. High scores indicate more of the concept being measured.

Table 7. Assessments by functional domain (may be modified based on individual study participant considerations) in study 102.

Study Participant Age			
		>5 Years	
<2 Years	2-5 Years	Ambulatory	Non-ambulatory
Motor Function			
• CHOP-INTEND • Developmental milestones	• HFMSE • NSAA • Developmental milestones • 6MWT • 10m walk/run	• HFMSE • NSAA • Developmental milestones • RULM • 6MWT • 100m walk/run • EK-R • PROMIS items for Physical Function (mobility and upper extremity) [a]	• HFMSE • Developmental milestones • RULM • EK-R • PROMIS items for Physical Function/upper extremity [a]
Respiratory Status			
• Ventilatory status [b]	• Ventilatory status [b]	• Ventilatory status [b] • Upright (sitting) and supine PFTs [c] • Nocturnal capnography and/or sleep study • CPET	
Feeding Status [d]			
• Use of feeding tube (nasogastric /gastrostomy)	• Use of feeding tube (nasogastric /gastrostomy)	• Use of feeding tube (nasogastric/gastrostomy)	

6MWT=6-Minute Walk Test; BiPAP=bilevel positive airway pressure; CHOP-INTEND=Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; CPET=cardiopulmonary exercise testing; EK=Egen Klassification; HFMSE=Hammersmith Functional Motor Scale-Expanded; NSAA=North Star Ambulatory Assessment; PFTs=pulmonary function tests; PROMIS=Patient-Reported Outcomes Measurement Information System; RULM=Revised Upper Limb Module

[a] PROMIS is for study participants aged ≥12 years. See the protocol for selected questions from PROMIS Bank v1.2 to be administered.

[b] Ventilatory status includes use of ventilatory assistance (such as BiPAP or mechanical ventilation), number of hours/day, daytime vs nighttime, and respiratory infections.

[c] Including maximal inspiratory and expiratory pressures, sniff nasal inspiratory pressure, and peak cough flow.

[d] Feeding status includes use of feeding tube (nasogastric/gastrostomy) and amount of supplemental feeding required.

Sample size

No sample size calculation was performed.

Statistical methods

Studies 101 and 102

Except for the survival analyses, data analyses were mainly descriptive.

Survival status at last contact was summarized for treated participants. The time to death was calculated from both the age of TK2d symptom onset and age of treatment initiation. Cox proportional hazards regression models and Kaplan-Meier estimates were calculated.

Summaries for motor milestones were provided broken out for pretreatment and post-treatment (i.e. after treatment initiation) periods. Patients at risk were counted in the denominators for percentage calculations. The net number of milestones gained was calculated as the number of new motor milestones gained minus the number of motor milestones lost for the pretreatment period (from disease onset) and post-treatment period separately.

Motor function endpoints (6MWT, HFMSE, NSAA, EK and CHOP INTEND) were tabulated. The NSAA met the criteria for interpretation on a linear scale, except for the question "Lifts Head", which was removed from the total linearised NSAA score.

Numbers and percentage of patients on ventilatory support were tabulated pre- and post-treatment, including increased and decreased hours of support over time, duration (days), and hours per day. Any addition or removal of ventilatory support was counted as a -1 or a +1 respectively; the net ventilatory support score for each patient was derived by summing these values.

The numbers and percentages of patients needing feeding support were presented; the presence and absence of a feeding nasogastric / gastric tube pretreatment, at treatment start, and any time post-treatment were reported. The approximate percentage of supplemental feeding administered via feeding tube were gathered (25%, 50%, 75%, or 100%), including dates for insertion (-1) or removal (+1). The net supplemental feeding score for each patient will be derived by summing these values.

No multiplicity correction was used.

Missing or partial dates for occurrence of motor milestone events, symptom onset, the first study drug dose, genetic testing, ventilatory and feeding support were imputed. If only the year or month of the event was available, and this was the same as the birth date, the end of the year or month was imputed. For dates with only the day portion missing, the day was replaced with '15.' For dates with the month and day portions missing, the month was replaced with 'June' and the day was replaced with '30.'

Patients had to initially achieve a motor milestone in order to be counted as a *loss* for that motor milestone: If a patient had a 'loss' record, it was assumed it had initially been achieved. For 'regain', a patient had to have initially achieved the motor milestone, lost it, and subsequently regained it at the last available assessment.

The following subgroups were presented for select analyses:

- Treatment type; dC/dT only, and dCMP/dTMP
- Age of TK2d symptom onset:
    - Category 1: ≤2.0 years of age, ≥2.0 and ≤12.0 years, >12 and <18 years of age, and ≥ 18 years of age;
    - Category 2: ≤12.0 years of age; >12.0 years of age.
- Age at first treatment (any treatment):
    - Category 1: ≤2.0 years of age; >2.0 and ≤12.0 years of age; >12.0 and 18.0 years of age;
    - Category 2: ≤18.0 years of age; >18.0 years of age.
- Treatment delay: ≤6.0 months; >6.0 months and ≤1.0 year; >1.0 year and ≤5.0 years; >5.0 years and ≤10.0 years; >10.0 years.
- Duration of treatment (from time of first treatment to time of last reported treatment: ≤1.0 year; >1.0 year and ≤3.0 years; >3.0 years and ≤5.0 years; >5.0 years.

Changes to SAP

The original Non-Survival SAP (Non-Survival MT-1621-101 SAP v1.0 [SAP-RETRO] was approved 01 May 2019.

- Addendum 1 to Non-Survival MT-1621-101 SAP v1.0 was approved on 28 June 2021. End of study date was changed from 31 December 2018 to the last "Date of Last Contact" and graphical displays were added to present the results in measures of efficacy over time.

- Addendum 2 to Non-Survival MT-1621-101 SAP v1.0 dated 01 May 2019 was driven by comments from the FDA. Responder and subgroup analyses and imputation rules were modified and additional analyses for developmental motor milestones, ventilatory support and feeding support were added.

Comparison of treated patients from studies 101 and 102 and untreated patients (MUPD)

The MUPD was used as the external control group in survival analyses of the treated patient dataset, for which patients in both groups were matched.

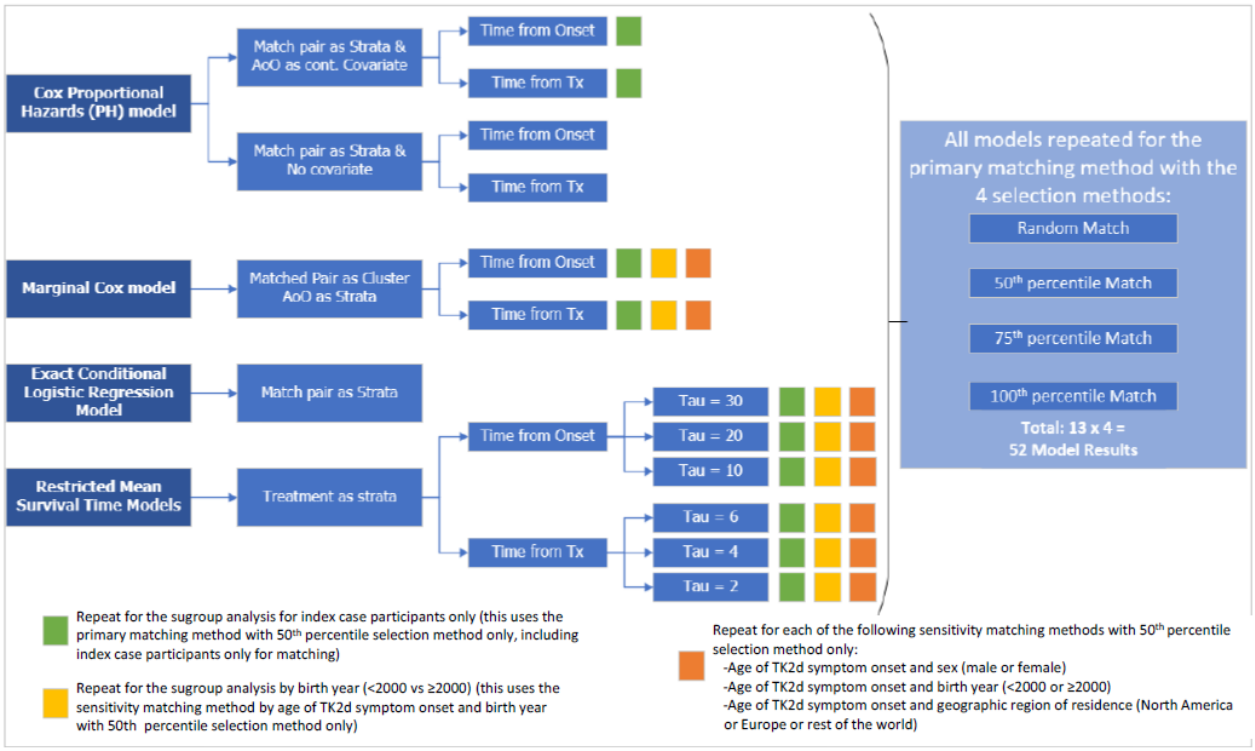
Post hoc, an estimand was defined based on the protocols and statistical analysis plans. The aim was to estimate the difference in time to death from either age of TK2d symptom onset or from treatment start, estimated by the HR, for adult and paediatric participants with genetically confirmed TK2d treated with pyrimidine nucleos(t)ides regardless of treatment discontinuation compared to matched untreated patients.

The primary endpoint was survival (time to death), calculated using matched data from treated and untreated patients. Survival was calculated from the age at onset of TK2d symptoms and the age of treatment initiation.

Matching was based on age at onset of TK2d symptoms (≤ 2.0, >2.0 to ≤12.0, and >12.0 years). Within each age group, treated patients were sorted in descending order (highest to lowest) according to their age of treatment initiation and within that, in descending order of age last known alive. For the first treated patients in the sorted list, all untreated patients who were alive beyond the age when the treated participant began treatment were identified, sorted in descending order according to their last known age and only those with their last known age in the upper half (50th percentile and higher) of the group were qualified for matching, from which untreated patient was selected and used as the match for the treated patient (random, upper 50^{th} percentile, upper 75^{th} percentile, and 100^{th} percentile).

Cox proportional hazards regression models were utilized to compare treatments for time to death. Separate analyses were done for each of the matching methods, and the models were stratified by these matched pairs. One model did not include any additional covariates and one model did include age of TK2d symptom onset continuous value as a covariate. For each model treatment was a time independent factor. Statistical differences in survival were assessed with the likelihood ratio test and a score test; p-values were not adjusted for multiplicity. As the number of events was small in some models, model instability was accounted for using the Firth correction. The HR and its 95% confidence interval were calculated. Kaplan-Meier figures for the time to death from TK2d symptom onset were provided, stratified by age of TK2d symptom onset. Participants who were still alive at the end of follow up were censored at their age last known alive.

The restricted mean survival times (RMST) by group were presented. Multiple cut points were utilized for the restricted mean survival time analysis (Tau). The restricted mean survival times were compared across treated and untreated participants using chi-squared tests. Tau values of 30, 20, and 10 years for time from TK2d symptom onset analyses and 6, 4, and 2 years for time from treatment analyses were utilized to determine restricted means. An exact conditional logistic regression model was included to overcome the challenges arising from the limited number of informative pairs available. The analysis allows for stratification on matched pair data. Figure 8 shows the analysis methods used for matched-pair survival data.



AoO=Age of TK2d symptom onset; cont.=continuous; TK2d=thymidine kinase 2 deficiency; Tx=treatment

Figure 8. Survival analysis models

Changes to SAP

Version 1.0 of the survival SAP was approved on 23 April 2019. Version 2.0 of the survival SAP was approved on 19 July 2019. Version 3.0 of the survival SAP was approved on 19 February 2020. Changes to the SAP were driven by comments from the FDA (eg, updates to matched-pairs analyses including removing time-dependent treatment effect analyses and including time to death from treatment initiation, removing composite endpoints, and removing responder analyses), the desire to be consistent with the analyses being performed in the integrated summary of efficacy, and to clarify existing analyses.

Results

Study MT-1621-101: A Retrospective Study of the Combination of Pyrimidine Nucleos(t)ides in Patients with Thymidine Kinase 2 deficiency (TK2d)

Participant flow

A total of 38 patients with TK2d receiving pyrimidine nucleos(t)ide treatment were included; 32 using dC/dT and 6 dCMP/dTMP. All patients were alive at last contact; 2 patients using dC/dT discontinued due to liver enzyme elevations (GGT and AST respectively).

Recruitment

The end of study date was planned on 15 December 2018; the actual end was on 28 March 2019 due to late enrolment of several patients.

Conduct of the study

Four protocol deviations were observed; in 2 cases this concerned signing the informed consent form after the date of last contact entered in the database, and in the other 2 cases an investigator rather than the patient / parent completed the date and / or name sections on the informed consent forms.

Numbers analysed

Data from thirty-eight patients were analysed.

Exposure to study drug

The dose of 800 mg/kg/day was prescribed in 73%, 79%, and 44% of the patients with an age at TK2d symptom onset of ≤ 2 years, > 2 to ≤ 12 years, and > 12 years respectively.

The overall mean duration of treatment was 2.2 years (standard deviation (sd) 1.94; range 0.3 – 7.2); 18% (n = 7) had a treatment duration < 6 months, 21% (n = 18) a duration between 6 and 12 months, 50% (n = 19) between 1 and 5 years, and 11% (n = 4) between 5 and 10 years. Total patient year of exposure (PYE) was 85.2 years. The patient group with an age at of TK2d symptom onset ≤ 2 years had a mean treatment duration of 3.0 years (sd 1.6, range 0.3 - 6.1), compared to 1.7 (sd 2.4, range 0.3 - 7.2) and 1.8 (sd 1.2, range 0.3 - 3.6) for the patient groups with age at TK2d symptom onset > 2 to ≤ 12 years and > 12 years. Patient Years of Exposure (PYEs) were 45.6, 23.3, and 16.3 for the three age groups respectively.

Concomitant treatment

Thirty-five of the 38 patients (92%) used at least one concomitant drug; most frequent were drugs in the Alimentary Tract and Metabolism ATC class (n = 25; 66%), of whom 20 (80%) with an age of TK2d symptom onset ⩽ 12 years. Mitochondrial supplements (n = 15, 40%) were included in this ATC class, as was loperamide (n = 4, 11%). Other common concomitant medications were in the Respiratory System ATC class (n = 16, 42%), mainly concerning salbutamol (sulphate) (n = 10; 26%). Systemic anti-infectives were used by 12 patients (32%), especially in patients with TK2d symptom onset ⩽ 12 years. Frequencies were generally rather comparable between the age categories.

Baseline data

Of the 38 included patients, 29 patients (76%) had an age at TK2d symptom onset of ≤ 12 years; 15 of these patients had an age at TK2d symptom onset of ≤ 2 years and 14 patients were > 2 and ≤ 12 years of age at TK2d symptom onset. The majority (n = 32) was treated with dC/dT, while 6 patients were treated with 'any monophosphate' (i.e. dCMP/dTMP).

The majority of patients was male (n = 21; 55%), except for the > 12 years age category in which the majority of patients was female (n = 7, 78%). Patients were predominantly Caucasian (n = 36, 95%) and residing in Spain (n = 22, 58%).

The overall median age at TK2d symptom onset was 2.5 years (range 0.14 - 60.3 years) and the overall median age at treatment initiation was 9.9 years (range 1.3 - 74.0). Of the 29 patients with an age at TK2d symptom onset of ≤ 12 years, 21 (72%) initiated treatment ≤ 12 years of age.

The majority of patients was ambulatory at baseline (n = 25; 66%); those with age at TK2d symptom onset ≤ 12 years were less often ambulatory compared to those with symptom onset > 12 years (55% versus 100%). Those in the latter group were also less likely to require the use of assistive devices compared to those with an age at TK2d symptom onset ≤ 12 years.

Ventilatory support was required in half of the patients (n = 19; 50%), most (n = 15, 40%) used non-invasive support (BiPAP). The average amount of support was 15.3 hours a day (sd 6.87). A total of 8 patients (21%) required a feeding tube. No clear differences were seen when stratified by treatment type of age of TK2d symptom onset. In the subgroup with an age at TK2d symptom onset ≤ 12 years, 11/14 patients on ventilatory support were also non-ambulant, and so were 5/6 patients on feeding support.

Baseline muscle biopsy findings were available from 28 patients (74%); 13% (n = 5) showed Cox deficiency, 24% (n = 9) Ragged red fibres, 34% (n = 13) showed both findings, and in 2.6% (n = 1) other abnormalities were seen.

The percentages mtDNA were available from 17 patients (45%); the mean percentage mtDNA was 36% (sd 38), ranging from 8% – 158%. TK2 activity was explored in 4 patients, with a median of 36% activity (ranging from 12% – 61%).

Deletion of mtDNA was investigated in 23 patients (61%), and confirmed in 11 of these patients.

Outcomes and estimation

Survival

Following initiation of treatment, no patient in study 101 died; survival analyses could not be performed. The median age last known alive was 12.4 years (range 1.6 – 74.9 years).

Developmental milestones

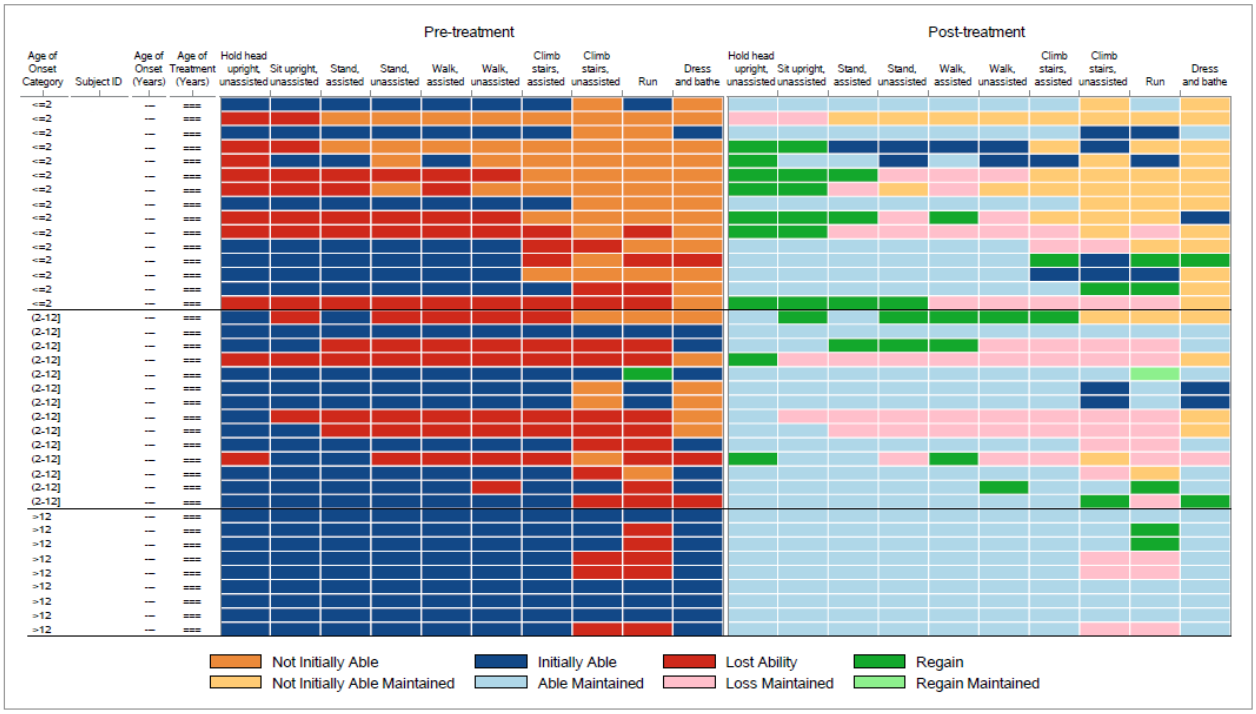
Developmental milestones by age at onset of TK2d symptoms are presented in a heatmap in Figure 9.

Data from the **pretreatment** period suggest that the youngest age at TK2d symptom onset group (≤ 2 years) was the most affected group, reflected in loss or never reaching motor milestones numerically more often than the other 2 age group. Motor milestones with higher complexity were more often not reached or lost prior to treatment compared to milestones with of lower complexity. One event of regain was documented in a patient with an age at TK2d symptom onset > 2 and ≤ 12 years (ability to run).

In the **post-treatment** period,

- 19 motor milestones were regained in 9 of the 15 patients with and age at TK2d symptom onset ≤ 2 years,
- 15 motor milestones were regained in 6 of the 14 patients with and age at TK2d symptom onset > 2 and ≤ 12 years, and
- 2 motor milestones were regained in 2 of the 9 patients with and age at TK2d symptom onset > 12 years age.

No patient lost a motor milestone while on treatment.



Note: Study participants must have initially achieved the developmental motor milestone in order to be counted as a loss for that development motor milestone. Study participants must have initially achieved the developmental motor milestone, lost the same developmental motor milestone, and subsequently regained the same developmental motor milestone in order to be counted as a regain for that development motor milestone.

Figure 9. Heatmap for developmental milestones (study 101)

Motor function

Motor function tests were obtained from a subset of patients only (Table 8); data from the 6MWT (n=22) and NSAA (n=13) are included in this report. Data from other assessments were available from 1 – 7 patients only.

For the **6MWT**, at the last assessment (3-84 months after treatment initiation), the mean (sd) change from baseline was 88.1 meters (sd 105.1) for all patients. Stratified by age at TK2d symptom onset, these differences were:

- ≤ 2 years: 171.9 meters (sd 118.6); n = 5,
- > 2 to ≤ 12 years: 112.5 meters (sd 106.1); n = 8,
- ≤ 12 years age: 135.3 meters (sd 110.2); n=13, and
- > 12 years: 19.8 meters (sd 43.7); n = 9.
- ≤ 12 years age: 135.3 meters (sd 110.2); n=13.

For the **NSAA**, the change from baseline to last assessment was 10.7 (sd 20.4). Data from only 2 patients were available in both groups age at onset of TK2d symptoms ≤ 2 years and > 2 to ≤ 12 years; these data are therefore not separately included in this report. For the > 12 years group, (n = 8) the change from baseline was 2.6 (sd 10.5, range -15 – 15).

Table 8: Change from baseline for 6MWT and NSAA – study 101

	Baseline	Last visit	Change from baseline
6MWT			
n	22	22	22
Mean (95% CI)	302.5 (241.9 – 363.1)	390.5 (336.1 – 444.9)	88.1 m (41.4 – 134.7)
Median (min, max)	327.0 (21.0 – 413.0)	405.0 (354.0 – 473.0)	67.0 (-40.0 – 327.0)
NSAA			
n	12	13	12
Mean (95% CI)	63.6 (51.7 – 75.5)	75.5 (65.1 – 85.9)	10.7 (-2.3 – 23.6)
Median (min, max)	64.0 (24 - 85)	73.0 (42 - 100)	8.5 (-15 – 67)

Ventilatory support

Ventilatory support was required in 50% (n = 19) of the patients in the pretreatment period. Of these, 2 patients (11%) completely stopped ventilatory support in the post-treatment period (after 464 days and 1082 days respectively) and 6 patients (32%) decreased the level of support (≥ 4 hours/day in 5 patients). All patients were ≤ 12 years of age at TK2d symptom onset; the age at treatment initiation varied from 1.29 to 25.9 years.

Two patients (11%) initiated ventilatory support in the post-treatment period (972 and 1069 days after start treatment), and one patient (5.3%) switched from BiPAP to full mechanical ventilatory support (24 days after start of treatment). Age at onset of TK2d symptoms was 12.4, 1.2, and 29.9 years respectively with a corresponding age at treatment initiation of 30.3, 1.8, and 59.6 years.

Baseline and last visit FVC values were available for 17 patients (45%). Mean FVC (in liters) was 1.45 (95% CI 1.02 – 1.88) at baseline and 1.67 (1.30 – 2.03) at last visit, with a mean difference of 0.27 (0.11 – 0.41). Stratified by age at TK2d symptom onset, these differences were

- ≤ 2 years: 0.31 (0.07 – 0.56); n = 7,
- > 2 to ≤ 12 years: 0.42 (0.20 – 0.63); n = 5, and
- > 12 years: 0.02 (-0.37 – 0.40); n = 5.

The % predicted FVC was available for 20 patients (53%) at baseline and at last visit. The mean % predicted FVC was 51.0 (95% CI 38.1 – 63.9) at baseline and 58.6 (44.8 – 72.4) at last visit, with a mean difference of 7.6 (3.4 – 11.8). Stratified by age at TK2d symptom onset, these differences were

- ≤ 2 years: 8.4 (-3.1 – 19.9); n = 7,
- > 2 to ≤ 12 years: 12.0 (5.9 – 18.1); n = 5, and
- > 12 years: 4.1 (-1.4 – 9.6); n = 8.

Feeding support

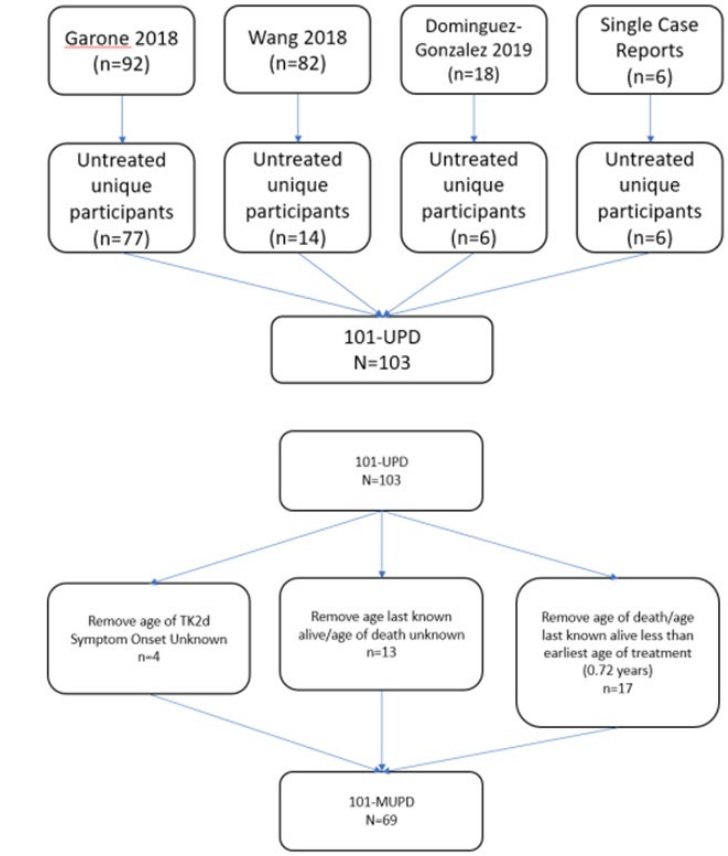
In the pretreatment period, 8 patients (21%) required feeding support via a tube (nasogastric or gastric, both 4 patients); 3 of these patients (38%) had their tube removed in the post-treatment period (days 203, 459, and 1462), while another patient got a tube inserted 15 days after treatment

initiation which was removed 90 days after treatment initiation. All of the aforementioned patients had an age at TK2d symptom onset ≤ 12 years.

Study MT-1621-101 and the Untreated Patient Database (101-(M)UPD)

Inclusion of studies and participant flow

A total of 9 studies was selected for inclusion of patients in the **MUPD** (Figure 10).



MUPD = Modified Untreated Patient Dataset; TK2d = thymidine kinase 2 deficiency; UPD = Untreated Patient Dataset

Figure 10. Source and compilation of the 101-(M)UPD

Baseline data

Baseline demographic data and disease characteristics for the MUPD and the treated patients from study 101 are presented in Table 9.

Table 9. Baseline demographics and disease characteristics in study 101 and 101-MUPD.

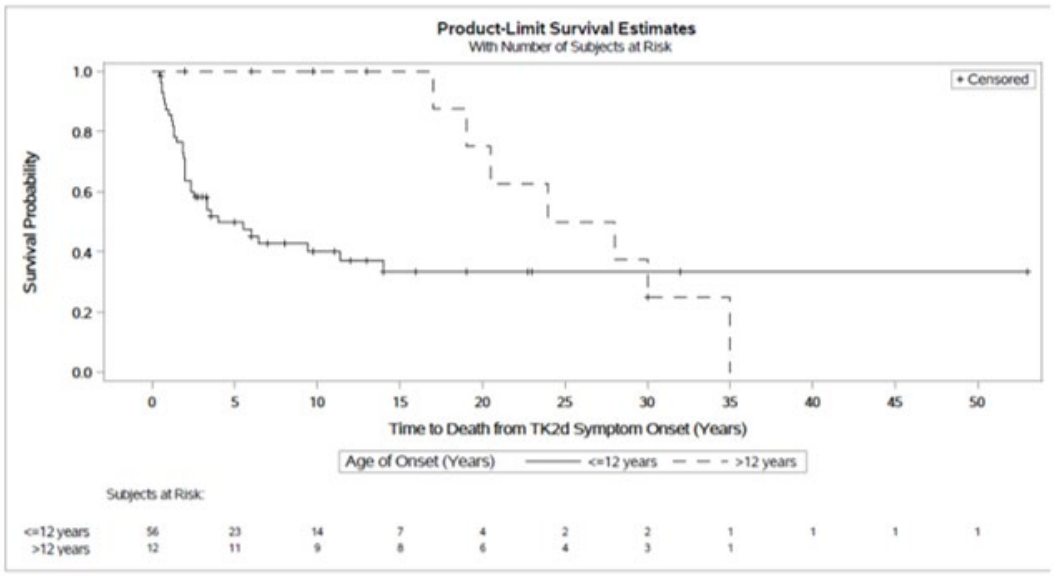
	Overall treated (n=38)	**MUPD (n = 69)**
Sex, n (%)		

	Overall treated (n=38)	MUPD (n = 69)
Male	21 (55%)	39 (57%)
Age of TK2d Symptom Onset (years)		
Mean (sd)	8.9 (14.3)	9.1 (15.5)
Median	2.5	2.0
Q1, Q3	1.4, 11.7	1.0, 9.0
Min, Max	0.5, 60.3	0.0, 72.0
Age of TK2d Symptom Onset, n (%)		
≤ 2 years	15 (40%)	40 (58%)
> 2 to ≤ 12 years	14 (37%)	16 (23%)
> 12 years	9 (24%)	13 (19%)
≤ 12 years	29 (76%)	56 (81%)
Number (%) of patients who died	0	40 (58%)
Age at death (years)		
Mean (sd)	-	13.2 (21.0)
Median	-	3.5
Q1, Q3	-	2.0, 9.3
Min, max	-	1.5, 70.0
Number (%) of patients alive	38 (100%)	29 (42%)
Age last known alive (years)		
Mean (sd)	22.0 (21.1)	25.3 (16.7)
Median	12.4	19.0
Q1, Q3	7.0, 31.9	9.0, 39.0
Min, max	1.6, 74.9	2.5, 74.0

Outcomes and estimation

Survival

Following treatment initiation, no treated study participant in 101 died, in contrast to 40 patients (58%) in the MUPD. Stratified by age at TK2d symptom onset, those without treatment (MUPD) and an age at TK2d symptom onset > 12 years more often died at a younger age compared to the > 12 years group (Figure 11).



101-MUPD = Modified Untreated Patient Database
Numbers displayed at the foot of graph represent number of participants still at risk.

Figure 11. Kaplan-Meier plot for survival in untreated patients, stratified by age of symptom onset (101-MUPD)

Hazard Ratio's (without correction for covariates) varied from 0.11 – 0.20 for the treated compared to the untreated (MUPD) patients in those with an age of TK2d symptom onset ≤ 12 years (Table 10). The HR for comparing treated with untreated patients (without correction for covariates) for the time from treatment to death varied between 0.09 to 0.14 (figure not shown).

Table 10. Cox proportional hazards modelling with matched pairs as stratum variable of time from TK2d symptom onset to death, comparing treated with untreated patients with symptom onset ≤12 years.

Matching	N Pairs	N Informative Pairs	AoO as Cont. Covariate		No Covariate	
			HR (CI) With Firth; Without Firth	LRT p-value With Firth; Without Firth	HR (CI) With Firth; Without Firth	LRT p-value With Firth; Without Firth
Random	28	4	0.1966 (0.0032, 1.2395); 0.0000 (NE)	0.0878; 0.0186	0.1111 (0.0008, 1.0405); 0.0000 (NE)	0.0550; 0.0185
50th Percentile	28	4	0.1818 (0.0020, 1.2750); 0.0000 (NE)	0.0951; 0.0207	0.1111 (0.0008, 1.0405); 0.0000 (NE)	0.0550; 0.0185
75th Percentile	28	3	0.2391 (0.0049, 1.7428); 0.0000 (NE)	0.2113; 0.0439	0.1428 (0.0011, 1.4728); 0.0000 (NE)	0.1342; 0.0414
100th Percentile	28	2	0.3333 (NE, 14.5629); 0.0000 (NE)	NE; 0.9947	0.2000 (0.0015, 2.4580); 0.0000 (NE)	0.3826; 0.0959

AoO = Age of thymidine kinase 2 deficiency symptom onset, CI = confidence interval, cont. = continuous, Firth = Firth correction, HR = hazard ratio; LRT = likelihood ratio test, N = number, NE = not estimable

Exact conditional logistic regression analyses were added to overcome computational challenges due to the absence of death in the treated patient group (study 101) and the limited number of pairs that could be matched (Table 11).

Table 11. Exact conditional logistic regression with matched pairs as stratum variable, of occurrence of death, comparing treated with untreated patients with symptom onset ≤12 years.

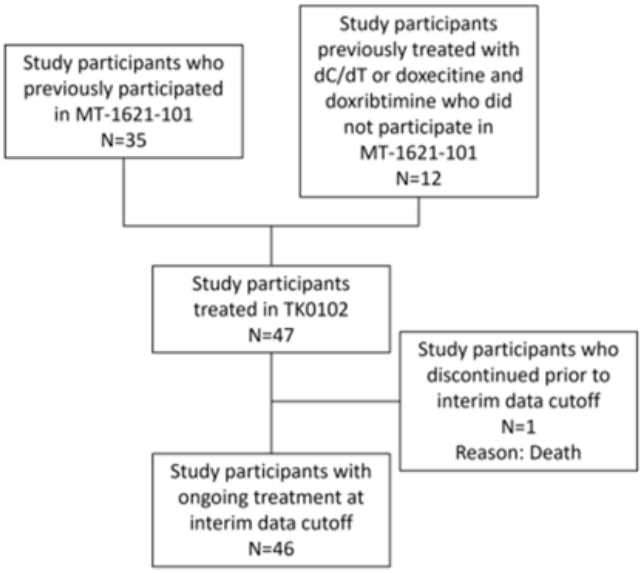
Age of TK2d Symptom Onset	Matching	N Pairs	N Informative Pairs	OR (95% CI)	P-value
All Participants	Random	36	16	0.044 (0.0, 0.206)	< 0.0001
	50th Percentile	36	15	0.047 (0.0, 0.221)	< 0.0001
	75th Percentile	36	16	0.044 (0.0, 0.206)	< 0.0001
	100th Percentile	36	16	0.044 (0.0, 0.206)	< 0.0001
≤ 12 years	Random	28	10	0.072 (0.0, 0.349)	0.0010
	50th Percentile	28	9	0.080 (0.0, 0.395)	0.0020
	75th Percentile	28	10	0.072 (0.0, 0.349)	0.0010
	100th Percentile	28	10	0.072 (0.0, 0.349)	0.0010

CI = confidence interval, N = number, OR = odds ratio

Study MT-1621-102: A phase 2, prospective, open label, single-arm, continuation treatment study of the efficacy and safety of doxecitine and doxribtimine in participants with TK2d

Participant flow

A total of 47 patients were enrolled in study 102; 35 patients originated from study 101, and 12 patients who did not participate in study 101 but received doxecitine and doxribtimine or non-GMP nucleos(t)ides via compassionate use (see Figure 12). The study was still ongoing with 46 patients; 1 patient died.



dC=deoxycytidine; dT=deoxythymidine
Data cutoff date: 15 Mar 2024.

Figure 12. Study participant disposition (safety population)

Recruitment

The start date of study 102 was on July 10th, 2019. Follow-up in study 102 is still ongoing, patient inclusion has ended. The date for the first data cut-off was 15 March 2024.

Conduct of the study

The original protocol, dated 07 January 2019, was amended 5 times. Most relevant were changed administration instructions to indicate that treatment should be taken with food, and to add CGI-I and PGI-I as efficacy assessments (Amendment 3, 29 April 2021), to incorporate a substudy (Spain only) of patients who discontinued dC/dT treatment due to elevated transaminases in study 101 (cancelled prior to initiation; Amendment 3.1, 09 June 2021), to add LFT discontinuation criteria for the main study and subsequent instructions for re-challenge (Amendment 4, 11 April 2022), and to provide guidance on potential dose changes due to weight changes in between clinic visits (Amendment 5, 18 August 2023).

There were 4 patients (8.5%) in whom at least 1 important protocol deviation was reported; 1 patient was included with bilirubin 2x ULN and 1 patient with ASL 3x ULN, 1 patient had 3 observations of drug compliance < 80%, one case of late reporting on SAE's, and dosing diary was not completed in another.

Numbers analysed

Data from 47 patients were analysed.

Exposure to study drug

Compliance to treatment was ≥ 80% in 36 patients (77%). Non-compliance was mainly due to adverse events (n = 5; 46%).

Overall, mean starting and last treatment dose were both slightly less than 700 mg/kg/day for doxecitine and doxribtimine. Dose interruptions were reported for 23 patients (49%), and dose decreases in 14 patients (30%). The highest frequency of dose interruptions was seen for the group of patients with an age at TK2d symptom onset of > 2 to ≤ 12 years (61%) compared to the groups with an age at TK2d symptom onset > 12 years (33%) and ≤ 2 years (45%). It is referred to the Safety section for more information on dose reductions and dose interruptions due to AEs.

The overall mean duration of treatment was 52.0 months (sd 4.7; range 23.0 – 55.0). Total patient year of exposure (PYE) was 203.8 years; 87.3 in patients with an age at TK2d symptom onset ≤ 2 years, 79.0 in those with an age at TK2d symptom onset of > 2 to ≤ 12 years, and 37.5 in patients with an age at TK2d symptom onset of > 12 years.

Concomitant medication

Thirty-six of the 47 patients (77%) used at least one concomitant drug; most frequent were analgesics (n = 35, 75%), systemic antibacterials (n = 31, 66%), vaccines (n = 30, 64%), and anti-inflammatory and antirheumatic products and vitamins (n= 27, 57% both). A total of 23 patients (49%) used drugs in the Alimentary Tract and Metabolism class, and antihistamines and drugs for obstructive airway disease were used each by 19 patients (40%). Frequencies were comparable between the age categories.

Baseline data

The majority of study participants was male (n = 27, 57%), except for the > 12 years age category in which the majority of patients was female (n = 7, 78%). Most patients were Caucasian (n = 44, 94%).

Patients mainly resided in Spain (n = 22, 47%) and the USA (n = 9, 19%); the remaining 16 patients originated from 9 different countries.

The overall median age at TK2d symptom onset was 2.33 years (range 0.31 to 60.30 years). The majority of patients (n = 38, 81%) had an age at TK2d symptom onset ≤ 12 years. Z-scores (in patients with TK2d symptom onset ≤ 12 years) for height and body mass index (BMI) were < 0.5 in 83% and 86% of the patients respectively.

The majority of patients (n = 37, 79%) was ambulatory (able to walk unassisted or assisted) at baseline; those > 12 years of age at TK2d symptom onset were all ambulatory (n = 9; 100%) compared to 70% in the ≤ 2 years and 79% in the > 2 to ≤ 12 years at TK2d symptom onset groups. Most patients in the > 12 years at K2d symptom onset group (n = 7; 78%) did not require assistive devices (55%), compared to 60% and 39% in the in the ≤ 2 years and > 2 to ≤ 12 years at TK2d symptom onset groups respectively

Ventilatory support was required in 78% of the > 12 years at onset group compared to 25% in the ≤ 2 years at TK2d symptom onset group and 39% in the > 2 to ≤ 12 years at TK2d symptom onset group. Of the 22 patients requiring ventilatory support, 19 used non-invasive support (BiPAP) and 3 required a tracheostomy. The average duration of support was 11 hours a day (sd 6.4). A total of 10 patients (21%) required a feeding tube; in 5 out of these 10 patients this served for 100% daily food intake, especially in the youngest age at TK2d symptom onset group (n = 4).

Outcomes and estimation

Survival

No formal survival analyses were performed. One patient (age at TK2d symptom onset > 12 years) died, after several years of treatment (treatment initiation with non-GMP dC/dT and transitioned to doxecitine and doxribtimine; death).

Developmental milestones

Data for study 102 are presented in the pooled analysis section below.

Motor function tests

Motor function tests were obtained in only a subset of patients (Table 12). Data from the 6MWT, NSAA, HFMSE, 100 m timed walk test, and the PROMIS were assessed in at least 15 patients and are included in this report.

Table 12. Motor function tests changes from baseline to month 48 in study 102

	Baseline / Day 1	Change from baseline at month 48
6MWT (m)		
N	30	26
Mean (sd)	400.3 (123.5)	50.4 (91.7)
Min - max	3 - 629	-121.0 – 232.0
NSAA total score		
N	16	15
Mean (sd)	67.8 (21.6)	3.1 (10.3)
Min - max	24 - 100	-15 – 30
HFMSE		
N	26	24
Mean (sd)	45.5 (19.9)	4.2 (14.1)
Min - max	7 - 66	-23 – 49

100 m timed walk test (sec)		
N	26	24
Mean (sd)	76.5 (34.1)	4.0 (41.0)
Min - max	42 - 184	-74 - 170
PROMIS total score		
N	25	19
Mean (sd)	31.9 (12.5)	-0.6 (10.68)
Min - max	12 - 57	-11 - 26

For patients with TK2d symptom onset ⩽ 12 years, numerical improvements over time were observed for the 6MWT (the median (min, max) change from Baseline to Month 48 was 58.0 m (-121.00, 232.00), CHOP INTEND (8.0 (-5, 41)), NSAA (2.0 (-15, 14)), and HFMSE (2.0 (-14, 49)) over all age strata. No change was found for the RULM. Worsening was observed for the Egen Klassification (1.0 (-8, 7) for EK 1 and 1.0 (-12, 9) for EK2).

Ventilatory support

At baseline, 22 patients (47%) required ventilatory support; of these, 19 (86%) required non-invasive support (e.g., BiPAP, CPAP) and 3 (14%) invasive support. Stratified by age at TK2d symptom onset, 40%, 50%, and 78% required ventilatory support at baseline (≤ 2 years, > 2 to ≤ 12 years, and > 12 years respectively).

One of the patients on non-invasive support at baseline switched to invasive support (age at TK2d symptom onset ≤ 2 years) and one patient discontinued ventilatory support during the study (age at TK2d symptom onset > 2 to ≤ 12 years). Four patients (18%) decreased the daily hours for ventilatory support, of whom 3 decreased support for at least 4 hours (all with an age at TK2d symptom onset > 2 to ≤ 12 years), while 5 patients (23%) increased the number of hours for ventilatory support of whom 1 with at least 4 hours (2 patients in the youngest and oldest age at TK2d symptom onset groups, and 1 in the age at TK2d symptom onset > 2 years to ≤ 12 years).

Of the 25 patients (53%) not on ventilatory support at baseline, 3 (12%) initiated ventilatory support during the study; 1 invasive (age at onset TK2d symptoms ≤ 2 years) and 2 non-invasive support (both age at onset of TK2d symptoms > 2 to ≤ 12 years). One of the patients who initiated ventilatory support during the study subsequently discontinued ventilatory support.

FVC (in Liters) and percentage predicted FVC were available from 29 patients at baseline and 32 patients at Month 48. The mean (sd) FVC change from baseline to Month 48 was 0.29 Liters (0.42), and -9.38 (17.69) for the % predicted FVC.

Feeding support

At baseline, 10 patients (21%) had feeding tubes (nasogastric/gastrostomy). Stratified by age at TK2d symptom onset, these percentages were 25% (n = 5), 17% (n = 3), and 22% (n = 2) at baseline (≤ 2 years, > > 2 to ≤ 12 years, and > 12 years respectively).

After start of study 102, no patient had the feeding tube removed but 3 patients (8.1%) required feeding tubes to be inserted; 1 (6.7%) in the ≤ 2 years of age group and 2 (13%) in the age at TK2d symptom onset group > 2 to ≤ 12 years. No differences were reported in percentage daily food intake during the study compared to baseline.

Clinical studies in special populations

Clinical data, as available, for subjects with renal impairment and data availability of those with hepatic impairment are described in the PK and safety sections. There is very limited experience in the elderly (>65 years of age).

In vitro biomarker test for patient selection for efficacy

Not applicable.

2.6.5.3. Supportive study(ies)

Study MT-1621-107

Methods

Study 107 was a retrospective data extraction study, aimed at collecting the vital status of untreated study participants with TK2d and those who have been treated with pyrimidine nucleos(t)ides outside of a sponsored study, as well as to characterise the natural course of TK2d. The original protocol concerned treated patients only, including a prospective 5-year follow-up as well, but this was amended to a retrospective chart review in both treated and untreated patients.

Medical records from patients with a genetic TK2 mutation with a known vital status were reviewed. Next to vital status (primary endpoint), data on demographics, disease characteristics, and clinical disease course were gathered, and for treated patients also data on study drug, dose, age at first treatment, treatment modifications and discontinuation, reasons for dose modifications, and adverse events were extracted, if available.

Results

A total of 61 patients was included, of whom 18 were treated (13 initially treated with dC/dT, 4 with dCMP/dTMP, and 7 with doxecitine/doxribtimine). Mean treatment duration was 96 weeks (range 2.9 – 473), mean dose 618 mg/kg/day (range 200 – 1200 mg/kg/day). Six patients (33%) discontinued treatment due to a TEAE, and another 3 (17%) discontinued due to serious TEAE's. Patient characteristics in both groups are listed in the table 19.

Table 13. Demographics and disease characteristics – Full Analysis Set

Parameter	Treated Study Participants (N=18)	Untreated Study Participants (N=43)	Overall (N=61)
Age at informed consent or waiver, years [a]			
n	13	32	45
Mean (SD)	14.4 (18.03)	28.0 (19.08)	24.1 (19.59)
Median	9.2	24.3	19.5
Age of TK2d symptom onset, years [b]			
n	18	40	58
Mean (SD)	10.7 (15.97)	13.0 (16.37)	12.3 (16.15)
Median	1.5	3.5	2.0
Age at TK2d symptom onset, n (%)			
≤ 2 years	12 (66.7)	18 (41.9)	30 (49.2)
> 2 and ≤ 12 years	1 (5.6)	9 (20.9)	10 16.4)
> 12 years	5 (27.8)	13 (30.2)	18 (29.5)
Age at first treatment received, years			
Mean (SD)	18.6 (24.77)	N/A	18.6 (24.77)
Age at treatment discontinuation/last treatment received (years)			
Mean (SD)	20.5 (23.89)	N/A	20.5 (23.89)
Sex, n (%)			
Male	11 (61.1)	19 (44.2)	30 (49.2)
Female	7 (38.9)	24 (55.8)	31 (50.8)

In the treated patient group, 5/18 (28%) **died**, versus 12/43 (28%) in the untreated group. Of the 5 treated patients who died, 2 were ≤ 2 years at age of TK2d symptom onset and these patients died at 9 and 11 months after symptom onset, after 23 and 44 days of treatment with nucleos(t)ides. The other three treated patients who died were > 2 years of age at symptom onset and discontinued treatment 5-29 months prior to death due to TEAE's. The mean age at death was 37.1 years (sd 33.5) in the treated and 10.6 (16.4) in the untreated group.

In the treated patient group, 11/14 (79%) patients lost at least one **motor milestone** and 1/11 (9.1%) of the patients regained a motor milestone prior to treatment initiation. No motor milestones were lost and six motor milestones were regained after treatment initiation.

Table 14. Developmental motor milestones for treated study patients – Full Analysis Set

Parameter, n (%)	Treated Study Participants (N=18)	
	Pre-treatment [a]	Post-treatment [b]
≥ 1 milestones initially achieved	14/17 (82.4)	0
≥ 1 milestones never initially achieved	10/17 (58.8)	10/10 (100)
≥ 1 milestones lost	11/14 (78.6)	0
≥ 1 milestones regained after loss	1/11 (9.1)	6/11 (54.5)

Note: Percentages are calculated using the number of study participants in each study participant group at risk for the milestone.
[a] Last assessment before initiating treatment.
[b] Last assessment post-treatment.

A total of 13 of the treated patients (72%) required **ventilatory support** (prior to treatment). Post-treatment, 1 patient initiated and 1 discontinued ventilatory support. In the untreated group, 23 patients (54%) were on ventilatory support, and 1 patient discontinued this due to intolerance.

A total of 9 of the treated patients (50%) was on **feeding support** prior to treatment initiation compared to 10 untreated patients (23%), mostly due to dysphagia. In one treated patient (11%), the feeding tube was removed post-treatment.

In the untreated patient group, 27/42 (64%) patients lost at least one motor milestone and 1/27 (3.7%) of the patients regained a motor milestone.

Of these untreated patients, data from 26 patients ≤ 12 years of age at symptom onset was available. From these patients, 14/26 (54%) never initially achieved at least 1 motor milestone, 20/26 (77%) lost at least one motor milestone, and 1/20 (5%) regained a milestone.

Table 15. Developmental motor milestones for untreated study patients – Full Analysis Set

Parameter, n (%)	Untreated Study Participants (N=43)
≥ 1 motor milestones initially achieved ability	42/42 (100.0)
≥ 1 milestones never initially achieved	15/42 (35.7)
≥ 1 milestones lost	27/42 (64.3)
≥ 1 milestones regained after loss	1/27 (3.7)

Note: Percentages are calculated using the number of study participants in each study participant group at risk for the milestone.

Table 16. Developmental motor milestones never initially achieved, lost, and regained in patients with age of TK2d symptom onset ≤12 years, untreated MT-1621-107 (107 untreated subpopulation)

	107 untreated subpopulation		
	Never Initially Achieved[b] n/N (%)	**Lost[c] n/N (%)**	**Regained[d] n/N (%)**
≥1 milestone abilities	14/26 (53.8)	20/26 (76.9)	1/20 (5.0)
Hold head upright, unassisted	0/23	11/23 (47.8)	0/11
Sit upright, unassisted	2/22 (9.1)	8/20 (40.0)	0/8
Stand, assisted	2/20 (10.0)	10/18 (55.6)	0/10
unassisted	3/21 (14.3)	10/18 (55.6)	0/10
Walk, assisted	8/20 (40.0)	7/12 (58.3)	0/7
unassisted	5/24 (20.8)	11/19 (57.9)	1/11 (9.1)
Climb stairs, assisted	9/18 (50.0)	5/9 (55.6)	0/5
unassisted	14/19 (73.7)	4/5 (80.0)	0/4
Run	13/18 (72.2)	3/5 (60.0)	0/3

ISE=Integrated Summary of Efficacy; TK2d=thymidine kinase 2 deficiency

[a] For the untreated summary, the denominator represents the number of participants that initially acquired a developmental motor milestone.

[b] The denominator represents the number of participants that initially acquired a developmental motor milestone and lost that milestone.

[c] For the pretreatment summary, the denominator represents the number of participants that initially acquired a developmental motor milestone in the pretreatment period.

[d] The denominator represents the number of participants that initially acquired a developmental motor milestone and lost that milestone in the pretreatment period.

Note: Results did not change for the 101+102 pretreatment population when the new data cut (ie, 14 Mar 2025) was used.

A total of 13 of the treated patients (72%) required **ventilatory support** (prior to treatment). Post-treatment, 1 patient initiated and 1 discontinued ventilatory support. In the untreated group, 23 patients (54%) were on ventilatory support, and 1 patient discontinued this due to intolerance.

A total of 9 of the treated patients (50%) was on **feeding support** prior to treatment initiation compared to 10 untreated patients (23%), mostly due to dysphagia. In one treated patient (11%), the feeding tube was removed post-treatment.

Study TK114

Methods

Study **114** was a non-interventional study, aimed at retrospective data collection from patients treated with doxecitine and doxribtimine in company-supported expanded access and compassionate use programs. Eligible patients for these programs were those with TK2d who were at risk of major disability or death resulting from TK2d, but who were not able to participate in a clinical study. Date of data cut-off was set at 1st March 2024. Collected data encompassed demographics, informed consent date, exposure details, vital status (survival), diagnosis data, and data related to family history.

Results

A total of 43 patients with TK2d was included in study 114. There were 9 patients who were also enclosed in study 107, and for these patients only data missing in study 107 were reported (if available).

Mean duration of treatment was 506 days; 12 patients were treated ≤ 6 months, 8 > 6 to ≤ 12 months, 21 patients > 1 to ≤ 5 years; maximum treatment duration was 7.4 years. Final treatment dose was 800 mg/kg/day for 34 (79%) of the patients.

One of the 43 patients deceased (2.3%) due to aspiration pneumonia. One patient discontinued the study due to a medical event (disease progression, drug ineffective). Of the 43 patients, 17 were each male or female (40% each), data for the other patient was missing. The majority was white (54%). At time of informed consent, median age was 7.4 years (range 0.7 to 56). Median age at TK2d symptom onset was 18 months (rage 4 to 240 months).

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

Pooling

A pooled analysis of efficacy, focusing on survival benefit, was performed with the **101+102 treated ISE population** which was a priori regarded as the most relevant data source. Methods and results of the separate studies are reported above. Data of the treated patients were compared with data of untreated patients originating from the **ISE-MUPD**. The focus is on patients with an age at TK2d symptom onset ≤ 12 years.

Outcomes/endpoints

Primary efficacy endpoint was survival (time to death), with matched-pair and unmatched data, including sensitivity analyses for matching. *Secondary* efficacy endpoints were developmental milestones, ventilatory support, and feeding support.

The primary model for the survival was the pre-specified matched-pairs Cox PH model, with convergence via the Firth method (see statistical analysis section above). Secondary efficacy endpoints were descriptively reported.

Numbers analysed

The 101+102 treated ISE population consisted of 50 patients, of whom 39 had an age at TK2d symptom onset ≤ 12 years. Treatment was ongoing in 47 patients; 1 patient died and 2 patients discontinued treatment due to adverse events (all with an age at TK2d symptom onset > 12 years). The ISE-MUPD group consisted of 114 patients, of whom 93 had an age at TK2d symptom onset ≤ 12 years.

Baseline demographic characteristics

Demographic data for the **101+102 treated ISE population** (n = 39) with an age at **TK2d symptom onset ≤ 12 years** is reported above for the separate studies.

The subgroup of patients with an age at **TK2d symptom onset ≤ 12 years** in the **ISE-MUPD** (n = 93) was predominantly male (n =F1 49, 53%), Caucasian (n = 24, 26%; race not reported for n = 67, 72%), had a median age of 1.3 (interquartile range (IQR) 0.8, 2.5), with 28% living in North America (n = 26), 22% in Europe (n = 20), and 23% (n = 21) in the Rest of World (not reported for 25 patients). Median age at TK2 symptom onset was 1.3 years (IQR 1.2, 2.5), with a median birth year of 2005 (IQR 1994, 2013).

Baseline disease characteristics

Baseline disease characteristics for the **101+102 treated ISE population**

(n = 39) with an age at **TK2d symptom onset ≤ 12 years** is reported above for the separate studies. In short, 21 (54%) were ambulatory. Further, 15 patients (39%) used wheeled support, 2 (5%) used standing/walking support, and 1 was bed ridden. Ventilatory support was required in 18 patients (46%). The majority (14/18) was on non-invasive support, with a median of 10 hours a day (IQR 8, 23). Eight patients (7%) had a feeding tube.

From the untreated patients with age at **TK2d symptom onset ≤ 12 years** in the **ISE-MUPD**, 10 (11%) were ambulatory, 15 (16%) lost ambulation while for the others (n = 68) it was not known. Data on ambulatory support were not available. Ventilatory support was required in 42 patients (45%). The majority was on non-invasive support, with a median of 24 hours a day (IQR 16, 24). Eight patients (8.6%) had a feeding tube.

Survival

In the **101+102 treated ISE population** (n = 50), 1 death was reported in a patient with an age at symptom onset > 12 years. The median age of the 49 patients last known alive was 14.4 years (range 5.1, 80.0). No patient died in the subgroup of patients with an age at **TK2d symptom onset ≤ 12 years** (n = 39).

In the **ISE-MUPD** (n = 114), 58 deaths were reported. Patients died at a median age of 2.9 years (range 0.8, 70.0). The median age of the 56 patients last known alive was 18.5 (range 0.8, 74.0). Of the subgroup of untreated patients with age at **TK2d symptom onset ≤12 years** (n = 93), 53 deaths were reported. Patients died at a median age of 4.6 years (range 0.8, 33.5); the median age of the 56 patients last known alive was 15.1 (range 0.8, 53.0).

Survival data from the **101+102 treated ISE population** and the **ISE-MUPD** were compared using Cox proportional hazard models (Table 17). Higher probabilities of survival of the treated compared to the untreated patients with age at **TK2d symptom onset ≤12 years** when considering time from TK2d symptom onset to death as well as time from treatment start to death were found: Hazard Ratios were 0.03 – 0.06 (dependent on the model) and 0.03 – 0.05 for time from treatment start (dependent on the model). Within the subgroup of patients with an age of symptom onset > 2 and ≤ 12 years, the Hazard Ratios varied from 0.08 – 0.33 and 0.08 – 0.26, respectively. In the total group, the HRs varied from 0.02 – 0.03 for time from TK2d symptom onset, and 0.02 – 0.09 for time from treatment start.

Table 17. Cox models of time to TK2d symptom onset to death, comparing treated patients (studies 101+102) with untreated patients (ISE-MUPD).

			Cox PH model, Matched-Pairs as Strata Variable, AoO as Continuous Covariate		Cox PH model, Matched-Pairs as Strata Variable, No Covariate		Marginal Cox PH Model with AoO category as Strata Variable	
Matching	**N Pairs**	**N Informative Pairs**	**HR (CI)** With Firth Without Firth	**LRT p-value** With Firth Without Firth	**HR (CI)** With Firth Without Firth	**LRT p-value** With Firth Without Firth	**HR (CI)**	**Wald Test p-value**
All participants								
Random	43	18	0.0298 (0.0001, 0.2228) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0270 (0.0002, 0.1973) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0289 (0.0040, 0.2096)	0.0005
50th Percentile	43	19	0.0284 (0.0002, 0.2019) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0256 (0.0002, 0.1864) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0271 (0.0037, 0.1994)	0.0004
75th Percentile	43	19	0.0290 (0.0001, 0.2226) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0256 (0.0002, 0.1864) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0297 (0.0052, 0.1709)	<0.0001
100th Percentile	43	20	0.0259 (0.0001, 0.2003) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0244 (0.0002, 0.1767) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0250 (0.0038, 0.1644)	0.0001
Age of TK2d symptom onset ≤12 years								
Random	36	16	0.0586 (0.0001, 0.2862) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0303 (0.0002, 0.2233) <0.0001 (NE, NE)	<0.0001 <0.0001	<0.0001 (<0.0001, <0.0001)	<0.0001
50th Percentile	36	18	0.0460 (<0.0001, 0.2549) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0270 (0.0002, 0.1973) <0.0001 (NE, NE)	<0.0001 <0.0001	<0.0001 (<0.0001, <0.0001)	<0.0001
75th Percentile	36	17	0.0298 (0.0002, 0.2243) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0286 (0.0002, 0.2095) <0.0001 (NE, NE)	<0.0001 <0.0001	<0.0001 (<0.0001, <0.0001)	<0.0001
100th Percentile	36	19	0.0256 (0.0002, 0.1993) <0.0001 (NE, NE)	<0.0001 <0.0001	0.0256 (0.0002, 0.1864) <0.0001 (NE, NE)	<0.0001 <0.0001	<0.0001 (<0.0001, <0.0001)	<0.0001

AoO=Age of TK2d symptom onset; CI=confidence interval; EP=Evaluable Population; Firth=Firth correction; HR=hazard ratio; ISE=Integrated Summary of Efficacy; LRT=likelihood ratio test; MUPD=Modified Untreated Patient Database; NC=non convergence; NE=not estimable; PH=proportional hazards; TK2d=thymidine kinase 2 deficiency

Note: EP includes all treated or untreated except those that died or were censored before the earliest age of treatment in study, have unknown age of TK2d symptom onset, are missing either age of death or age last known alive, or have age of TK2d symptom onset equal to the age of event.

Note: Number of informative pairs is not applicable for the marginal Cox models.

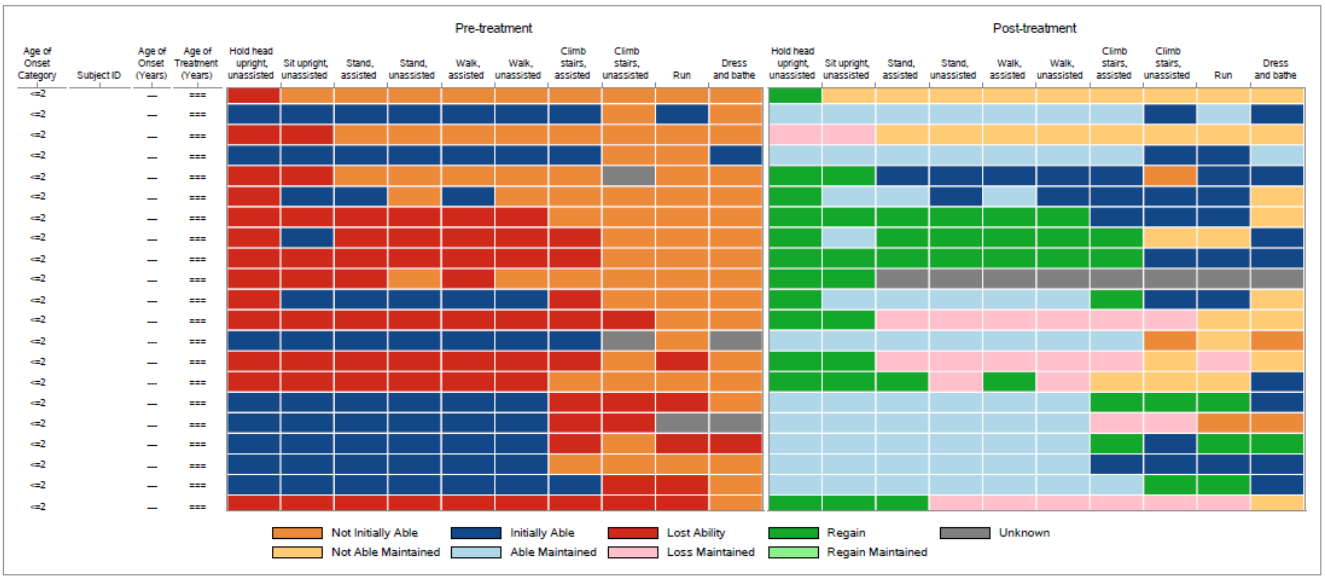
The Exact Conditional Logistic regression analyses showed a statistically significant lower risk of death for the treated patients compared to the matched untreated patients with age at **TK2d symptom onset ≤12 years** of approximately 96% (OR=0.039-0.053).

RMST analyses indicated that in the 30 years following disease onset, mean survival time among the patients with an age of **TK2d symptom onset ≤12 years** was 30.0 years, compared to a maximum of 15.4 years in the untreated patients. A statistically significant increase in the mean survival time was found for this subgroup of treated patients over the 6, 4, and 2 years following treatment start: Over the 6 years following treatment start, mean survival time among treated patients was 6.0 years compared to a maximum of 3.4 years among the untreated patients.

Additional survival analyses are presented below in section 2.6.5.5.

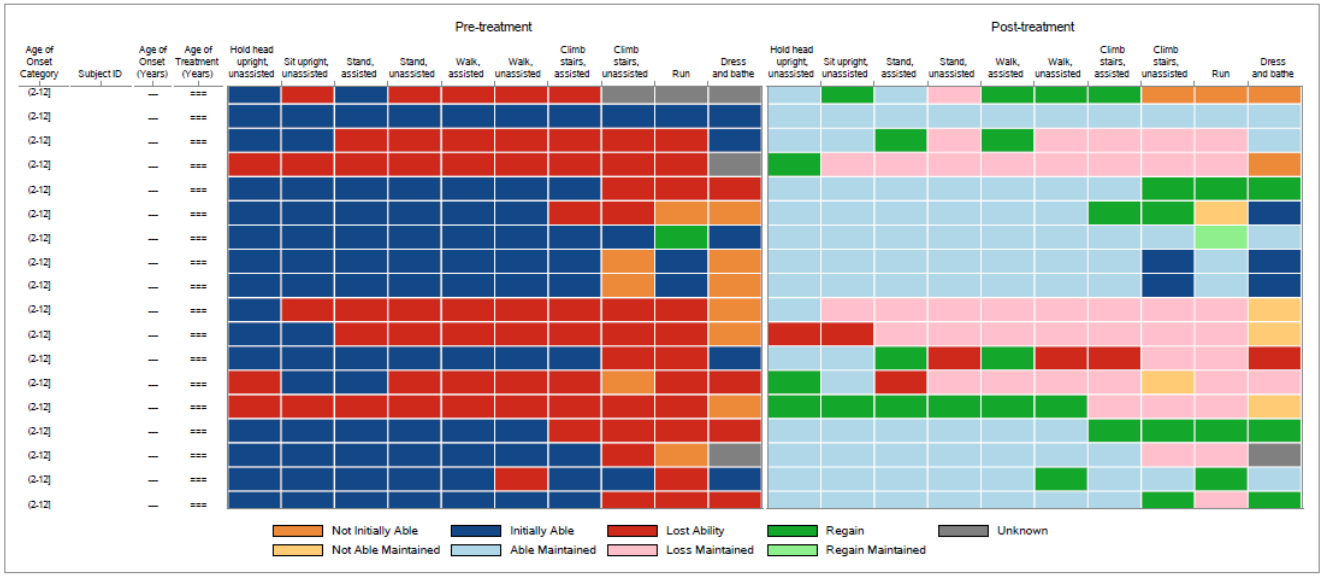
Motor milestones

Data on motor milestones were only available for treated patients (**101+102 ISE population**). The data are stratified by age at TK2d symptom onset (≤ 2 years and > 2 and ≤12 years).



ISE=Integrated Summary of Efficacy; TK2d=thymidine kinase 2 deficiency
Note: Participants must have initially achieved the developmental motor milestone in order to be counted as a loss for the developmental motor milestone.
Note: Participants must have initially achieved the developmental motor milestone, lost the same developmental motor milestone, and subsequently regained the same developmental motor milestone, in order to be counted as a regain for the developmental motor milestone.

Figure 13. Heat map for motor milestones during pre- and post-treatment (101+102 treated ISE population, age of TK2d symptom onset: ≤2 years)



ISE=Integrated Summary of Efficacy; TK2d=thymidine kinase 2 deficiency
Note: Participants must have initially achieved the developmental motor milestone in order to be counted as a loss for the developmental motor milestone.
Note: Participants must have initially achieved the developmental motor milestone, lost the same developmental motor milestone, and subsequently regained the same developmental motor milestone, in order to be counted as a regain for the developmental motor milestone.

Figure 14. Heat map for motor milestones during pre- and post-treatment (101+102 treated ISE population, age of TK2d symptom onset: >2 to ≤12 years)

The net gain of motor milestones in patients with an age of onset of TK2d symptoms ≤ 12 years was - 2.2 (sd 3.5, range -5.0 – 0) prior to treatment initiation compared to 2.3 (sd 2.75, range 0.0 – 3.0) after treatment initiation. The largest difference was however seen for the youngest age at TK2d symptom onset group (≤ 2 years) compared to the > 2 to ≤ 12 years group (mean gain prior and after treatment -1.8 versus 3.4, compared to -2.7 versus 1.1 respectively) (data cut off March 2024).

Table 18 provides an overview of lost and regained motor milestones pre- and post-treatment with data cut off of 14 March 2025. Without treatment, spontaneous (re)gain of motor milestones is very rare (1/32, 3.1%) while loss is common (32/39, 82%). After treatment initiation, (re)gain of motor milestone is commonly observed (26/31, 84%), while loss of motor milestones still occurs but substantially less often than in the pretreatment period (10/38, 26%).

Please note that the numbers in the Table do not always align with the numbers counted in the heat maps; this is due to the fact that patients may lose and regain during the post-treatment period and while these observations are both counted and integrated in the Table, only the last observation is included in the heat map.

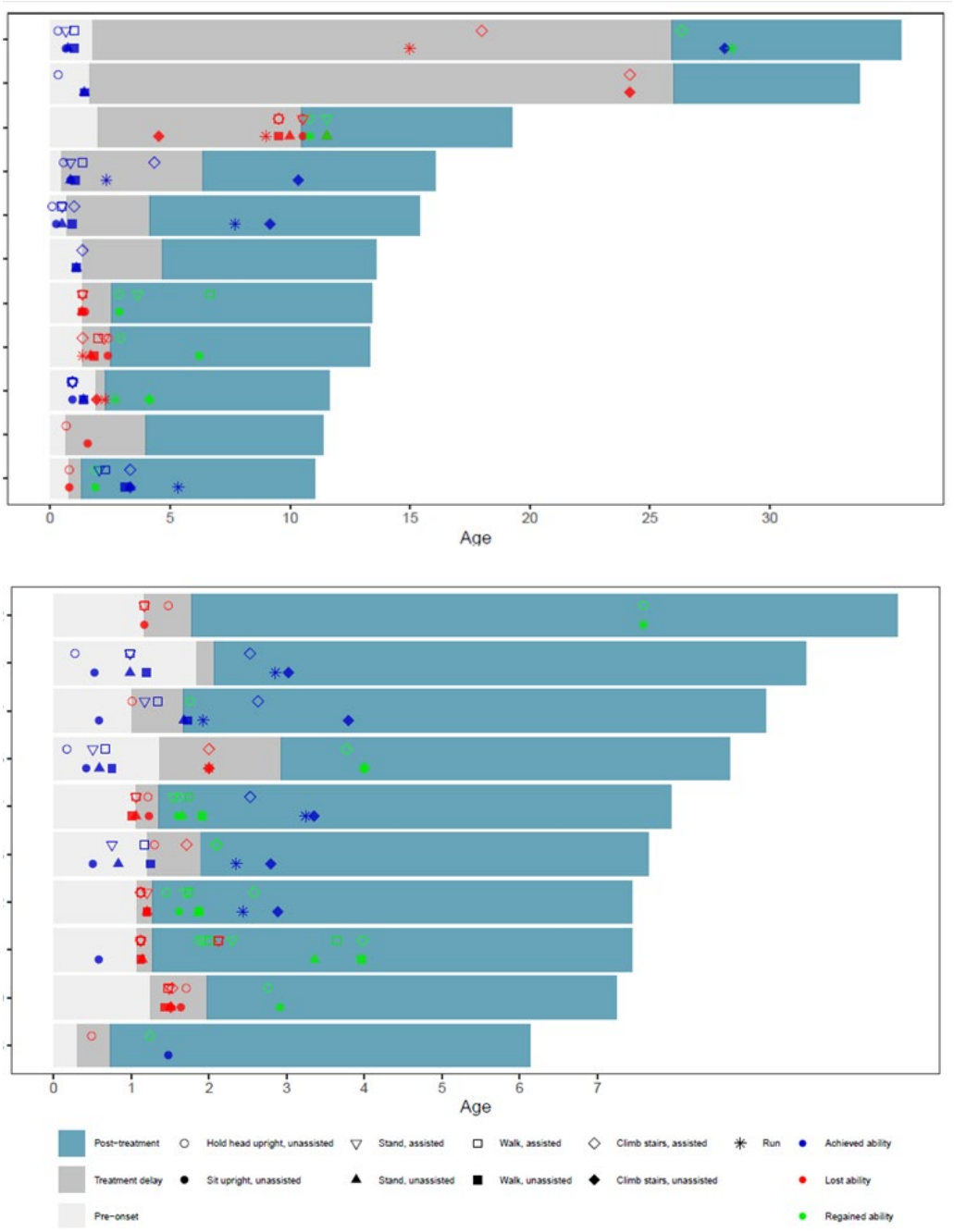
Table 18. Motor milestones lost and regained pre- and post-treatment, stratified by age of symptom onset, in the 101+102 ISE treated population

Category	All treatment durations											
	101+102 treated ISE population Age of TK2d symptom onset ⩽2 years N=21				101+102 treated ISE population Age of TK2d symptom onset >2 to ⩽12 years N=18				101+102 treated ISE population Age of TK2d symptom onset ⩽12 years N=39			
	Pretreatment		Post-treatment		Pretreatment		Post-treatment		Pretreatment		Post-treatment	
Item	**Lost n (%)**	**Regained n (%)**	**Lost n (%)**	**Regained n (%)**	**Lost n (%)**	**Regained n (%)**	**Lost n (%)**	**Regained n (%)**	**Lost n (%)**	**Regained n (%)**	**Lost n (%)**	**Regained n (%)**
Not at risk for milestone[a]	0/21	4/21 (19.0)	1/21 (4.8)	4/21 (19.0)	0/18	3/18 (16.7)	0/18	4/18 (22.2)	0/39	7/39 (17.9)	1/39 (2.6)	8/39 (20.5)
At risk for milestone	21/21 (100)	17/21 (81.0)	20/21 (95.2)	17/21 (81.0)	18/18 (100)	15/18 (83.3)	18/18 (100)	14/18 (77.8)	39/39 (100)	32/39 (82.1)	38/39 (97.4)	31/39 (79.5)
⩾1 milestones abilities lost/regained	**17/21 (81.0)**	**0/17**	**2/20 (10.0)**	**15/17 (88.2)**	**15/18 (83.3)**	**1/15 (6.7)**	**8/18 (44.4)**	**11/14 (78.6)**	**32/39 (82.1)**	**1/32 (3.1)**	**10/38 (26.3)**	**26/31 (83.9)**
Milestones[a,b]												
Ability to hold head upright, unassisted	13/21 (61.9)	0/13	0/20	12/13 (92.3)	3/18 (16.7)	0/3	1/18 (5.6)	3/4 (75.0)	16/39 (41.0)	0/16	1/38 (2.6)	15/17 (88.2)
Ability to sit upright, unassisted	9/20 (45.0)	0/9	**0/20**	8/9 (88.9)	4/18 (22.2)	0/4	1/16 (6.3)	2/5 (40.0)	13/38 (34.2)	0/13	**1/36 (2.8)**	10/14 (71.4)
Ability to stand, assisted	8/18 (44.4)	0/8	1/16 (6.3)	5/8 (62.5)	5/18 (27.8)	0/5	2/15 (13.3)	3/7 (42.9)	13/36 (36.1)	0/13	3/31 (9.7)	8/15 (53.3)
Ability to stand, unassisted	7/16 (43.8)	0/7	1/15 (6.7)	4/7 (57.1)	7/18 (38.9)	0/7	3/14 (21.4)	3/8 (37.5)	14/34 (41.2)	0/14	4/29 (13.8)	7/15 (46.7)
Ability to walk, assisted	8/18 (44.4)	0/8	1/15 (6.7)	4/8 (50.0)	7/18 (38.9)	0/7	2/15 (13.3)	5/8 (62.5)	15/36 (41.7)	0/15	3/30 (10.0)	9/16 (56.3)
Ability to walk, unassisted	7/16 (43.8)	0/7	0/14	3/7 (42.9)	8/18 (44.4)	0/8	1/13 (7.7)	3/9 (33.3)	15/34 (44.1)	0/15	1/27 (3.7)	6/16 (37.5)
Ability to climb stairs, assisted	9/13 (69.2)	0/9	0/13	5/9 (55.6)	9/18 (50.0)	0/9	2/13 (15.4)	4/10 (40.0)	18/31 (58.1)	0/18	2/26 (7.7)	9/19 (47.4)
Ability to climb stairs, unassisted	5/5 (100)	0/5	0/11	2/5 (40.0)	11/14 (78.6)	0/11	0/9	4/11 (36.4)	16/19 (84.2)	0/16	0/20	6/16 (37.5)
Ability to run	5/6 (83.3)	0/5	0/11	3/5 (60.0)	12/15 (80.0)	1/12 (8.3)	2/9 (22.2)	4/12 (33.3)	17/21 (81.0)	1/17 (5.9)	2/20 (10.0)	7/17 (41.2)

	All treatment durations											
	101+102 treated ISE population Age of TK2d symptom onset ⩽2 years N=21				101+102 treated ISE population Age of TK2d symptom onset >2 to ⩽12 years N=18				101+102 treated ISE population Age of TK2d symptom onset ⩽12 years N=39			
Category	Pretreatment		Post-treatment		Pretreatment		Post-treatment		Pretreatment		Post-treatment	
Item	Lost n (%)	Regained n (%)	Lost n (%)	Regained n (%)	Lost n (%)	Regained n (%)	Lost n (%)	Regained n (%)	Lost n (%)	Regained n (%)	Lost n (%)	Regained n (%)

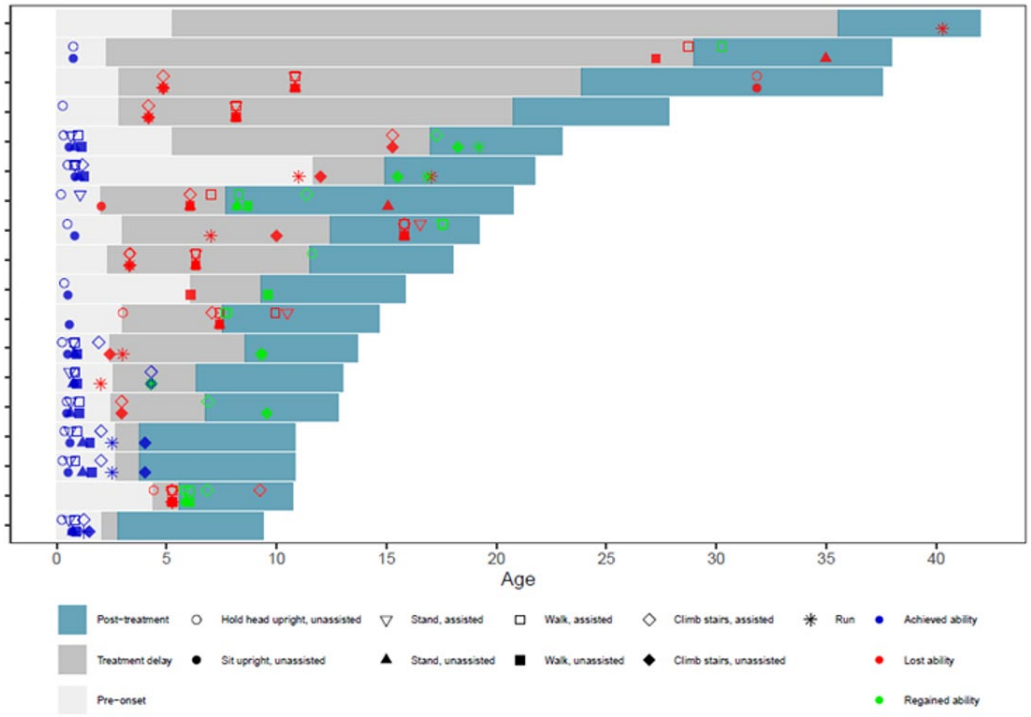
FAS=Full Analysis Set; ISE=Integrated Summary of Efficacy; TK2d=thymidine kinase 2 deficiency
Note: Imputation rules used for this table are described in the Development Program Imputation Rules. The last assessment post-treatment was utilized to assign a participant.
[a] A participant may be counted in more than 1 developmental motor milestone item, but only once within each line of the table.
[b] Percentages are based on the number of participants at risk for the specific developmental motor milestone.
Note: TK0102 data cutoff date: 14 Mar 2025

The following Figures provide swimmer plots for motor milestones in the 101+102 ISE treated population over time, stratified by age at onset of TK2d symptoms.



FAS=Full Analysis Set; ISE=Integrated Summary of Efficacy; TK2d=thymidine kinase 2 deficiency
Note: Participants are omitted if there is no developmental motor milestone record where date and/or age at event is available.
Note: Achievement occurring prior to a loss is not presented here (ie, it is accepted that a developmental motor milestone must have been initially achieved to be lost; and for achievement shown on the figure, there is no reported loss).
Note: Age is provided in years.
Note: TK0102 data cutoff date: 14 Mar 2025

Figure 15. Swimmer plots of motor milestones in patients with age at TK2d symptom onset ≤2 years in the 101+102 ISE treated population



FAS=Full Analysis Set; ISE=Integrated Summary of Efficacy; TK2d=thymidine kinase 2 deficiency
Note: Participants are omitted if there is no developmental motor milestone record where date and/or age at event is available.
Note: Achievement occurring prior to a loss is not presented here (ie, it is accepted that a developmental motor milestone must have been initially achieved to be lost; and for achievement shown on the figure, there is no reported loss).
Note: Age is provided in years.
Note: TK0102 data cutoff date: 14 Mar 2025

Figure 16. Swimmer plot of motor milestones in patients with age at TK2d symptom onset >2 and ≤12 years in the 101+102 ISE treated population

Ventilatory support

In the group of patients **≤12 years at TK2d symptom onset** of the **101+102 treated ISE patients**, 18 of the 39 (46%) initiated ventilatory support prior to treatment, and 4 (19%) initiated ventilatory support post treatment initiation. Of these 22 patients, 5 (23%) were discontinued ventilatory support.

The median number of hours of support was 10 (range 8, 24) pretreatment, versus 10 (range 0, 24) post-treatment. Similar hours of support were observed prior and post treatment initiation for the ≤ 2 years of age at TK2d symptom onset group (12.5 and 13 hours) and the > 2 to ≤ 12 years group (8 and 8 hours).

Feeding support

In the group of patients **≤12 years at TK2d symptom onset** of the **101+102 treated ISE patients**, 12 of the 39 (31%) had a feeding tube inserted prior to treatment; in 1 patient it was also removed prior to treatment. After treatment initiation, 4 had a feeding tube inserted and 4 patients had the feeding tube removed.

2.6.5.5 Analysis performed across trials (pooled analyses and meta-analysis): Additional survival analyses

Methods

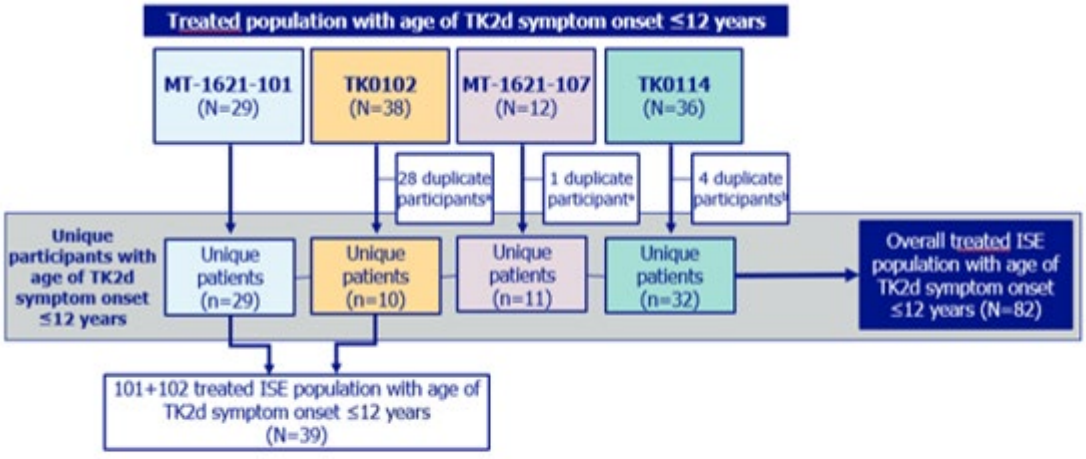
Additional survival analysis were performed to explore the impact of bias; the most relevant analyses are presented in this section. Statistical approaches were identical to previously described for studies 101 and 102.

First, both the treated population (study 101 + 102) and the untreated population were ***restricted*** to those alive in the second half of 2018. Alternatively, the treated patient population was ***expanded*** to the overall treated population (pooling data from studies 101 + 102 + 107 + 114) compared to all patients in the untreated population. These survival analyses used time from symptom onset or time from treatment as time zero. The analyses were also performed by matching patients by age of TK2d symptom onset, time from treatment start, and birth year, and using models with and without age of symptom onset as a continuous variable to address confounding effect (data not shown).

Secondly, alternative approaches were adopted for sensitivity analyses:

- An additional censoring step using the **100th percentile matching** sensitivity analyses was performed on the expanded patient populations (treated and untreated). This is considered the most conservative matching, where treated patients are sorted in descending order according to their age of treatment initiation; the first treated patient in the sorted list is matched with the untreated patient having the highest age last known alive within the same age of TK2d symptom onset category. Untreated patients who died are censored at the same time the treated patient is censored, within each matched pair of treated/untreated patient.
- An alternative matching approach was adopted using **sequential emulation** following principles of target trial emulation: Control patients were emulated per month of age and treated patients were randomly matched to a control patient that was eligible in the same month of age.

Patient flow for studies including all treated patients (studies 101, 102, 107, and 114) is shown below.



ISE=Integrated Summary of Efficacy; TK2d=thymidine kinase 2 deficiency

a Removed duplicate patient (started treatment in MT-1621-101).

b Removed duplicate patients (started treatment in MT-1621-107).

Figure 17. Flow chart for patients with an age at TK2d symptom onset ≤ 12 years at study level to treated ISE populations (101 + 102 and overall)

Results

The ***restricted*** dataset comprised of 20 matched pairs, including 2 informative pairs. In the treated population with an age of TK2d symptom onset ≤12 years, there were no deaths (0/20; 0%) while there were 3 deaths (3/20; 15%) in the untreated population; the corresponding hazard ratios ranged from <0.0001 to 0.33.

Table 19. Cox models – Time from TK2d symptom onset to death (EP; 101+102 treated ISE population and matched *restricted* ISE-MUPD) - Age of TK2d symptom onset ≤12 years

			Cox PH model, Matched-Pairs as Strata Variable, AoO as Continuous Covariate		Cox PH model, Matched-Pairs as Strata Variable, No Covariate		Marginal Cox PH Model with AoO category as Strata Variable	
Matching	**N Pairs**	**N Informative Pairs**	**HR (CI)** With Firth	**LRT p-value** With Firth	**HR (CI)** With Firth	**LRT p-value** With Firth	**HR (CI)**	**Wald Test p-value**
Random	20	2	0.3333 (0.0080, NE)	NE	0.2000 (0.0015, 2.4580)	0.3826	<0.0001 (<0.0001, <0.0001)	<0.0001
50th Percentile	20	2	0.3334 (0.0085, NE)	NE	0.2000 (0.0015, 2.4580)	0.3826	<0.0001 (<0.0001, <0.0001)	<0.0001
75th Percentile	20	2	0.3334 (0.0085, NE)	NE	0.2000 (0.0015, 2.4580)	0.3826	<0.0001 (<0.0001, <0.0001)	<0.0001
100th Percentile	20	2	0.3334 (0.0085, NE)	NE	0.2000 (0.0015, 2.4580)	0.3826	<0.0001 (<0.0001, <0.0001)	<0.0001

AoO=age of TK2d symptom onset; CI= ; EP=Evaluable Population; Firth=Firth correction; HR=hazard ratio; ISE=Integrated Summary of Efficacy; LRT=likelihood-ratio test; MUPD=Modified Untreated Patients Database; N=number; NC=nonconvergence; NE=not estimable; PH=proportional hazards; TK2d=thymidine kinase 2 deficiencyηNote: EP includes all treated or untreated participants except those that died or were censored before the earliest age of treatment in study, have unknown age of TK2d symptom onset, are missing either age of death or age last known alive, or have age of TK2d symptom onset equal to the age of event.ηNote: Number of informative pairs is not applicable for the marginal Cox models.
Note: TK0102 data cutoff date: 14 Mar 2025

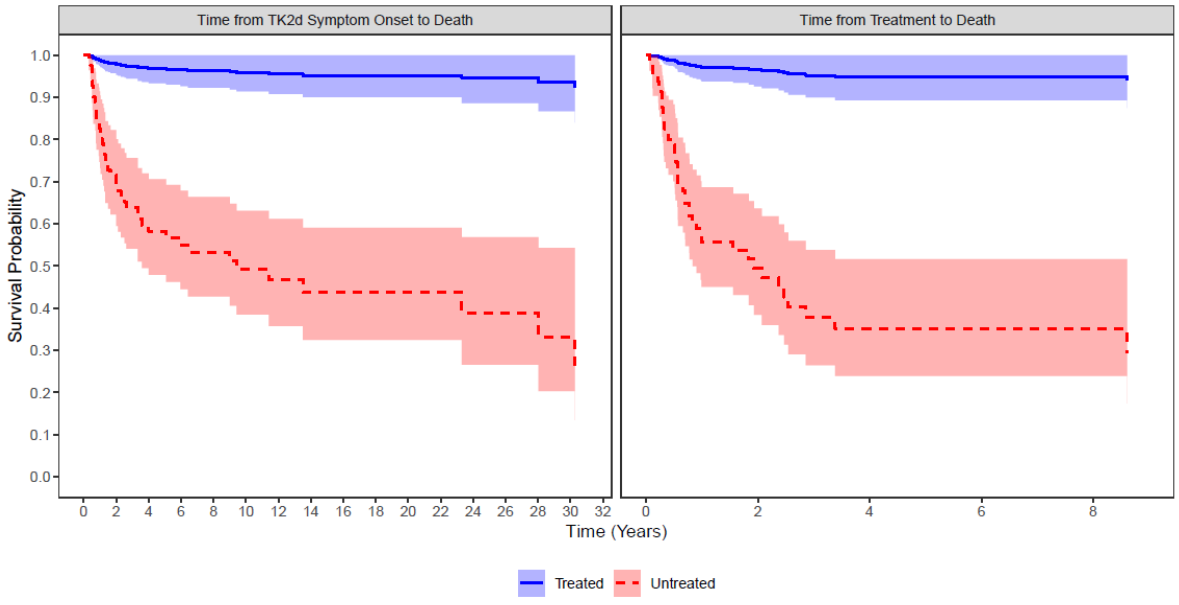
The HRs for all treated patients compared to all untreated patients with an age of TK2d symptom onset ≤ 12 years (***expanded***) vary between 0.059 and 0.141 for time to onset from TK2d symptoms and treatment start (Table 20).

Table 20. Cox models – Time fromTK2d symptom onset to death (EP; treated participants [ISE] and untreated participants [ISE-MUPD]) - Age of TK2d symptom onset ≤12 years

Matching	N pairs	N informative pairs	AoO Cont. Covariate		No Covariate		Marginal Cox	
			HR(CI)	LRT p-value	HR(CI)	LRT p-value	HR(CI)	Wald p-value
Random	77	29	0.1054 (0.0224, 0.3006)	<0.0001	0.0909 (0.0185, 0.2757)	<0.0001	0.0620 (0.0192, 0.2002)	<0.0001
50th percentile	77	32	0.0636 (0.0065, 0.2290)	<0.0001	0.0820 (0.0167, 0.2466)	<0.0001	0.0593 (0.0188, 0.1867)	<0.0001
75th percentile	77	29	0.0909 (0.0185, 0.2757)	<0.0001	0.1041 (0.0221, 0.3046)	<0.0001	0.0607 (0.0193, 0.1915)	<0.0001
100th percentile	78	30	0.1407 (0.0382, 0.3699)	<0.0001	0.1273 (0.0343, 0.3401)	<0.0001	0.0608 (0.0193, 0.1921)	<0.0001

AoO=age of TK2d symptom onset; CI=confidence interval; HR=Hazard Ratio; LRT=Likelihood ratio test
Note: Results are reported for the Cox model using Firth Correction.
Note: Informative Pairs are only used for the Cox Proportional Hazard Model. The marginal Cox model uses all matched pairs.
Note: TK0102 data cutoff date: 14 Mar 2025

The survival curves for the expanded populations are presented in the Figure below.



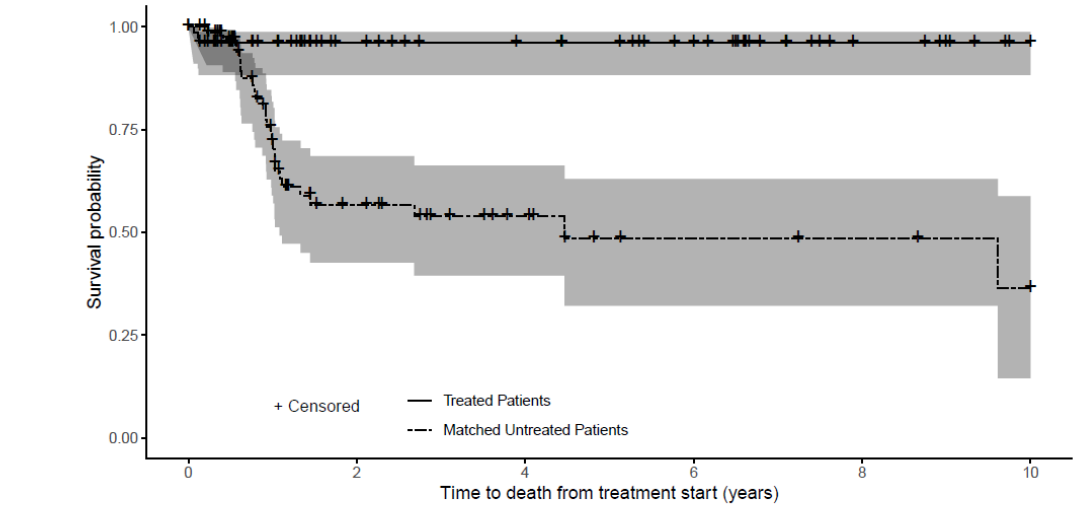
EP=Evaluable Population; ISE=Integrated Summary of Efficacy; MUPD=Modified Untreated Patients Database; TK2d=thymidine kinase 2 deficiency. Note: Marginal model with age of TK2d symptom onset category as strata variable, 50th percentile, no covariate, treatment as time independent variable. Note: TK0102 data cutoff date: 14 Mar 2025

Figure 18. Direct adjustment curves (EP; all treated participants and untreated [ISE-MUPD] participants) - Age of TK2d symptom onset ≤12 years

Results of the sensitivity analysis, using the **100th percentile matching** on the time from treatment start adding an additional censoring step for untreated patients at the same time as the matched treated patient were censored are presented below. The Kaplan-Meier curves with 95% confidence limits are provided in Figure 19 for patients with age of TK2d symptom onset ≤12 years, Figure 20 for patients with an age of TK2d symptom onset ≤2 years, and Figure 21 for patients with an age of TK2d symptom onset >2 to ≤12 years.

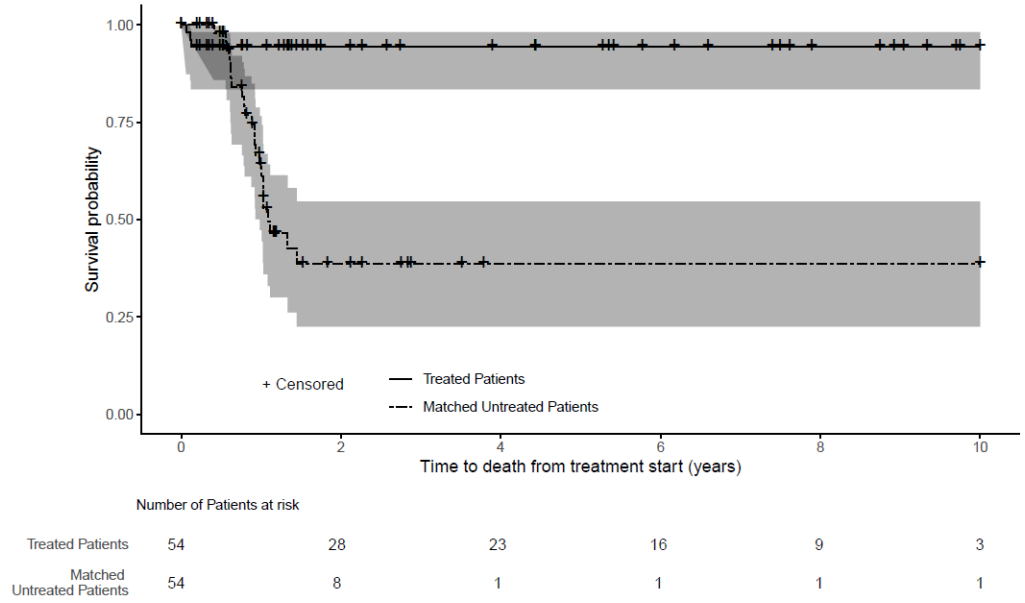
Among patients with age of TK2d symptom onset ≤12 years, 3 patients (3.9%) in the treated group experienced death compared to 28 patients (36.4%) in the untreated group leading to a hazard ratio (95% confidence interval [CI]) of 0.1448 (0.0396, 0.3808), using the Cox PH model with age of TK2d symptom onset as continuous variable. Among patients with age of TK2d symptom onset ≤2 years, 3 patients (5.6%) in the treated group died compared to 23 patients (42.6%) in the untreated group; Hazard Ratio 0.1767 (95% CI 0.0481, 0.4700; Cox PH model with age of TK2d symptom onset as continuous variable. Among patients with age of TK2d symptom onset >2 and ≤12 years, no patient in the treated group died compared to 5 patients (21.7%) in the untreated group (HR 0.1347 ; 95% CI 0.001, 1.0131) (Table 21).

The Kaplan-Meier curves are overlapping at the start of treatment for approximately the first 6 months showing that the 100th Percentile matching may have reduced potential heterogeneity at treatment start between the treated and untreated group. In the untreated group, there is a rapid and significant drop of the survival curve, confirming that the risk of death for these patients is very high without treatment. This is mainly driven by the subgroup of age of TK2d symptom onset ≤2 years, whereas the risk of death is less rapid over time for the subgroup of age of TK2d symptom onset >2 and ≤12 years.



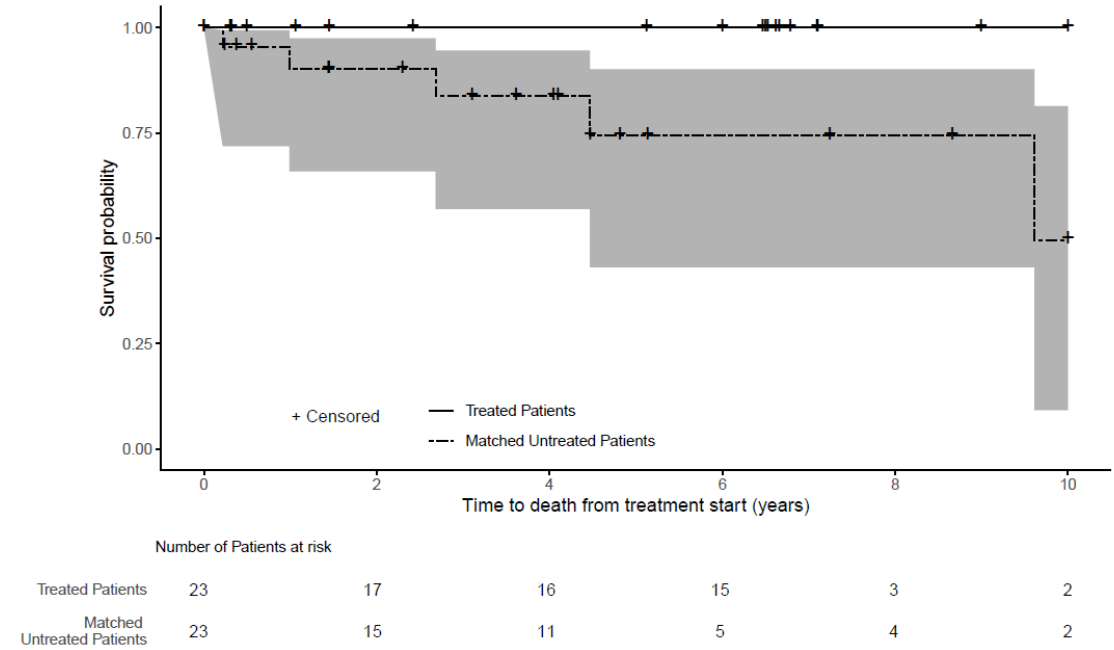
ISE=Integrated Summary of Efficacy; TK2d=thymidine kinase 2 deficiency

Figure 19. ISE: Kaplan-Meier curve with 95% confidence limits for time to death from treatment start using the 100th percentile matching with additional censoring step, age of TK2d symptom onset ≤12 years



ISE=Integrated Summary of Efficacy; TK2d=thymidine kinase 2 deficiency

Figure 20. ISE: Kaplan-Meier curve with 95% confidence limits for time to death from treatment start using the 100th percentile matching with additional censoring step, age of TK2d symptom onset ≤2 years



ISE=Integrated Summary of Efficacy; TK2d=thymidine kinase 2 deficiency

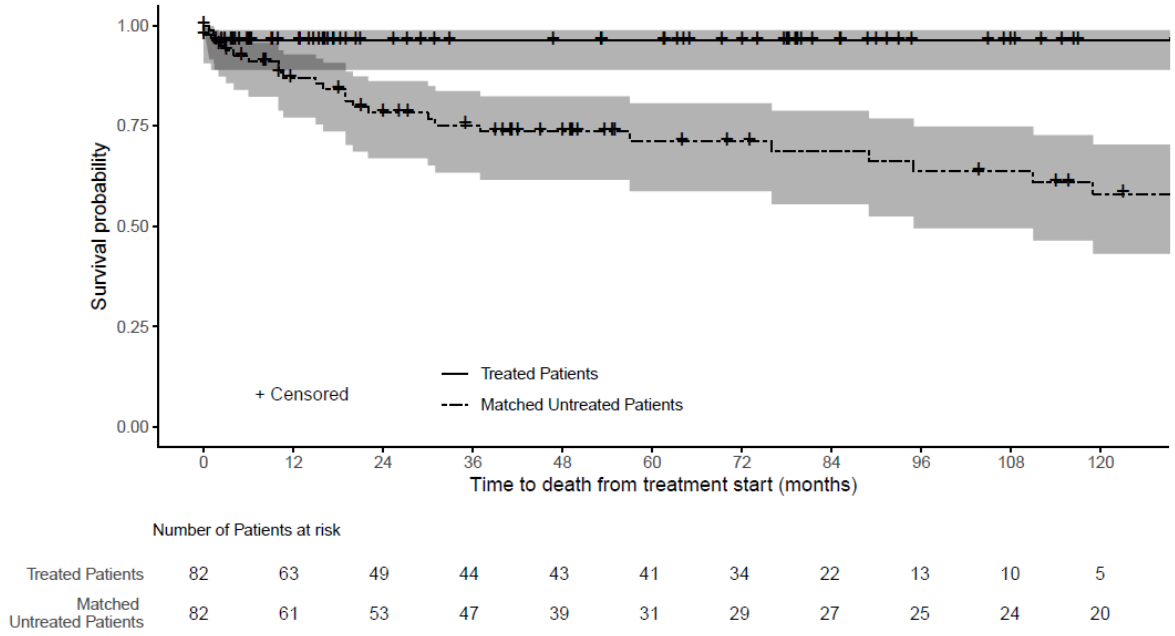
Figure 21. ISE: Kaplan-Meier curve with 95% confidence limits for time to death from treatment start using the 100th percentile matching with additional censoring step, age of TK2d symptom onset >2 to ≤12 years

Table 21. Sensitivity 1: Cox Proportional Hazard model – Time from treatment start to death using the 100th percentile matching with additional censoring step (ISE; treated participants [ISE] and untreated participants [ISE-MUPD])

Matching	N matched pairs	Number (%) of participants who died		N informative pairs	Age of TK2d symptom onset cont. covariate[a]	
		Treated	Untreated		HR(CI)	LRT p-value
Age of TK2d symptom onset ≤12 years						
100th percentile	77	3 (3.9)	28 (36.4)	29	0.1448 (0.0396, 0.3808)	<0.0001
Age at TK2d symptom onset ≤2 years						
100th percentile	54	3 (5.6)	23 (42.6)	23	0.1767 (0.0481, 0.4700)	0.0001
Age at TK2d symptom onset >2 to ≤12 years						
100th percentile	23	0	5 (21.7)	5	0.1347 (0.001, 1.0131)	0.0487

Cont.=continuous; ISE=Integrated Summary of Efficacy; LRT=likelihood-ratio test; MUPD=Modified Untreated Patient Database; TK2d=thymidine kinase 2 deficiency
a A matched pair is informative if at least 1 participant experiences a death and the participant who died must have the shorter observed time to event between the 2 participants. Only informative pairs contribute to the partial likelihood estimator of the hazard ratio.
c Cox Proportional Hazard Model with Firth Correction with age of TK2d symptom onset as continuous variable.
Note: If the untreated participant within a pair dies after the matched treated participant is censored, the untreated participant is censored at the follow-up time of the treated participant.

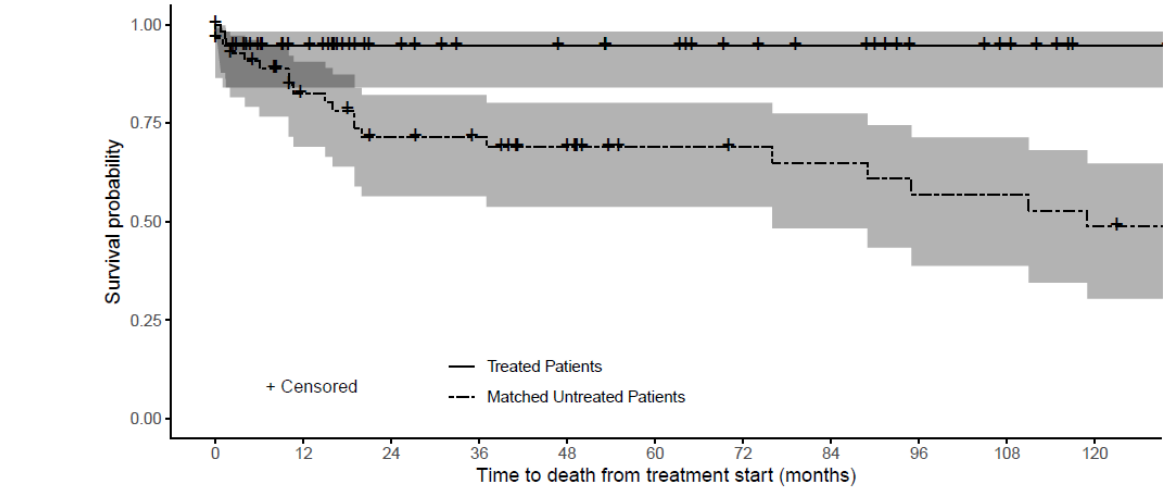
Sensitivity analyses using **sequential emulation** matching and using Cox PH model with treatment as time dependent variable were generally consistent with the other sensitivity analyses. The Kaplan-Meier curves with the 95% confidence limits with this alternative matching algorithm (sequential emulation with 1:1 matching) are provided in Figure 22 for the age of TK2d symptom onset category ≤ 12 years, Figure 23 for age of TK2d symptom onset ≤2 years, and Figure 24 for age of TK2d symptom onset >2 and ≤12 years.



Number of Patients at risk

Treated Patients	82	63	49	44	43	41	34	22	13	10	5
Matched Untreated Patients	82	61	53	47	39	31	29	27	25	24	20

Figure 22. ISE: Kaplan-Meier curve with 95% confidence limits for time to death from treatment start using sequential emulation and 1:1 matching in participants with age of TK2d symptom onset ≤12 years



Number of Patients at risk

Treated Patients	56	41	30	26	25	23	18	15	10	8	3
Matched Untreated Patients	56	38	31	29	24	18	17	16	14	14	12

Figure 23. ISE: Kaplan-Meier curve with 95% confidence limits for time to death from treatment start using sequential emulation and 1:1 matching in participants with age of TK2d symptom onset ≤2 years

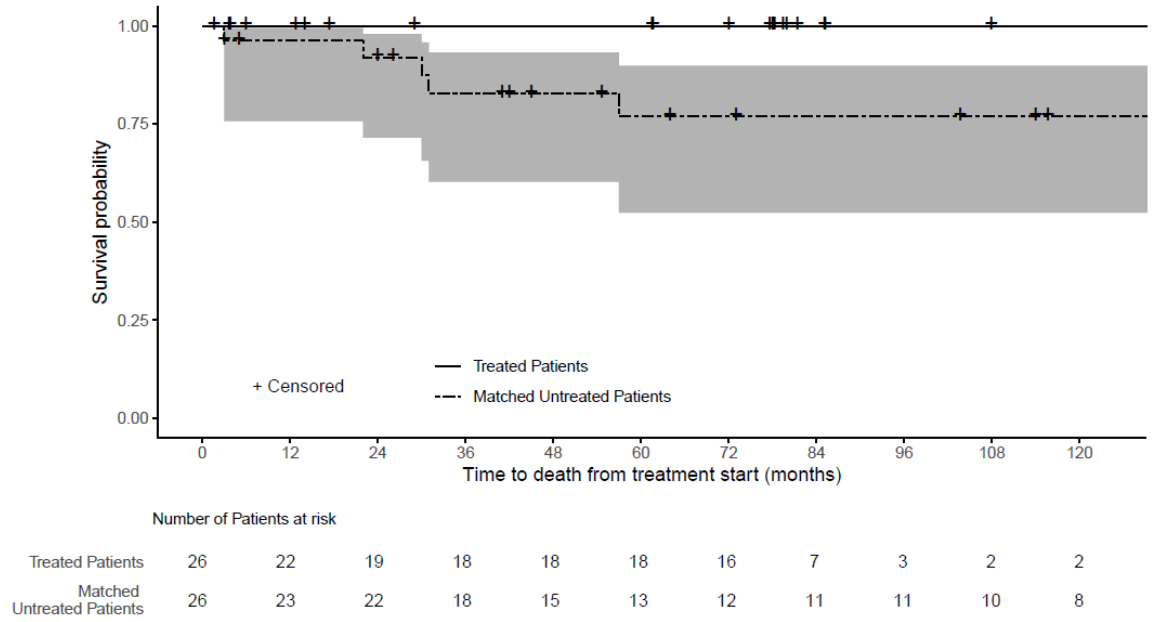


Figure 24. ISE: Kaplan-Meier curve with 95% confidence limits for time to death from treatment start using sequential emulation and 1:1 matching in participants with age of TK2d symptom onset >2 and ≤ 12 years

Using the Cox PH model with age of TK2d symptom onset as continuous variable, the HR is 0.2197 (0.0613, 0.5925) among patients with age of TK2d symptom onset ≤12 years, HR is 0.2895 (0.0783, 0.8232) among patients with age of TK2d symptom onset ≤2 years, and HR is 0.2427 (<0.0001, 2.9510) among patients with age of TK2d symptom onset >2 and ≤12 years (Table 22).

Table 22. Sensitivity 2: Cox Proportional Hazard model – Time from treatment start to death using sequential emulation with 1:1 matching (ISE; treated participants [ISE] and untreated participants [ISE-MUPD])

Matching	N matched pairs	Number (%) of participants who died		N informative pairs	Age of TK2d symptom onset cont. covariate[a]	
		Treated	Untreated		HR(CI)	LRT p-value
Age of TK2d symptom onset ≤12 years						
Sequential emulation 1:1 matching	82	3 (3.7)	30 (36.6)	20	0.2197 (0.0613, 0.5925)	0.0008
Age at TK2d symptom onset ≤2 years						
Sequential emulation 1:1 matching	56	3 (5.4)	22 (39.3)	16	0.2895 (0.0783, 0.8232)	0.0089
Age at TK2d symptom onset >2 to ≤12 years						
Sequential emulation 1:1 matching	26	0	8 (30.8)	4	0.2427 (< 0.0001, 2.9510)	0.6332

Cont.=continuous; ISE=Integrated Summary of Efficacy; LRT=likelihood-ratio test; MUPD=Modified Untreated Patient Database; TK2d=thymidine kinase 2 deficiency
a An untreated patient may be counted several times if this patient was matched to different treated patients.
b A matched pair is informative if at least 1 participant experiences a death and the participant who died must have the shorter observed time to event between the 2 participants. Only informative pairs contribute to the partial likelihood estimator of the hazard ratio.
c Cox Proportional Hazard Model with Firth Correction with age of TK2d symptom onset as continuous variable.

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The development program of doxecitine and doxribtimine consists of several phase 2 studies, including only patients with TK2d. Study MT-1621-101 was a retrospective chart study of patients with TK2d treated with non-commercial pyrimidine nucleos(t)ides, that was followed-up by the ongoing, prospective study MT-1621-102 in which all patients were treated with doxecitine and doxribtimine, at doses aligned with the proposed posology. Data from studies 101 and 102 were (pooled and) compared with data from an external control group derived from the (Modified) Untreated Patient Database (M)UPD.

Additional studies were performed to obtain data on treated (and untreated) patients outside of company-supported studies (study MT-1621-107) and in extended access programs (study TK0114), as well as more data from affected family members from patients included in the clinical studies (study TK0110) and untreated patients to be additionally included in the MUPD (TK0112).

Main studies

TK2d is an ultra-rare disease and this was reflected in the atypical development program. Studies 101 and 102 were considered the main clinical studies supporting this application, with the MUPD as external control group for comparison of survival. Study MT-1621-107, a non-interventional chart review to collect survival data of treated and untreated patients with TK2d outside company-support studies, and study 114, a non-interventional study including the most severely affected patients with TK2d in company supported compassionate use programs, were considered supportive.

Study 101 is a retrospective chart review describing the safety, tolerability, and efficacy of treatment of TK2d patients with non-commercial formulations of doxecitine and doxribtimine (non-GMP dC/dT or non-GMP dCMP/dTMP). Study 102 prospectively studied the patients also included in study 101, as well as other patients with TK2d who were treated with pyrimidine nucleos(t)ides; those on non-GMP dC/dT or non-GMP dCMP/dTMP were switched to doxecitine and doxribtimine at start of the study with doses corresponding to the proposed posology. As such, study 102 is a single arm trial (SAT). As discussed in the *Reflection paper on establishing efficacy based on single-arm trials submitted as pivotal evidence in a marketing authorisation* (EMA/CHMP/564424/2021), evidence from SATs may in some occasions be acceptable for an MA, when several requirements with regard to the inclusion of the target and patient population, the selection of appropriate endpoints, statistical analyses, and the role of external (control) data are adequately dealt with. These issues are addressed below in the corresponding sections.

The average treatment duration in study 102 is 52 months (range 23 – 55), and the study is still ongoing. Because study 102 is mainly a follow-up of patients included in study 101, baseline assessments of study 102 were performed while on treatment and this makes that interpretation of this study is most informative when combined with the pretreatment data of study 101. However, these pretreatment data were retrospectively gathered and inherently prone to unstandardised assessments and missing values. It was addressed in the Scientific Advices that a small RCT would be the ideal approach, but as the worldwide patient pool is very small, the current development program

was considered potentially adequate to generate data in support of a Marketing Authorisation under Exceptional Circumstances.

To illustrate the natural clinical course of the disease especially with regard to survival, the (M)UPD was composed of cases identified in literature completed with untreated patient data from the chart review studies in this application. External data are a crucial design element in SATs and in exceptional cases it may be used for direct comparison for efficacy assessment (EMA/CHMP/564424/2021); please see discussion below on the adequacy of the external data used in the current application.

Altogether, studies 101 and 102 comprise the principal sources of data supporting this application for survival and non-survival endpoints. The (M)UPD provided data on survival in untreated patients; non-survival data was mainly absent (motor function) or largely missing (ventilatory support (25%) and feeding support (72%)).

Study participants

The in- and exclusion criteria of the main studies were basically in line with the target population as defined in the indication, i.e. '*…for the treatment of paediatric and adult patients with genetically confirmed thymidine kinase 2 deficiency (TK2d) with an age of symptom onset on or before 12 years*'. An age restriction was not included in the eligibility criteria. This can be understood given the rarity of the disease and the search for as much data of as many patients as possible; data from patients with a TK2d symptom onset ≥ 12 years were considered supportive evidence and were not intended for extrapolation purposes to this subpopulation, which is endorsed, especially when considering the total body of evidence in this application.

Patients in study 101 (and herewith a large sample of study 102) were eligible for inclusion if they had a confirmed TK2 gene mutation, availability of medical records from time of onset of TK2d symptoms, use of pyrimidine nucleos(t)ides, and most recent visit between 01 June and 15 December 2018. With the last requirement (most recent visit between 1 June and 15 December in 2018), only patients who were alive at time of inclusion were eligible; those who died or did not have visits in this period were not eligible. Herewith, selection bias (more specifically: immortal time bias) was introduced. Additional analyses using restricted populations, expanded populations, as well as specific matching approaches were adopted to address this issue (see statistical analysis section below).

Study treatment

Doxecitine and doxribtimine is an oral formulation that '*… should be administered every day in 3 equal doses with food. Kygevvi is titrated and dosed based on individual patient tolerability, up to a maximum recommended maintenance dose of 800 mg/kg/day*' according to the proposed posology.

Patients in study 101 were treated with non-GMP dC/dT or non-GMP dCMP/dTMP (all pyrimidine nucleos(t)ides); the non-commercial formulations of the proposed product doxecitine and doxribtimine. The small differences between the non-commercial and the commercial formulations were not expected to affect clinical results (see Quality AR). On this aspect, all data from patients using pyrimidine nucleos(t)ides can be extrapolated to the proposed commercial product doxecitine and doxribtimine, as used in study 102.

No dedicated dose finding nor dose response studies were performed. The proposed posology was based on pre-clinical data and early data in healthy volunteers and patients with TK2d suggesting a starting dose of 260 mg/kg/day to increase plasma concentration over endogenous concentrations, and a maximum dose of 800 mg/kg/day limited by intolerability. No dose-response relation was observed. Data supporting the proposed posology is thus limited. According to the ICH E4 guidance (CPMP/ICH/378/95), '*dose-response data are desirable for almost all new chemical entities entering the market'* and '*…informative dose-response data, like information on responses in special*

populations, on long-term use, on potential drug-drug and drug-disease interactions, is expected, but might, in the face of a major therapeutic benefit or urgent need, or very low levels of observed toxicity, become a deferred requirement.'

In both studies 101 and 102 the majority of patients was treated with the maintenance dose of 800 mg/kg/day as aligned with the proposed posology in section 4.2 of the SmPC. This maximal maintenance dose is stratified by body weight. The proposed weight bands vary from 0.1 kg for children of 3.0 kilograms steadily increasing up to weight bands of 1.5 kg for patients of 83.5 kg. This means that for the youngest children, the dose needs to be adjusted very frequently as weight gain of just a hundred grams can be reached within weeks.

The proposed indication refers to age categories at time of TK2d symptom onset, but no lower or upper age limits for patients at the time of treatment were included in the SmPC. Due to the fact that severe disease symptoms may start early in life, postponing treatment is considered inadequate. Moreover, there are no safety data that would suggest necessity of an upper age restriction.

As there is currently no treatment for TK2d, there is no recommendation for a specific line of treatment.

Outcome measures

There are no specific guidelines or literature on endpoint / measurement tools or sets to be used in studies on TK2d. The endpoints chosen in the main studies were based on the CHMP Scientific Advices and literature.

The **primary endpoint** in studies 101 and 102 was survival; main secondary endpoints were in the domains of motor function (including motor milestones), and ventilatory and respiratory support. As a primary endpoint, survival theoretically complies with the requirements of 1) being clinically relevant and convincing evidence (*ICH 9 guideline*), and 2) isolating treatment effects, i.e. observations of the desired outcome would occur only to a negligible extent in the absence of active treatment (*Reflection paper on establishing efficacy based on single-arm trials submitted as pivotal evidence in a marketing authorization*; EMA/CHMP/564424/2021). Furthermore, survival has been accepted as (primary) endpoint in marketing authorisations for other diseases with childhood manifestation and high mortality rates, including Spinal Muscle Atrophy. As such, survival as primary endpoint in the main studies is acceptable.

Secondary endpoints were defined in line with the CHMP Scientific Advices as well, in three (non-survival) domains, i.e. motor function (including motor milestones), ventilatory support, and feeding support. Motor milestones is considered a relevant endpoint in this application because of the within-subject comparison of pre- and post-treatment initiation, motor milestones are widely recognized, and there are international references available from the WHO. Further, motor milestones represent a range of motor functions relevant across development, are commonly tracked in medical charts, and have been used widely in international growth studies. In patients with a progressive disease as TK2d, motor milestone development is impaired, characterised by failure to acquire motor milestones, as well as loss of initially acquired milestones. Regain of an initially lost milestone is anticipated to be very rare. Thus, when a motor milestone is lost and subsequently found to be regained after treatment initiation, this might support treatment effect on disease course. For firm conclusions on beneficial treatment effect, however, it should be demonstrated that regain of motor milestones does not or rarely occur in patients with TK2d prior to treatment (again, see *Reflection paper on establishing efficacy based on single-arm trials submitted as pivotal evidence in a marketing authorization*; EMA/CHMP/564424/2021).

The selection of endpoints for the domain motor function (the 6MWT, the Hammersmith Functional Motor Scale Expanded (HFMSE), the North Star Ambulatory Assessment (NSAA), the Egen

Klassifikation (EK), the Revised Upper Limb Module (RULM), and the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND), were in line with the CHMP Scientific Advices. It was accepted that these endpoints were not validated for the TK2d population, because of the rarity and only recent discovery of the disease. It is inherent to the design of the main studies that physicians were not trained, which makes the assessment of the motor function endpoints prone to bias and results should be interpreted with care. Interference of TK2d-related encephalopathy (present in up to 30% of the (younger) patients with TK2d) on motor function endpoints was considered small: Encephalopathy was not explicitly assessed in the main studies, but exploration of the summary of medical history with SMQ non-infectious encephalopathy / delirium and the eCRF for patients included in study 101, yielded only three events which potentially affected two assessments. Altogether, the data on motor function endpoints are informative at most.

The remaining two non-survival domains were *ventilatory* and *feeding support*. Both are considered the resultant of muscle weakness, and impairment at these domains impacts the quality of life and life expectancy; their relevance is acknowledged. There were no guidelines on ventilatory and feeding support specifically for patients with TK2d in the main studies; decisions on this aspect were up to the discretion of the treating physician and local practice. The impact on efficacy endpoints will remain uncertain (not pursued).

Additional endpoints were biomarkers, muscle biopsy findings, including percentages mtDNA. There are no formally validated biomarkers for TK2d. The selected biomarkers in study 102 were venous lactate, creatine kinase, FGF-21, and GDF-15. Especially for GDF-15, there is cumulating evidence on its relevance in mitochondrial diseases, and TK2d in particular.

Sample size, randomization and blinding

Sample size calculations were not performed, which is justified. Inherent to the design of the main studies, randomisation and blinding were not applicable.

Statistical analyses

The SAP and its amendments for study 101, and to a lesser extent study 102 and the comparison against MUPD, were all finalized when data was already present and potentially known. Because of the retrospective nature of part of the studies, this is understandable, but analysis plans will be assessed as such.

Post hoc, an estimand was defined for studies 101 and 102 and for the comparison with MUPD. Treatment discontinuation was identified as intercurrent event and handled with a treatment policy strategy, which is acceptable.

The study population was defined as all enrolled patients. The definition of the analysis population is acceptable, however, as discussed above, the enrollment criteria introduced immortal time bias and this study population will likely provide more favorable results than the average patient population.

Initial survival was analysed using matched data between studies 101 + 102 and the MUPD. Prior to matching, untreated patients who died or were censored before the youngest age at which a treated patient began treatment were excluded, as a mean to reduce survival bias. This can be understood. Alternative analyses were performed to address the potential sources of bias in the comparison between treated (studies 101+102) and untreated patients (MUPD). Given the fact that analysis plans were defined while (part of) the data was already known, this could lead to data-driven choices, therefore, using a range of possible methods is appreciated. Using different onset times, as well as restricting the control patients to those alive at the same age as their matched patient started treatment, address the influence of selection bias, in particular some of the immortal time bias, and lead time bias.

Additional survival analyses were performed to explore the impact of immortal time bias by 1) ***restricting*** both the treated population (study 101 + 102) and the untreated population to those alive in the second half of 2018, and 2) ***expanding*** the treated patient population to the overall treated population (studies 101 + 102 + 107 + 114) compared to the untreated population. Patients were additionally matched by age at TK2d symptom onset, time from treatment start, and birth year. Further, sensitivity analyses were performed using the **100th percentile matching** and **sequential emulation**.

The matching process was based on age at onset of TK2d symptom, categorized as ≤ 2.0 years of age, >2.0 to ≤12.0 years of age, and >12.0 years of age. Within each age group, treated patients were sorted in descending order according to their age of treatment initiation and subsequent last known alive age. From this ordered database, matches were created at random as well as from the upper 50th, 75th, and 100th percentile. This approach can be accepted.

Survival was analysed using descriptive statistics and planned to be analysed using Kaplan-Meier (KM) estimates. The KM-estimates were not presented for study 101 itself but, given the fact that no patient died and KM-estimates were provided for the comparison with the untreated patient data sets, this is acceptable.

Time to death was calculated from symptom onset as well as from treatment initiation. The latter handles part of the immortal time bias, as it excludes the time until treatment onset and potential deaths when compared to similar historical patients.

After matching, survival as from the age at TK2d symptom onset and the age at treatment initiation was compared using Cox proportional hazard regression analyses for each of the matched pairs separately (random, 50th, 75th, or 100th percentile approach) and Kaplan-Meier curves. Both unadjusted models and models including age at onset, sex, birth year, or geographic region were performed. These methods are considered standard methods for the type of data and are acceptable. However, because of the low number of patients and because of the separation observed in the data, Cox regression using Firth correction is preferred, as standard Cox regression may not converge and provide reliable estimates. Exact conditional regression modelling and restricted mean survival time analysis was also planned, which is agreed given the potential problems of Cox regression in case of low numbers. Further sensitivity analyses were performed using sequential emulation and Cox regression with treatment as time dependent variable. In the sequential emulation, following principles of target trial emulation, control patients were emulated per month of age and treated patients were randomly matched to a control patient that was eligible at the same month of age. Together these analysis methods can be accepted for giving an impression of the treatment effect across several assumptions.

Other endpoints were summarised descriptively, which is acceptable as well, given the uncontrolled design and low number of patients in the study due to the rarity of the disease. No adjustment for multiplicity was performed but given the fact that all analyses were planned after (part of) the data collection and the analyses encompass the range of potential analyses for this data, this is not a concern. It does mean results will be assessed with this multiplicity in mind.

For NSAA, the item "lifts head" was removed, because in literature, Rasch analyses indicated a misfit for this item (*Mayhew et al., 2011*). As NSAA data in TK2d from other studies are not available and comparison of data is thus not aimed for / possible, exclusion of the item from the NSAA was accepted.

A set of imputation rules were defined to fill in unknown or partial missing dates. For example, if only the year was known, the day and month were imputed as 30 June. This implies a large margin / lack of accuracy, which is relevant especially in young children when motor milestones develop rapidly. For the primary endpoint survival, no imputation was required, and thus outcome is not affected. For

motor milestones, the percentage of missing records (about 10%) was not negligible but acceptable, especially given the applied imputation rules which are reasonable and supported by literature on motor development in healthy children. For 'date of TK2d onset', the date for day and month has been imputed in 6 patients (21%); only 1 patient might have been misclassified to the target population (≤ 12 years of age). It is unlikely that this has affected outcome in terms of survival, as was illustrated by sensitivity analyses.

If a patient had a loss of motor milestone record, it was assumed that the patient must have initially reached this milestone and subsequently lost it. The same reasoning was used for the regain of motor milestones. Whether this is reasonable depends on how accurate and consequent the treating physicians entered the data in the patients' chart. Because this issue addresses both loss and regain (i.e. negative and positive effect of treatment) of milestones, and because the issue is inherent to retrospective data gathering, it is not further pursued.

Conduct of the studies

There were several protocol amendments among which two (study 102) were considered potentially affecting study results. Protocol Amendment 3.0 included medication administration with food and dose adjustment based on changing body weight. Only a few pharmacokinetic samples were collected after the protocol amendment and therefore the impact of this amendment on efficacy and safety cannot be estimated based on the pharmacokinetics (and the lack of a quantified exposure-response relationship).

Efficacy data and additional analyses

Study population

The majority of patients (81%) enclosed in studies 101 and 102 had an age at onset of TK2d symptoms ≤ 12 years and the analysis is focused on this subpopulation. This is aligned with CHMP Scientific Advices; these patients generally have the most severe disease presentation and an eventual treatment effect is most likely to be demonstrated in this subgroup of patients; these patients reflect the claimed indication.

Gender was rather balanced with a slight dominance of males. Over 95% appeared of white race and 64% was not hispanic or latino; based on PK, data extrapolation of data to non-white / hispanic and latino patients seems justified. Data on height and weight / BMI indicated a tendency for failure to thrive, reflected in a substantial percentage of patients with Z-scores for height and BMI ≤ 0.

Baseline disease characteristics confirmed the severity of the disease, with about 50% of the patients being non-ambulatory, 40% requiring ventilatory support, and 20% of them requiring feeding support. Being non-ambulatory coincided with the need for ventilatory support and / or feeding support.

Baseline demographics of the untreated patient population (MUPD) with an age at onset of TK2d symptoms ≤ 12 years were rather comparable to the treated patients in the main studies, although demographics were not available for a substantial number of untreated patients. Disease characteristics also were missing in a large number of untreated patients, and therefore not readily interpretable. The available data suggest that the treated patients were generally born later than the untreated patients (median birth year 2009 versus 1999). This may be the resultant of the selection bias/immortal time bias introduced by only including patients in study 101 being alive just prior to inclusion in the study. See section on statistical analysis above for additional statistical measures to control for this bias.

Patient flow of treated patients at study level was clearly presented. The applicant made reasonably clear that the capture rate of treated patients with TK2d symptom onset ≤12 years was close to complete within participating centres, which reduces the impact of selection bias from this source. The

search and selection process of (published) untreated cases was well described, which suggests complete capture of untreated patients in literature as well. Complete capture, however, does not protect against publication bias and can, as such, not be excluded.

The sample of treated patients with TK2d symptom onset > 12 years was small (19%). Compared to the younger age at onset group, patients with an age at onset > 12 years were all ambulatory (100%) but required ventilatory support more often (about 80%). This suggests that clinical manifestations of TK2d in this group indeed may be different, although this may well be biased by selection (i.e the most severely affected / deceased patients not included). This issue is not further pursued as this group of patients is not included in the claimed indication.

Dose

See section on study treatment above.

Adherence and drug exposure

Overall, drug continuation rates were high (94%). Patient-years of exposure (PYE) for study 102 (thus including all except 3 patients from study 101 as well) were slightly more than 4 years for the target population, which comparable equal for both age at onset of TK2d symptom categories (≤ 2 years, and > 2 to ≤ 12 years).

Adherence and drug exposure data from study 102 are considered more reliable compared to study 101, because these were prospectively follow-up. Overall, time on treatment was 52 months, without differences among the age at onset of TK2d symptoms groups, which is considered sufficiently long to study the course of efficacy over time. Median starting and last doses in study 102 were comparable (800 mg/kg/day) and aligned with the maximum maintenance dose of the proposed posology in the indicated population. As such, these data are considered informative for the current application.

Treatment compliance (defined as use of study drug > 80%) was suboptimal (74% in study participants with an age of TK2d symptom onset ≤ 12 years), and lowest (56%) in the patients with an age at onset of TK2d symptoms > 2 to ≤ 12 years mostly due to adverse events (depression and diarrhea in two cases each, one case with femur fracture). Treatment was restarted in each case and the time interval of treatment interruption was relatively short with a maximum duration of three months. The low compliance rate in this subgroup is related to the small sample with a few patients having large impact; it is unlikely that the subgroup of patients with an age at onset of TK2d symptoms > 2 to ≤ 12 years is associated with treatment non-compliance in essence; this has no implications for the claimed indication.

The adherence data of retrospective study 101 were less detailed, as can be expected from the design. Compliance data were not available. Slightly less than 80% of patients with an age at onset of TK2d symptoms ≤ 12 years was treated according to the claimed posology. This difference with the claimed posology can be understood because in this study patients also at the beginning of the drug developmental process were included in which physicians may have been less experienced with the drug and more prone to lower the dose. The issue is not further addressed.

Concomitant medication

In studies 101 and 102, 77% and 92% of the patients (respectively) used concomitant medication, especially related to the symptomatology of TK2d, such as antibacterials for preventing (airway) infections, vitamins / supplements, and salbutamol. Higher rates of concomitant medication observed in study 102 compared to study 101 are presumably the resultant from more accurate monitoring in study 102 (being prospective in nature). There was no clear trend in concomitant medication use. Data on concomitant medication in the untreated population is not available. Impact on efficacy and safety outcomes cannot be established.

Treatment effects

Treatment effects will subsequently be discussed for treated patient (pooled data), and then compared with data from untreated patients when available (unless specified otherwise).

Survival analyses suggested a substantial survival benefit for those treated versus those untreated. In the ***restricted*** dataset for treated (study 101 + 102) and untreated patients (MUPD) with an age of TK2d symptom onset ≤12 years, i.e. both datasets aligned in terms of in- and exclusion criteria, one patient died in the treated population and three in the untreated population. Hazard Ratios (HRs) for mortality using various analyses and matching methods, were small (≤ 0.33), and generally statistically significant. A comparable pattern was seen when comparing survival curves of all treated and untreated patients (***expanded*** dataset); again, HRs were small (≤ 0.141). However, the onset of the separation of the survival curves raised concerns given the steep survival decline in the untreated patients. When the survival analyses were performed for the subgroups with age at TK2d symptom onset ≤2 years and >2 and ≤12 years separately, the steep decline in the Kaplan-Meier curve for matched untreated patients was visible for the subgroup ≤2 years but not in the subgroup >2 and ≤12 years. Additional analyses using the most conservative matching strategy (***100th percentile matching***) with an additional censoring step (untreated patients who died were censored at the same time the treated patient was censored), although to a lesser extent, still showed a steep drop in the first part of the curve, especially for the subgroup ≤2 years; HRs were ≤ 0.1767. The ***sequential emulation*** following principles of target trial emulation, resulted in Kaplan-Meier curves showing sustained separation but more stable hazards over time, i.e. without the steep drop observed in previous analyses (HRs ≤ 0.2895). Irrespective of the analyses, the HRs for the two age subgroups were comparable to the overall HRs.

Altogether, the results of all the survival analyses are consistent in suggesting a treatment benefit. However, although selection and time-related bias are partly addressed, selection bias cannot be considered to be sufficiently eliminated or even understood given the fundamental differences in the datasets compared, especially when considering that the untreated patients from the literature are likely to present worse mortality outcomes compared to untreated patients in centres that were not involved with the sponsored studies from retrospective chart review. It is concluded that bias will remain a problem in the comparison of survival.

The main secondary endpoint **motor milestones** is considered valuable as it is less affected by bias: Comparisons are made within-subject. Pretreatment data of studies 101+102 in patients with an age at TK2d symptom onset ≤ 12 years showed 1 regained motor milestone in 1 patient. In another cohort, untreated patients from study 107 (n = 26), also 1 regained motor milestone in 1 patient was observed. Despite the limited study sizes, these data suggest that spontaneous regain, thus without treatment, is a rare observation in the natural course of TK2d. This is relevant, as in single arm trials it is required that the desired outcome should be known to occur only to a negligible extent in the absence of effective treatment (*Reflection paper on establishing efficacy based on single-arm trials submitted as pivotal evidence in a marketing authorisation application EMA/CHMP/458061/2024*). The contrast is large when compared to the post-treatment phase (studies 101+102), in which 84% of the patients regained a milestone. Notably, regains were documented even when treatment was initiated decades after symptom onset. A large contrast was also observed when comparing losses of previously acquired milestones pre- and post-treatment (i.e. 82% versus 26%). Notably, regains were documented even when treatment was initiated decades after symptom onset. Altogether, the data on motor milestones are considered in support of the efficacy of doxecitine and doxribtimine in the treatment TK2d.

Motor function test results over time were only available in small patient samples which results in high levels of uncertainty for the specific scales. High-over, a trend for improvement is seen for the

6MWT, CHOP INTEND, NSAA, and HFMSE. It remains unclear how these tests change over time in untreated patients but a deterioration is expected based on the natural history of the disease. Considering the small number of observation and the lack of validation of these endpoints in the TK2d population, these data provide at best, if any, weak evidence for stabilisation of motor function in patients with TK2d. Data on the other two domains, **ventilatory and feeding support**, were not convincingly in support of stabilization or improvement due to treatment: Some patients improved, some deteriorated, but the numbers were small.

Data on **growth** (weight, height and BMI) for patients starting treatment before 18 years of age, tended to increase over time during treatment, which might be in support of continuing growth.

Biomarkers for TK2d are not formally defined. Venous lactic acid and CK showed a tendency for decreasing values over time during treatment and this might suggest improvement in mitochondrial function. Data for FGF-21 and GDF-15 showed low values during treatment, but there are no pretreatment data. It is known from literature that GDF-15 values in untreated patients with TK2d are 15-30-fold increased compared to healthy volunteers and that GDF-15 values are related to TK2d disease severity and treatment effect. The observation that the treated patients with TK2d (study 102) consistently show close to normal values may be in support of treatment effect of doxecitine and doxribtimine.

No human muscle histology data after treatment initiation was available to demonstrate the hypothesised increase in muscular mtDNA; this leaves the MOA unsupported with human data. Although this is unfortunate, the issue is not further pursued as data were not gathered. The remaining uncertainty is mitigated by a treatment effect in patients with TK2d.

Onset of effect and long-term treatment

There are no explicit data on the onset of effects. Long-term treatment data were available from study 102; median time on treatment was 52 months which is considered sufficiently long to study the course of efficacy over time. For survival, potential treatment effect was reflected in divergence of curves, which occurred early after treatment initiation, i.e. at about 6 months. The rapid onset of effect was mainly observed in the youngest age group. This finding aligns with the knowledge that the youngest patients represent the most severe phenotype. On the other hand, rapid divergence of survival curves may also reflect selection bias which hampers interpretation. The course of motor milestones over time suggests that regains may appear just shortly after as well as years after treatment initiation.

Treatment withdrawal

No data on treatment withdrawal is available, but treatment is anticipated to be required lifelong. The absence of data on withdrawal is therefore not further pursued.

Subgroups

Main subgroups were age at onset of TK2d symptoms; the data for these subgroups were included throughout the discussion above.

Pooled analyses

Data from studies 101 + 102 (and studies 101, 102, 107, and 114 for survival data only) were presented combined throughout the discussion above.

Additional expert consultations

In view on the uncertainties regarding the robustness of the comparison of survival data between the ISE treated population with the (ISE-)MUPD detailed in previous sections of this AR, the

methodological working party (MWP) was requested by CHMP to elaborate comprehensively on whether they consider that **selection bias and time-related bias, including immortal time bias,** has sufficiently been eliminated in the comparative survival analyses of treated versus untreated TK2d patients with symptom onset ≤12 years of age, given the shape of the Kaplan-Meier curve of the control group. The MWP provided the response below.

MWP response to CHMP request regarding MAA Kygevvi (EMEA/H/C/005119), October 2025

We do not consider that selection bias and immortal time bias have been sufficiently eliminated in the comparative survival analyses of treated *versus* untreated TK2d patients with symptom onset ≤12 years of age. Therefore, the survival analyses estimates (hazard ratio and corresponding confidence intervals) cannot be considered reliable.

Quantitative evaluation of both selection bias and immortal time bias is difficult. However, detailed critical analysis concerning the handling of selection bias and immortal time bias is presented below.

Handling of selection bias:

- Selection of study participants that were alive as of 01 Jun 2018 may have introduced selection bias. This was acknowledged and the overall treated population included additional unique patients for which data was collected regardless of vital status. Therefore, this source of selection bias has been mitigated.
- However, other potential sources of selection bias are likely to operate. Especially the untreated group consists of different cohorts, one from literature, with an almost immediate drop in survival, which seems difficult to explain from a biological rationale perspective. Selection processes applied to the control cohort, that might have introduced selection bias could be: the exclusion of patients who participated in the interventional trials (MT-1621-101, 102) and the sampling of controls without replacement in the matching process. In the latter case, first matching controls to treated patients who initiated treatment later in their TK2d disease course, and not (re)using these controls for earlier treatment initiators whom they might have also matched would deplete the controls matched to earlier treatment initiators of longer-term survivors. Both are examples in which there is conditioning (i.e., selection) based on "future events".

Given the fundamental differences between the datasets compared, selection bias cannot be considered to be sufficiently eliminated or even understood. This does also play a role in the accuracy of the survival curve estimates. This could especially be aggravated when considering untreated patients from the literature who presented worse mortality outcomes compared to untreated patients in centres that were not involved with the sponsored studies from retrospective chart review from the applicant.

Handling of immortal time bias:

Two approaches were proposed by the applicant to handle immortal time bias (2), as follows:

- The first approach, matching treated and untreated patients on age at TK2d symptom onset, would mainly control for confounding, not for immortal time bias. Methods used to deal with confounding would not correct time-related biases.
- The second approach, alignment of start of follow-up, only partially handled immortal time bias. Follow-up starts at age at treatment start in the treated and at same age in the matched untreated control. Start of follow-up at time of treatment start and time of symptom onset in the treated should ideally be aligned with the start of follow-up and the time of symptom onset in the untreated matched pair. This would be achieved by e.g. prescription time distribution matching (3): a control group is matched to an exposed group so that both groups have a similar starting point of follow-up in relation to the time point of being at risk, i.e. the time point of symptom onset. Selection at random (with

replacement) from a list of age matched individuals would be the preferred approach to reduce immortal time bias.

The sensitivity analysis that is based on matching on both age at TK2d symptom onset and year of birth category (<2000, ≥2000) could be seen as a form of prescription time matching and has contributed to mitigate partly this source of bias, but the year of birth category is imprecise. Another potential problem might be that the time of symptom onset is difficult to assess, and it is not clear how this was done for the different patient cohorts included in the analysis. An alternative analysis that classifies treatment time-varying (e.g. a time-dependent Cox model), with starting follow-up at time of symptom onset might have been more appropriate to prevent immortal time-bias but will not mitigate the problem of selection bias.

When looking at the Kaplan Meier curves, there is an early steep survival decline in the control group which suggests that the data generating mechanism is not fully understood and, therefore, the proposed methods do not prevent this either. Furthermore, the fact that the two analyses lead to roughly similar Kaplan-Meier curves, cannot be explained.

Conclusion

In summary, we conclude that selection bias is a fundamental problem in the comparative survival analyses of treated versus untreated TK2d patients with symptom onset ≤12 years of age that cannot be sufficiently mitigated whichever way the data is analysed to achieve reliable estimates of hazard ratio and corresponding confidence intervals.

A SAG (Scientific advisory group) Neurology (SAG-N) was also convened on the 2nd of December 2025 to respond to several questions raised by CHMP during the assessment.

FINAL SAG-N ANSWERS (dd 11-12-2025)

1. **Concerning the natural course of the disease:**
   a. **Do you consider the survival curves of the untreated patient population, representative of the variability in the natural course of TK2d?**

The SAG-N experts considered by consensus that the pattern in the survival curves - including the steep decline in survival observed at the beginning of the follow up - can be considered roughly representative of the variability in the natural course of TK2d. Variability could be explained by factors such as the severity of depletion of mitochondrial DNA (i.e. early onset patients have severe depletion whereas later onset patients have less severe depletion) and the occurrence of intercurrent illnesses.

   b. **Do you consider the motor milestone development observed during the pre-treatment period of patients in study 101 representative of the variability of natural course of TK2d?**

The vast majority of the SAG-N experts considered that the pattern of motor milestone development observed during the pre-treatment period of patients in study 101 may be representative of the variability of natural course of TK2d. At the same time, the majority of experts agreed that there is uncertainty and that it is not possible to attain a firm conclusion based on the data provided. The uncertainty comes from the fact that the time at which the motor milestone is gained and lost is not reported and from the potential impact of selection bias. To illustrate this second source of uncertainty, it was discussed that 86% of patients in the study with an age of onset below 12 years reached the milestone 'able to walk unassisted', which appears not to be aligned with the known motor development evolution and could invoke a selection bias towards a less impaired enrolled population, the population who reached an age to receive the treatment.

c. It appears that there are significant differences in the natural course of the Tk2D. What are the main predictors of prognosis in this disease?

The SAG-N experts agreed by consensus that the main predictor of prognosis is the age at onset (i.e. the lower the age, the worse the severity). Another relevant predictor is the velocity of the progression (i.e. the higher the velocity, the worse the severity). It was also noted that some literature data indicate that certain mutations could be associated with a less severe phenotype.

2. Do you consider it plausible that treatment with doxecitine/doxribtimine (dC/dT) results in mtDNA restoration and subsequent benefit on survival and motor milestones to the extent shown?

The SAG-N experts agreed that it is plausible that treatment with doxecitine/doxribtimine (dC/dT) improves mitochondrial function. This is directly supported by results from non-clinical studies and indirectly supported by results in biomarkers of mitochondrial function for which, on the other hand, it was noted that they could have also been obtained if the medicinal product works through a different pathway from mtDNA restoration (e.g. TK1 and dCK). It was discussed that there is little evidence in humans (e.g. no muscle biopsies) and that no clear dose-response was observed.

It was agreed that it is plausible that the effect on improvement of mitochondrial function could subsequently lead to benefit on survival and motor milestones. It is however uncertain to which extent, depending on the effect of bias, which cannot be quantified. For the effect on survival, most SAG members considered selection bias to be a relevant confounder. Some SAG members considered that selection bias, particularly immortal time bias, was potentially of major impact, given the fact that no treated patients in the 101 study died. Most SAG members agreed that the effect of bias on motor milestones is probably less severe than on survival. The retrospective data collection relying on what treating physicians wrote down in the chart following an open label treatment with a new drug, for which there may be high expectations, may have positively biased the motor milestones registration to some extent.

Further, the SAG-N experts discussed the therapeutic lag of the medicinal product and it was mentioned that the effect might be twofold: a fast effect on the mitochondrial respiratory chain that could explain some very rapid effect on motor capacities and a long-lasting effect on muscle restoration.

3. What is your view on the strength of evidence that the beneficial effects observed for motor milestones are due to treatment with dC/dT rather than by natural variation?

It was agreed by consensus that regaining motor milestones is very unusual in the natural history of the condition. Some experts were concerned that there is some uncertainty based on the potential impact of selection bias (potentially less impaired participants enrolled in the studies) and observational bias (potential risk of overestimation for motor milestones assessment due to the open-label nature of the study). Overall, most experts considered that the magnitude of the effect may be smaller as the direction of the bias could have led to an overestimation.

The patient representatives were of the opinion that the pattern of motor milestone development observed during the pre-treatment period of patients in study 101 is representative of the variability of natural course of TK2d and the observed effects on motor milestones and survival are meaningful. They considered it essential to start the treatment as soon as possible after diagnosis.

4. Are you aware of the existence of any registry including patients with TK2d?

a. If so, do you consider this registry suitable for use as a (post-marketing) data source?

The SAG-N members commented that there are efforts undertaken to prepare / compile data from existing patients in different EU countries including Italy, France, Germany, and Spain. There are also registries within the European Reference Networks (ERN), which could include patients with TK2d, including ERN-EURO-NMD (which has a subgroup for mitochondrial diseases and a registry for

neuromuscular disease (NMD) patients), ERN-RND and MetabERN; it was considered of utmost importance that a collaboration between different registries is accomplished.

b. If not, do you consider a prospective registry-based study including patients with genetically confirmed TK2d as from treatment start with dC/dT would be feasible to determine the impact of treatment on overall survival and development of motor milestones, and what patient data would necessarily need to be included in this registry?

The SAG-N considered that a prospective registry-based study including patients with genetically confirmed TK2d as from treatment start with dC/dT is feasible to determine the impact of treatment on overall survival and development of motor milestones. The Applicant's proposal (i.e. collecting the following clinical outcomes: developmental motor milestones, respiratory function, ventilatory support and feeding support) is considered acceptable with some suggestions for improvement: all new diagnosed patients should be included irrespective of treatment, to avoid selection bias; a core outcome set should be created and weight should be added as an outcome variable.

Additional efficacy data needed in the context of a MA under exceptional circumstances

The applicant applied for a Marketing Authorisation under Exceptional Circumstances because of the ultra-rare nature of the disease, its heterogeneity, and the benefit of treatment. An RCT is considered not feasible due to the rarity of the disease. The proposed post-authorisation safety study (PASS; study TK109) is primarily focused on safety; secondary objectives are to study efficacy / clinical outcomes including survival, developmental motor milestones, ventilatory and feeding support, growth, ADL, and quality of life during 8 years. It is recommended that muscle biopsy and / or MRI and biomarker data, obtained in at least a subset of patients, should be gathered as well. Patients originating from study 102 will be included as well as newly treated patients with onset of TK2d symptoms on or before 12 years of age; it is expected that at least 20 newly treated patients per year will be eligible for inclusion.

2.6.7. Conclusions on the clinical efficacy

Treatment with doxecitine and doxribtimine is indicated for patients with genetically confirmed TK2d, with an age at symptom onset at or before 12 years. The unmet medical need for treatment in this population is high, as it is associated with substantial morbidity and mortality, especially in the youngest age at onset group (≤ 2 years of age at symptom onset).

The rarity of this mitochondrial disease resulted in an atypical clinical development program in terms of study designs and patient numbers. The main studies comprised one retrospective chart review of treated patients, and one ongoing follow-up, longitudinal study up to over 50 months with a total of 49 patients with TK2d, of whom 39 were aligned with the claimed indication (age at onset of TK2d symptoms ≤ 12 years). As an RCT was not performed, an untreated patient database was extracted from medical literature and a retrospective chart review to serve as an external control group. The assessment of the primary endpoint, **survival**, was complicated by the comparison of two different data sources and thus patient populations, introducing selection and time-related bias. This was dealt with by performing variety of analyses, including, but not limited to, the use of a restricted population applying the same selection criteria for untreated and treated patients, the use of an expanded population comprising the entire database of treated and untreated patients, additional matching approaches (e.g. 100th percentile matching), and sequential emulation. These analyses all resulted in a substantial survival benefit for the treated population over the untreated population, but the magnitude of the observed benefit as well as the timing of onset of the separation of survival curves,

raise concerns that residual bias remained. This concern was also shared by the consulted SAG and MWP. It is concluded that the differences between the data sources of treated and untreated patients cannot be solved or statistically controlled for, and the bias can thus not be mitigated.

The main secondary endpoint, **motor milestones**, assessed pre- and post-treatment in study 101+102, was supportive for the efficacy of doxecitine and doxribtimine. Regain of motor milestones appeared very rare in the natural course of this progressive disease; the high frequency of regains in the post-treatment period is thus plausibly due to treatment with dC/dT. The substantially reduced frequency of losses post-treatment compared to pretreatment is in full support of this. The relevance of these findings was endorsed by the SAG.

Results on the domains of **ventilation and feeding** at best support an absence of disease progression in a few patients. The value of (unvalidated) **biomarker** data over time remains uncertain. The study designs introduced uncertainty which could only partially be solved. Data are not comprehensive, and this will also not be achieved given the rarity of the disorder.

The main study is still ongoing; patients currently treated will eventually also be followed-up in the PASS (study TK109).

To address the missing efficacy data in the context of a MA under Exceptional Circumstances, the PASS (study TK109) will also (but not limited to) include secondary efficacy endpoints including survival, motor milestones, muscle histology / imaging, and biopsies in at least a subset of patients.

2.6.8. Clinical safety

2.6.8.1. Patient exposure

The pooled analysis includes studies MT-1621-101, TK0102, and MT-1621-107 (where data were collected) that enrolled participants with TK2d, referred to hereafter as the ISS pooled safety population. Retrospective data collection for MT-1621-107 was limited to AEs or SAEs leading to treatment discontinuation, interruption, or reduction in dose. Studies 101 and 107 are complete. TK0102 is ongoing and a data cutoff date of 15 Mar 2024 was used. Retrospective study TK0114 in patients of the expanded access program has very limited safety data and it is not included in the ISS pooled safety population, but it is reported separately.

In the ISS pooled safety population of 67 participants of studies 101, 102, 107, the median (Q1 – Q3) exposure to pyrimidine nucleos(t)ide therapy for participants with TK2d was 5.5 (3.9 - 6.7) years with most of the participants in the categories of ≥5 years duration of exposure (60%) followed by 2 to <5 years (19%). The median (Q1 - Q3) daily dose received by participants was 777 (603 - 794) mg/kg/day. The overall cumulative duration of exposure in the 67 participants was 345 participant-years.

In the population of 39 participants with an age of TK2d symptom onset of ≤12 years, the median (Q1 - Q3) duration of treatment was 72 (57 - 92) months, which is approximately 6 years (Table 23). The majority of participants had at least 2 years of exposure and of the 39 participants with an age of TK2d symptom onset of ≤12 years in studies 101 and 102, all participants had at least 2 years of exposure. The median (Q1 - Q3) dose received in the ≤12 years age of TK2d symptom onset group was 789 (672 - 795) mg/kg/day (sum of each API, see footnote Table 23).

In the total pooled population of 67 participants, 44 (66%) were ≤18 years at first treatment. The median (Q1 - Q3) duration of treatment exposure was similar in participants with an age at first

treatment of ≤18 years and participants who were >18 years. Most participants had treatment for ≥5 years: 59% in participants ≤18 years of age and 61% who were >18 years of age at first treatment. The median (Q1 - Q3) daily dose received by participants in the ≤18 years of age at first treatment category was 793 (715 - 795) mg/kg/day.

Table 23. Drug exposure in participants with age of TK2d symptom onset of ≤12 years, ISS pooled safety population

Category	All Formulations (101, 102) N=39	All Formulations (101, 102, 107) N=50
Duration of exposure (months)		
Median	78.445	71.789
Q1, Q3	67.305, 104.000	57.339, 92.717
Duration of exposure n (%)		
At least 1 dose	39 (100)	50 (100)
2 to <5 years	8 (20.5)	12 (24.0)
⩾5 years	31 (79.5)	32 (64.0)
Average daily dose received (mg/kg/day)		
n	39	50
Median	393.59	394.73
Q1, Q3	335.96, 396.85	335.96, 397.27

API=active pharmaceutical ingredient; ISS=Integrated Summary of Safety; Q1=25th percentile; Q3=75th percentile; TK2d=thymidine kinase 2 deficiency
Note: Doses are presented in this table using original nomenclature. For example, a dose presented as 200mg/kg/day is 200mg/kg/day doxecitine and 200mg/kg/day doxribtimine; within the body of the report, doses are presented in the current nomenclature that is the sum of each API.
N represents the total number of participants in each column. n represents the number of participants contributing to the summary.
A month is defined as 28 days.
Duration of exposure (subject-months) was calculated in one of two ways:
1. For participants with no dose interruptions, duration of treatment was calculated in months as (last dose date - first dose date +1)/(365.25/13).
2. For participants with dose interruptions, duration of treatment was calculated in months as the sum of (end dose date - start dose date) + 1 for each individual dosing period divided by (365.25/13). Overlap between periods of exposure are taken into account when calculating duration.

Participants with missing dose information are excluded.
One participant's data was reported in both the MT-1621-101 and MT-1621-107 studies. Data for this participant are summarized under each study separately but counted once in the All Formulations column. Average daily dose in mg/day could not be derived for MT-1621-107 due to missing weight data.

2.6.8.2. Adverse events

Summary of Adverse Events

In the ISS pooled safety population of 39 participants with TK2d symptom onset ≤12 years of age, most participants (94%) reported at least 1 treatment-emergent AE; and for the majority of patients (71%) these were considered treatment-related. There were no treatment-emergent AEs that led to drug discontinuation and for 5 (16%) participants a dose reduction was applied. Occurrence of Adverse Events of Special Interest was numerically lower as compared to the full sample of N=50 participants (Table 24). Adverse Events of Special Interest occurred frequently: non-infectious diarrhoea in 33 of 39 (85%, 124 events), drug related hepatic disorders in 17 of 39 (43%), abdominal pain (including abdominal pain upper) in 12 of 39 (31%), and vomiting in 13 of 39 (33%) participants (Table 24). Safety topics of interest are discussed further in the next section on ADRs. There were 13 participants (42%) with a treatment-emergent SAE, that was considered related in 4 (13%) participants. No SAEs led to study drug discontinuation or dose reduction.

In the ISS pooled safety population of 50 participants, the occurrence of AEs was overall similar as compared to the occurrence in the target population (Table 24).

As indicated above, limited safety information is available from TK0114. SAEs occurred in 7/43 (16%) patients; 1 patient permanently discontinued medication, there were 2 patients with AEs that were considered to be drug-related, and there was 1 death.

Table 24. Overall summary of Treatment-emergent Adverse Events, ISS pooled safety population (101, 102, 107)

	All Formulations (101, 102) TK2d onset ≤ 12 years of age N=39		**All Formulations (101, 102) N=50**		**All Formulations (101, 102, 107) N=67**	
	n (%)	**Events**	**n (%)**	**Events**	**n (%)**	**Events**
At least 1 TEAE	39 (100%)	968	50 (100)	1140	-	-
TEAE related to study drug	32 (82%)	241	43 (86.0)	284	-	-
TEAE leading to study drug discontinuation	0	0	3 (6.0)	3	9 (13.4)	10
TEAE leading to dose reduction	9 (23%)	23	14 (28.0)	31	16 (23.9)	36
Safety Topics of Interest						
Noninfectious diarrhoea	33 (85%)	124	43 (86.0)	147	-	-
Drug related hepatic disorders	17 (43%)	38	22 (44.0)	56	-	-
Abdominal pain (including abdominal pain upper)	12 (31%)	30	13 (26.0)	32	-	-
Vomiting	13 (33%)	48	14 (28.0)	49	-	-
At least 1 TESAE	13 (41.9)	41	28 (56.0)	107	-	-
TESAE related to study drug	4 (10%)	9	4 (8.0)	9	-	-
TESAE leading to study drug discontinuation	0	0	1 (2.0)	1	4 (6.0)	5
TESAE leading to dose reduction	0	0	0	0	0	0
TESAE leading to death	0	0	1 (2.0)	1	3 (4.5)	3[a]

ISS=Integrated Summary of Safety; TEAE=treatment-emergent adverse event; TESAE=treatment-emergent serious adverse event. N represents the total number of participants in each column. n represents the number of participants contributing to the summary; percentages are based on N.
Participants reporting more than 1 TEAE are counted once in the participants column.
All occurrences of an event are counted in the Events column.
Data collection for MT-1621-107 was limited to fatal TESAEs or TE(S)AEs leading to treatment discontinuation, interruption, or dose reduction.
One participant's data was reported in both the MT-1621-101 and MT-1621-107 studies. Data for this participant are counted once in the All Formulations column.
Related to study drug=events reported as possibly related or related to study drug per the Investigator.

[a] In MT-1621-107 there were 5 deaths, 3 of which occurred **after** treatment discontinuation and hence are not included in this table.

2.6.8.3. Common Adverse Events

For the ISS pooled safety population of 50 participants, most AEs occurred in the SOC of Gastrointestinal disorders (92%), Infections and infestations (86%), Investigations (70%), Respiratory, thoracic and mediastinal disorders (68%), General disorders and administration site conditions (58%); AEs in the SOC of Musculoskeletal and connective tissue disorders occurred in 44%.

The most frequently reported treatment-emergent AEs in the patients with age of TK2d symptom onset ≤12 years (Table 25) were diarrhoea in 33 of 39 participants (85%), pyrexia in 18 of 39 participants (46%), COVID-19 in 17 of 39 participants (44%), upper respiratory tract infection in 16 of 39 participants (41%), and rhinorrhoea in 15 of 39 participants (39%).

Table 25. Summary of TEAEs by SOC and PT in ≥20% study participants with age of TK2d onset ≤12 years of age, ISS pooled safety population

Preferred term	Non-GMP formulations N=31 n (%)	Doxecitine and doxribtimine N=38 n (%)	All Formulations N=39 n (%)
Diarrhoea	20 (64.5)	26 (68.4)	33 (84.6)
Pyrexia	5 (16.1)	17 (44.7)	18 (46.2)
COVID-19	0	17 (44.7)	17 (43.6)
Upper respiratory tract infection	3 (9.7)	13 (34.2)	16 (41.0)
Rhinorrhoea	2 (6.5)	13 (34.2)	15 (38.5)
Vomiting	4 (12.9)	9 (23.7)	13 (33.3)
Cough	3 (9.7)	11 (28.9)	11 (28.2)
Headache	1 (3.2)	10 (26.3)	11 (28.2)
ALT increased	5 (16.1)	7 (18.4)	11 (28.2)
Abdominal pain	4 (12.9)	7 (18.4)	10 (25.6)
Gastroenteritis	1 (3.2)	8 (21.1)	9 (23.1)
AST increased	4 (12.9)	6 (15.8)	9 (23.1)
Respiratory tract infection	2 (6.5)	6 (15.8)	8 (20.5)
Blood CPK increased	8 (25.8)	0	8 (20.5)

ALT=alanine aminotransferase; AST=aspartate aminotransferase; COVID-19=Coronavirus disease 2019; CPK=creatine phosphokinase; GMP=Good Manufacturing Practice; ISS=Integrated Summary of Safety; TEAE=treatment-emergent adverse event; TK2d=thymidine kinase 2 deficiency
Note: N represents the total number of participants in each column; Percentages are based on N; preferred terms are sorted by decreasing frequency within All Formulations.
Note: All Formulations column does not equal the sum of non-GMP nucleos(t)ides and doxecitine and doxribtimine formulations due to participants transitioning from MT-1621-101 to TK0102 studies.

For the ISS pooled safety population of 50 participants, the most frequently reported TEAE overall was diarrhoea in 43 of 50 participants (86%). The next most frequently reported TEAEs were: pyrexia in 20 of 50 (40%) followed by upper respiratory tract infection in 19 of 50 (38%), COVID-19 in 18 of 50 (36%), abdominal pain (upper) in 15 (30%), and rhinorrhoea in 15 of 50 (30%) participants.

Dysphagia and gastro-oesophageal reflux disorder occurred in 8 (16%) and 6 (12%) patients. Gastroenteritis occurred in 9 (18%) of patients. Most infections were of the airways: Covid-19, pneumonia, (upper) respiratory tract infection, nasopharyngitis. The most frequent AEs in the investigations SOC were: ALT increased (28%), AST increased (22%), blood CPK increased (20%).

The majority, 39 of 50 (78%) participants, had more than 5 years of treatment. According to the analysis of frequently occurring AEs by time of occurrence, diarrhoea was reported more frequently during the first 3 months (74%) and then stabilized overall over the intervals analysed (18% between 3-6 months and 24% between 6-12 months, 37% after 12 months). Except for gastroenteritis (18%, all >12 months), there were no other large numerical differences in occurrence over time.

Adverse drug reactions

ADRs identified by the applicant are: diarrhoea, vomiting, ALT increased, AST increased, and abdominal pain/upper abdominal pain (Table 26); these AEs were also considered by the applicant as adverse events of special interest (safety topics of interest).

Table 26. Adverse drug reactions and their occurrence, ISS pooled safety population

Preferred Term	**All Formulations (101, 102)** **N=50** **n (%)**
Diarrhoea	43 (86.0)
Vomiting	14 (28.0)
ALT increased	14 (28.0)
AST increased	11 (22.0)
Abdominal pain (including abdominal pain upper)	13 (26.0)

ALT=alanine aminotransferase; AST=aspartate aminotransferase; ISS=Integrated Summary of Safety; TEAE=treatment-emergent adverse event
Note: N represents the total number of participants in each column; Percentages are based on N.
At each level of summation (any event, system organ class, and preferred term), participants reporting more than 1 TEAE are counted only once.

Diarrhoea

In the ISS pooled safety population of 50 participants, 39 participants were within the proposed indication of TK2d symptom onset of ≤12 years. In this age of TK2d symptom onset category, 33 of 39 participants (85%) had 124 "noninfectious diarrhoea" events, compared to 10 of 11 participants (91%) in the >12 years of age subgroup.

In the ISS pooled safety population of 50 participants, 86% had in total 147 events of diarrhoea. Events of diarrhoea were mild (48%) to moderate (24%) or severe (14%), and 1 event was serious in 1 of 50 participants (2.0%) who continued treatment without further reported events. The majority of TEAEs of diarrhoea resolved either without dose change or with temporary dose reduction. Diarrhoea did not appear to be associated with weight loss. There are no drug-related events of failure to thrive in the ISS pooled safety population. Events of diarrhoea and events of abdominal pain (including abdominal pain upper) did generally not coincide, nor were events of diarrhoea and events of vomiting. Diarrhoea was considered study drug-related per the investigator in 40 of 50 participants (80%). There were 14 of 67 participants (21%) who had a dose reduction due to events of diarrhoea. Diarrhoea led to treatment discontinuation in 2 of 67 participants (3.0%); these were non-serious mild events of diarrhoea reported in retrospective study MT-1621-107.

Diarrhoea occurred early after treatment initiation, <3 months in 37 of 50 participants (74%); the cumulative incidence was lower (<40%) after 12 months of treatment. The median (Q1 - Q3) time to first occurrence of "noninfectious diarrhoea" was 20 (2 - 171) days, and the median (Q1 - Q3) duration was 8 (3 - 170) days.

Vomiting

In the 39 participants within the proposed indication of TK2d symptom onset of ≤12 years, 13 of 39 participants (33%) had a total of 48 vomiting events. In the ISS pooled safety population of 50 participants, 14 of 50 participants (28%) had vomiting with 49 events reported. Most events of vomiting were mild in intensity and in 3 participants it had been severe. Instances of vomiting were all self-limiting. Events of vomiting and diarrhoea did generally not coincide. There have been 2 SAEs of vomiting (inpatient hospitalization). One event was reported in a participant in TK0102. The event was not related to treatment in the opinion of the Investigator, no change in dosing occurred, and the event resolved. The other event was reported on Day 1 in an adult with Grade 3 events of nausea and vomiting that led to treatment discontinuation. Dose reduction due to vomiting was applied in 1 participant.

The median (Q1 - Q3) time to first occurrence of vomiting was 90 (44 - 1451) days, and the median (Q1 - Q3) duration was 2.0 (1 - 3) days. For the ISS pooled safety population, 8 of 50 participants (16%) had vomiting <3 months after start of treatment; 4 of 50 participants (8.0%) between ≥3 to <6 months; 1 of 50 participant (2.0%) between ≥6 to <12 months and 8 of 49 participants (16%) after ≥12 months. Of the 14 participants that experienced an event of vomiting, 2 participants experienced concurrent events of abdominal pain and diarrhoea each showing that these events generally did not coincide.

Hepatic disorders

In the 39 participants within the proposed indication of TK2d symptom onset of ≤12 years, 17 of 39 participants (44%) experienced at least 1 event reported in the "drug related hepatic" disorders comprehensive search SMQ. In the ISS pooled safety population of 50 participants, 22 of 50 participants (44%) had TEAEs within the "drug related hepatic disorders" comprehensive search SMQ with 56 events reported.

The most frequently reported events in the SMQ were ALT increased in 14 of 50 participants (28%) and AST increased in 11 of 50 participants (22%). Most of these events were mild in severity, were transient, were not associated with direct bilirubin elevation and resolved over time with continuation of doxecitine and doxribtimine. None were serious. There were no events of potential drug-induced liver injury (PDILI) or Hy's law events reported throughout the program; there have been no cases of severe liver injury or jaundice. No treatment-related exacerbation with clinically significant injury following hepatic enzyme increased has been observed. Out of 56 events of "drug related hepatic disorders", 47 events resolved, no event resolved with sequelae, 2 events were resolving, the outcome of 3 events was unknown, 4 events were not resolved at the time of the data cutoff, and no events were fatal while on treatment.

In the ISS pooled safety population of 67 participants, 2 of 67 participants (3.0%) experienced TEAEs within the "drug related hepatic disorders" SMQ which led to permanent treatment discontinuation. Both participants had an age of TK2d symptom onset >12 years. The events were: a nonserious Grade 1 event of gammaglutamyltransferase (GGT) increased on Day 204, the drug was withdrawn, and the event resolved on Day 267; and a Grade 1 event of hepatic enzyme increased on Day 110, the drug was withdrawn, and the event resolved on Day 464. Upon restarting treatment at a reduced dose, both

participants transaminase levels subsequently elevated again approximately 1 month after reinitiation of study drug, which resulted in the study drug being permanently discontinued.

The median (Q1 - Q3) time to event in the "drug related hepatic disorders" comprehensive search was 199 (78 - 980) days, and the majority of events occurred after 12 months and resolved while participants continued on treatment. The cumulative incidence for "drug related hepatic disorders" went up from <1 month (0.040) through 1 to <3 months (0.123), 3 to <6 months (0.191), and 6 to <12 months (0.313); thereafter it remained relatively consistent until 18 months to <2 years (0.370) at which it increased until 2 years to <5 years (0.493) and ≥5 years (0.631).

Mitochondrial diseases including TK2d are known to be associated with transaminase elevations either potentially through a mitochondria-related effect on the liver and/or disease-related muscle injury. Elevated enzymes are among the 10 most frequently reported clinical manifestations of patients with TK2d, having been reported in up to 82% of patients (Wang et al, 2018). At baseline of study 101, 87% of study participants had one or more investigation AEs, with the most common being blood creatine phosphokinase increased in 31 of 38 study participants (82%); AST and ALT increased were registered for 1 instance each.

Abdominal pain

In the 39 participants within the proposed indication of TK2d symptom onset of ≤12 years, 12 of 39 participants (31%) experienced "abdominal pain" with 30 events. In the ISS pooled safety population of 50 participants, 13 of 50 participants (26%) experienced a total of 32 "abdominal pain" (including abdominal pain upper) events.

The severity of events of "abdominal pain" (including abdominal pain upper) ranged from mild to severe. Most events have been self-limiting. Grade 1 (mild) events were reported for abdominal pain in 4 of 50 participants (8.0%) and abdominal pain upper in 3 of 50 participants (6.0%). Grade 2 (moderate) events were reported for abdominal pain in 2 of 50 participants (4.0%) and abdominal pain upper in 1 of 50 participants (2.0%). Grade 3 (severe) events were reported for abdominal pain in 4 of 50 participants (8.0%) and abdominal pain upper in 1 of 50 participants (2.0%). Of the 50 participants in the ISS pooled safety population, a single participant experienced an SAE of abdominal pain (inpatient hospitalization), the event was not related to study drug in the opinion of the Investigator, no change in dosing occurred, and the outcome was resolved at the time of study completion. Of the 67 participants in the ISS pooled safety population, no participant had "abdominal pain" events which required permanent treatment discontinuation. Three of 67 participants (4.5%) had "abdominal pain" (including abdominal pain upper) TEAEs that led to dose reduction. Events of "abdominal pain" (including abdominal pain upper) did generally not coincide with events of diarrhoea.

The median (Q1 - Q3) time to first occurrence of "abdominal pain" (including abdominal pain upper) was 812 (123 - 1245) days, and the median (Q1 - Q3) duration was 1.5 (1 - 4) days.

2.6.8.4. Serious adverse event/deaths/other significant events

Serious Adverse Events

In TK2d participants with age of symptom onset ≤12 years, 23 of 39 participants (59%) had at least one treatment-emergent SAE. Pneumonia and acute respiratory failure were the most frequently reported SAEs occurring in 5 of 39 participants (13%) each (Table 27).

In the ISS pooled safety population (N=50), SAEs occurred in 28 of 50 participants (56%), and in 4 of 50 participants (8.0%) these were study drug-related per investigator. The most frequently reported treatment-emergent SAEs were: pneumonia in 7 of 50 participants (14%), acute respiratory failure in 5 of 50 participants (10%), femur fracture in 4 of 50 participants (8.0%) as well as dysphagia and pneumonia aspiration in 3 of 50 participants (6.0% each). Of these, femur fracture was the only SAE that was considered study drug-related according to the Investigator. Overall, 4 of 67 participants (6.0%) permanently discontinued pyrimidine nucleos(t)ide treatment due to treatment-emergent SAEs.

Table 27. Treatment-emergent SAEs in ≥2 participants with TK2d symptom onset ≤12 years of age, ISS pooled safety population

Preferred term	≤12 years N=39 n (%)
At least 1 TESAE	23 (59.0)
Acute respiratory failure	5 (12.8)
Pneumonia	5 (12.8)
Femur fracture	4 (10.3)
Nephrolithiasis	2 (5.1)
Pneumonia influenzal	2 (5.1)
Pyrexia	2 (5.1)
Bronchitis	2 (5.1)
COVID-19	2 (5.1)
Chest pain	2 (5.1)
Chronic respiratory failure	2 (5.1)
Dysphagia	2 (5.1)
Dyspnoea	2 (5.1)
Failure to thrive	2 (5.1)
Gastrostomy	2 (5.1)
Pneumonia aspiration	2 (5.1)
Respiratory failure	2 (5.1)
Viral upper respiratory tract infection	2 (5.1)

COVID-19=Coronavirus disease 2019; ISS=Integrated Summary of Safety; TESAE=treatment-emergent serious adverse event; TK2d=thymidine kinase 2 deficiency
Note: N represents the total number of participants; Percentages are based on N; preferred terms are sorted by decreasing frequency.

Deaths

Of the 67 participants in the ISS pooled safety population, 3 participants (4.5%) had a fatal treatment-emergent SAE (unknown cause/respiratory failure, TK2d disease progression, seizures). In study 107, 3 deaths occurred, >5 months after treatment discontinuation. All 6 deaths were considered not related to the study drug. (See efficacy section on survival for more details.)

One additional death occurred in study 114, due to aspiration pneumonia. Food aspiration occurred 2 days after discontinuation of doxecitine and doxribtimine.

AEs leading to dose reduction

In the ISS pooled safety population of 67 participants, 50 participants were within the proposed indication of TK2d symptom onset of ≤12 years. Of the participants, 10 of 50 (20%) reported at least 1 TEAE that led to dose reduction. The most common cause of dose reduction was diarrhoea or frequent bowel movements, in 11 of 50 participants (22%). All other TEAEs leading to dose reduction were reported for 1 – 2 participants. No patient reduced dose due to hepatic enzymes increased or hepatic events.

In the ISS pooled safety population of 67 participants, 16 of 67 treated study participants (24%) had at least 1 TEAE that led to dose reduction. The most common cause of dose reduction was diarrhoea in 14 of 67 participants (21%) which was generally Grade 1 (mild) or Grade 2 (moderate) and resolved with dose reduction. All other reasons for dose reductions were single or few instances, including proposed ADRs (abdominal pain, vomiting, ALT/AST increased).

2.6.8.5. Laboratory findings

Laboratory evaluations were collected at each scheduled visit in TK0102, collected when available in retrospective study MT-1621-101, and were not collected in MT-1621-107 or TK0114.

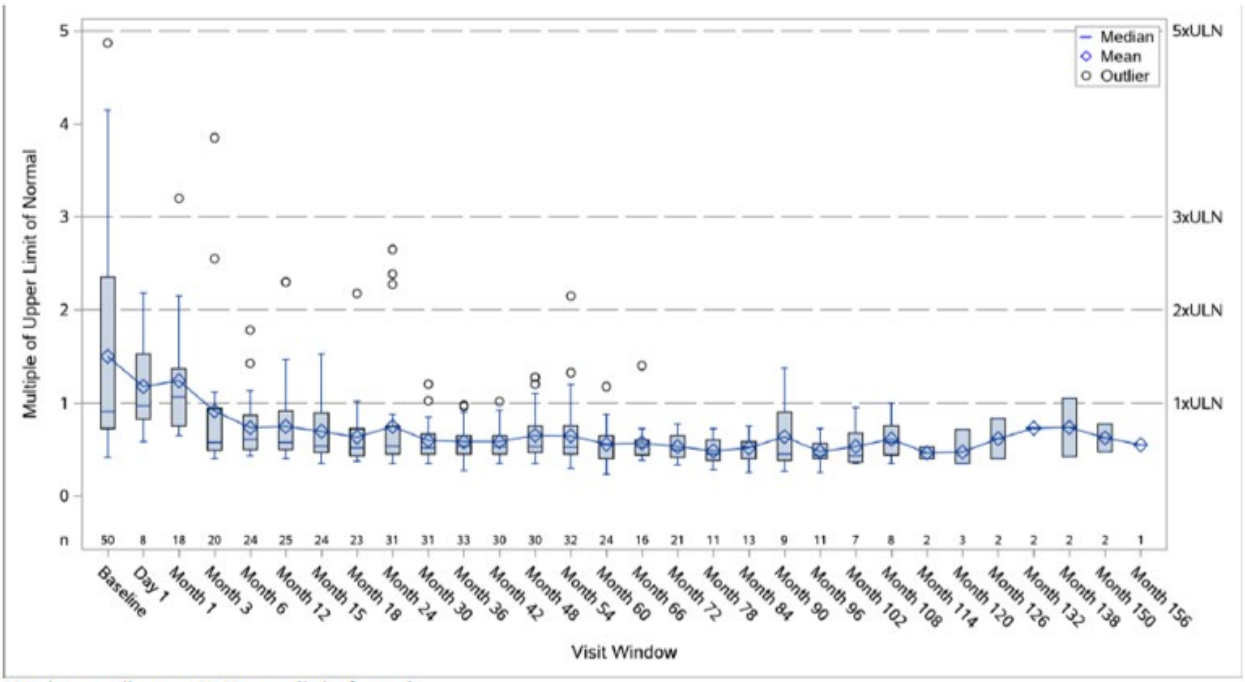
Hematology

For the 50 participants in the ISS pooled safety population, the mean values for the haematology parameters generally remained stable over time. Most participants did not experience shifts in CTCAE grades from baseline to worst post-baseline values in haematology results. Shifts remained Grade 2 or less for all parameters with little exceptions of a Grade 3 shift. Persistent haematology abnormalities while on study drug that occurred in >10% of participants with TK2d included haematocrit (12%) and lymphocytes (14%). There were no marked haematology laboratory abnormalities reported.

Chemistry

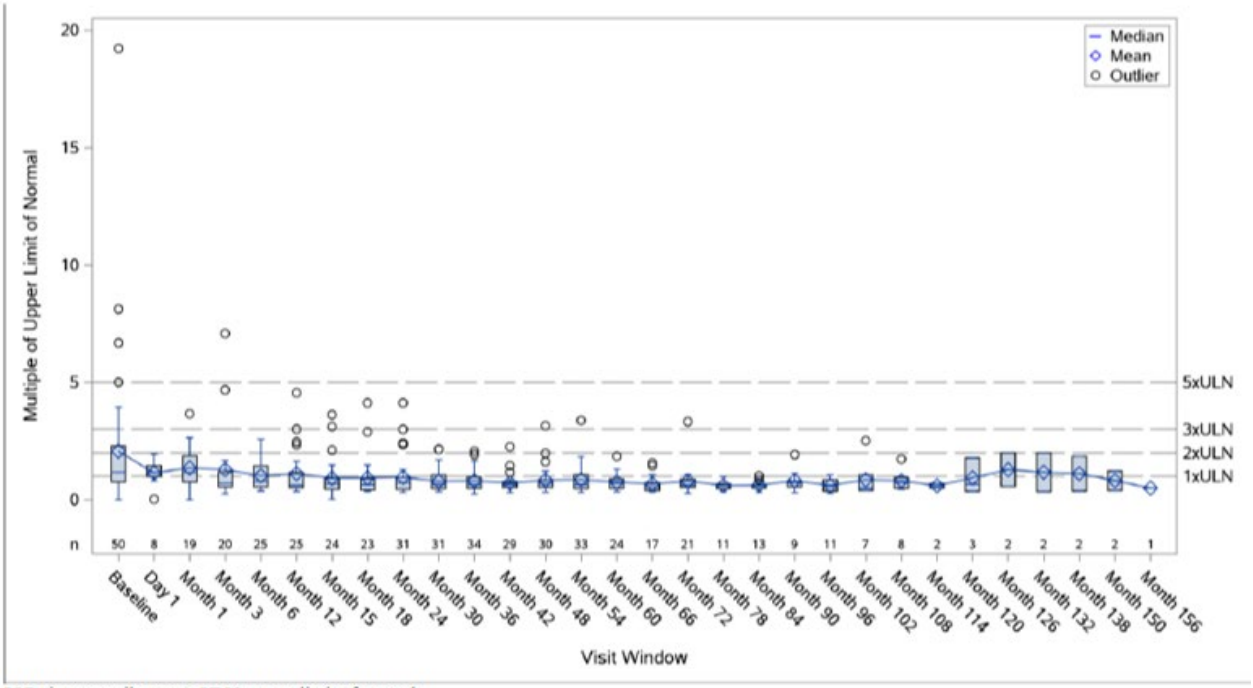
Most participants experienced shifts in CTCAE grades from baseline to worst post-baseline values in clinical chemistry results; overall shifts remained Grade 3 or less for all parameters with the exception of single instances of a shift to Grade 4 for calcium (n=1), CK (n=3), glucose (n=1), and potassium (n=1). Persistent chemistry abnormalities while on study drug that occurred in >10% of participants included direct bilirubin in 12 of 50 participants (24%], creatinine in 7 of 50 participants (14%), as well as lactic acid and lactate dehydrogenase in 6 of 50 participants (12% each).

Liver abnormalities were considered as Adverse Events of Special Interest. There were 27 of 50 participants (54%) with elevated **ALT** at baseline and 21 of 50 participants (42%) with elevated **AST** at baseline (which may include earliest post-treatment measure when pretreatment is not available). In the ISS pooled safety population, the majority of events of ALT increased (93%) or AST increased (82%) resolved without dose change. No study participant had last post-baseline ALT or AST values ≥Grade 3, and there were no SAEs associated with AST or ALT elevations. The majority of transaminase elevation AEs were mild in intensity, and none were concomitantly reported with events of direct bilirubin elevation. In total, 9 participants (18%, event rate: 5.2) had ALT or AST >3×ULN, and 4 participants (8.0%, event rate: 1.2) had ALT or AST >5×ULN.



IQR=interquartile range; ULN=upper limit of normal
Notes: For each participant, the largest reported value (standardized to the ULN) is presented for each visit window.
The upper and lower fences (whiskers) are drawn from the box to the most extreme point that is less than or equal to 1.5 times the IQR.
The bottom and top edges of the box indicate the IQR, defined as the range of values between the first and third quartiles.
Outliers are observations that are more extreme than the upper and lower fences.

Figure 25. Aspartate aminotransferase over time, ISS pooled safety population



IQR=interquartile range; ULN=upper limit of normal
Notes: For each participant, the largest reported value (standardized to the ULN) is presented for each visit window.
The upper and lower fences (whiskers) are drawn from the box to the most extreme point that is less than or equal to 1.5 times the IQR.
The bottom and top edges of the box indicate the IQR, defined as the range of values between the first and third quartiles.
Outliers are observations that are more extreme than the upper and lower fences.

Figure 26. Alanine aminotransferase over time, ISS pooled safety population

Vital signs and physical observations

There were no clinically meaningful changes in vital signs over time. A review of the ECGs collected in the ISS pooled safety population showed no evidence of clinically significant effects of doxecitine and doxribtimine on QT interval corrected for heart rate (QTc) or other ECG parameters.

2.6.8.6. In vitro biomarker test for patient selection for safety

Not applicable.

2.6.8.7. Safety in special populations

Age at first treatment

Generally, the profile of treatment-emergent **common AEs** was similar across the participants, but in the younger age at first treatment subgroup (≤18 years), there was a higher frequency of AEs of pyrexia, rhinorrhoea, vomiting, and ALT/AST increase, as compared to the subgroup ≥18 years. ALT increased and AST increased occurred in 33% and 27% of participants ≤18 years of age, and in 18% and 12% of older participants. Vomiting occurred in 39% of participants ≤18 years of age and in a single older participant. Diarrhoea was frequent in participants ≤2 years at start of treatment (67%), in patients between 2 – 12 years (86%), in patients 12 – 18 years (100%, n=3), and patients >18 years at first age of treatment (94%).

The treatment-emergent **SAEs** occurring in the patients with age of first treatment ≤2 years of age, and of >2 and ≤12 years of age were: acute respiratory failure, pneumonia, pyrexia, Covid-19, pneumonia aspiration, femur fracture (Table 28).

AEs concerning **paediatric growth, neurodevelopment, behaviour, and endocrine-related events** (PGNBE) were pre-defined by the applicant. In the ISS pooled safety population, 9 of 50 participants (18%) had an age at first treatment of ≤2 years. Of these 9 participants, a total of 3 participants (33%) had PGNBE AEs: one participant with femur fractures, non-serious scoliosis, and mental state changes; one participant with failure to thrive, one participant with non-serious scoliosis. Of these events, only femur fracture was considered as possibly related by the investigator, all events resolved.

For data and discussion on **growth, weight, and BMI**, it is referred to the efficacy section. There is little experience in patients of >65 years of age (n=2).

Table 28. Treatment-emergent SAEs by age of first treatment in ≥2 participants in any subcategory, ISS pooled safety population

Preferred term	Age at First Treatment			
	≤2 years of age N=9 n (%)	>2 and ≤12 years of age N=21 n (%)	>12 and <18 years of age N=3 n (%)	≥18 years of age N=17 n (%)
At least 1 TESAE	7 (77.8)	9 (42.9)	2 (66.7)	10 (58.8)
Acute respiratory failure	3 (33.3)	1 (4.8)	0	1 (5.9)
Pneumonia	2 (2.22)	3 (14.3)	0	2 (11.8)
Pyrexia	2 (22.2)	0	0	0
COVID-19	2 (22.2)	0	0	0
Pneumonia aspiration	2 (22.2)	0	0	1 (5.9)
Femur fracture	1 (11.1)	2 (9.5)	1 (33.3)	0
Nephrolithiasis	0	0	0	2 (11.8)
Restrictive pulmonary disease	0	0	0	2 (11.8)

COVID-19=Coronavirus disease 2019; ISS=Integrated Summary of Safety; TESAE= treatment-emergent serious adverse event
Note: N represents the total number of participants in each column; Percentages are based on N.

Sex

There were no clear differences in the proportions of females and males experiencing the most frequently reported treatment-emergent AEs overall by sex subgroup (Table 29). There were no notable numerical differences between female and males in the proportions of diarrhoea, vomiting, abdominal pain (upper), ALT increased, but the proportion of AST increased was larger in males as compared to females (32% *versus* 9%). More males (68%) than females (41%) had treatment-emergent SAEs. No notable numerical differences in the kind of treatment-emergent SAEs were observed between male and female participants, except for acute respiratory failure (1 female and 4 males) and femur fracture (0 females and 4 males).

Table 29. Top 4 of treatment-emergent AEs by sex, ISS pooled safety population

Preferred term	All Formulations (101, 102)	
	Male N=28 n (%)	Female N=22 n (%)
Diarrhoea	24 (85.7)	19 (86.4)
Upper respiratory tract infection	9 (32.1)	10 (45.5)
Pyrexia	13 (46.4)	7 (31.8)
COVID-19	12 (42.9)	6 (27.3)

COVID-19=Coronavirus disease 2019; ISS=Integrated Summary of Safety; TEAE=treatment-emergent adverse event; TK2d=thymidine kinase 2 deficiency
Note: N represents the total number of participants in each column; percentages are based on N.

Renal impairment

In MT-1621-**106** in **non**-TK2d patients, moderate renal impairment (estimated glomerular filtration rate [eGFR] ≥30mL/min but ≤59mL/min) and severe (eGFR ≥15mL/min but ≤29mL/min) was associated with a substantial increase in exposure to dC and dT following a single oral administration of doxecitine and doxribtimine and was characterized by high interindividual variability. A single oral dose of 266.6mg/kg (133.3mg/kg doxecitine and 133.3mg/kg doxribtimine) was well tolerated by all participants in MT-1621-106. No safety issues were identified in these participants with moderate and severe renal impairment; all events reported were consistent with manifestations or complications of renal impairment.

Hepatic impairment

There is no experience with the use of doxecitine and doxribtimine in patients with TK2d who have impaired hepatic function. Of all the participants who started treatment with elevated AST/ALT (CTCAE grades) at baseline, nearly all had last post-baseline AST/ALT grades and worst post-baseline grades, that were of equal or lower than the baseline grade of AST and ALT.

2.6.8.8. Immunological events

Not applicable.

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

No specific drug-drug interaction study with doxecitine and doxribtimine has been conducted. The drug-drug-interaction (DDI) potential is low based on nonclinical testing, clinical experience and the endogenous nature of the compounds. A standard battery of in vitro nonclinical studies did not indicate the potential for clinically relevant pharmacokinetic DDI (see non-clinical AR). Systemic concentrations of dC and dT following doxecitine and doxribtimine are expected to be within the range of their normal endogenous levels in healthy adult participants (see Pharmacokinetic section).

2.6.8.10. Discontinuation due to adverse events

In the ISS pooled safety population of 67 participants, 50 participants were within the proposed indication of **TK2d symptom onset of ≤12 years**. In this age of TK2d symptom onset category, 2 of 50 participants (4.0%) discontinued pyrimidine nucleos(t)ide treatment.

For the 67 participants in the ISS pooled safety population, there were 14 of 67 participants (21%) who had a dose reduction due to events of diarrhoea, and diarrhoea led to treatment discontinuation in 2 of 67 participants (3.0%). There were 2 participants who discontinued due to mild events of hepatic enzyme elevation within 1 month after rechallenge. All other AEs were single reasons.

2.6.8.11. Post marketing experience

Not applicable.

2.6.9. Discussion on clinical safety

Safety data collection

The main source of clinical safety information is the pooled data set (ISS) from studies 101, 102 and 107. Study 102 essentially is a follow-up study of study 101. In study 101 the data were

retrospectively collected through chart review and in study 102 data were prospectively collected. Also in study 107, data were retrospectively collected by chart review, but data collection was limited to the more severe events by protocol.

The study population (N=67) is larger than the target population of patients with TK2d symptom onset ≤12 years of age (n=39). The wider study population is considered as supportive safety data.

The way of data collection of these studies appears to be reasonably reliable. For all studies, a study protocol was established before start of data collection. The protocols detailed the objectives, design, the safety data to be collected, the way of data collection and the quality control; this all appears to be complying to standard levels and appears as adequate. However, retrospective collection of prospectively collected data (in charts) inherently comes with uncertainties about correctness and completeness/missingness.

The main uncertainty/limitation for the analysis of safety data is the lack of a control group. For the assessment of (potential) ADRs therefore, the occurrence of AEs must be gauged against the occurrence of manifestations and sequels or complications of TK2d, and against what can be expected from the mode of action and administration route of doxecitine/doxribtimine.

It is considered unlikely that there was a major influence of selection bias on the safety data, and, from the available (non-)clinical safety data, it is not obvious that important potential risks or ADRs are missed.

Patient exposure

The study population (N=67) is larger than the target population of patients with TK2d symptom onset ≤12 years of age (n=39, 58%). The majority of patients (n=44, 66%) were paediatric at treatment start. Due to the relatively low patient numbers, it also relied on extrapolation of safety information from study population to target population.

The size of the safety database is limited in patient numbers, and is below the usual standard for long-term treatments, of at least 100 patients from the target population treated for at least 12 months (ICH Guideline E1). This is to some extent compensated by the long exposure times of the patients: most (n=31, 80% of studies 101 + 102) patients of the target population had an exposure time ≥5 years and drop-out and permanent discontinuations were infrequent. A considerable portion of the patients was paediatric, which is in line with the target population. However, there is little (n=9) information regarding patients with symptom onset ≤2 years of age. There is very little experience with patients >65 years of age (n=2), which is sufficiently reflected in the SmPC.

Adverse drug reactions

In the study population of N=50 participants and in the target population of n=39 participants, the safety profile of doxecitine/doxribtimine was generally manageable.

All patients had at least one treatment-emergent AE, which is not surprising, given the long observation period. The majority of AEs was however considered **drug-related** by the investigators. This aligns with the frequent occurrence of: diarrhoea, vomiting, abdominal pain (upper) and ALT/AST increased, which were a priori suspected as ADRs by the applicant. **SAEs** occurred in a considerable portion (42%) of patients from the target population, but these were usually not considered as treatment-related, which is agreed (see next section).

Study drug **discontinuations** due to AEs did infrequently occur, though **dose reductions** were necessary in a considerable portion (23%) of patients from the target population, commonly due to diarrhoea (18%). The number of permanent discontinuations was overall low and not concerning, especially in the proposed target population of patients with TK2d symptom onset ≤12 years of age,

where only 2 (4%) patients permanently discontinued treatment due to AEs. There was no pattern in AEs leading to permanent discontinuation, including only 2 cases of diarrhoea, which is the most common ADR.

The applicant initially proposed 5 **Adverse Drug Reactions** to be included in the SmPC (section 4.8): diarrhoea, vomiting, ALT increased and AST increased, (upper) abdominal pain. These AEs were a priori considered by the applicant as Safety Topics of Interest and are among the most common AEs in the integrated safety sample of patients exposed and followed in studies 101 and 102. The common AEs in the study sample are a mixture of: manifestations, sequels, or complications of TK2d; AEs that are independently occurring comorbidities; AEs that can be seen as ADRs. It is agreed that diarrhoea, vomiting, and abdominal pain (upper) are considered as ADRs of doxecitine/doxribtimine. Besides these, the safety data, including values from haematology and chemistry and the vital signs and including non-clinical evidence, do currently not give rise to consider additional ADRs.

ALT and AST increased with a frequency 'very common' were considered as ADRs by the applicant, but this was not supported. ALT/AST increased is reflected in a warning (section 4.4) in the SmPC and is appropriately covered by the important potential risk of liver dysfunction in the RMP. From a mechanism of action point of view there is no obvious link between doxecitine and doxribtimine and liver enzyme elevations. It is correct that ALT/AST increased occurred frequently in the studies. Accordingly, at baseline about half of the patients had elevated ALT/AST, and the average levels of ALT/AST decreased over time (see section on laboratory values). Most of the events of ALT/AST increased, were mild in severity, were transient, were not associated with direct bilirubin elevation and resolved over time with continuation of doxecitine and doxribtimine. No study participant had last post-baseline ALT or AST values ≥Grade 3, and there were no SAEs associated with AST or ALT elevations. Study drug discontinuations due to increased hepatic enzymes occurred in 2 cases, both cases (TK2d onset >12 years) had a second event of mild elevations after reinitiating treatment. This is the only evidence available, and is considered insufficient evidence as it also may have occurred by chance.

From a mechanism of action point of view there is no obvious link between doxecitine and doxribtimine and the ADR of **diarrhoea.** The additive magnesium stearate is of such a low amount that it is an unlikely cause of diarrhoea. It seems that diarrhoea is caused by 'overload' of the gastro-intestinal system by the relatively large amount of drug product. An impairment on weight and growth has not been observed and it did not appear that diarrhoea lowered the systemic exposure to doxecitine/doxribtimine. A warning concerning the severity and course, the reversibility, and the possibilities to (temporarily) lower the dose to stop diarrhoea was included in the SmPC.

Vomiting and abdominal pain (upper) can be considered as manifestations of an underlying more general GI disturbance, caused by a relative 'overload' of a normally endogenously produced substance in the GI tract. From the non-clinical studies there were no safety signs for gastro-intestinal AEs. Vomiting and abdominal pain do not show clear overlap with TK2d symptoms, but are not uncommon in children in general. Mainly due to their frequency of occurrence, there is a reasonable possibility that vomiting and abdominal pain (upper) are ADRs. Also, palatability is not favourable from the patient perspective and may lead to vomiting. Vomiting and abdominal pain usually did not coincide.

There were no safety signals concerning **haematology, chemistry, or vital signs**. In the ISS pooled safety population, the mean values for the haematology parameters generally remained stable over time, parameter shifts remained Grade 2 or less for all parameters with little exceptions. There were no consistent trends in chemistry variables, except for ALT/AST for which there were relatively high averaged baseline levels with a trend of decreasing values over time.

Serious AEs (SAEs) and deaths

A considerable portion of patients had at least one **SAE**, in both the study population (N=50) as well as in the target population of patients with TK2d symptom onset ≤12 years of age (n=39). These SAEs were generally not considered study drug related and this is agreed. Although without a control group for reference, the SAEs can generally be understood as manifestations or consequences of TK2d (e.g. dysphagia, pyrexia, failure to thrive, respiratory failure), can be understood as complications of TK2d (e.g. airway infections), or as comorbidities (e.g. femur fracture). Although femur fracture was considered as study drug related by the investigator, the mode of action of doxecitine/ doxribtimine does not make it a likely candidate for ADR. There were 3 **deaths** while patients were exposed to study drug. It is unlikely that these deaths are related to the study drug, because the death causes were explained by the nature of TK2d, by respiratory failure, or by seizures.

Special populations

There are no safety concerns that are relevantly different for special populations, notably age (at first treatment) and sex. There is no experience with TK2d patients with renal impairment or hepatic impairment, which is reflected in the SmPC and as missing information in the safety specifications of the RMP. However, a lack of information alone does not qualify these uncertainties as missing information (see Safety Specifications discussion). No dose adjustments are proposed by the applicant for patients with impaired renal or hepatic function (see discussion on clinical pharmacology). There is little experience in the elderly, which is appropriately reflected in the SmPC.

Some numerical differences between paediatrics and adults were observed in the occurrence of the proposed ADRs: vomiting, and AST/ALT elevated. This is not of special concern. Vomiting was not considered as severe (see ADR section) and may generally be more prevalent in the paediatric population, as compared to adults. It is not certain that AST/ALT increased may also be caused by TK2d, the higher occurrence in paediatrics may reflect the disease severity in younger participants.

AEs concerning paediatric growth, neurodevelopment, behaviour, and endocrine-related events (PGNBE) were pre-defined by the applicant. There were 3 PGNBE-related events in the 9 of 50 participants (18%) with an age at first treatment of ≤2 years. These events were not considered to be treatment-related and this is agreed. Generally, there is a lack of information on safety in young children, which however also accounts for the complete target population (see Safety Specifications). There also is a lack of information on safety in the elderly (age >65 years of age but TK2d symptom onset <12 years of age), but this is not considered to be of special concern, given the overall safety profile and because the weight of the target population is in the paediatric population and younger adults.

Product information

One **contra-indication** (section 4.3) was included in the SmPC. Hypersensitivity issues to the active substance are not expected but a contra-indication was included for sensitivity to excipients, in line with the guidance. No need for other contra-indications was apparent. A history of liver disease including ALT / AST / total bilirubin increases up to 2x ULN, and other significant medical conditions that might interfere with the clinical course of TK2d, was an exclusion criterion for study 102 (not for study 101). These exclusion criteria currently do not translate into a meaningful contra-indication.

The **warnings** (section 4.4) that is currently included are: a warning that elevated **liver** enzymes and liver dysfunction/failure have been observed as a clinical manifestation of TK2d, and that in clinical studies elevations in ALT/AST have also been observed in patients treated with Kygevvi. The warning does not point to ALT/AST elevations as ADR of doxecitine/doxribtimine and this agreed. A warning was included concerning **diarrhoea**, describing the severity, reversibility, and the possibilities to (temporarily) lower the dose to stop diarrhoea.

On the basis of the mechanism of action, the pharmacodynamic and pharmacokinetic profile and reported adverse drug reactions, it is considered that doxecitine/doxribtimine has no or negligible influence on the **ability to drive and use machines** (section 4.7).

As **Adverse Drug Reactions** (section 4.8), the applicant initially proposed: diarrhoea, vomiting, alanine aminotransferase (ALT) increased, aspartate aminotransferase (AST) increased, and abdominal pain (including abdominal pain upper). The consideration of AST/ALT increased as ADR was not agreed but inclusion of liver dysfunction/liver injury as important potential risk was agreed.

There are no data on symptoms associated with an **overdose** (section 4.9). The text is quite general and appropriate.

Additional safety data needed in the context of a MA under exceptional circumstances

It was applied for a MA under exceptional circumstances. The rarity of the disease makes it difficult to perform a randomised controlled trial with sufficient size and follow-up in a reasonable time frame. This means that safety data were collected from a cohort of TK2d patients who were all treated, without being able to contrast safety data against a control group. Moreover, the cohort of treated patients is heterogeneous regarding age at TK2d symptom onset and age at first treatment, the latter most relevant for safety. As already pointed to in Scientific Advice (EMEA/H/SA/3933/1/2018/PA/PED/SME/PR/III), given the rarity of the disease and the need for long-term (presumably life-long) treatment it is needed to collect long-term data to further evaluate the expected long-term efficacy and safety data. In response, the applicant agreed to perform a PASS (study TK0109) as a **Specific Obligation** as an additional pharmacovigilance activity (also see Safety Specifications discussion). In order to ensure adequate monitoring of safety and efficacy of Kygevvi in the treatment of patients with thymidine kinase 2 deficiency (TK2d), the MAH shall also provide yearly updates on any new information concerning the safety and efficacy of Kygevvi.

2.6.10. Conclusions on the clinical safety

The safety profile of doxecitine/doxribtimine is not without patient impact, but it appears to be overall manageable.

In the study population of 50 patients, of which 39 patients aligned with the claimed indication of TK2d symptom onset ≤ 12 years of age, the safety profile of doxecitine/doxribtimine was generally manageable.

The lack of a control group hampers causal interpretation for assessing ADRs. The most relevant ADRs to consider in the Benefit-Risk assessment are gastrointestinal disturbances: Diarrhoea, vomiting, and abdominal pain (upper). Although these AEs were generally not serious, their very common frequency makes them relevant for the assessment of the B-R. In addition, for diarrhoea it counts that patients may have chronic diarrhoea or may have repeated episodes of diarrhoea. Apart from discomfort, diarrhoea frequently led to dose reductions to stop diarrhoea. Diarrhoea did not lead to reduced exposure through increased passage/less time for absorption. ALT/AST increased also occurred frequently, but its occurrence is difficult to discriminate from ALT/AST increased as manifestation of TK2d and it was considered not to be an ADR.

In the safety specifications of the RMP, liver dysfunction is included as important potential risk. Use during pregnancy and breastfeeding and long-term safety are included as missing information in the RMP.

Performance of a PASS (study TK0109) is considered a Specific Obligation.

2.7. Risk Management Plan

2.7.1. Safety concerns

Table 30. Summary of safety concerns (RMP version 0.4)

Summary of safety concerns	
Important identified risks	None
Important potential risks	Liver dysfunction
Missing information	Long-term safety in patients with TK2d symptom onset ≤2 years and symptom onset >2 to ≤12 years Use during pregnancy and breastfeeding

2.7.2. Pharmacovigilance plan

Table 31. On-going and planned additional pharmacovigilance activities (RMP version 0.4)

Study **Status**	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
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				
TK0109 - A post-authorization noninterventional study Planned	To describe the safety and clinical outcomes of doxecitine and doxribtimine treatment	Important potential risk: Liver dysfunction Missing information: Long-term safety in patients with TK2d symptom onset ≤2 years and symptom onset >2 to ≤12 years; use during pregnancy and breastfeeding	Protocol submission	Within 6 months of marketing authorization in Europe
			Intermediate report	Annually

LPLV=last patient last visit; TK2d=thymidine kinase 2 deficiency

2.7.3. Risk minimisation measures

Table 32. Summary table of pharmacovigilance activities and risk minimization activities (RMP version 0.4)

Safety concern	**Risk minimization measures**	**Pharmacovigilance activities**
Important potential risk		
Liver dysfunction	**Routine risk minimization measures:** Kygevvi is intended for use with the instructions and supervision of specialist healthcare professionals experienced in the management of patients with mitochondrial disorders SmPC Section 4.2 (Posology and method of administration)	**Routine Pharmacovigilance activities beyond adverse reactions reporting and signal detections:** None **Additional Pharmacovigilance activities:** Post authorization safety study TK0109

Safety concern	Risk minimization measures	Pharmacovigilance activities
	SmPC Section 4.4 (Special warnings and precautions for use) PL Section 3 (How to take Kygevvi) **Additional risk minimization measures:** None	
Missing Information		
Long-term safety in patients with TK2d symptom onset ≤2 years and symptom onset >2 to ≤12 years	**Routine risk minimization measures:** Kygevvi is intended for use with the instructions and supervision of specialist healthcare professionals experienced in the management of patients with mitochondrial disorders (SmPC Section 4.2 Posology and method of administration) PL Section 3 (How to take Kygevvi) **Additional risk minimization measures:** None	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** None **Additional pharmacovigilance activities:** Post authorization safety study TK0109
Use during pregnancy and breastfeeding	**Routine risk minimization measures:** Kygevvi is intended for use with the instructions and supervision of specialist healthcare professionals experienced in the management of patients with mitochondrial disorders (SmPC Section 4.2 [Posology and method of administration]) SmPC Section 4.6 (Fertility, Pregnancy, and Lactation) PL Section 2 (What you need to know before you take Kygevvi) PL Section 3 (How to take Kygevvi) **Additional risk minimization measures:** None	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** None **Additional pharmacovigilance activities:** Post authorization safety study TK0109

PL=patient information leaflet; SmPC=summary of product characteristics; TK2d=thymidine kinase 2 deficiency

2.7.4. Conclusion

The CHMP considers that the risk management plan version 0.4 is acceptable.

The applicant is reminded that in case of a Positive Opinion, the body of the RMP and Annexes 4 and 6 (as applicable) will be published on the EMA website at the time of the EPAR publication, so considerations should be given on the retention/removal of Personal Data (PD) and identification of Commercially Confidential Information (CCI) in any updated RMP submitted throughout this procedure.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the

requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 03.11.2025. 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, Kygevvi (Doxecitine / Doxribtimine) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU and it is approved under a conditional marketing authorisation [REG Art 14-a].

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

Kygevvi (doxecitine/doxribtimine) is an orally administered replacement therapy to compensate for the intra-mitochondrial shortage of thymidine kinase 2 (TK2) occurring in patients with thymidine kinase 2 deficiency (TK2d). The primary mechanism of action of Kygevvi is the incorporation of nucleosides deoxycytidine (dC) and deoxythymidine (dT) into skeletal muscle mtDNA to restore mtDNA copy number and improve skeletal muscle function in TK2d patients. Doxecitine and doxribtimine utilize residual TK2 activity as well as the cytosolic phosphorylation pathway via thymidine kinase 1 and deoxycytidine kinase to increase mtDNA precursors (dCTP and dTTP in the mitochondria).

The drug product is a 1:1 mixture of doxecitine and doxribtimine (2 g each) powder for oral solution in a single 4 g sachet. The maintenance dose thus is 800mg/kg/day (400 mg/kg/day of doxecitine and 400 mg/kg/day of doxribtimine). This maintenance dose is reached after a short titration with 2 lower dose levels (2 weeks each for 260 mg/kg/day and 520 mg/kg/day) to ensure tolerability.

3.1.1. Disease or condition

Kygevvi is recommended to be indicated *'for the treatment of paediatric and adult patients with genetically confirmed thymidine kinase 2 deficiency (TK2d) with an age of symptom onset on or before 12 years'.* The applicant is pursuing marketing approval under exceptional circumstances.

Thymidine Kinase 2 deficiency (TK2d), a rare disorder first identified in 2001, is one of several mitochondrial deoxyribonucleic acid (mtDNA) depletion and deletion syndromes, leading to insufficient synthesis of mitochondrial respiratory chain enzyme complexes (Berardo et al, 2022; Hirano et al, 2001; Saada et al, 2001). MtDNA depletion is defined as a residual mtDNA copy number of <30% compared with age-matched controls (Rahman and Poulton, 2009). In TK2d, the skeletal muscle is particularly impacted, likely due to reduced thymidine kinase 2 activity in muscle tissue and the high energy demand of skeletal muscle tissue (Alberio et al, 2007). The gold standard for confirming TK2d is based on genetic testing which may reveal pathologic variants in the nuclear TK2 gene (de Barcelos et al, 2019).

The natural course of TK2d is relentlessly progressive without spontaneous improvement in symptoms. It is associated with high mortality, especially in patients with symptom-onset ≤12 years of age; patients with symptom onset ≤2 years of age have the worst prognosis. Due to its pathophysiology, TK2d impacts motor function, with progressive proximal muscle weakness that results in impairment of ambulation and activities of daily living, respiratory muscle changes leading to restrictive lung disease, and bulbar muscle changes leading to dysphagia (Berardo et al, 2022; Garone et al, 2018; Wang et al, 2018).

3.1.2. Available therapies and unmet medical need

There are no medicinal products currently approved for the treatment of TK2d. Disease management is limited to supportive measures, such as ventilatory and feeding support and devices to assist with mobility. The disorder leads to an increased mortality and has a large impact on quality of life for patients, their caregivers and families (Amtmann et al, 2023; UMDF, 2022). Consequently, in the target population of patients with symptom onset ≤12 years of age, there is a substantial unmet medical need.

3.1.3. Main clinical studies

The clinical development program for doxecitine/doxribtimine comprises 3 'phase 1' studies for biopharmaceutics and clinical pharmacology in healthy volunteers and a series of observational studies in treated and untreated patients with TK2d. A randomised controlled trial in patients with TK2d has not been performed. Main clinical studies comprise a retrospective chart review (study **101** with n=38) in patients treated with (precursors of) doxecitine/doxribtimine and one ongoing prospective single arm 'follow-up' trial with doxecitine/doxribtimine (study **102** with n=47). In studies 101+102 together (**101+102 ISE treated population)**, there were 39 paediatric or adult TK2d patients with an age of TK2d symptom onset ≤12 years, which is the intended target population.

These data are supported by a second retrospective chart review (study **107** including n=18 treated and n=43 untreated patients) and a noninterventional study including patients using pyrimidine nucleos(t)ides within company supported expanded access programs (study **114** with n=34). Data from treated patients from studies **101, 102, 107, and 114** were enclosed in the **ISE treated population** (n = 82).

Untreated patient data was mainly derived from literature publications (**MUPD**; n = 54 with an age ≤ 12 years of age at symptom onset), and an updated literature search completed with untreated patient data from study 107 (**ISE**-**MUPD**; n = 93 with an age ≤ 12 years of age at symptom onset).

Data from studies 101, 102, and 107 were pooled for safety analysis (**ISS**).

3.2. Favourable effects

The favourable effects described below originate from data in patients with age at TK2d symptom onset ≤12 years (i.e. the claimed indication), unless stated otherwise.

Survival

Across all treated patients with age of TK2d-symptom onset ≤12 years (studies 101+102, 107, 114; ISE treated population; n = 82) 3 patients died (3.9%), compared to 53 patients (57%) across all untreated patients (ISE-MUPD; n = 93). All performed survival analyses yielded similar curves with survival benefit in treated versus untreated patients, also when only comparing patients included in the ISE-treated 101+102 population with the MUPD.

Motor milestones

The development of motor milestones was evaluated within patients over time. Data were available from studies 101+102 (pretreatment and post-treatment), and a retrospective chart review study 107 (untreated patients). Pretreatment and untreated patient data showed that spontaneous regain of motor milestones is rare in the natural course of TK2d (up to 5%) while losses were observed in 82% of the patients. In the post-treatment period (i.e. while on treatment), 84% of the patients regained a milestone while losses were observed in 26% of the patients.

Ventilatory support

In the group of treated patients, 18 of the 39 (46%) patients initiated ventilatory support in the pretreatment period, and 4 (19%) patients initiated ventilatory support in the post-treatment period. Of those 22 patients on ventilatory support, 5 (23%) discontinued ventilatory support during the post-treatment period. The median hours of support were similar pre- and post-treatment periods.

Feeding support

In the group of treated patients, 12 of the 39 (31%) had a feeding tube inserted in the pretreatment period; in 1 patient it was also removed in this period. In the post-treatment period, 4 patients had a feeding tube inserted and 4 patients had the feeding tube removed.

Biomarkers

Treated patients (study 102) consistently showed close to normal GDF-15 values; baseline values were absent. A comparable pattern was found for FGF-21. Venous lactic acid and CK showed a tendency for decreasing values over time during treatment.

3.3. Uncertainties and limitations about favourable effects

TK2d is an ultra-rare disease and this was reflected in the atypical development program. The clinical studies in support of this application are cohort studies of treated patients with retrospective and/or prospective data collection; (survival) data from untreated patients was predominantly derived from literature and a retrospective chart review. The main endpoint of interest, survival, was compared between treated and untreated cohorts using various statistical approaches with correction for, amongst others, age at symptom-onset, the use of several stringent matching algorithms, and target trial emulation.

The data suggest a survival benefit in doxecitine and doxribtimine treated patients with TK2d compared to untreated patients, overall and in both age subgroups (age at TK2d symptom onset <2 years, and > 2 to ≤12 years). The benefit appears large, and generally statistically significant. However, although selection and time-related bias are addressed in the various statistical analysis approaches, selection bias cannot be considered to be sufficiently eliminated or even understood given the fundamental differences in the datasets compared, especially when considering that the untreated patients from the literature are likely to present worse mortality outcomes ('worst cases are published in literature') compared to untreated patients in centres that were not involved with the sponsored studies from retrospective chart review. Bias thus remains a problem in the comparison of survival, and this cannot be mitigated whichever way the data are analysed. This conclusion is supported by the MWP. The SAG-Neurology similarly concluded on the unknown impact of bias on the survival data but additionally stated that it is plausible that the effect on improvement of mitochondrial function could lead to benefit on survival. Altogether, survival benefit is not unequivocally demonstrated; there is remaining uncertainty due to bias. Comparative data in a randomised fashion are not to be expected (see 'Additional considerations on the benefit-risk balance').

Data on **ventilatory and feeding support** were available only for those requiring this support; patterns in changes in support comparing pre- and post-treatment data were not convincingly in support of stabilization or improvement due to treatment: Some patients improved, some deteriorated, but the numbers were small.

Mouse models demonstrated improvement in survival and transient effects in body weight maintenance, mtDNA depletion, motor function, and respiratory function. Further, mtDNA incorporation in animal models was shown within 10 days after administration. These findings support the Mode of Action, but were not replicated in humans. The mice models are considered valid models for mitochondrial disease in humans and the outcomes in the mice models are relevant for humans. The remaining uncertainty about the MoA can be solved by clinical trial data.

The posology is based on extrapolation from preclinical studies. No dose(-exposure)-response relationships have been quantified in humans, variability in the pharmacokinetic profile is high and poorly understood. Furthermore, it is unknown whether the pharmacokinetic profile in plasma is indicative for the pharmacokinetic profile at the site of action, which prevents justifying an optimal

dosing regimen for patients with TK2d and prevents providing dosing recommendations to accommodate the influence of intrinsic and extrinsic factors that influence the pharmacokinetic profiles of dC and dT.

It can currently not be excluded that potential maturation processes in paediatric patients less than 2 years of age will affect the pharmacokinetic profiles of dC and dT. The ontogeny of critical transporters and enzymes and rate-limiting steps in the pharmacokinetics are unknown.

3.4. Unfavourable effects

The pooled analysis (ISS) includes studies 101, 102, and 107 (where data were collected), in participants with TK2d who were all treated with doxecitine/doxribtimine. The target population of patients with an age of TK2d symptom onset of ≤12 years (n=39) is a subset of the total study population (N=67); studies 101/102 to assess common AEs included n=50 patients. Retrospective study 114 in patients of the expanded access program has limited safety data.

In the sample of the target population of 39 participants with TK2d symptom onset ≤12 years of age, most participants (94%) reported at least 1 treatment-emergent AE; and for the majority of patients (71%) these were considered treatment-related. There were no treatment-emergent AEs that led to drug discontinuation; for 5 (16%) participants a dose reduction was applied. In this sample there were 13 participants (42%) with a treatment-emergent SAE, that were commonly single adverse events that were not considered as study-drug related by the investigator. No SAE led to study drug discontinuation or dose reduction. In the total sample (N=67) there were 3 deaths while on treatment, the causes were not considered to be study drug related and deaths did not occur in the sample of patients with TK2d onset ≤12 years of age.

The most common AEs in the target population (n=39) included: non-infectious diarrhoea in 33 of 39 (85%, 124 events), drug related hepatic disorders in 17 of 39 (43%), abdominal pain (including abdominal pain upper) in 12 of 39 (31%), and vomiting in 13 of 39 (33%) participants. The occurrence in the target population (n=39) and the 101+102 total population (n=50) did not proportionally differ.

Diarrhoea

In the population of 50 participants, 86% had in total 147 events of diarrhoea, which could be mild (48%) or moderate (24%) or severe (14%), and 1 event was serious. In most cases (80%), diarrhoea was considered study drug-related. Diarrhoea led to treatment discontinuation in 2 of 67 participants (mild severity). The majority of cases resolved without dose change, or with temporary dose reduction (21%). Diarrhoea did not appear to be associated with weight loss or failure to thrive. Events of diarrhoea and events of abdominal pain (including abdominal pain upper) did generally not coincide, nor did events of diarrhoea and events of vomiting.

Abdominal pain

In the population of 50 participants, 26% had a total of 32 events of abdominal pain (upper). The severity ranged from mild to severe, it was severe in 5 participants. Most events have been self-limiting. In few (n=3) instances, abdominal pain led to dose reduction and it did not lead to permanent discontinuations.

Vomiting

In the population of 50 participants, 28% had vomiting with 49 events reported. Most events of vomiting were mild in intensity and in 3 participants it had been severe. Instances of vomiting were all self-limiting. Events of vomiting and diarrhoea did generally not coincide.

Hepatic events

In the population of 50 participants, 44% had 'drug related hepatic disorders' with 56 events reported. The most frequently reported were ALT increased in 28% and AST increased in 22% of participants. Most of these events were mild in severity, were transient, were not associated with direct bilirubin elevation and resolved over time with continuation of doxecitine/doxribtimine. None were serious and there were no events of potential drug-induced liver injury (PDILI) or Hy's law events. ALT/AST increased led to dose reductions or treatment discontinuation in single cases.

3.5. Uncertainties and limitations about unfavourable effects

The main source of clinical safety information is the pooled data set from studies 101, 102, and 107. Study 102 essentially is a follow-up study of study 101 and does not contribute a substantial number of new patients. Only study 102 has prospective data collection.

The main limitation for the analysis of safety data is the lack of a control group. For the assessment of (potential) ADRs therefore, the occurrence of AEs must be gauged against the occurrence of manifestations, sequels and complications of TK2d, and against what can be expected from the mode of action of doxecitine/doxribtimine.

The size of the safety database is limited in patient numbers and is below the usual standard for long-term treatments of at least 100 patients from the target population treated for at least 12 months (ICH Guideline E1). The study population (N=67) is larger than the target population of patients with TK2d symptom onset ≤ 12 years of age (n=39). Due to the low patient numbers, it is therefore also relied on extrapolation of safety information from study population to the included representatives of the target population. There is little experience with patients >65 years of age (n=2), and no experience with patients with TK2d and hepatic impairment or renal impairment. Also, the experience in children with age at first treatment <2 years of age (n=9) is limited. More long-term safety data in a larger population of both subpopulations will be collected post-marketing (PASS).

3.6. Effects Table

Table 33. Effects Table for Kygevvi for the treatment of patients with TK2d with an age of symptom onset on or before 12 years.

Effect	Short Description	Unit	Kygevvi	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Motor milestones	≥1 milestones abilities	n/N (%)	Lost: 10/38 (26.3%) Regained: 26/31 (83.9%)	--	**Unc**: No external control group, includes retrospective data. **SoE**: Before treatment, spontaneous (re)gain of motor milestones is very rare (1/32, 3.1%) while loss is common (32/39, 82%).	ISE
Unfavourable Effects						
Diarrhoea		%	85	--	**Unc**: No control group.	

Effect	Short Description	Unit	Kygevvi	Control	Uncertainties/ Strength of evidence	References
Vomiting		%	33	--	**SoE**: High frequency, usually considered drug related, no overlap with known TK2d manifestations.	ISS
Abdominal pain		%	31	--		

Abbreviations: --=Not available; ISE=Integrated Summary of Efficacy database; MUPD=modified untreated patients database; ISS=Integrated Summary of Safety database.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Favourable effects

TK2d is an ultra-rare disease and this was reflected in the atypical development program, which is without randomised controlled pivotal trials but with retrospective and prospective cohorts. Data collection is ongoing and a PASS (TK109) is agreed for safety and efficacy data gathering post-marketing.

There are no specific guidelines on endpoint / measurement tools or sets to be used in studies on TK2d. The endpoints chosen in the main studies were in agreement with the CHMP Scientific Advices and aligned with medical literature. The major endpoints survival, motor milestones, and ventilatory and respiratory support are considered clinically relevant for the intended target population of patients with TK2d symptom onset ≤12 years. Survival has been accepted as (primary) endpoint in marketing authorisations for other diseases with childhood manifestation and high mortality rates. Especially in children with symptom onset ≤2 years, the most seriously affected subgroup of patients, an effect of treatment on survival would be most impactful and probably best evaluable. In line with the Scientific Advices, a survival benefit in the youngest patients could be extrapolated to the older patients with symptom onset ≤12 years, supported by a regain of lost motor milestones in all patients with symptom onset ≤12 years. The advantage of survival and motor milestones as outcomes, in the light of retrospectively collected data, is that these outcomes are in principle objectively observable. Still, their value is determined by the reliability of documentation in the charts. Imputation of missing data from the charts was well defined and considered reasonable. Potential misclassification of (a few) patients to the respective age at onset of TK2d symptom onset-group, i.e. the target population, due to missing data unlikely impacted the data.

When comparing the cohorts of all treated (ISE-treated population) and all untreated (ISE-MUPD) patients with TK2d symptom onset ≤12 years, three patients with symptom onset ≤12 years in the treated cohort died compared to more than half of the untreated patients. The analyses suggested a large survival benefit in the cohort of treated patients, which would undoubtedly be important for patients and their families. However, despite a variety of statistical analysis approaches, selection bias cannot sufficiently be eliminated or even understood given the fundamental differences in the datasets compared and survival benefit is not considered unequivocally demonstrated. This conclusion was in line with the conclusion of the MWP and also supported by the SAG-Neurology. The latter group however, considered a treatment effect plausible, directly due to results from non-clinical studies and indirectly supported by results in biomarkers. Given these uncertainties, survival data do not allow inclusion in SmPC section 5.1, aligned with the guidance on SmPC section 5.1 assessment (*EMA/CHMP/566497/2023*)

Data supporting the MoA merely depends on animal studies. No patient data confirm mtDNA restoration upon treatment. However, the TK2 mutant mice are valid models for mitochondrial disease, reproducing key features of TK2d—particularly the infantile-onset form. These models replicate the mitochondrial dysfunction, muscle pathology, and mtDNA depletion seen in patients, with alternative pathways (TK1, dCK, residual TK2) able to partially compensate for TK2 loss. Treatment in mice improved survival, mtDNA content, and motor, respiratory, and weight outcomes, aligning with key clinical endpoints in humans. The importance of the findings in mice models on the MoA was supported by the SAG-Neurology. The waning efficacy in mice is likely species-specific. The relevance of these data in mice was acknowledged by the SAG-Neurology.

Dosing was also mainly derived from animal data, no dose-response studies were performed and an exposure-response relationship in humans was not quantified. Consequently, no therapeutic window was defined and the optimal dose remains unclear, which also includes an uncertainty for dosing in patients with renal and hepatic impairment and the need for the precise weight bands as currently proposed for the posology.

Given the uncertainty with regard to the survival data, motor milestones is a key endpoint for the efficacy assessment. Pre- and post-treatment (i.e. while on treatment) comparison was based on uncontrolled, retrospective study 101 and prospectively collected chart data of study 102 that may be imprecise; the comparison itself is not affected by selection bias as these are made within-subject. Further, the natural course of TK2d is characterised by motor milestones that are not gained, lost over time, and seldom regained (pretreatment period of studies 101+102, 107). The high frequency of regains observed in the post-treatment period is plausibly due to treatment with doxecitine and doxribtimine and highly relevant for daily functioning of patients. The substantially reduced frequency of losses post-treatment compared to pretreatment is in support of treatment effect and equally important. The SAG-Neurology confirmed the relevance of these statements. The pre- versus post-treatment effect was highest in the youngest children (symptom onset <2 years of age) who have the worst prognosis but also became affected in their crucial developmental period; the relevance of this finding was also confirmed by the SAG-Neurology.

Biomarker data, especially for GDF-15, is relevant as it provides additional support for treatment effect on markers indicative for mitochondrial distress. Although not validated, the low and close to normal values observed during treatment, may be supportive for the favourable effects of doxecitine and doxribtimine. The biomarker data may indirectly support the efficacy of doxecitine / doxribtimine, according to the SAG-Neurology.

The use of feeding tubes and ventilatory support were a priori regarded as clinically relevant outcomes, reflecting patient impact of dysphagia and respiratory difficulties. However, there was no clear change in these outcomes before treatment and after treatment initiation. These outcomes therefore do not weigh in the B-R.

Unfavourable effects

The safety data are considered representative for the intended target population, despite small numbers. In the study population of N=50 participants and in the target population of n=39 participants, the safety profile of doxecitine/doxribtimine was generally manageable. A considerable portion of the patients was paediatric, which is in line with the target population. The number of included patients falls below the usual standard of N=100 patients followed for at least 12 months. This is to some extent compensated by the long exposure times of the patients: most (80%) patients of the target population had an exposure time ≥5 years, and drop-out and permanent discontinuations were infrequent. This latter supports that the ADRs are manageable in the target population.

Among the most frequent AEs were: diarrhoea, vomiting, abdominal pain (upper) and ALT/AST increased, which were a priori suspected as ADRs by the applicant. It is relevant for patient burden that ADRs of GI disturbance (diarrhoea, vomiting, abdominal pain) were frequent but usually did not coincide and resolved with or without (temporarily) dose reduction. Dose reductions were necessary in a considerable portion (23%) of patients from the target population, commonly due to diarrhoea. Events of diarrhoea, vomiting and abdominal pain (upper) were generally mild or moderate, transient and resolved. Consequently, these 3 unfavourable effects appear as manageable and do not discount heavily on the benefits in the B/R assessment; recurrent or chronic diarrhoea may weigh heaviest due to frequency/recurrence and patient impact.

It is uncertain that ALT/AST elevations are ADRs. As these are also a frequent manifestation of TK2d, did not lead to hepatic failure in the study, and resolved while on treatment, it is not considered that this uncertainty should have bearings on the B/R assessment.

Other frequent AEs were considered as manifestations of TK2d or as comorbidities and do not count in the safety considerations of doxecitine/doxribtimine. SAEs occurred in a considerable portion (42%) of patients from the target population, but the SAEs and deaths that occurred are not considered as treatment-related and therefore do not negatively impact B/R.

3.7.2. Balance of benefits and risks

The benefit-risk balance of Kygevvi (doxecitine/doxribtimine) is positive.

The main body of evidence supporting efficacy of doxecitine / doxribtimine is derived from motor milestones data. The regain of motor milestones during treatment is considered plausibly related to treatment, considering the natural course of the disease. The reduced frequency of new losses of motor milestones during treatment compared to pretreatment is in support of this. Biomarker data are in support of a regain of mitochondrial function. The proof-of-concept of restoration of mtDNA levels by treatment with Kygevvi is supported by valid mouse models, which are considered representative for treatment effects in humans with TK2d.

A survival benefit is suggested, but the data are biased due to insuperable differences in patient populations and study designs. As a result, a clear survival benefit cannot be established. Data on feeding and ventilatory support were limited due to small numbers and not adding clear evidence on treatment effect.

The unfavourable effects (ADRs) are disturbances of the gastro-intestinal tract with diarrhoea, vomiting and abdominal pain, which appear to be manageable with or without dose reductions.

During the assessment, the applicant was requested to reword the indication to ensure genetic confirmation of the disease, aligned with the population studied in this application. The applicant agreed to the request from CHMP and revised the indication in section 4.1 of the SmPC as follows: *'KYGEVVI is indicated for the treatment of paediatric and adult patients with genetically confirmed thymidine kinase 2 deficiency (TK2d) with an age of symptom onset on or before 12 years'.*

Knowledge of the disease is still limited and the small patient sample inherently leaves some uncertainties in the clinical data. Collection of relevant data post-authorisation in a PASS (as a Specific Obligation) is agreed (study TK109), which will include endpoints to support efficacy secondary to safety of doxecitine and doxribtimine.

3.7.3. Oral explanation

During an oral explanation at the CHMP plenary meeting in December 2025, the applicant argued that section 5.1 of the SmPC should include a statement on survival benefit. The applicant argued that all statistical models to mitigate bias confirm robustness of survival benefit.

The applicant argued that adding at the end the statement saying "the magnitude of survival benefit could however not be determined" as proposed by the CHMP will raise question on any impact on mortality.

The applicant therefore made the following proposal for Section 5.1 of the SmPC:

Survival

In the MT-162-101 and TK0102 studies, where all patients were treated for more than five years, no deaths were observed in the 39 patients with an age of symptom onset ≤ 12 years. The uncontrolled nature of the studies does not allow for a fully unbiased estimate of the magnitude of survival benefit. However, when comparing treated patients to matched, untreated patients, there was a reduction of risk of death ranging from 78% to 95% across various survival models and using different types of matching.

In case the above proposal would not be acceptable for the CHMP, the applicant made the following alternative proposal:

No deaths were observed in the 39 patients with an age of symptom onset ≤ 12 years. The uncontrolled nature of the studies does not allow for a fully unbiased estimate of the magnitude of survival benefit. However, data indicated a survival benefit in patients with TK2d treated with doxecitine and doxribtimine compared to matched untreated patients.

As a justification for the proposal, the applicant stated that:

- Premature death is a defining and clinically significant feature of TK2d for patients with symptom onset ≤ 12 years of age,
- Survival outcomes are critical for understanding disease prognosis and for guiding treatment decisions,
- The proposed wording is strictly factual, transparently reports observed data, and explicitly acknowledges the limitations of the evidence due to the uncontrolled nature of the studies,
- This approach aligns with the SmPC's purpose to provide healthcare professionals with accurate, balanced, and clinically relevant information. Including survival data ensures that prescribers and patients have access to the totality of evidence available at the time of authorisation, enabling informed decision-making regarding therapy initiation and continuation.

During the OE, the applicant also provided an overview on how they plan to collect post-approval data via independent registries and a proposed PASS study (TK0109).

The CHMP considered that survival data should not be included in section 5.1 of the SmPC, since it is substantially biased and not methodologically robust (see uncertainties); as such, it does not adequately inform prescribers on the efficacy of the medicine, which is among the essence of section 5.1. The applicant was therefore explicitly requested to remove the text on survival from section 5.1 of the SmPC. In its response, the applicant proposed to include only factual data on survival without comparison with untreated patients, in section 5.1 of the SmPC. The proposed text '*Up to the clinical cutoff date of 14 March 2025, no deaths were observed in this group*' was proposed to be included at the end of the section on the description of the patient population. This was not considered acceptable

by the CHMP, mainly due to the fact that a statement on the absence of deaths will be interpreted as a beneficial outcome, which is not considered justified based on the biased survival data. The applicant eventually agreed to remove the text on survival from section 5.1 of the SmPC.

3.7.4. Additional considerations on the benefit-risk balance

Marketing Authorisation under exceptional circumstances

The applicant requested consideration of its application for a Marketing Authorisation under exceptional circumstances in accordance with Article 14(8) of the above mentioned Regulation (section 2.5.4). The applicant based the proposal on the following ground: *"Inability to provide comprehensive efficacy and safety data due to rarity of the indication"* (see EMEA/357981/2005).

1. *Quality of evidence*

   A randomised controlled trial has not been performed and a large part of the efficacy data were retrospectively collected, in the treated as well as in the untreated patients. Specifically, a positive selection may have occurred in selecting treated patient data and a negative selection may have occurred in sampling comparative data from untreated patients. The dose-response relationship has not been quantified.

   Performing a randomised controlled trial in the target population, with the highest unmet medical need, is not considered feasible. This is due to the rarity of the disorder, as well as the long time it takes to ascertain data for the most relevant outcomes: survival and motor function.

   Long term follow up of treated patients with TK2d is an acceptable way to ascertain data concerning the fate of these patients. This is feasible, because these patients have a high medical need and are treated and followed in specialist centres.

2. *Efficacy: precision of effect size*

   The effect size of treatment versus being untreated, is estimated using expectations from the natural course, as well as a by comparison with an external matched cohort of untreated patients. Under these conditions, it must be assessed whether or not the main favourable effects are present or absent. The precise size of the treatment effect cannot be reliably estimated.

3. *Efficacy: clinical meaningfulness*

   Given the nature of TK2d in the target population of patients with symptom onset ≤12 years, motor development and survival are clinically relevant outcomes, as assessed. There are no established biomarkers or intermediate outcomes that could act as proxy outcomes, with the aim of shortening observation periods of a trial.

4. *Efficacy: duration of efficacy*

   TK2d is a genetic defect and as such the shortage of TK2 is life-long, although it is not clear whether the effect of treatment or even the treatment need is constant over time. There are some data of adults who started treatment >18 years but had symptom onset ≤12 years of age. There is little experience of long-term treatment in patients of the target population. Long term follow up of treated patients is an acceptable way to ascertain data concerning the fate of these patients.

5. *Safety: exposure*

The sample of treated patients (N=67) falls below the standard of at least N=100 patients treated for at least 12 months. This is partly compensated by the average observation time of 5 years on treatment. However, unexpected/infrequent ADRs cannot be discovered with a small sample and without a control group. Long term follow-up of treated patients is an acceptable way to ascertain safety data in an increasing number of patients, from the two subpopulations (symptom onset <2 years and symptom onset 2≤12 years of age) of the target population.

6. *Safety: length of follow-up*

   The average observation time of 5 years on treatment is relatively long, but the sample is small. While treatment in principle is life-long, lengthening observation time in a registry/ observational study could compensate for a lack of available participants, if there is little withdrawal.

7. *Target population versus study population*

   The target population was part of the study population, the data from the total sample were in line. However, the sizes of the two relevant subpopulations of the target population (symptom onset <2 years and symptom onset 2≤12 years of age) were quite small accordingly. Long term follow up of a larger group of treated patients is an acceptable way to ascertain more data in these subgroups.

8. *Pharmacological rationale*

   The pharmacological rationale is strong. Kygevvi can be seen as orally taken replacement therapy due to lacking TK2 at mitochondrial level. However, it is unclear whether doxecitine/ doxribtimine arrives in a physiologically normal level at the site of action, and the mode of action within the mitochondrion has not been confirmed in humans. The mouse models were considered valid and relevant and are supportive for the rationale.

9. *Natural history*

   A cohort of untreated patients with TK2d within (and outside) the target population was established. However, the cohort was assembled using retrospective data collection of published cases mainly. This may have led to an overestimation of mortality in untreated patients. It is unclear whether a larger and unbiased historical cohort could have been assembled using chart review in specialist centres. If Kygevvi is approved, establishing a prospective cohort of untreated TK2d patients is unethical.

Based on the above, the clinical data are not considered comprehensive. With the now available knowledge, the benefit-risk balance is expected to be the same over the time of treatment.

Marketing authorisation under exceptional circumstances

As comprehensive data on the product are not available, a marketing authorisation under exceptional circumstances was requested by the applicant in the initial submission.

The CHMP considers that the applicant has sufficiently demonstrated that it is not possible to provide comprehensive data on the efficacy and safety under normal conditions of use, because the applied for indication is encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence in form of a randomised controlled trial of sufficient size. Given the lack of proxy outcomes and the observation times needed to assess the outcomes of primary interest (survival and motor development), it would be contrary to generally accepted principles of medical ethics to

collect such information using a randomised controlled trial. Therefore, recommending a marketing authorisation under exceptional circumstances is considered appropriate.

3.8. Conclusions

The overall benefit-risk balance of Kygevvi is positive, subject to the conditions stated in section 'Recommendations'.

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 Kygevvi is favourable in the following indication(s):

Kygevvi is indicated for the treatment of paediatric and adult patients with genetically confirmed thymidine kinase 2 deficiency (TK2d) with an age of symptom onset on or before 12 years.

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

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

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.

Specific Obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measures:

Description	Due date
Non-interventional post-authorisation safety study (PASS): TK0109: to describe the safety and clinical outcomes of doxecitine and doxribtimine treatment in patients with thymidine kinase 2 deficiency (TK2d) with age of symptom onset on or before 12 years.	Annually (with annual re-assessment)
In order to ensure adequate monitoring of safety and efficacy of Kygevvi in the treatment of patients with thymidine kinase 2 deficiency (TK2d), the MAH shall provide yearly updates on any new information concerning the safety and efficacy of Kygevvi.	Annually (with annual re-assessment)

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

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that Doxecitine 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.

The new active substance claim for Doxribtimine has been withdrawn.

Doxecitine/doxribtimine has not been authorised in the Union and considering that doxecitine can be qualified as NAS under the first indent of the Annex I to the NtA Chapter 1, the medicinal product will be considered as containing a new active substance.

Refer to Appendix on new active substance (NAS).

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0287/2022 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.