NDA/BLA Multi-Disciplinary Review and Evaluation

Application Type	NDA Efficacy Supplement SE1
Application Number(s)	018342/S-015
Priority or Standard	Priority
Submit Date(s)	11/24/2025
Received Date(s)	11/24/2025
PDUFA Goal Date	5/24/2026
Division/Office	Division of Neurology 1 / Office of Neuroscience
Review Completion Date	March 9, 2026
Established/Proper Name	Leucovorin calcium
(Proposed) Trade Name	Wellcovorin
Pharmacologic Class	Folate analog
Code name	
Applicant	GlaxoSmithKline (GSK)
Dosage form	Tablet
Applicant proposed Dosing Regimen	Initiate oral dose based on body weight and titrate based on clinical review of patient response: o Less than 40 kg: 1 to 2 mg/kg/day and titrate up to the maximum recommended dosage of 8.5 mg/kg/day o 40 kg or more: 1 to 2 mg/kg/day and titrate to the maximum recommended dosage of 330 mg/day Administer once daily or in divided doses up to 6 times per day. Individual doses should be maintained at no more than 75 mg, and preferably at 25 mg or below.
Applicant Proposed Indication(s)/Population(s)	For the treatment of cerebral folate deficiency with folate receptor 1 mutation (CFD FOLR1) in adult and pediatric subjects
Applicant Proposed SNOMED CT Indication Disease Term for each Proposed Indication	711403001 | Cerebral folate transport deficiency (disorder)
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s) (if applicable)	For the treatment of cerebral folate transport deficiency in adult and pediatric patients who have a confirmed variant in the folate receptor 1 gene (FOLR1-CFTD)
Recommended SNOMED CT Indication Disease Term for each Indication (if applicable)	

Recommended Dosing Regimen	As above

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OPQ=Office of Pharmaceutical Quality
OPDP=Office of Prescription Drug Promotion
OSI=Office of Scientific Investigations
OSE= Office of Surveillance and Epidemiology
DEPI= Division of Epidemiology
DMEPA=Division of Medication Error Prevention and Analysis
DRISK=Division of Risk Management

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Glossary

AC	advisory committee
ADME	absorption, distribution, metabolism, excretion
ADR	adverse drug reaction
AE	adverse event
AI	acceptable intake
API	active pharmaceutical ingredient
ASD	autism spectrum disorder
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDC	Centers for Disease Control and Prevention
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CFD	cerebral folate deficiency
CFTD	cerebral folate transport deficiency
CMC	chemistry, manufacturing, and controls
COA	clinical outcome assessment
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CPCA	Carcinogenic Potency Categorization Approach
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSF	cerebrospinal fluid
CSR	clinical study report
CSS	Controlled Substance Staff
DEPI	Division of Epidemiology
DHOT	Division of Hematology Oncology Toxicology
DMC	data monitoring committee
DPV	Division of Pharmacovigilance
ECG	electrocardiogram
eCTD	electronic common technical document
ETASU	elements to assure safe use
FAERS	FDA Adverse Event Reporting System
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act

FDCA	Federal Food, Drug, and Cosmetic Act
FOLR1	folate receptor alpha gene
FRAA	folate receptor autoantibodies
GCP	good clinical practice
GRMP	good review management practice
HFM	hereditary folate malabsorption
ICH	International Conference on Harmonisation
IM	intramuscular
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
MRI	magnetic resonance imaging
MTHF	5-methyltetrahydrofolate
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application
NDSRI	nitrosamine drug substance-related impurities
NME	new molecular entity
OCS	Office of Computational Science
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PAS	prior approval supplement
PBRER	Periodic Benefit-Risk Evaluation Report
PCFT	proton-coupled folate transporter
PD	pharmacodynamics
PeRC	Pediatric Review Committee
PI	prescribing information
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert (also known as Patient Information)
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan

SGE	special government employee
SOC	standard of care
TEAE	treatment emergent adverse event

1 Executive Summary

1.1. Product Introduction

Wellcovorin (leucovorin calcium) is a folate analog initially approved in 1983 under New Drug Application (NDA) 018342 for the indication "to diminish the toxicity and counteract the effects of impaired methotrexate elimination and inadvertent overdosages of folic acid antagonists."

Glaxo Wellcome (hereafter GSK or the Applicant) discontinued marketing and manufacturing of Wellcovorin (leucovorin calcium) tablets in the US in 1997 and NDA 018342 was withdrawn in 1999.

On April 28, 2017, FDA announced in the Federal Register that Wellcovorin (leucovorin calcium) tablets, EQ 5 mg base and EQ 25 mg base, were not withdrawn from sale for reasons of safety or effectiveness under 21 CFR 314.161.

On September 24, 2025, the Agency issued a Federal Register notice to approve the previously withdrawn NDA 018342 for Wellcovorin on the basis of new data.

On October 10, 2025, FDA issued a Prior Approval Supplement (PAS) request to GSK requesting that the Applicant submit a PAS for NDA 018342 for the treatment of cerebral folate deficiency.

With this submission, the Applicant is seeking an indication for Wellcovorin (leucovorin calcium) tablets, for oral use, for the treatment of cerebral folate deficiency in adult and pediatric subjects with folate receptor 1 mutation. The proposed oral dosage for subjects with cerebral folate deficiency is 1 to 2 mg/kg/day initially for all subjects, with a maximum daily dosage of 8.5 mg/kg/day for subjects weighing less than 40 kg and 350 mg/day for subjects weighing 40 kg or more, administered once daily or in divided doses, with individual doses preferably maintained at 25 mg or below to optimize bioavailability, as doses should be rounded to the nearest tablet strength or combination of strengths. The available dosage forms and strengths include 5 mg and 25 mg tablets.

1.2. Conclusions on the Substantial Evidence of Effectiveness

Cerebral folate deficiency (CFD) is a distinct neurological disorder characterized by decreased cerebrospinal fluid (CSF) folate levels with normal systemic folate status, fundamentally differing from systemic folate deficiency by selectively impacting brain function through impaired folate transport across the blood-brain barrier or disrupted central nervous system folate metabolism. The primary cause of CFD is impaired folate transport into the central nervous system, and the most well characterized cause is due to variants in the folate receptor alpha (FOLR1) gene. Cerebral folate transport deficiency due to a variant in FOLR1 (FOLR1-

CFTD) is an extremely rare autosomal recessive progressive neurologic disorder with an estimated prevalence of less than 1 in 1,000,000 individuals. Although there may be transient variability in some symptoms such as seizures, substantial improvement or complete resolution of neurologic symptoms is not expected in the course of FOLR1-CFTD in the absence of treatment.

In addition to supportive care and symptomatic treatments (e.g., medications for seizures), leucovorin is used in clinical practice to treat FOLR1-CFTD and is considered standard-of-care. There is strong scientific and mechanistic rationale for the use of leucovorin, a folate analog that crosses the blood-brain barrier, to treat this condition which causes folate deficiency within the central nervous system. Leucovorin is an FDA-approved product that has been marketed for many decades to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosages of folic acid antagonists. It has a well-established safety profile, with generally good tolerability.

Given the strong prior foundational knowledge about the pathophysiology of FOLR1-CFTD and the reported benefits of leucovorin in this condition, the Agency undertook a systematic review of the published scientific literature to assess the evidence of effectiveness for leucovorin for the treatment of FOLR1-CFTD. Although leucovorin is already used as standard-of-care, there is public health benefit to establishing the safety and effectiveness of a therapy as it may increase recognition and diagnosis of a treatable disorder and potentially improve access to treatment.

The review identified 46 unique subjects based on prespecified eligibility criteria who had genetically confirmed variants in the FOLR1 gene, received treatment with leucovorin, and had available clinical outcome data. Patient-level data was extracted from the case reports and case series.

Descriptive analyses were conducted based on changes in relevant clinical symptom domains (i.e., seizure control, motor function, developmental abilities and behavioral features). Changes in clinical symptom domains was categorized by number of domains impacted and magnitude of effects so that a change in scoring by one category would clearly represent a clinically meaningful change. Clinical symptom domains were evaluated as a change from pre-treatment baseline and were compared to an estimate of no change/decline in the trajectory of these domains that was based on the natural history of FOLR1-CFTD in the absence of treatment with leucovorin. This estimate of the anticipated disease trajectory was generally derived from descriptions of the clinical course in patients prior to initiation of treatment with leucovorin or who did not receive treatment with leucovorin. As the initiation of treatment with leucovorin was often delayed in patients, this allowed for characterization of the relentlessly progressive nature of the condition, over years in some cases, to serve as a control for the study.

Of the 46 identified subjects in the literature, 30 (65%) experienced complete recovery or substantial improvement in symptoms, while 6 sujects (13%) showed no improvement including

3 subjects with very prolonged treatment delays exceeding 13 to 15 years. When compared to an estimate of no change or decline in these symptom domains in the natural history of the disease, the consistent finding of substantial improvement or complete recovery in a majority of treated patients is highly unlikely to have occurred spontaneously, is inconsistent with the known natural history of the disease, and cannot reasonably be attributed to alternative treatments or natural variability in disease phenotype. The magnitude of the treatment effect is sufficiently large and convincing to overcome biases inherent to the design of the study and retrospective collection of data from published literature. Overall, the Agency determined that the data from this systematic review are adequate to support the efficacy of leucovorin for FOLR1-CFTD.

Confirmatory evidence is provided by strong mechanistic evidence of the treatment effect of leucovorin on the established pathophysiology of cerebrale folate transport deficiency. In subjects with available CSF samples, the levels of CSF 5-methyltetrahydrofolate (MTHF), the predominant physiological form of folate in the CSF, were normalized in 80% of subjects after treatment with leucovorin.

Despite the lack of standardization in dosing regimens across the case reports, the dosing regimen for FOLR1-CFTD was assessed by evaluating the range of doses administered across all 46 subjects, considering factors such as age, body weight, clinical response, and safety outcomes reported in the literature. The Agency analyzed dosing patterns from the published FOLR1-CFTD cases as well as dosing information from related conditions. Based on this comprehensive assessment, the recommended dosage regimen is to initiate treatment at 1 to 2 mg/kg/day based on body weight and titrate based on clinical response, with maximum recommended dosages of 8.5 mg/kg/day for patients less than 40 kg or 330 mg/day for patients 40 kg or more, administered once daily or in divided doses up to 6 times per day with individual doses maintained at no more than 75 mg (preferably 25 mg or below).

Given the use of leucovorin as a standard of care for FOLR1-CFTD and extreme rarity of the condition, it would not be feasible to conduct a randomized, controlled trial. With these challenges in mind, it is appropriate to consider data from published case reports and case series, if sufficient information can be obtained from these publications to meet the evidentiary standards for approval.

With consideration of the need for substantial flexibility in this extremely rare and serious condition and given the very strong scientific and mechanistic rationale for use of leucovoin in FOLR1-CFTD, the systematic review of the literature and the descriptive analysis of published case reports may be considered to meet the criteria for an adequate and well-controlled study. The review was designed with a clearly stated objective to evaluate the evidence for the efficacy of leucovorin in subjects with FOLR1-CFTD with a description of the prespecified methods used for the selection of reports and and assessment of the data. Requirements for verification of variants in the FOLR1 gene allowed for certainty in the patient population that

was included in the review. Although the clinical assessments for efficacy and safety were not standardized or prospectively specified, the review methodology specified coarse criteria for the assessment of relevant clinical symptom domains that allowed for quantification of large and clinically meaningful improvements in symptoms to minimize the potential for bias. An estimate of no change/decline in symptoms was capable of serving as a valid control based on a characterization of the relentlessly progressive decline in patients prior to initiation of treatment with leucovorin, which spanned many years in some cases. Additionally, descriptive analytical methods were found to be adequate to assess efficacy in this study given that the treatment effects were so large that they would be unexpected in the natural history of the disease. Additionally, there was consistency of these findings with 65% of the treated patients reported in the literature experiencing substantial improvement or complete resolution of symptoms.

There are substantial limitations and potential biases in the systematic review that include, but are not limited to, the lack of standardization of assessments and dosing regimens, retrospective analyses, and potential for positive reporting bias. The Division considered these limitations as part of its review, but nonetheless, finds the data from the review, together with confirmatory evidence based on strong mechanistic data, to be compelling and adequate to demonstrate substantial evidence of effectiveness for leucovorin for the treatment of FOLR1-CFTD.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

Cerebral folate transport deficiency (FOLR1-CFTD) is a rare autosomal recessive progressive neurologic disorder (prevalence <1 in 1,000,000) caused by variants in the FOLR1 gene that encodes the receptor responsible for folate transport into the central nervous system. Laboratory testing demonstrates decreased cerebrospinal fluid folate levels despite normal systemic folate status due to impaired folate transport into the central nervous system. The disease is characterized by severe, progressive, neurological deterioration including developmental delays, refractory seizures, and motor dysfunction that can progress to severe immobility and ultimately death without treatment. Although there may be transient variability in some symptoms such as seizures, substantial improvement or complete resolution of neurologic symptoms is not expected in the course of the disease in the absence of treatment. Currently, there are no approved treatments for cerebral folate deficiency of any etiology, creating a significant unmet medical need, particularly for the severe and fatal FOLR1-CFTD condition.

Leucovorin is a folate analog that crosses the blood-brain barrier and is considered standard-of-care alongside supportive and symptomatic treatment, for managing FOLR1-CFTD in clinical practice, based on strong scientific and mechanistic rationale. As an FDA-approved product marketed for decades to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosages of folic acid antagonists, leucovorin has a well-established safety profile and generally good tolerability.

The evaluation of leucovorin for the treatment of FOLR1-CFTD was based on a systematic review of the published scientific literature The review identified 46 unique subjects with genetically confirmed FOLR1-CFTD treated with leucovorin. The study population was predominantly female (67%) with early disease onset (mean age 2.2 years) and severe baseline manifestations including near-universal white matter changes on MRI (98%), motor dysfunction (96%), and refractory seizures (85%); 33 of the 46 subjects (72%) presented with significant neurodevelopmental and behavioral delays.

Baseline CSF 5-MTHF levels (7.17±9.30 nmol/L, Mean±SD) were available for 32 subjects (70% of the cohort), serving as both a diagnostic biomarker for FOLR1-CFTD and a pharmacodynamic biomarker of treatment response. Among those tested, 84% had severely reduced CSF 5-MTHF levels below 10 nmol/L, confirming the diagnosis.

Treatment with leucovorin was administered orally (59%) or in a combination of oral and intravenous (IV) administration (30%). Oral leucovorin

dosing ranged from 0.5 mg/kg/day to 11 mg/kg/day, with the majority of subjects receiving doses between 2-6 mg/kg/day, while weight-based IV doses ranged from 6 mg/kg administered weekly to 25 mg/kg given monthly. Dose escalation strategies were commonly employed, and the majority of subjects appeared to require indefinite treatment based on follow-up data spanning 1 to 5 years. Leucovorin demonstrates high solubility and bioavailability greater than 95% for oral and intramuscular formulations compared to intravenous administration up to 25 mg, with adequate PK bridging between different formulations.

Descriptive analyses were conducted based on changes in relevant clinical symptom domains (i.e., seizure control, motor function, developmental abilities and behavioral features). Changes in the clinical domains were categorized by number of domains impacted and magnitude of effects so that a change in scoring by one category would clearly represent a clinically meaningful change. Clinical symptom domains were evaluated as a change from pre-treatment baseline and were compared to an estimate of no change/decline in the trajectory of these clinical domains that was based on the natural history of FOLR1-CFTD in the absence of treatment with leucovorin.

This estimate of the anticipated disease trajectory was generally derived from description of the clinical course in patients prior to initiation of treatment with leucovorin or who did not receive treatment with leucovorin. As the initiation of treatment with leucovorin was often delayed in patients, this allowed for characterization of the relentlessly progressive nature of the condition, over years in some cases, to serve as a control for the study.

The efficacy assessment framework categorized treatment responses as complete recovery (normal or near-normal neurological function with resolution of major symptoms), substantial improvement (clinical gains across two or more functional domains), partial improvement (gains in one domain with persistent deficits in others), or no improvement (stable without clinical benefit or worsening).

Overall, 40 of 46 subjects (87%) experienced some degree of clinical benefit from leucovorin therapy, with 30 subjects (65%) achieving substantial improvement or complete recovery. Complete recovery was achieved in 3 subjects (7%), all of whom received treatment with minimal delay after symptom onset. Substantial improvement, the largest response category, was observed in 27 subjects (59%) who demonstrated clinical gains including seizure freedom or significant reduction combined with motor and developmental progress. Partial improvement was documented in 9 subjects (20%), while no improvement occurred in 6 subjects (13%), including three with very prolonged treatment delays exceeding 13 to 15 years, one with concurrent POLG1 mutations, and two who remained stable without demonstrable clinical benefit.

The therapeutic effect was further substantiated by levels of 5-MTHF in CSF serving as a biomarker of treatment response. Pre and post-

treatment CSF 5-MTHF levels were available for 15 subjects. The pre-treatment CSF 5-MTHF levels were 7.72±12.58 nmol/L (Mean±SD); and post treatment CSF levels for the same subjects were 66.37±33.28 nmol/L (Mean±SD), showing substantial improvements in central nervous system folate availability following leucovorin therapy. All 15 subjects showed some improvement in CSF 5-MTHF levels but normalization of CSF levels to values above 40 nmol/L was achieved in 12 out of 15 subjects (80%). Subjects with normalized 5-MTHF levels also demonstrated greater clinical improvement, providing strong mechanistic evidence that leucovorin's restoration of CSF folate levels was directly responsible for the observed clinical outcomes.

When compared to an estimate of no change or decline in these symptom domains in the natural history of the disease, the consistent finding of substantial improvement or complete recovery in a majority of treated subjects is highly unlikely to have occurred spontaneously, is inconsistent with the known natural history of the disease, and cannot reasonably be attributed to alternative treatments or natural variability in disease phenotype. The magnitude of the treatment effect is sufficiently large and convincing to overcome biases inherent to the design of the study and retrospective collection of data from published literature. Overall, the Agency determined that the data from this systematic review are adequate to support the efficacy of leucovorin for FOLR1-CFTD.

Leucovorin demonstrates an acceptable safety profile for treatment of FOLR1-CFTD, consistent with its well-established safety profile in approved indications. No deaths or treatment-related serious adverse events were reported across all reviewed cases. The limited systematic adverse event reporting available showed minimal safety concerns, with only mild leukopenia possibly related to intravenous treatment in one case. Post-marketing surveillance over more than 70 years identified no new safety signals relevant to the FOLR1-CFTD indication, with the few adverse reactions primarily consisting of hypersensitivity reactions. However, given the small database of 46 subjects with FOLR1-CFTD, existing warnings and precautions in the labeling cannot be excluded in this population and will be maintained.

The substantial clinical benefits that have been demonstrated, including potential for complete recovery in a rare fatal condition, and confirmatory evidence provided by strong mechanistic evidence of the treatment effect of leucovorin on the established pathophysiology of FOLR1-CFTD outweigh the minimal safety risks associated with leucovorin therapy in FOLR1-CFTD such that the benefit-risk is favorable.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Cerebral folate deficiency (CFD) is a distinct neurological disorder characterized by decreased cerebrospinal fluid (CSF) folate levels with normal systemic folate status, fundamentally differing from systemic folate deficiency by selectively impacting brain function through impaired folate transport across the blood-brain barrier or disrupted central nervous system folate metabolism. • The primary cause of CFD is impaired transport into the central nervous system and the most well characterized syndrome is due to variants in the folate receptor alpha (FOLR1) gene (FOLR1-CFTD). • FOLR1-CFTD is an extremely rare autosomal recessive neurometabolic disorder (prevalence <1 in 1,000,000), resulting from genetic defects in the FOLR1 gene encoding FRα, a glycosylphosphatidylinositol-anchored protein that facilitates transport of 5-methyltetrahydrofolate (5-MTHF), the prevalent physiologic form of folate, across the choroid plexus into cerebrospinal fluid. • FOLR1-CFTD is a severely debilitating and life-threatening condition with early childhood onset and presenting with developmental delays, and progressive neurological deterioration including cognitive decline, refractory seizures, and motor dysfunction that can progress to severe immobility without treatment. • Subjects typically have extremely low CSF 5-MTHF levels of less than 10 nmol/L.	• FOLR1-CFTD is an extremely rare, severely debilitating, and life-threatening autosomal recessive disorder caused by FOLR1 gene mutations. The condition is characterized by extremely low cerebrospinal fluid (CSF) folate levels despite normal systemic folate concentrations. FOLR1-CFTD manifests with progressive neurological deterioration including developmental delays, uncontrolled seizures, and motor dysfunction, ultimately leading to a fatal outcome without treatment. Although there may be transient variability in some symptoms such as seizures, substantial improvement or complete resolution of neurologic symptoms is not expected in the course of the disease in the absence of treatment.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Current Treatment Options	• There is currently no treatment approved for CFD of any etiology. • Leucovorin (folinic acide), is a folate analog that crosses the blood-brain barrier approved to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosages of folic acid antagonists. • Leucovorin, , is commonly used off-label in conjunction with supportive case and symptomatic treatment, for treatment of FOLR1-CFTD.	FOLR1-CFTD is a severe debilitating and fatal disease; there is a high unmet need in this population for which there are no approved therapies. Although leucovorin is already used as standard-of-care, there is public health benefit to establishing the safety and effectiveness of a therapy as it may increase recognition and diagnosis of a treatable disorder and potentially improve access to treatment.
Benefit	• Efficacy evaluation was based on a systematic review and descriptive analysis of published cases and case series available in the scientific literature. Given the extreme rarity of the condition and the strong prior foundational knowledge about the pathophysiology of FOLR1-CFTD including the established use of leucovorin,, well-characterized case reports were deemed an adequate source of clinical data. • Forty-six (46) unique subjects were identified with FOLR1-CFTD with genetically confirmed FOLR1 variants, who underwent treatment with leucovorin (folinic acid) and had available outcomes. • Of the 46 FOLR1-CFTD cases, 31 (67%) were females with an age ranging from 2 to 33 years and average age at onset of 2.2 (SD 1.4) years. • At baseline almost all subjects had white matter changes on brain MRI	The systematic review and descriptive analysis of the literature cases demonstrates effectiveness of leucovorin in the treatment of FOLR1-CFTD in adult and pediatric patients. Treatment with leucovorin, administered orally or in combination with intravenous routes at doses ranging from 0.5-11 mg/kg/day resulted in complete recovery in 3 subjects (7%) and substantial improvement in 27 subjects (59%) across relevant clinical symptom domains such as seizure control, motor function, developmental abilities and behavioral features. No improvement occurred in 6

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	(44/45 of those scanned), 44/46 (96%) had motor signs, 39/46 (85%) had uncontrolled seizures refractory to standard of care, and 33/46 (72%) presented with neurodevelopmental and behavioral delays. • Baseline CSF 5-MTHF levels (7.17±9.30 nmol/L, Mean±SD) were available for 32 subjects (70% of the cohort), serving as both a diagnostic biomarker for FOLR1-CFTD and a pharmacodynamic biomarker of treatment response. Among those tested, 84% had severely reduced CSF 5-MTHF levels below 10 nmol/L. • Treatment with leucovorin was administered orally (59%) or orally in combination with intravenous (IV) administration (30%). Oral leucovorin dosing ranged from 0.5 mg/kg/day to 11 mg/kg/day, with the majority of subjects receiving doses between 2-6 mg/kg/day. Weight-based IV doses ranged from 6 mg/kg administered weekly to 25 mg/kg given monthly. Dose escalation strategies were commonly employed based on response to treatment. Treatment duration data varied considerably across the case reports, with the majority of subjects appeared to require indefinite treatment. • Leucovorin demonstrates high solubility and bioavailability greater than 95% for oral and intramuscular formulations compared to intravenous administration up to 25 mg, with adequate pharmacokinetic bridging between different formulations. • Descriptive analyses were conducted based on changes in relevant clinical symptom domains (i.e., seizure control, motor function, developmental abilities and behavioral features). Changes in clinical domains were categorized by number of domains impacted and magnitude of effects so that a change in scoring by one category would clearly represent a clinically meaningful change. Clinical	subjects (13%), half of whom experienced prolonged treatment delays exceeding 13 to 15 years. The delayed initiation of leucovorin treatment in many patients allowed for characterization of the relentlessly progressive nature of FOLR1-CFTD over years, serving as a natural history control, while also demonstrating that earlier intervention is associated with better clinical outcomes. CSF 5-MTHF levels increased from mean baseline CSF values of 7.72±12.58 nmol/L to 66.37±33.28 nmol/L post-treatment, with 80% of sampled subjects achieving normalization above 40 nmol/L. The consistent finding of substantial improvement or complete recovery is highly unlikely to have occurred spontaneously, is inconsistent with the known natural history of the disease, and cannot reasonably be attributed to alternative treatments or natural variability. The magnitude of the treatment effect is sufficiently large to overcome biases inherent to retrospective data collection from published literature. Confirmatory evidence is provided by strong mechanistic evidence of leucovorin's effect on

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	symptom domains were evaluated as a change from pre-treatment baseline and were compared to an estimate of no change/decline in the trajectory of these clinical domains that was based on the natural history of FOLR1-CFTD in the absence of treatment with leucovorin. • CSF 5-MTHF levels were considered predictive biomarker of treatment effect. When available pre-treatment values were compared to post-treatment values and clinical outcomes. • Complete recovery was achieved in 3 subjects (7%), all of whom appeared to receive treatment with minimal delay after symptom onset, including one patient treated pre-symptomatically. • Substantial improvement was observed in 27 subjects (59%), who demonstrated clinical gains across multiple functional domains ranging from seizure freedom to developmental gains such as language recovery and independent ambulation. • No improvement was observed in 6 subjects (13%), with 3/6 subjects experiencing prolonged treatment delays exceeding 13-15 years. One subject presenting with concurrent POLG1 mutations and two subjects remained stable but showed no further improvement. • Post-treatment CSF 5-MTHF levels (66.37±33.28 nmol/L; Mean±SD) were available for 15 subjects out of 32 who had baseline values. Twelve subjects (80%) achieved normalization of CSF 5-MTHF levels above 40 nmol/L. Subjects achieving CSF 5-MTHF normalization demonstrated greater levels of clinical improvement. • The analysis demonstrates substantial improvement in symptoms and changes in disease trajectory that are not expected in the natural history of FOLR1-CFTD and cannot reasonably be attributed to alternative treatments or natural variability in disease phenotype. The	CSF folate levels, which is the established cerebral folate transport deficiency pathway.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	magnitude of the treatment effect is sufficiently large and convincing to overcome biases inherent to the retrospective collection of data from published literature to establish the efficacy of leucovorin for FOLR1-CFTD • Confirmatory evidence is provided by strong mechanistic evidence of the effect of leucovorin on cerebral folate levels, which is the established pathophysiology of cerebral folate transport deficiency.	
Risk and Risk Management	• The overall understanding of the safety profile for leucovorin is informed by the toxicology, safety pharmacology, and clinical safety review of NDA 018342. • Because leucovorin is an approved and marketed product, the safety assessment also included information obtained in the postmarketing setting. • The safety assessment for leucovoin within the FOLR1-CFTD population is based on clinical safety data extracted from the published case reports in subjects with FOLR1-CFTD treated with leucovorin. The safety database includes 46 subjects with FOLR1-CFTD. • No deaths were reported and and no treatment-related serious adverse events were documented across the reviewed studies. • Notably, only three cases from the literature provided systematic adverse event reporting, with two subjects reporting no side effects and one experiencing mild leukopenia possibly related to intravenous treatment. Laboratory monitoring data were limited but revealed no clinically significant abnormalities. • Post-marketing surveillance over more than 70 years of leucovorin use identified no new safety signals relevant to the FOLR1-CFTD	The safety profile of leucovorin is well characterized when considering the entire safety database across all development programs. Based on the reviewed cases and studies, leucovorin demonstrates an acceptable safety profile for the treatment of FOLR1-CFTD, consistent with its established safety profile in approved indications. The absence of new safety signals, with consideration of the severity of untreated FOLR1-CFTD, supports a favorable risk-benefit assessment for this indication. Continued post-marketing surveillance remains appropriate to monitor for any emerging safety concerns as clinical experience expands in this population. If GSK decides to restart commercial distribution of this product, the Applicant

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	indication. • The few adverse reactions identified were primarily hypersensitivity reactions in three subjects, supporting the retention of existing warnings and precautions in product labeling. • GSK discontinued commercial marketing of its product in 1997. Because no GSK products exist for testing and GSK further indicates that it does not intend to market or manufacture the product following approval of this PAS, the risk of nitrosamine formation in the drug product cannot be assessed at this time.	would need to submit a supplement with information sufficient to demonstrate that the to-be-marketed product meets all current legal and regulatory requirements. Such a supplement will need to include any chemistry, manufacturing and controls changes needed to comply with relevant compendial requirements or to address current recommendations for control of impurities, including nitrosamine-related impurities along with supporting data as described in the Nitrosamine Guidance and RAIL Guidance.

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

				Section of review where discussed, if applicable
☐	The patient experience data that were submitted as part of the application include:			
	☐	Clinical outcome assessment (COA) data, such as		
		☐	Patient reported outcome (PRO)	
		☐	Observer reported outcome (ObsRO)	
		☐	Clinician reported outcome (ClinRO)	
		☐	Performance outcome (PerfO)	
	☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Natural history studies		
	☐	Patient preference studies (e.g., submitted studies or scientific publications)		
	☐	Other: (Please specify):		
☐	Patient experience data that were not submitted in the application, but were considered in this review:			
	☐	Input informed from participation in meetings with patient stakeholders		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Other: (Please specify):		
☒	Patient experience data was not submitted as part of this application.			

2 Therapeutic Context

2.1. Analysis of Condition

Cerebral folate deficiency (CFD) represents a distinct category of neurological disorders that fundamentally differs from systemic folate deficiency in its pathophysiology and clinical presentation. First conceptualized by Ramaekers and Blau in 2004 (Ramaekers, 2004), CFD is defined as any neuropsychiatric or neurodevelopmental disorder characterized by decreased CSF folate levels, in the presence of normal folate status outside the nervous system. This condition specifically affects the central nervous system while leaving peripheral folate metabolism intact, creating a unique clinical challenge that requires specialized diagnostic approaches and targeted therapeutic interventions.

Unlike systemic folate deficiency, which affects multiple organ systems and presents with characteristic hematological abnormalities, CFD selectively impacts brain function through impaired folate transport across the blood-brain barrier or disrupted folate metabolism within the central nervous system.

Folate, also known as vitamin B9, is an essential micronutrient derived from dietary sources that supports normal cellular function (Scaglione, 2014). The predominant physiological form of folate is 5-methyltetrahydrofolate (5-MTHF), an essential cofactor for the re-methylation of homocysteine to methionine, generating S-adenosylmethionine, which serves as the primary methyl donor for numerous methylation reactions in cells (Scaglione, 2014; Pope, 2019). In the nervous system, folate metabolism is critical during development and throughout life for maintaining proper myelination, neurotransmitter synthesis, and cellular repair mechanisms (Naninck, 2019).

A key mechanism underlying CFD involves dysfunction of the folate receptor alpha (FRα), a glycosylphosphatidylinositol-anchored protein encoded by the FOLR1 gene. FRα facilitates the transport of 5-MTHF across the choroid plexus into the cerebrospinal fluid compartment, maintaining significantly higher concentrations in CSF than in plasma (Djukic, 2007). This transport system is essential for ensuring adequate folate levels in the brain, where folate serves critical roles in neurotransmitter synthesis, DNA methylation, and various metabolic pathways essential for proper neurological function.

Primary forms of CFD are caused by genetic defects in folate transport or metabolism, or may result from autoantibodies directed against FRα; however, the role of autoantibodies has not been confirmed or fully elucidated. The primary form of CFD that is the focus of this review is cerebral folate transport deficiency due to pathogenic FOLR1 variants (FOLR1-CFTD), which is described below.

Cerebral Folate Transport Deficiency with Folate Receptor 1 Mutation (FOLR1-CFTD)

FOLR1-CFTD is a rare autosomal recessive neurometabolic disorder characterized by extremely low CSF 5-MTHF levels (typically less than 10 nmol/L) with normal peripheral folate levels. Symptom onset typically occurs in early childhood with initially subtle clinical signs such as developmental delays, particularly in cognition, speech, and gait. The natural history of untreated disease includes progressive neurological deterioration with developmental regression and cognitive decline, behavioral issues and autistic features, refractory seizures, and motor dysfunction that can progress to severe immobility (Goldman, 2024). Although there may be transient variability in some symptoms such as seizures, substantial improvement or complete resolution of neurologic symptoms is not expected in the course of the disease in the absence of treatment. Estimated prevalence is less than 1 in 1,000,000 individuals. Leucovorin (folinic acid) has been used off-label and has been reported to provide benefit to patients by bypassing the defective transport mechanism and providing readily available folate to the central nervous system (Pope, 2019; Potic, 2023). However, there are no currently approved therapies for FOLR1-CFTD.

Hereditary Folate Malabsorption (HFM)

It is important to distinguish FOLR1-CFTD from hereditary folate malabsorption (HFM), a rare autosomal recessive disorder caused by mutations in the SLC46A1 gene encoding the proton-coupled folate transporter (PCFT) protein, a member of the superfamily of solute carriers (Goldman, 2008). PCFT is highly expressed in the small intestine and is required for intestinal folate absorption. In addition to FRα, PCFT is also expressed in the choroid plexus, and both appear to be required for transport of folate into the CSF (Qiu et al., 2006; Zhao et al., 2017). While HFM does result in profoundly low CSF folate levels due to impaired folate transport across the choroid plexus, it simultaneously causes systemic folate deficiency affecting multiple organ systems. Unlike FOLR1-CFTD, which presents with isolated central nervous system manifestations, HFM typically manifests within the first few months of life with megaloblastic anemia, frequently pancytopenia, immune deficiency with recurrent infections, and failure to thrive, in addition to progressive neurological deterioration if untreated (Goldman, 2008). The presence of folate-deficiency anemia or other systemic manifestations of folate deficiency excludes a diagnosis of FOLR1-CFTD. Furthermore, the intestinal absorption defect in HFM necessitates parenteral leucovorin therapy, as oral formulations are ineffective due to the underlying PCFT transporter dysfunction (Goldman, 2008).

Patients with hereditary folate malabsorption were not included in this review.

Secondary Causes of Low CSF Folate

Low CSF folate can also occur secondarily in the context of other disease processes. In primary mitochondrial diseases such as Kearns-Sayre syndrome caused by large-scale mitochondrial DNA deletions, low CSF folate has been recognized in the presence of normal peripheral folate levels, meeting the definition of secondary CFD (Serrano, 2010). The underlying mechanisms

remain unclear but are hypothesized to involve compromise of choroidal integrity due to accumulation of deleted mitochondrial DNA molecules in the choroid plexus, leading to impaired 5-MTHF transport into the brain (Tanji, 2000). While leucovorin therapy can correct CSF 5-MTHF levels and improve cerebral white matter lesions, it does not prevent the progressive multisystem decline associated with mitochondrial diseases (Quijada-Fraile, 2014).

Low CSF folate has also been documented in association with various other conditions, including serine deficiency disorders, dihydropteridine reductase deficiency, aromatic L-amino acid decarboxylase deficiency, and pyridoxine-dependent epilepsy (Pope, 2019). Additionally, low CSF folate has been linked to certain genetic syndromes such as Rett syndrome and variants of Aicardi-Goutières syndrome, though the precise mechanisms underlying these associations remain under investigation.

Secondary causes of low CSF folate were not included in this review.

CFD Due to Folate Receptor Alpha Autoantibodies (CFD-FRAA)

Serum autoantibodies against folate receptor alpha (FRα autoantibodies or FRAA) have been reported to cause cerebral folate deficiency in subsets of patients with autism spectrum disorders (ASD) and other neuropsychiatric conditions (Ramaekers, 2007a; Ramaekers, 2007b; Ramaekers, 2013; Frye, 2013; Ramaekers, 2014; Wells, 2024). It has been proposed that FRAA may impair folate transport across the choroid plexus and contribute to CFD. However, the pathophysiological significance of these antibodies remains uncertain for several reasons. First, studies examining FRAA prevalence demonstrate remarkable inconsistency, with prevalence ranging from 6% to 89% in ASD subjects and 0% to 55% in controls across different methodologies (Frye, 2013; Zhou, 2018; Shoffner, 2016; Shi, 2024). Second, there are no standardized methods for qualitative or quantitative FRAA testing. Third, the relationship between serum FRAA titers and CSF 5-MTHF levels is inconsistent across studies, and the natural history of CFD due to FRAA remains incompletely characterized.

A literature search identified two randomized placebo-controlled trials of leucovorin in patients with ASD and evidence of FRAA (Frye, 2018; Panda, 2024); however, the Panda et al. 2024 study was retracted due to concerns with the statistical analyses performed (Retraction note: Panda, 2026). Given the uncertain pathophysiological role of FRAA, the substantial methodological inconsistencies in FRAA testing across studies, and the limited controlled trial evidence, studies of leucovorin for the treatment of CFD-FRAA did not form the basis for the efficacy determination in this review. A review of the Frye et al. 2018 study may be found in the Appendix in Section 19.2.

2.2. Analysis of Current Treatment Options

There is currently no product approved for the treatment of any form of cerebral folate deficiency, including FOLR1-CFTD, which is the focus of this review.

3 Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

NDA 018342 for Wellcovorin (leucovorin calcium) tablets was initially approved in 1983 and was indicated to diminish the toxicity and counteract the effects of impaired methotrexate elimination and inadvertent overdosages of folic acid antagonists.

3.2. Summary of Presubmission/Submission Regulatory Activity

The Applicant, Glaxo Wellcome (the Company merged with SmithKline Beecham in 2000 forming GlaxoSmithKline, commonly know, and hereafter referred to as GSK) discontinued marketing and manufacturing of Wellcovorin tablets in the US in 1997, and following GSK's notification that the product was no longer marketed, the Agency announced withdrawal of the NDA approval in the Federal Register on September 22, 1999, under 21 CFR 314.150(c).

In the Federal Register of April 28, 2017, FDA announced its determination that Wellcovorin tablets were not withdrawn from sale for reasons of safety or effectiveness under 21 CFR 314.161.

On September 22, 2025, FDA announced action to initiate the approval of Wellcovorin (leucovorin calcium tablets) for subjects with cerebral folate deficiency (CFD), a neurological condition associated with impaired folate transport into the brain, in accordance with 21 CFR 314.160, which provides, in relevant part, that FDA may, on the basis of new data, approve an application for which it had previously withdrawn approval.

On September 24, 2025, the Agency issued a Federal Register notice to approve the previously withdrawn NDA 018342 for Wellcovorin (leucovorin calcium) tablets, EQ 5 mg base and EQ 25 mg base on the basis of new data.

FDA conducted a systematic review of case reports and case series published between 2009 and 2024, followed by a descriptive systematic analyses of those cases, and determined that the information supports the conclusion that orally administered leucovorin tablets improve certain symptoms in adults and pediatric subjects with FOLR1-CFTD.

On October 10, 2025, FDA issued a Prior Approval Supplement (PAS) request to GSK requesting submission of a PAS for NDA 018342 to revise the prescribing information to include a new indication for the treatment of CFD in adults and pediatric subjects, providing case studies of subjects with FOLR1-CFTD as evidence of the essential scientific information to support safe and effective use and a draft label conforming with PLR content and format requirements.

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

Not Applicable

4.2. Product Quality

Product quality information was not submitted with this sNDA as GSK will not be manufacturing the oral tablet.

In 2020, FDA issued guidance to industry, *Control of Nitrosamine Impurities in Human Drugs* (Rev. 2, Sept. 2024) (Nitrosamine Guidance), recommending that manufacturers of APIs and drug products take steps to detect and prevent unacceptable levels of nitrosamine impurities in drug products. The Nitrosamine Guidance recommends that manufacturers and applicants follow a three-step mitigation strategy: (1) conduct risk assessments for nitrosamines in their APIs and drug products; (2) conduct confirmatory testing if risks are identified; and (3) report changes implemented to prevent or reduce the presence of nitrosamine impurities in APIs and drug products in approved and pending NDAs and ANDAs. That mitigation strategy was also recommended in 2023, when FDA addressed nitrosamine drug substance-related impurities (NDSRIs) in the FDA published the guidance for industry, *Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities* (NDSRIs) (Aug. 2023) (RAIL Guidance). To reflect the evolving and highly technical nature of nitrosamines, a nitrosamine guidance webpage (Nitrosamine Guidance Webpage) was created and publishes recommended acceptable intake (AI) limits for nitrosamine impurities.[1]

To address the challenges posed by NDSRIs, FDA, in collaboration with international drug regulators, developed the predicted Carcinogenic Potency Categorization Approach

(CPCA) methodology for determining an AI limit based on an NDSRI's potency category. The RAIL Guidance formalized the CPCA methodology, which uses structural features of NDSRIs to predict their carcinogenic potency and assign corresponding AI limits across the following five categories: Category 1 (26.5 ng/day) (most potent), Category 2 (100 ng/day), Category 3 (400 ng/day), and Categories 4 and 5 (1500 ng/day each) (least potent).[2]

[1] See FDA website, *CDER Nitrosamine Impurity Acceptable Intake Limits,* at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cder-nitrosamine-impurity-acceptable-intake-limits.
[2] RAIL Guidance at 8.

As recommended in the Nitrosamine Guidance and RAIL Guidance, applicants should conduct risk assessments, followed by testing for those nitrosamines identified as posing potential risks in the drug product. Potential nitrosamine risks may be identified either by FDA by publishing specific nitrosamines on the Nitrosamine Guidance Webpage, or by the applicant during their risk assessment. Not all identified nitrosamine impurities warrant establishing a specification limit. In accordance with the guidance, "a manufacturer or applicant may demonstrate in the risk assessment that subjecting the drug to nitrosating conditions (i.e., targeted forced degradation) will not form nitrosamine impurities in the drug product. In those instances, omission of confirmatory testing may be justified by the risk assessment."[3] Applicants may also omit routine testing for an NDSRI if it is present at less than 10% of the AI in drug product.[4] However "if the confirmatory testing indicates nitrosamine levels exceed 10 percent of the recommended AI limit but are within the recommended AI limit, a control for nitrosamines should be established in the release and stability specifications."[5]

Wellcovorin (leucovorin calcium) tablets, EQ 5 mg base and EQ 25 mg base, are the subject of NDA 018342, initially approved on July 8, 1983, and held by GSK. On November 24, 2025, GSK submitted a PAS pursuant to section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act (FDCA) for Wellcovorin (leucovorin calcium) tablet (NDA 018342/Supplement 15). This PAS provides for a new indication for the treatment of cerebral folate deficiency with folate receptor 1 mutation in adults and pediatric patients.

GSK further indicated that it does not intend to market or manufacture Wellcovorin (leucovorin calcium) tablets following approval of this supplement and that it intends to withdraw NDA 0188342 following approval of this supplement.

As published on the Nitrosamine Guidance Webpage, leucovorin may contain two NDSRIs: N-nitroso-leucovorin-1 and N-nitroso-leucovorin-2. Using the CPCA methodology, FDA placed these impurities in Potency Category 4 (one of the least potent categories), which has a recommended AI limit of 1500 ng/day.[6]

Because the commercial marketing of Wellcovorin (leucovorin calcium) tablets was discontinued in 1997, upon submission of NDA 018342/Supplement 15 on November 24, 2025, there was no product for the Applicant to test in accordance with the three-step mitigation strategy outlined in the RAIL Guidance and Nitrosamine Guidance. Because no GSK products exist for testing and GSK further indicates that it does not intend to market or manufacture the product following approval of this PAS, we recommend that this supplement can be approved in

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[3] Nitrosamine Guidance at 21.
[4] *Id.*
[5] Nitrosamine Guidance at 22.
[6] Table 1: FDA Recommended AI Limits for Certain Hypothetical NDSRIs and Other Identified Nitrosamine Impurities, CDER Nitrosamine Impurity Acceptable Intake Limits webpage (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cder-nitrosamine-impurity-acceptable-intake-limits).

the absence of nitrosamine confirmatory testing. If GSK decides to restart commercial distribution of this product in the future, the Applicant should submit a supplement with information sufficient to demonstrate that the product meets all current legal and regulatory requirements. Such a supplement will need to include any chemistry, manufacturing and controls changes needed to comply with relevant compendial requirements or to address current recommendations for control of impurities, including nitrosamine-related impurities along with supporting data as described in the Nitrosamine Guidance and RAIL Guidance.

4.3. Clinical Microbiology

Not Applicable

4.4. Devices and Companion Diagnostic Issues

Not Applicable

5 Nonclinical Pharmacology/Toxicology

No new nonclinical studies were submitted with this supplement. The Applicant's proposed labeling for Sections 8 (Use in Specific Populations), 12.1 (Clinical Pharmacology, Mechanism of Action), and 13 (Nonclinical Toxicology) is acceptable from a nonclinical perspective.

6 Clinical Pharmacology

6.1. Executive Summary

Wellcovorin (leucovorin calcium), a leucovorin oral tablet formulation, is approved to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosages of folic acid antagonists. In the current efficacy supplement, the Applicant is seeking approval for the treatment of cerebral folate transport deficiency (CFTD) in adults and pediatric patients with a FOLR1 genetic mutation (FOLR1-CFTD). No new clinical studies were conducted by the Applicant to evaluate the safety and efficacy of leucovorin for the treatment of FOLR1-CFTD. The efficacy and safety of leucovorin for the treatment of FOLR1-CFTD are primarily supported by the published case reports identified following a systematic literature search (refer to Section 7.2). Data from a total of 46 subjects, aged 0.2 years to 33 years, primarily pediatric subjects (38 out of 46), with FOLR1-CFTD were used for the clinical pharmacology assessment.

Further, a systematic literature search has also been conducted to assess the pharmacokinetic (PK) characteristics of leucovorin and its metabolites across different leucovorin formulations (tablet, capsule, solution, intravenous (IV) and intramuscular (IM) injections). Subsequently, PK bridging between the different formulations used in the literature and the approved product, Wellcovorin, were assessed. Additionally, the effect of food and administration instructions for subjects unable to swallow whole tablets were also evaluated to provide appropriate administration instructions in the United States prescribing information (USPI).

Leucovorin is a highly soluble and well-absorbed drug after oral administration. The bioavailability of different oral formulations and the IM injection of leucovorin are greater than 95% compared to the IV formulation for doses up to 25 mg of oral leucovorin. Therefore, the different oral formulations or parenteral formulations (IM or IV injections) of leucovorin used in the literature are not anticipated to impact the bioavailability of leucovorin. The impact from food, crushing and mixing the tablet with liquid/soft food are not expected to have clinically significant impact. The Office of Clinical Pharmacology review finds that the PK bridging between the other oral formulations or parenteral formulations (IV or IM injections) of leucovorin and Wellcovorin is adequate. Therefore, Wellcovorin can rely on the PK, safety and effectiveness information reported in the literature to support the approval for the treatment

of cerebral folate transport deficiency in adult and pediatric patients with FOLR1 genetic mutation.

6.2. Summary of Clinical Pharmacology Assessment

PK studies reported in the literature showed that the serum exposures of total folates and/or 5-MTHF (active metabolites after leucovorin) are similar across different oral leucovorin formulations and parenteral injections. After oral leucovorin administration or parenteral injections (IM or IV injections) at doses of 25 mg or less, the bioavailability is reported to be greater than 95%. As leucovorin is a highly-soluble and well-absorbed drug and available data from literature support that the formulation differences are not expected to impact the bioavailability of leucovorin, the PK bridging between different oral formulations or parenteral formulations (IM and IV) of leucovorin used in the literature and Wellcovorin at the highest strength, 25 mg, is deemed adequate.

Dosage and administration of leucovorin for the treatment of CFTD-FOLR1 has been assessed based on the reported dosing information in the published cases of CFTD-FOLR1 which were also used in for the efficacy and safety reviews, and published reports related to other indications (folinic acid responsive seizures and toxoplasmosis infections) in the literature. A body weight based and titration-based dosage regimen of Wellcovorin is recommended for the treatment of FOLR1-CFTD in adults and pediatric patients. Refer to Section 6.2.2 for additional information.

A dedicated food effect study was not conducted with Wellcovorin. However, the reported oral formulations (tablets, capsules and solutions) are immediate release and showed high oral bioavailability (≥95%). Additionally, as leucovorin was reported to be administered by crushing the tablets and mixing with food in the case reports, food is not expected to significantly impact the PK of leucovorin. Therefore, Wellcovorin can be administered with or without food.

6.2.1. Clinical Pharmacokinetics

Leucovorin is a racemic mixture of (l)- or levoleucovorin and (d)- or dextroleucovorin. Following oral administration of leucovorin to healthy adults, dextroleucovorin, levoleucovorin, and 5-MTHF exposures increased in a dose proportional manner with doses up to 25 mg, but in a less than dose proportional manner with doses greater than 25 mg.

Absorption

Following oral administration of leucovorin in adults, the apparent bioavailability of levoleucovorin is 97% for 25 mg, 75% for 50 mg, and 37% for 100 mg, and dextroleucovorin is approximately 19% for 25 mg, 20% for 50 mg, and 7% for 100 mg. After a single oral 15 mg (7.5 mg/m2) dose of WELLCOVORIN, time to peak serum folate concentration is 1.7 hours.

Effect of Food: The effect of food on the pharmacokinetics of WELLCOVORIN has not been evaluated. As leucovorin is a highly soluble and well absorbed drug, and different immediate-release oral formulations of leucovorin (oral tablet and oral solution) showed relatively higher bioavailability of total folates (>95%), food is not expected to have a clinically significant effect on the pharmacokinetics of leucovorin or 5-MTHF. Leucovorin tablets were crushed and mixed with food or liquid in clinical studies reported in literature.

Distribution

Levoleucovorin is minimally bound to human serum albumin. The reported human serum albumin binding of 5-MTHF ranges from 42-49%.

Levoleucovorin is not observed in CSF and 5-MTHF is reported to accumulate in CSF in children with leukemia.

Elimination

After intravenous administration of leucovorin in adults, the reported mean plasma elimination half-life in the literature was 0.5-1.3 hours for levoleucovorin and 3-7 hours for 5-MTHF.

Metabolism: Following administration of oral leucovorin, levoleucovorin undergoes significant metabolism in intestinal cells via methenyltetrahydrofolate synthetase (MTHFS) and methylenetetrahydrofolate reductase (MTHFR) to 5-MTHF. 5-MTHF is the main active metabolite in plasma after oral administration of leucovorin.

Excretion: Leucovorin is mainly excreted by the kidney as unchanged dextroleucovorin, levoleucovorin, or as 5-MTHF, the metabolic product of levoleucovorin.

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

The recommended oral dosage of Wellcovorin for patients with FOLR1-CFTD is based on the patient's weight (see Table 1). Adjust dosage based on clinical response *[see Clinical Studies (14.1)]*.

Table 1. Recommended Wellcovorin Dosage for Patients with FOLR1-CFTD

Patient Weight	Initial Total Daily Dosage[a]	Maximum Total Daily Dosage[a]	**Frequency of Administration[b]**
Less than 40 kg	1 to 2 mg/kg/day	8.5 mg/kg/day	Administer the total daily dosage once daily or in divided doses up to 6 times per day. Single doses of 25 mg or less are preferred; do not administer more than 75 mg as a single dose.
40 kg or more	1 to 2 mg/kg/day	330 mg/day	

[a] Round doses to the nearest tablet strength or combination of strengths.

[b] Bioavailability is reduced with individual leucovorin doses above 25 mg in adults .

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. Clinical Pharmacology Questions

1. Is PK bridging between leucovorin oral tablet (Wellcovorin) and other leucovorin oral or parenteral formulations (IM and IV) reported in literature adequately established?

Yes. The PK bridging between Wellcovorin and other oral formulations or parenteral formulations of leucovorin used in the literature is adequate.

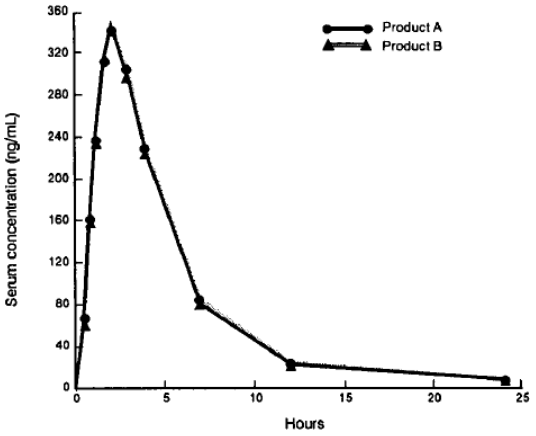
Of the 46 case reports, 27 subjects with FOLR1-CFTD received oral only leucovorin treatment, 16 subjects received oral and IV leucovorin treatment, 2 subjects received intramuscular leucovorin, 1 subject received oral, IV and intrathecal leucovorin treatment. Some case reports lack information on the specific type of formulation used. Based on the results from a systematic literature search, the PK bridging between Wellcovorin and the available PK data from different oral or parenteral (IM and IV) formulations reported in literature were assessed.

Leucovorin (folinic acid) is a racemic mixture of the diastereoisomers of the 5-formyl tetrahydrofolic acid (5-formyl-THF). The l-isomer is the biologically active isomer, while the d-isomer is minimally and slowly metabolized. The increase in plasma or serum folate activity observed after administration of leucovorin is predominantly due to 5-methyltetrahydrofolate (5-MTHF) metabolized from l-leucovorin. A significant portion of oral leucovorin is converted, via multiple steps of enzymatic metabolism, to its major circulating active metabolite, 5-MTHF, in the gastrointestinal (GI) tract prior to the absorption. Hence, the systemic exposures of the parent drug, leucovorin, is lower than those observed with IV or IM leucovorin injections (Mehta et al. 1978, Straw et al. 1984, McGuire et al. 1987, McGuire et al. 1988, Greiner et al.

1989, Priest et al. 1991). Significant differences in the levels of reduced total folates and 5-MTHF were not observed across the literature when leucovorin is given orally or parenterally at doses 25 mg or less. As a result, the exposures of total folates and/or 5-MTHF are generally used when determining the bioavailability of oral leucovorin products.

De Vito et al. 1989 conducted a study comparing Lederle leucovorin (leucovorin oral tablet from Lederle Labs which is associated with Pfizer) and Wellcovorin after a single dose of 25 mg (5 x 5 mg). The results showed superimposable PK profiles for total folates (Figure 1) and comparable key pharmacokinetic parameters for l-leucovorin, 5-MTHF, and total folates (Table 2) between Lederle and Wellcovorin oral tablet formulations. The results support that PK bridging between the oral tablet formulation from Lederle labs and Wellcovorin is adequate.

Figure 1. Mean Serum Concentrations of Total Folates after Single Doses of Lederle (A) and Wellcovorin (B) Leucovorin Oral Tablet Formulations, at 25 mg (5 x 5 mg) Doses (n=36)



Source: Figure 1, De Vito et al. 1989

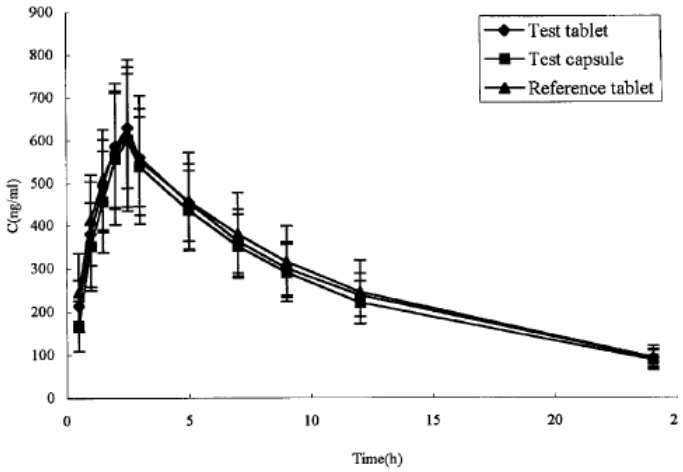
Table 2. Comparison of Pharmacokinetic Parameters of l-Leucovorin and Metabolites after Single Doses of Lederle (A) and Wellcovorin (B) Leucovorin Oral Tablet Formulations, at 25 mg (5 x 5 mg) Doses (n=36)

Pharmacokinetic Parameter	l-Leucovorin		Total Folates		5-MTHF	
	A	B	A	B	A	B
AUC_{0-24} (ng×hr/mL)	59.2	62.4	1899	1854	1834	1784
AUC_{inf} (ng×hr/mL)	66	67.4	1978	1937	1910	1866
C_{max} (ng/mL)	28.5	31.8	357	348	348	335
T_{max} (hr)	0.6	0.7	2.1	2.1	2.1	2.1
$t_{½}$ (hr)	6.3	7.2	5.3	5.3	5.2	5.3

AUC_{inf}: area under the concentration-time curve from 0 to infinity; AUC_{24}: area under the concentration-time curve from 0 to 24h; C_{max}: peak serum concentration; t_{max}: time to peak serum concentration; $t_{1/2}$: terminal elimination half-life
Source: Table 1, De Vito et al. 1989. Concentrations were determined by microbiologic folate assays.

In a relative bioavailability study by Duan et al. 2002, the PK of a single dose of oral capsule formulation (75 mg; 3 x 25 mg capsules) was compared with two different oral tablet formulations (test tablet (75 mg; 5 x 15 mg) and reference tablet (a generic drug, Antrex, for Lederle leucovorin oral tablet, 75 mg; 5 x 15 mg)). The PK profile of the test capsule was similar to the test and reference oral tablet formulations (Figure 2), and comparable bioavailability between capsule and tablet formulations of leucovorin was also demonstrated (Table 3). Considering Antrex is a generic product for Lederle leucovorin oral tablet and the adequate PK bridging between Lederle and Wellcovorin oral tablets, the oral capsule formulation is considered to have comparable exposures to Wellcovorin.

Figure 2. Mean Concentration-Time Profiles of l-Leucovorin After Single Doses of Oral Tablet (Test), Oral Capsule (Test) and Reference Tablet at 75 mg in Healthy Volunteers (n = 12)



Source: Figure 2, Duan et al. 2002.

Table 3. Mean Pharmacokinetic Parameters of l-Leucovorin After Single Doses of Oral Tablet (Test), Oral Capsule (Test) and Reference Tablet at 75 mg in Healthy Volunteers (n = 12)

Parameter	Test tablet	Test capsule	Reference tablet
AUC_t (ng×hr/mL)	(6.6 ± 1.3) × 10^3	(6.2 ± 1.3) × 10^3	(6.7 ± 1.7) × 10^3
AUC_{inf} (ng×hr/mL)	(7.7 ± 1.5) × 10^3	(7.3 ± 1.6) × 10^3	(7.9 ± 2.0) × 10^3
C_{max} (ng/mL)	(6.5 ± 1.5) × 10^2	(6.2 ± 1.5) × 10^2	(6.4 ± 1.6) × 10^2
T_{max} (hr)	2.5 ± 0.3	2.5 ± 0.3	2.4 ± 0.3
F (%)	99.7 ± 13.7	93.9 ± 11.7	-

Source: Adopted from Table 1, Duan et al. 2002. Concentrations were determined by HPLC-UV.

In a study by Mehta et al. 1978, single 15 mg doses of oral solution, oral tablet and IM injection (all from Lederle Labs) were administered in healthy adult volunteers. The results suggest that there was no significant difference in the serum levels of l-leucovorin and 5-MTHF following administration of oral solution, the oral tablet and IM injection (Table 4).

Table 4. Mean Pharmacokinetics for l-Leucovorin and 5-MTHF After Oral Solution, Oral Tablet and IM Administration of 15 mg Leucovorin Calcium in Healthy Volunteers

Mode of administration	l-leucovorin		5-MTHF	
	$t_{1/2}$ (hr)	$AUC_{0\text{-}6h}$ (ng×hr/mL)	$t_{1/2}$ (hr)	$AUC_{0\text{-}6h}$ (ng×hr/mL)
Oral solution	0.64 ± 0.07	9.5 ± 1	2.28 ± 0.23	937 ± 128
Oral tablet	0.67 ± 0.07	12 ± 1	2.18 ± 0.23	1117 ± 153
IM	0.75 ± 0.03	371 ± 28	2.24 ± 0.21	1157 ± 231

$AUC_{0\text{-}6h}$: area under the curve from time 0 to 6 hours postdose; $t_{1/2}$: half-life; IM: intramuscular injection
Source: Adopted from Table 1, Mehta, et al. 1978. Microbiologic folate assays were used for bioanalysis.

In a PK study by Greiner et al. 1989, a single dose of 25 mg leucovorin oral capsule was compared with 25 mg leucovorin IV injection. The systemic exposures of total folates were similar after both routes of administration (Table 5), indicating that leucovorin is a well absorbed drug and its oral bioavailability is relatively similar to IV administration.

Table 5. Mean Pharmacokinetic Parameters for Total Folates after Oral Capsule and Intravenous Administration of 25 mg Leucovorin

Parameter	Intravenous	Oral
C_{max} (ng/mL)	-	363 ± 121
$AUC_{0\text{-}24}$ (ng×hr/mL)	2107 ± 651	2308 ± 891
$t_{1/2}$ (hr)	7.59 ± 3.72	7.26 ± 4.04

Source: Adapted from Table 1, Greiner et al. 1989

The PK after IV, IM, and oral administration of a 25 mg dose of leucovorin were reported by McGuire et al. 1989. Exposures of total folates and 5-MTHF after intramuscular injection and oral administration are shown to be similar to those after intravenous injection (Table 6).

Table 6. Mean Pharmacokinetic Parameters for Total Folates and 5-MTHF after IV, IM and Oral Administration of 25 mg Leucovorin

	Intravenous	Intramuscular	Oral
	Total folates		
C_{max} (ng/mL)	1259 ± 205	436 ± 123	393 ± 96
AUC_{inf} (ng×hr/mL)	2354 ± 444	2392 ± 489	2149 ± 612
	5-MTHF		
C_{max} (ng/mL)	258 ± 55	226 ± 52	367 ± 87
AUC_{inf} (ng×hr/mL)	1563 ± 366	1649 ± 406	2012 ±567

Source: Adopted from Table 1, McGuire et al. 1988

In summary, systemic exposures of different oral formulations (i.e., tablet, capsule and solution) and parenteral formulations (IM and IV injections) are relatively similar. Additionally,

leucovorin is a highly soluble and well-absorbed drug and the reported absolute bioavailability of different oral formulations (Straw et al. 1984, McGuire et al. 1987, and Priest et al. 1991) is ≥95% at doses 25 mg or less. This suggests that the formulation differences are not expected to impact the bioavailability of different immediate release oral formulations of leucovorin. Collectively, the physicochemical characteristics and available PK data of leucovorin support that the PK bridge between different immediate release oral formulations or parental formulations (IM and IV) used in the literature and the listed drug, Wellcovorin, is adequate. Hence, Wellcovorin can rely on the published case reports on safety and effectiveness of oral leucovorin for the treatment of FOLR1-CFTD.

2. Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes.

The dosage recommendation in subjects with FOLR1-CFTD is a collective consideration based on the dosing regimen reported in the case reports as well as safety information from literature associated with other indications such as ASD, folinic acid (i.e., leucovorin) responsive seizures and toxoplasmosis infection.

Of the 46 case reports in subjects with FOLR1-CFTD who initiated leucovorin treatment, age information was described for 41 subjects, and 38 were pediatric subjects with an age range of 0.2 years (2.4 months) to 17 years (13 years and older (6/38); children 6 to 12 years old (15/38); children 2 to <6 years old (15/38); children <2 years old (2/38)). Further, literature reports suggest that oral leucovorin is also used for the treatment of folinic acid responsive seizures (Torres et al. 1999, Frye et al. 1999, Nicolai, et al. 2006, and Gallagher et al. 2009) and toxoplasmosis infections (Centers for Disease Control and Prevention (CDC)) in neonates, infants, children and older pediatric subjects and adults.

The recommended starting dose in subjects with FOLR1-CFTD is 1-2 mg/kg/day. The information about initial doses were available for 25 out of 44 subjects who received oral leucovorin. Of the 25 subjects, 14 subjects received a starting dosage of 1-2 mg/kg/day, 9 subjects received an initial dosage greater than 2 mg/kg/day and 2 patients-initiated dosage lower than 1 mg/kg/day. The starting dosage and maintenance dosage for 7 out of 14 subjects were 1-2 mg/kg/day. However, the dosages were escalated in the remaining 7 subjects who initiated on the 1-2 mg/kg/day regimen. Of the 42 subjects with the maintenance dosage reported, none received a dosage less than 1 mg/kg/day. Further, the CDC's recommendation on the use of oral leucovorin in newborns (5 mg), infants >1 month (10 mg), and children (≥10 mg) with toxoplasmosis infections for 6 months or longer supports the safe use of leucovorin in pediatric subjects (See Section 8.4.10. for additional safety assessment including long-term use of leucovorin).

Of the 42 subjects with known maintenance oral dose, the median dose administered was 5 mg/kg/day, 79% (33/42) of the subjects received doses ≤6 mg/kg/day, and 4 subjects (0.2 and 11 years of age) received doses of 8.0 to 9.0 mg/kg/day. Hence, a maximum dose of 8.5 mg/kg/day is recommended. Although the doses reported in the case reports were body weight-based, the body weight values were not reported. Therefore, a maximum daily dose of approximately 330 mg was estimated based on the 50th percentile body weight for the U.S. children and adults reported in the CDC's growth chart. As the 50th percentile body weight for 12 years old is approximately 40 kg, the dosage regimens were stratified as <40 kg and ≥40 kg.

Although this efficacy supplement is primarily focused on oral leucovorin for the treatment of FOLR1-CFTD, literature reports indicate that oral leucovorin was also used at doses up to 11 mg/kg/day or approximately 330 mg/day in combination with IV or IM injections for the treatment of subjects with FOLR1-CFTD for oral leucovorin.

Wellcovorin is recommended to be administered once daily or in divided doses up to 6 times per day. The unit dose (i.e., the dose administered at each time of a day) should be maintained at no more than 75 mg, preferably at 25 mg or below. Given that the elimination half-lives of l-leucovorin and 5-MTHF are 0.5-1.3 hour and 3-7 hours, respectively, and unit doses above 25 mg have also been shown to have reduced oral bioavailability, doses should generally be divided to allow a relatively high oral bioavailability and maintain the plasma concentration of the active moieties of leucovorin. In the absolute bioavailability study by McGuire et al., the total folate exposures of single oral doses of leucovorin from 20 mg to 200 mg were compared to 200 mg IV leucovorin. The results shown in Table 7 indicate that the absolute bioavailability of oral solution was gradually reduced with increasing the oral dose from 20 mg to 200 mg (98% at 20 mg, 61% at 60 mg and 41% at 100 mg). Similar results were reported in the previously approved label for Wellcovorin and a publication by Straw et al. 1984. With the 75 mg oral dose, the absolute oral bioavailability of leucovorin is expected to be approximately 50%. Thus, the review team recommends the maximum unit dose of oral leucovorin as 75 mg.

To explore the bioavailability of split doses, Schilsky et al. 1990 conducted a study in which volunteers received Wellcovorin oral tablets at 1000 mg (administered as 100 mg every hour for the first 5 doses followed by 100 mg every 4 hours for the remaining 5 doses), the absolute bioavailability of 100 mg oral leucovorin was 37% (Refer to Section 15, OCP Appendix for details). The absolute bioavailability of 100 mg oral leucovorin following administration as divided doses (37%) are relatively similar to that reported in another study where the absolute bioavailability of single 100 mg oral leucovorin was 41% (Table 7, McGuire et al., 1987). The results from these studies suggest that either split dosing (up to 10 times a day) or single dose administration of oral leucovorin at 100 mg did not appreciably affect its absolute oral bioavailability. As majority of subjects with FOLR1-CFTD (approximately 70%) required 150 mg or lower doses of daily oral leucovorin and the unit dose of 25 mg dose would achieve higher oral bioavailability, the review team recommends dosing up to six times daily in divided doses of Wellcovorin (e.g., 6 x 25 mg = 150 mg).

Table 7. Mean Systemic Exposures of Total Folates and Bioavailabilities after Single Doses of Oral Leucovorin[a] vs IV Injection

Dose, mg	N	AUC_{inf} ng×hr/mL	Relative bioavailability vs 20 mg PO	Relative bioavailability vs 200 mg IV
20	24	2,184	(100%)	98%
40	16	3,408	78%	76%
60	16	4,056	62%	61%
80	24	4,288	49%	48%
100	16	4,600	42%	41%
200 po	6	6,848	31%	31%
200 iv	6	22,298	—	(100%)

[a] Leucovorin Calcium cryodesiccated powder reconstituted with aromatic elixir USP to a final concentration of 1 mg leucovorin free acid per mL solution.
AUC_{inf}: area under the curve from time 0 to infinity; po: oral; iv: intravenous injection
Source: Table 1, McGuire et al. 1987. Concentration determined by microbiologic folate assays.

As the oral bioavailability of leucovorin begins to reduce at doses above 25 mg, less than dose proportional increases in the exposures of l-leucovorin, 5-MTHF and total folates were reported following administration of oral leucovorin at doses greater than 25 mg. Therefore, it is recommended that the individual dose preferably remains at 25 mg or lower. However, given the doses reported in the case reports were up to 330 mg/day, it might be necessary for some subjects to receive the unit doses of above 25 mg. The prescribers may determine the dosing frequency based on the clinical response, tolerability and total daily dose required for a patient.

3. Is there clinically relevant food-drug interaction, and what is the appropriate management strategy?

No. Food is not expected to impact the pharmacokinetics of leucovorin. Leucovorin can be administered with or without food.

The interaction of leucovorin oral tablet with food has not been formally evaluated. However, a dedicated food effect study by Furuhata et al. 2009 suggests that low-fat meal did not affect the Cmax and AUCt of 25 mg oral leucovorin tablet in subjects with colorectal cancer when compared to the fasted state. Zhang et al. 2025 reported in a 12-week randomized clinical trial conducted that oral leucovorin tablet can be swallowed directly or crushed and mixed with food or drink for ease of consumption. Furthermore, literature reports suggest that 5-MTHF, a major active metabolite after leucovorin administration, can be added to the infant formula, baby food, and processed cereal-based food as a source of folate for nutritional purposes (EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), 2019). As leucovorin is a highly soluble and well absorbed drug, the doses were titrated to clinical response, oral leucovorin was reported to be tolerated at doses as high as 28 mg/kg/day (Lubout et al. 2020), and the

available literature reports suggest that different formulations of oral leucovorin can be administered with food, the impact of food on the PK and safety of leucovorin is expected to be minimal. The review team recommends administering leucovorin oral tablet with or without food.

4. Can oral leucovorin tablets be crushed and mixed with water or other liquid food?

Yes.

A dedicated PK study to compare the PK of leucovorin following administration as whole tablet versus crushed and mixed with water or other liquid has not been conducted. As the oral solution and oral tablets have similar bioavailability (refer to the response to Question 2), crushing leucovorin tablet and mixing with food or a liquid before administration is not expected to affect the bioavailability of leucovorin. Zhang et al. 2025 reported that leucovorin oral tablets can be swallowed directly or crushed and mixed with food or drink. Dill et al. 2011 also reported crushing oral tablets and mixing them with water to assist administration in a pediatric patient with FOLR1-CFTD. The use of oral leucovorin has been reported in multiple case reports in pediatric subjects (including an indication other than FOLR1-CFTD) down to the age of 14 days (Gallagher et al. 2009). To allow ease of administration of leucovorin oral tablets to pediatric subjects and those who are having swallowing difficulties, crushing and mixing with food or liquid are required. Based on the physico-chemical properties of leucovorin, immediate-release dosage form of leucovorin oral tablets, similar bioavailability between tablets and solution formulations, and literature reports on the use of leucovorin oral tablets by crushing and mixed with food or liquid, the review team concluded that leucovorin oral tablets can be crushed and mixed with soft food or an age-appropriate liquid (e.g., water, breast milk, or infant formula) before administration.

7 Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

Table 8 Clinical Studies Relevant to this NDA

Trial Identity	NCT no.	Trial Design	Regimen/ schedule/ route	Study Endpoints	Treatment Duration/ Follow Up	No. of subjects enrolled	Study Population	No. of Centers and Countries
N/A	N/A	26 case reports and case series	Leucovorin, variable dose/schedule/route	Primary: descriptive clinical response to treatment Biomarkers: CSF levels of 5-MTHF (as available)	Variable	46 subjects	Subjects with FOLR1 pathogenic variants Age range: 2-33 years	N/A, 20 Countries

7.2. Review Strategy

The review of efficacy and safety of leucovorin for the treatment of FOLR1-CFTD is based on a comprehensive review and descriptive analysis of published case reports and case series available in the scientific literature. The Agency was able to rely on published literature for several reasons. First, leucovorin is used in clinical practice to treat FOLR1-CFTD and is considered standard-of-care; second, there is a strong mechanistic rationale for the use of leucovorin as a folate analogue which crosses the blood brain barrier for a condition caused by impaired folate transport into the central nervous system; third FOLR1-CFTD is an extremely rare, well-defined, and progressively debilitating condition with high unmet medical need, and there is public health benefit to establishing the safety and effectiveness of a therapy as it may increase recognition and diagnosis of a treatable disorder and potentially improve access to treatment. Moreover, the Agency may rely on data from published literature when the reports contain adequate information necessary for approval.

Lastly, leucovorin is an FDA-approved product that has been marketed for many decades to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosages of folic acid antagonists. It has a well-established safety profile, with generally good tolerability. Therefore, the safety of leucovorin is also partly informed by existing clinical safety information in the oncologic population for the oncology indication, although the population with cerebral folate deficiency differs substantially. The review of safety is complemented by an analysis of post-marketing data.

Table 16 in the Appendix (Section 19.1) provides an overview of case series and case reports reviewed to support the efficacy and safety evaluation of leucovorin for the treatment of FOLR1-CFTD.

7.2.1. Systematic Review of cases of FOLR1-CFTD

Literature Search
A comprehensive literature search was conducted to identify all published clinical trials, case reports, and case series of subjects with genetically confirmed FOLR1 variants treated with leucovorin. The search strategy included:

Databases Searched:
- PubMed/MEDLINE
- Cochrane Library
- ClinicalTrials.gov

Search Terms:

- ("FOLR1" OR "folate receptor alpha" OR "folate receptor 1" OR "folate receptor alpha deficiency" OR "folate receptor 1 deficiency" OR "FOLR1 deficiency" OR "FOLR1 mutations" OR "FOLR1 variants")
- AND ("cerebral folate deficiency" OR "CFD" OR "cerebral folate transport deficiency" OR "folate transport deficiency")
- AND ("leucovorin" OR "leucovorin" OR "5-formyltetrahydrofolate" OR "calcium folinate" OR "calcium levofolinate")

Additional Search Strategy:

- Hand-searching of reference lists from identified studies

Search Inclusion Criteria:

- Study design: randomized clinical trials, case reports, and case series
- Population: Subjects with genetically confirmed FOLR1 mutations
- Intervention: Leucovorin treatment (any formulation or route)
- Outcomes: descriptive clinical outcome data available
- Publication: Peer-reviewed journals, English language
- Time Period: 2000-2025

Search Exclusion Criteria:

- CFD due to other causes (folate receptor autoantibodies, other genetic defects)
- Case reports of subjects not treated with leucovorin
- Case reports without genetic confirmation
- Duplicate publications of the same subjects
- No outcome data reported

Results of Literature Search

Consistent with the rare nature of the disease, a literature search revealed no clinical trials enrolling subjects with FOLR1-CFTD.

The literature search revealed 28 peer reviewed case reports and case series including 49 subjects with genetically confirmed FOLR1 variants. One case report was excluded as the subject, a 47-year-old Italian male with adult onset of symptoms, was not treated with leucovorin (Manco, 2023). Another study that included two cases of adolescent Japanese female siblings did not include clinical outcome data and was excluded (Ohba, 2013).

Table 9 shows the study characteristics of the case report literature that met eligibility criteria and are included in this review. Studies included a total of 46 eligible subjects with FOLR1-CFTD, reported across 26 studies published between 2009 and 2024 (Cario, 2009; Steinfeld, 2009; Pérez-Dueñas, 2010; Dill, 2011; Grapp, 2012; Al-Baradie, 2014; Toelle, 2014; Akiyama, 2016; Delmelle, 2016; Ferreira, 2016; Karin, 2017; Kobayashi, 2017; Pope, 2019; Tabassum, 2019;

Mafi, 2020; Zhang, 2020; Brunetti, 2021; Gowda, 2021; Papadopoulou, 2021; Jaafar, 2022; Almahmoud, 2023; Kanmaz, 2023; Potic, 2023; Dreha-Kulaczewski, 2024; Girgis, 2024; Wang, 2024). Thirty cases were described in more than one publication. The geographic diversity spans multiple countries and three continents, reflecting the worldwide recognition of this rare condition. Notably, there were no subjects in the United States that were included in the published case reports.

Table 9 Study Characteristics Meeting Eligibility Criteria

Study Characteristics	Value
Total Studies	26
Total Subjects	46
Publication Years	2009 - 2024
Countries Represented	China, UAE, Egypt, Italy, Lebanon, India, Algeria, Greece, Turkey, Saudi Arabia, Germany, Belgium, France, Gambia, Ghana, Russia, Japan, Finland, Azerbaijan, Serbia
Continents	Africa, Europe, Asia

Abbreviations: UAE: United Arab Emirates

8 Statistical and Clinical and Evaluation

8.1. Review of Relevant Individual Trials Used to Support Efficacy

8.1.1. FOLR1-CFTD Case Reports

Patient Demographics

Table 10 shows the demographic characteristics of the 46 subjects with FOLR1-CFTD included in this efficacy analysis. Female subjects are 67% of the population, however the significance of the female predominance is unclear. The age range at time of case reporting extended from 2 to 33 years, indicating the prevalence of FOLR1-CFTD across pediatric and adult populations and supporting the need for treatment options suitable for both age groups.

Table 10: Patient Demographics of Included Studies

Patient Demographics	Value
Total Subjects	46

Patient Demographics	Value
Sex	
Female	31 (67%)
Male	15 (33%)
Age†	
Mean (SD)	10.4 years (6.6)
Median	8
Range	2 years - 33 years
Nationality Distribution	Turkish 7 (15%), Finnish 5 (11%), Chinese 3 (7%), Algerian 3 (7%), Saudi 3 (7%), German 3 (7%), Italian 3 (7%), UAE 2 (4%), Egyptian 2 (4%), Lebanese 2 (4%), Indian 2 (4%), Greek 2 (4%), Belgian 2 (4%), Serbian 2 (4%), Other* 5 (11%)

Data collated from published case reports by reviewer.
†Patient age at time of case reporting.
*Other includes French, Gambian, Ghanaian, Russian, and Azerbaijani subjects (1 each).

FOLR1 Variants

Examination of the specific FOLR1 variants reported across the 46 subjects revealed significant allelic heterogeneity, with 26 different mutations identified across the cohort. At least 40% of the subjects were born to consanguineous parents, consistent with the autosomal recessive inheritance pattern of this condition. The most frequently observed mutation was c.506G>A (p.C169Y), occurring in 5 subjects, including cases with homozygous mutations and compound heterozygous combinations. Several mutations were observed in multiple subjects, including c.428G>A (p.W143*) in 3 subjects, c.195C>G (p.C65W) in 3 subjects, c.665A>G (p.N222S) in 3 subjects, and the splice site variant g.3576 T>G in 3 subjects.

Most subjects carried homozygous FOLR1 mutations while several subjects were compound heterozygotes carrying two different pathogenic variants. FOLR1 mutations encompassed various types of genetic alterations, including missense variants, nonsense mutations, splice site variants, and small deletions and insertions, indicating that loss-of-function mutations throughout the FOLR1 gene can result in FOLR1-CFTD. Two subjects also carried mutations in other genes, including the POLG1 gene and FGF3 gene. Overall, the genetic diversity observed across this international cohort reflects the global distribution of FOLR1-CFTD and suggests that the condition results from diverse loss-of-function mechanisms rather than being concentrated in specific mutational hotspots within the gene.

Natural History of FOLR1-CFTD

Since initial recognition that pathogenic FOLR1 variants can cause CFD, leucovorin has been considered standard of care treatment and has been used off-label. However, most reports describe a substantial delay between symptom onset and treatment initiation, providing a unique opportunity to characterize the natural history of untreated disease, which could be used as a baseline for comparison with effect of treatment with leucovorin. The majority of case reports included detailed descriptions of clinical manifestations both at symptom onset and at pre-treatment baseline, thus capturing disease progression during the untreated period.

The following descriptive analysis of disease onset characteristics versus pre-treatment baseline characteristics demonstrates the natural history of FOLR1-CFTD, illustrating the progressive deterioration that occurs without treatment. This untreated natural history is used to create an estimate of the disease course in the untreated state that can serve as a control against which treatment responses can be evaluated.

Disease Onset Characteristics

Table 11 shows the disease onset characteristics for the 46 subjects with FOLR1-CFTD included in this analysis. Data showed that FOLR1-CFTD typically presents in early childhood with a mean age of onset of 2.2 years (range 2 months to 7 years), with the vast majority of subjects developing symptoms in the first three years of life.

Neurodevelopmental symptoms were a predominant presenting feature, occurring in 72% of subjects at disease onset. The most common developmental symptoms included developmental delay, language or speech delay, and developmental regression. Motor symptoms were the second most common initial manifestation, affecting 48% of subjects, with ataxia and tremor being the most frequently reported motor signs. Seizure-related symptoms occurred as presenting features in 20% of cases, including absence seizures with staring episodes, myoclonic seizures, and drop attacks with falling. Behavioral problems or autistic features were evident at disease onset in 11% of subjects and symptoms included hyperactivity, irritability, and diminished eye contact with verbal communication difficulties. Other neurological symptoms also occurred in 11% of subjects and included strabismus, sleep disorders, microcephaly, and congenital deafness.

Table 11 Disease Onset Characteristics

Characteristic	Value	Details
Age at Onset (N=43)*		
Mean (SD)	2.2 years (1.4)	

Characteristic	Value	Details
Median (range)	2.0 years (2 months - 7.0 years)	
Birth to 1 year	6 (14%)	
>1 to 2 years	21 (49%)	Most common presentation period
>2 to 3 years	12 (28%)	
>3 years	4 (9%)	
First Symptoms (N=46)		Most Common Symptoms
Developmental	33 (72%)	Developmental delay, language or speech delay, developmental regression
Motor symptoms	22 (48%)	Ataxia, tremor
Seizure-related symptoms	9 (20%)	Absence seizures and staring episodes, myoclonic seizures, drop attacks with falling
Behavioral/Autistic features	5 (11%)	Hyperactivity, irritability, diminished eye contact and verbal communication
Other neurological features	5 (11%)	Strabismus, sleep disorder, microcephaly, congenital deafness

Data collated from published case reports by reviewer.
*Age at onset data available for 43 of 46 subjects.
Note: Subjects may have had multiple types of first symptoms; percentages reflect proportion of subjects with each symptom category.

Pre-Treatment Baseline Disease Characteristics

Table 12 shows pre-treatment baseline clinical and diagnostic characteristics, demonstrating consistent and severe neurological involvement that is characteristic of this condition. Motor signs were nearly universal, present in 96% of subjects, and typically manifested as progressive motor involvement including ataxia, tremor, spasticity, hypotonia, and dyskinesia. Seizures affected 85% of subjects and were often daily and refractory to antiseizure medications, with myoclonic and generalized tonic-clonic seizures being the most common types. Developmental

regression was documented in 78% of subjects and was often characterized as severe. Behavioral problems and autistic features were present in 43% of subjects and included reduced or absent verbal communication, decreased social interaction, and irritability.

Pre-treatment MRI was available for 45 subjects and neuroimaging abnormalities were found in nearly all subjects (98%). The most common neuroimaging features included hypomyelination, cerebellar atrophy, and nonspecific white matter changes, reflecting the underlying disruption of normal brain development and myelination processes. Brain magnetic resonance spectroscopy (MRS) was performed in 22 subjects, with 73% showing abnormal findings characterized by low choline and inositol levels, providing additional evidence of disturbed myelin metabolism.

Baseline CSF 5-MTHF levels (7.17±9.30 nmol/L, Mean±SD) were available for 32 subjects (70% of the cohort), serving as both a diagnostic biomarker for FOLR1-CFTD and a pharmacodynamic biomarker of treatment response. Among those tested, 84% had severely reduced CSF 5-MTHF levels below 10 nmol/L, confirming the diagnosis. Reference ranges of normal for CSF 5-MTHF have not been fully established, but appear to vary by age, with 40 being the low end of normal across all ages, and up to 120 nmol/L within range for adults and up to 240 nmol/L for children (Mayo Clinic Laboratories). This profound reduction in central nervous system folate availability provided the biological rationale for leucovorin supplementation therapy and served as an objective biomarker confirming the diagnosis of FOLR1-CFTD as well as a baseline for comparison of treatment effect.

Table 12 Pre-Treatment Clinical and Diagnostic Characteristics

Characteristic	n/ N(%)	Details
Clinical Manifestations	N = 46	
Motor Signs	44/46 (96%)	Progressive motor involvement with ataxia, tremor, spasticity, hypotonia, dyskinesia
Seizures	39/46 (85%)	Seizures often daily and refractory to antiseizure medication and most often myoclonic and generalized tonic-clonic
Developmental Regression	36/46 (78%)	Often severe
Autistic Features	20/46 (43%)	Reduced/absent verbal communication, decreased social interaction, irritability

Characteristic	n/ N(%)	Details
Neuroimaging Findings	N = 45	
Pre-treatment MRI abnormal	44/45 (98%)	Hypomyelination, cerebellar atrophy, nonspecific white matter changes
Brain MRS Findings	N = 22	
Pre-treatment MRS abnormal	16/22 (73%)	Low choline and inositol
CSF 5-MTHF	N = 32	
Pre-treatment CSF 5-MTHF available	32/46 (70%)	
CSF 5-MTHF <10 nmol/L	27/32 (84%)	Normal: above 40 nmol/L

Data collated from published case reports by reviewer.
Abbreviations: MRS, magnetic resonance spectroscopy; CSF, cerebrospinal fluid; 5-MTHF, 5-methyltetrahydrofolate.

Summary of Natural History Data

The natural history analysis demonstrates that FOLR1-CFTD typically presents in early childhood with a mean age of onset of 2.2 years (range 2 months to 7 years), with neurodevelopmental symptoms (72%) and motor symptoms (48%) being the most common initial manifestations. Without treatment, the disease shows progressive deterioration, with pre-treatment baseline characteristics revealing nearly universal motor signs (96%), frequent seizures (85%), developmental regression (78%), and neuroimaging abnormalities (98%) including hypomyelination and cerebellar atrophy. Baseline CSF 5-MTHF levels were severely reduced (mean 7.17±9.30 nmol/L), with 84% of tested subjects showing levels below 10 nmol/L compared to normal ranges of 40-240 nmol/L, confirming the profound central nervous system folate deficiency. This untreated natural history of relentless progression provides a control against which treatment responses can be evaluated, demonstrating the substantial deterioration that occurs between disease onset and treatment initiation.

Treatment with Leucovorin

Timing of Treatment Initiation

Treatment was initiated across a wide age range, with data on the age at treatment initiation available for 41 of 46 subjects. As shown in Table 13, the mean age at treatment initiation was 9

years (range 2 months to 33 years). The majority of subjects began treatment during childhood, with 69% starting treatment before age 10 years. However, treatment was initiated in adolescence or adulthood in some cases, reflecting the challenges in recognizing this rare condition and the evolution of diagnostic capabilities over the study period.

A critical finding was the substantial delay between symptom onset and treatment initiation observed for many subjects. Treatment delay data were available for 38 subjects and revealed a mean delay of 6.8 years (range 0 to 31 years). Only 11% subjects experienced minimal delay (<6 months), while 79% subjects experienced delays of 2 years or longer. The longest treatment delays exceeded 20 years in some of the adult-diagnosed cases. This pattern of delayed treatment initiation has important implications for therapeutic outcomes, as early intervention appears critical for optimal clinical response in this progressive condition.

Table 13 Treatment Timing Characteristics

Characteristic	Value	Details
Age at Treatment Initiation		
Subjects with data available	41/46 (89%)	
Mean (SD)	9 years (6.9)	
Median (range)	8 years (2 months - 31 years)	
Age categories		
<2 years	2 (5%)	
2 to <5 years	8 (20%)	
5 to <10 years	18 (44%)	
10 to <18 years	10 (24%)	
≥18 years	3 (7%)	
Treatment Delay		
Subjects with data available	38/46 (83%)	
Mean (SD)	6.8 years (7.0)	
Median (range)	5 years (0 - 31 years)	
Delay categories		

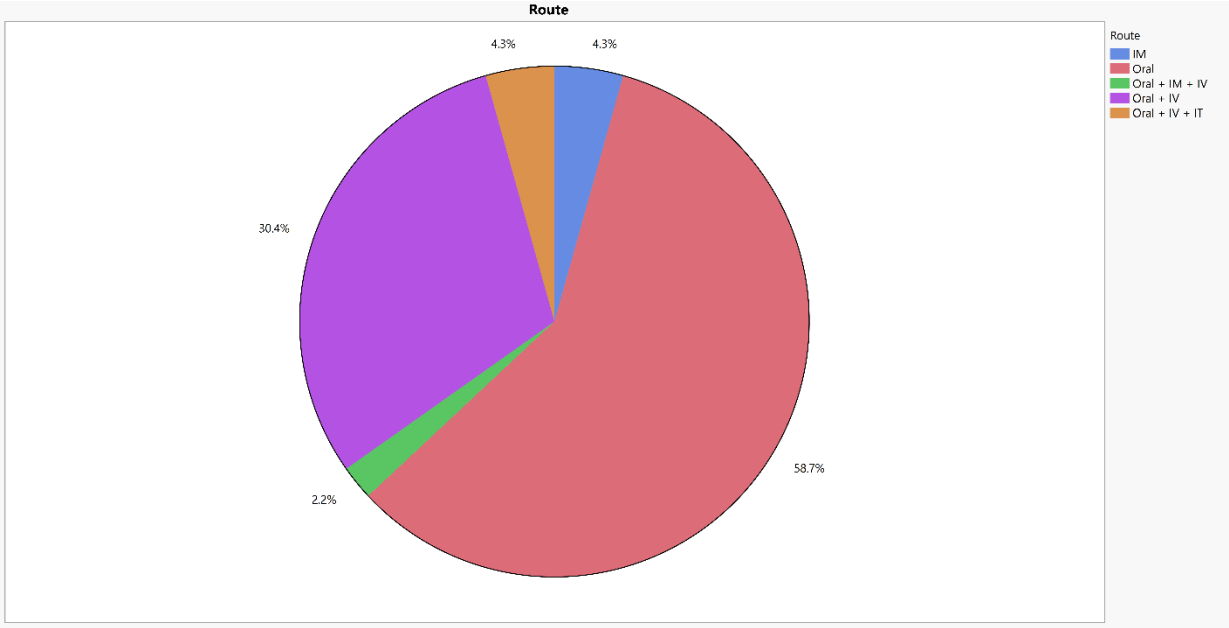
Characteristic	Value	Details
<6 months	4 (11%)	Early treatment
6 months to <2 years	4 (11%)	
2 to <5 years	10 (26%)	
≥5 years	20 (53%)	Substantial delay

Data collated from published case reports by reviewer.

Route of Administration

Figure 3 shows frequency of routes of administration for the leucovorin treatment approaches used across the case report literature. The majority of treatment approaches included oral (59%) or oral + intravenous (IV) (30%) dosage forms. A few treatment approaches included oral + intramuscular (IM) leucovorin, with or without IV administration. Dreha-Kulaczewski et al. included a 9-year-old female patient treated with daily oral, weekly IV, and every two months intrathecal (IT) administration and a 17-year-old female patient treated with daily oral, weekly IV, and monthly IT leucovorin (Dreha-Kulaczewski, 2024).

Figure 3 Route of Administration



Data collated from published case reports and figure generated by reviewer.
Abbreviations: IM, intramuscular; IV, intravenous; IT, intrathecal

Dosing Ranges and Patterns

Dosage regimens of leucovorin, as reported in the cases series, are considerably different, reflecting the absence of established treatment guidelines and the empirical nature of dose

selection in clinical practice. Of the 59% (n = 27) subjects who received oral leucovorin only, 25 subjects had information for the starting dose used, ranging from 0.5 to 3 mg/kg/day. The starting oral dose was 2 mg/kg/day in 56% (n = 14/25) of subjects. Of the 20 subjects with dose escalation reported, 85% (n = 17) had a maximum dose ≤6 mg/kg/day (reported range: 1.7 to 8.5 mg/kg/day).

IV leucovorin dosing varied significantly in both dose and frequency of administration. The most frequently reported IV regimen was 20 mg/kg administered monthly. Weight-based IV doses ranged from 6 mg/kg administered weekly to 25 mg/kg given monthly, with most centers using intermittent high-dose approaches rather than continuous infusion. Several subjects received combination therapy with daily oral dosing supplemented by weekly or monthly IV administration.

Dose escalation strategies were commonly employed. Typical escalation patterns included increasing oral doses from 2-3 mg/kg/day to 5-8 mg/kg/day or adding IV therapy to existing oral regimens. Some centers implemented systematic dose titration based on clinical response and CSF 5-MTHF levels, while others used empirical dose increases based on seizure control or developmental progress. The highest reported doses reached 11 mg/kg/day orally in combination with monthly IV therapy, suggesting that some subjects may require aggressive dosing approaches to achieve optimal clinical benefit.

IM dosing was less commonly reported, with doses ranging from 2-4 mg/kg/day when used. IT administration was employed in only rare cases using doses of 2-8 mg administered every other month or monthly.

Treatment Duration

Information on treatment duration was not consistently reported across case reports. While the majority of reports provided follow-up data spanning 1 to 5 years, the specific duration of leucovorin treatment could not always be distinguished from the overall follow-up period. However, treatment duration could be inferred from the follow-up timeframes, which appeared to indicate indefinite therapy. This inference was supported by observations that discontinuation of leucovorin in several reported cases led to clinical deterioration and return of symptoms, suggesting the need for lifelong treatment.

Efficacy

Parameters for systematic evaluation of treatment effect were not consistently reported across case reports. Clinical assessments of the treatment effect focused primarily on seizure frequency and severity, motor function improvements, and developmental progress. CSF 5-MTHF levels served as the primary biochemical parameter for treatment monitoring when available, with some centers using CSF folate normalization as a target for dose optimization. Neuroimaging follow-up with brain MRI or MRS was not routinely performed, and results of routine laboratory monitoring were rarely detailed. The heterogeneity in evaluation

approaches reflects the absence of established treatment guidelines and the evolving understanding of optimal management strategies for FOLR1-CFTD over the study period.

Key patient-level clinical and biomarker data from the 46 subjects with FOLR1-CFTD included in this review can be found in the Appendix (Section 19.1, Table A1).

Efficacy Assessment Framework for This Review

The review team developed a structured approach to evaluate treatment efficacy across the case series and literature for the purposes of this regulatory analysis. This framework was necessary due to unique methodological challenges inherent in the available evidence, including the absence of standardized assessments of efficacy, variable follow-up periods, and heterogeneous reporting practices across different studies and treatment centers. This approach differs from the clinical evaluations conducted by individual case report authors, which were described in the previous section and varied considerably in their methods and parameters.

Response categorization was established based on the clinical improvements compared to baseline reported across multiple functional domains, recognizing that FOLR1-CFTD affects multiple neurological systems. Treatment response was evaluated across three primary domains (seizure control, motor function, and developmental abilities) representing clinically meaningful concepts. The post-treatment clinical condition across these domains was compared with the pre-treatment baseline condition. Such efficacy analysis compares observed changes from baseline to an estimate of what would have happened without intervention.

Complete recovery was defined as subjects achieving normal or near-normal neurological function with resolution of major symptoms and restoration of age-appropriate developmental milestones. Substantial improvement was characterized by clinical gains in two or more domains, such as seizure freedom or significant seizure reduction combined with notable motor function recovery or developmental progress. Partial improvement included gains in clinical function in one domain with or without persistent deficits in other domains. No improvement included subjects who remained stable without demonstration of clinical benefit or worsened. The use of these coarse criteria for the assessment of relevant clinical symptom domains allows for quantification of large and clinically meaningful improvements in symptoms to minimize the potential for bias; a change in scoring by one category would clearly represent a clinically meaningful change.

This framework acknowledges several important limitations inherent to case report literature. Assessments of efficacy were not standardized across studies, with different investigators using varying criteria and terminology for assessing improvement. Follow-up duration was inconsistent, ranging from months to years, which limited the ability to assess long-term durability of treatment effects. Additionally, the subjective nature of many efficacy assessments, combined with the potential for publication bias toward positive results,

necessitated careful consideration in the interpretation of aggregate findings. To address these limitations, the review team's efficacy framework prioritized outcomes that are both relatively objective and clinically meaningful such as seizure freedom or restoration of age-appropriate developmental milestones. This approach was undertaken to provide the most reliable evaluation of treatment efficacy despite the inherent constraints of the available evidence.

CSF 5-MTHF levels were considered a predictive biomarker of treatment effect. When available, pre-treatment values were compared to post-treatment values and clinical outcomes.

Magnetic resonance spectroscopy (MRS) represents another potential objective biomarker of treatment response (Dreha-Kulaczewski, 2024), as pre-treatment brain MRS frequently demonstrated abnormal findings characterized by low choline and inositol levels, reflecting disturbed myelin metabolism (Table 12). However, post-treatment MRS data were not available to a sufficient degree to systematically evaluate MRS as a treatment response biomarker in this review. The limited availability of post-treatment MRS data in the case report literature highlights an important gap in the current evidence base and represents an opportunity for more systematic biomarker assessment in prospective treatment protocols.

Results

Application of the efficacy assessment framework to the 46 subjects with FOLR1-CFTD treated with any formulation of leucovorin yielded the following results (Table 14).

Complete Recovery was reported for 3 subjects (7%). All three subjects received treatment with minimal delay after symptom onset, including one patient treated pre-symptomatically. These subjects demonstrated normal or near-normal neurological function with resolution of major symptoms and restoration of age-appropriate developmental milestones.

Substantial Improvement was reported for 27 subjects (59%), representing the largest response category. These subjects demonstrated clinical gains across two or more functional domains. Response patterns were diverse, ranging from seizure freedom with marked motor and social improvements to significant seizure reduction (often >50%) combined with developmental gains such as language recovery and independent ambulation. Several subjects achieved complete seizure freedom, while others experienced substantial seizure reduction accompanied by meaningful motor and cognitive improvements. Some subjects showed differential responses to treatment routes, with better outcomes achieved through intravenous compared to oral administration.

Partial Improvement was reported for 9 subjects (20%). These subjects demonstrated gains in clinical function in one domain with persistent deficits in other domains. Examples included subjects with continued ataxia despite achieving seizure control, or cases where treatment provided benefit in one domain (e.g., motor function) while other symptoms (e.g., cognitive impairment or ongoing seizures) remained unchanged.

No Improvement occurred in 6 subjects (13%). Three subjects showed no appreciable improvements in the setting of prolonged treatment delays exceeding 13-15 years from onset of symptoms to treatment initiation. One patient with concurrent POLG1 mutations also showed no response. Two subjects remained stable without demonstration of clinical benefit.

Unable to Assess: One subject (2%) could not be categorized within this framework. This patient experienced clinical benefit described as "slow improvement in clinical condition," but the treatment response could not be definitively assigned to a specific category.

Overall, 40 of 46 subjects (87%) experienced some degree of clinical benefit from leucovorin therapy, with 30 subjects (65%) achieving substantial improvement or complete recovery.

A key point is that compared to the progressive natural history of FOLR1-CFTD, both the observed clinical improvements and stabilized symptoms suggesting lack of disease progression are unexpected.

Please refer to Section 19.1 Table 16 of the Appendix for a description of outcomes at an individual level for all 46 subjects reviewed.

Table 14 Efficacy Assessment - Results by Category (N=46)

Category	N (%)	Definition
Complete recovery	3 (7%)	Normal function, resolution of major symptoms
Substantial improvement	27 (59%)	Clinical gains in two or more domains
Partial improvement	9 (20%)	Clinical gain in one domain with persistent deficits
No improvement	6 (13%)	No discernible clinical benefit or stable condition
Unable to assess	1 (2%)	Insufficient outcome data[†]

Data collated from published case reports by reviewer.
[†]Treatment response described as “slow improvement in clinical condition”

Results in the subgroup treated with oral leucovorin

As stated in Section 6.3.1, systemic exposures of leucovorin formulations (oral tablet, capsule, solution, and parenteral IM and IV) are relatively similar, with absolute bioavailability ≥95% for oral formulations at doses ≤25 mg. However, because tablets for oral use are the dosage form

object of this review, the review team evaluated efficacy results for the subgroup of individual subjects who received leucovorin exclusively via the oral route.

Twenty-seven out of 46 (59%) subjects received leucovorin only via the oral administration route. A range of clinical improvements in various neurological symptoms following treatment with oral leucovorin was reported for 24 of the 27 subjects (e.g., reduction in severity or number of seizures; improvements in motor function, communication, and/or behavior). The remaining 3 subjects showed either no change or no progression of symptoms (these 3 subjects were counted as an outcome of "no improvement" above). Both the observed clinical improvements and the lack of disease progression are unexpected when compared to the progressive natural history of patients with FOLR1-CFTD. The observed favorable outcomes after treatment with oral leucovorin are in agreement with the favorable outcomes observed after treatment across leucovorin formulations.

Descriptive analysis of the relationship between treatment timing and outcome in selected cases.

Table 1515illustrates the relationship between treatment timing and clinical outcomes across representative cases, demonstrating patterns that support early initiation of leucovorin treatment for patients with FOLR1-CFTD.

All three subjects who achieved complete recovery received treatment within 6 months of symptom onset or pre-symptomatically, with treatment initiated between 2 months of age and 2 years, 3 months of age. Two subjects had resolution of ataxia and the subject treated presympomatically did not develop symptoms within the duration of follow-up.

Outcomes in subjects categorized as substantial improvement showed more variable delays in initiation of treatment but generally received therapy within the first few years after symptom onset. Notable examples include the subject described by Al-Baradie et al. (2014), who became seizure-free with marked motor and social improvements despite a 2 to 5-year treatment delay, and the subject described by Delmelle et al. (2016) who achieved seizure freedom, independent ambulation, and language recovery after adding monthly IV dosing in addition to daily oral dosing. Even subjects with longer delays of 5 years or more, such as cases described by Mafi et al. (2020) and Pérez-Dueñas et al. (2010), demonstrated substantial improvements including seizure freedom and enhanced psychomotor development, indicating that meaningful clinical benefit remains possible despite delayed treatment initiation.

Outcomes in subjects categorized as partial improvement illustrate the most likely outcomes of delayed treatment, with subjects showing meaningful but incomplete responses. A 17-year-old subject described by Gowda et al. 2021 achieved seizure control but had persistent ataxia and cognitive decline despite treatment, while the subject described by Toelle et al. (2014) experienced significant seizure reduction but continued to have motor deficits. These cases suggest that although leucovorin can provide clinical benefit even with substantial treatment

delays, the degree of improvement may be limited when treatment is initiated years after symptom onset.

Outcomes in subjects categorized as no improvement demonstrate the critical importance of early intervention and highlight potential confounding factors. Three subjects described by Brunetti et al. (2021) with treatment delays of 13 to 15 years showed no clinical improvement despite leucovorin therapy. These cases underscore the progressive nature of untreated FOLR1-CFTD and the diminishing potential for recovery with prolonged treatment delays, reinforcing the urgent need for early recognition and prompt therapeutic intervention in this condition.

Table 1515 Individual Subject Outcomes by Treatment Delay (Selected Cases)

Study	Age at Treatment	Treatment Delay	Seizures	Motor	Development	Overall Response
Steinfeld et al. 2009	2 years 3 months	<6 months	No baseline seizures	Resolved ataxia	No baseline developmental problems	Complete
Potic et al. 2023	2 years	<6 months	No baseline seizures	Resolved truncal ataxia and intention tremor	No baseline developmental problems	Complete
Dreha-Kulaczewski et al. 2024	2 months	Presymptomatic†	No baseline seizures	No baseline motor problems	No baseline developmental problems	Complete
Al-Baradie et al. 2014	5 years 8 months	2 to <5 years	Seizure free	Markedly improved motor skills	Markedly improved social interaction	Substantial
Delmelle et al. 2016	3 years 1 month	<6 months	Seizure free	Walking independently	Language recovery	Substantial
Mafi et al. 2020	11 years	≥5 years	>50% seizure reduction	Improved motor coordination	Improved social interactions	Substantial

Study	Age at Treatment	Treatment Delay	Seizures	Motor	Development	Overall Response
Pérez-Dueñas et al. 2010	7 years	≥5 years	Seizure free	Improved psychomotor development	Improved psychomotor development	Substantial
Gowda et al. 2021	17 years	≥5 years	Seizure control	Persistent ataxia	Persistent cognitive decline	Partial
Toelle et al. 2014	5 years	2 to <5 years	50-75% reduction in drop attacks, persistent myoclonus	Persistent ataxia	Baseline developmental regression; outcome not specified	Partial
Brunetti et al. 2021	17 years	≥5 years	Baseline daily refractory tonic-myoclonic seizures	Baseline progressive ataxia, spasticity, dystonia	Baseline developmental regression	None
Brunetti et al. 2021	15 years	≥5 years	Baseline tonic and myoclonic seizures	Baseline progressive ataxia, spasticity, dystonia	Baseline developmental regression	None
Brunetti et al. 2021	14 years	≥5 years	Baseline daily refractory tonic-myoclonic seizures	Baseline progressive ataxia, spasticity, dystonia	Baseline developmental regression	None

Data collated from published case reports by reviewer.
† 2-month-old patient from Dreha-Kulaczewski et al. had no symptoms throughout course.

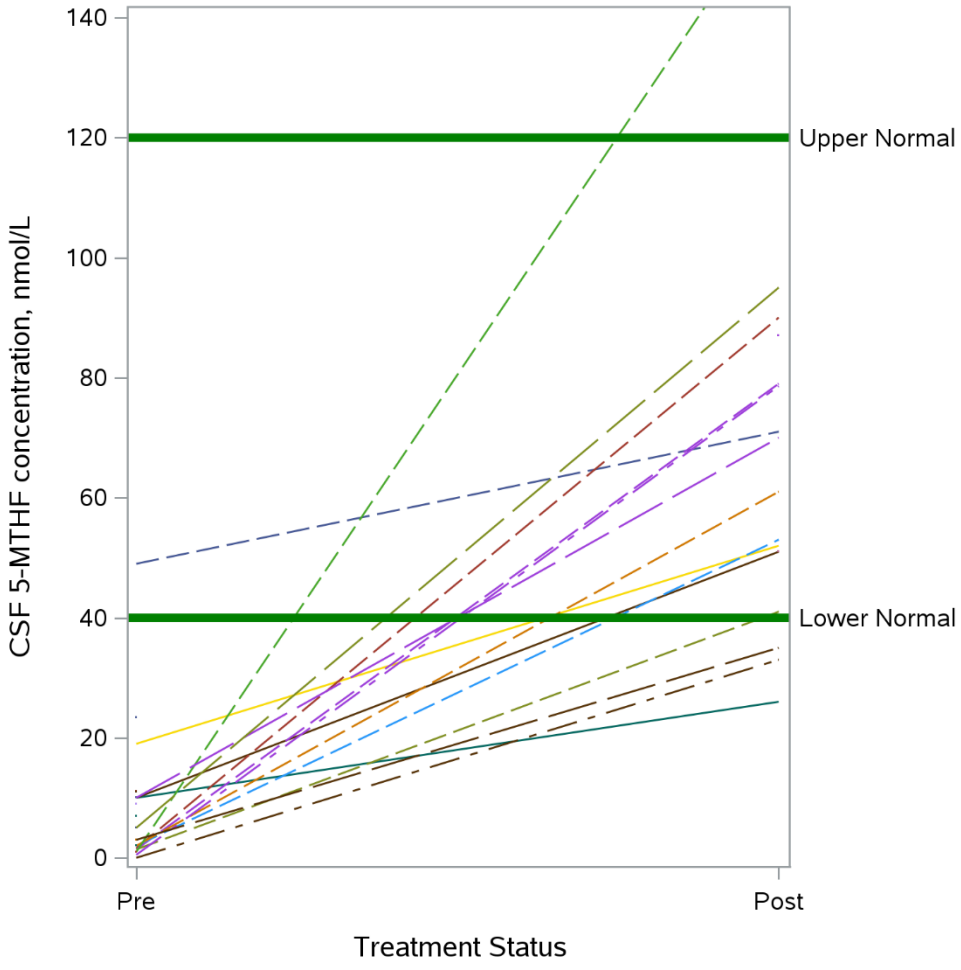
As stated above, baseline CSF 5-MTHF levels (7.17±9.30 nmol/L, Mean±SD) were available for 32 subjects (70% of the cohort). However, pre and post-treatment CSF 5-MTHF levels were only available for 15 subjects. The pre-treatment CSF 5-MTHF levels were 7.72±12.58 nmol/L (Mean±SD); and post treatment CSF levels for the same subjects were 66.37±33.28 nmol/L; Mean±SD), showing substantial improvements in central nervous system folate availability following leucovorin therapy. All 15 subjects showed some improvement in CSF 5-MTHF levels

but in 12 out of 15 subjects (80%) normalization of CSF 5-MTHF levels was achieved with values above 40 nmol/L. The magnitude of improvement varied considerably, with some subjects achieving CSF levels well within the normal range while others showed partial correction of the biochemical defect.

Figure 4 shows the biochemical response to leucovorin treatment in subjects with available follow-up CSF data.

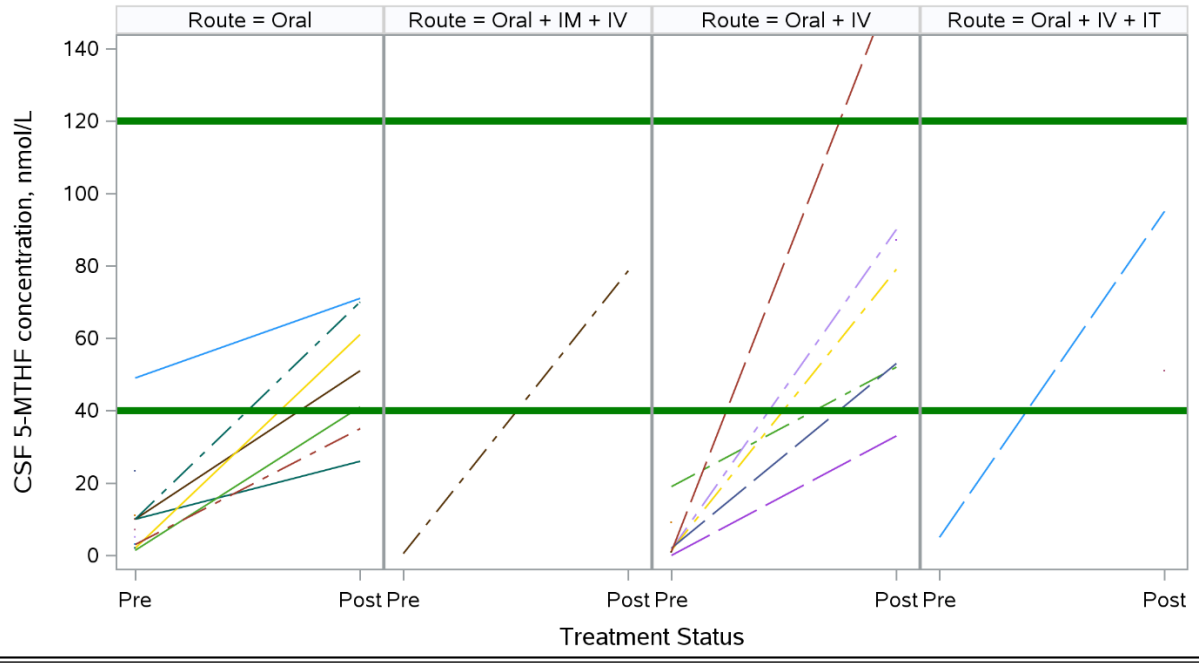
Figure 5 shows the biochemical response to leucovorin treatment in subjects with available follow-up CSF data by route of administration.

Figure 4 Pre- and Post-Treatment CSF 5-MTHF Levels



Data shown are from individual subjects. Data collated from published case reports and figure generated by reviewers.
Lower and upper normal levels are based on listed normal reference range of 40 to 120 nmol/L.
Abbreviations: Pre, Pre-treatment; Post, Post-treatment

Figure 5 Pre- and Post-Treatment CSF 5-MTHF Levels. Data shown by route of administration



Data shown are from individual subjects. Data collated from published case reports and figure generated by reviewers.
Lower and upper normal levels are based on listed normal reference range of 40 to 120 nmol/L.
Abbreviations: Pre, Pre-treatment; Post, Post-treatment

The descriptive analysis of the relationship between treatment timing and outcome in selected cases showed generally concordant responses, with subjects achieving CSF 5-MTHF normalization demonstrating higher rates of clinical improvement. However, the analysis remained descriptive, as there were insufficient data to establish a mathematical correlation coefficient. Observationally, some subjects showed meaningful clinical benefits despite CSF 5-MTHF levels below values considered normal, while others reached normal CSF 5-MTHF levels with more limited clinical gains. These patterns indicate that while CSF 5-MTHF levels serve as a valuable diagnostic and pharmacodynamic/response biomarker demonstrating target engagement, clinical response is influenced by additional factors including treatment delay (the most critical determinant), baseline disease severity, and individual patient characteristics.

Conclusions on Effectiveness in FOLR1-CFTD

Assessment of efficacy was based on a descriptive analysis of 46 genetically confirmed cases of CFTD with FOLR1 genetic variants treated with leucovorin for which clinical outcomes were available.

The patient population was predominantly female (67%); ages ranged from 2 months to 33 years, with early disease onset (average age at onset 2.2 years) and severe baseline manifestations including near-universal white matter changes on MRI (98%), motor dysfunction (96%), and refractory seizures (85%).

The review team developed a structured efficacy assessment framework evaluating treatment response across relevant clinical symptom domains (seizure control, motor function, developmental abilities, and behavioral features), categorized by number of domains impacted and magnitude of effects so that a change in scoring would clearly represent a clinically meaningful change.

The review methodology specified coarse criteria for the assessment of these clinical symptom domains that allowed for quantification of large and clinically meaningful improvements in symptoms to minimize the potential for bias. Clinical symptom domains were evaluated as a change from pre-treatment baseline and were compared to an estimate of no change/decline in the trajectory of these clinical domains that was based on the natural history of FOLR1-CFTD in the absence of treatment with leucovorin.

Post-treatment clinical condition was compared with pre-treatment baseline, with outcomes categorized as complete recovery, substantial improvement, partial improvement, or no improvement based on neurological function, symptom resolution, and developmental milestone achievement.

Results demonstrated that 40 of 46 subjects (87%) experienced clinical benefit compared to baseline, with 30 subjects (65%) achieving substantial improvement or complete recovery. Three subjects (7%) achieved complete recovery (all treated with minimal delay), 27 subjects (59%) showed substantial improvement ranging from seizure freedom with motor gains to significant seizure reduction with developmental progress, 9 subjects (20%) demonstrated partial improvement in one domain, and 6 subjects (13%) showed no improvement (including three with treatment delays exceeding 13-15 years). Both the observed clinical improvements and disease stabilization are inconsistent with the progressive natural history expected without treatment intervention. In the subgroup of 27 subjects receiving oral leucovorin exclusively, 24 (89%) demonstrated clinical improvements or lack of disease progression compared to baseline, consistent with the overall sample.

CSF 5-MTHF levels served as both a diagnostic and response biomarker. Paired pre- and post-treatment data, available in 15 subjects, showed substantial improvements from baseline with 12 subjects (80%) achieving normalization of 5-MTHF levels above 40 nmol/L. Although subjects achieving CSF normalization generally demonstrated higher rates of clinical improvement, some subjects showed meaningful clinical benefits despite incomplete biochemical correction, indicating that clinical response is influenced by multiple factors including treatment delay (the most critical determinant), baseline disease severity, and individual patient characteristics.

Treatment timing analysis demonstrated that all three subjects achieving complete recovery received treatment within 6 months of symptom onset, while subjects with delays exceeding 13-15 years showed no improvement, underscoring the critical importance of early intervention.

In the context of an extremely rare, progressive disease and constraints that preclude the conduct of traditional randomized controlled trials, the descriptive analysis meets the criteria of an adequate and controlled investigation comparing subjects' post-treatment states with their pre-treatment baseline conditions and demonstrates clinically meaningful improvements that are unlikely to have occurred spontaneously. Normalization of CSF 5-MTHF levels following leucovorin treatment provides strong mechanistic evidence supporting the treatment effect beyond clinical observations alone.

Substantial evidence of effectiveness is demonstrated for leucovorin for the treatment of FOLR1-CFTD based on a single adequate and well-controlled study plus the confirmatory evidence provided by the biomarker demonstrating mechanistic evidence of an effect of leucovorin on the underlying pathophysiology of cerebral folate transport deficiency.

8.2. Assessment of Efficacy Across Trials

N/A

8.3. Integrated Assessment of Effectiveness

N/A

8.4. Review of Safety

8.4.1. Safety Review Approach

The overall understanding of the safety profile for leucovorin is informed by the toxicology, safety pharmacology, and clinical safety review of NDA 018342. Because leucovorin is an approved and marketed product, the safety assessment also included information obtained in the postmarketing setting.

According to the current USPI, leucovorin is contraindicated in patients with a history of hypersensitivity reaction to leucovorin (folinic acid), levoleucovorin, folic acid, or any component of the formulation, as anaphylactic reactions have been reported. The primary safety concern is hypersensitivity reactions, including anaphylaxis and urticaria, which may require permanent discontinuation based on severity. The most commonly reported adverse reactions identified during postmarketing use include dermatologic reactions (pruritus and rash), respiratory symptoms (dyspnea), and other clinical events such as rigors and temperature change. Additionally, important drug interactions exist with certain antiepileptic drugs

(phenobarbital, phenytoin, and primidone), where high-dose leucovorin may reduce antiepileptic effectiveness and increase seizure frequency. Although the patient population with FOLR1-CFTD differs substantially from the oncologic population in terms of disease characteristics, treatment duration, and patient demographics, the extremely limited safety data available from the 46 published cases of FOLR1-CFTD necessitates reliance on the well-established safety profile of leucovorin.

8.4.2. Review of the Safety Database in FOLR1-CFTD

Overall Exposure
The safety database for leucovorin in FOLR1-CFTD includes 46 subjects with FOLR1-CFTD.

CFD due to FOLR1 Variants (Case Report Literature)

- 46 subjects with genetically confirmed FOLR1 mutations
- Age range: 2 months to 31 years at treatment initiation
- Treatment duration: Variable, ranging from months to years
- Dosing regimens: Oral doses 0.5-8.5 mg/kg/day
- Geographic distribution: 20 countries across multiple continents

Adequacy of the Safety Database
The safety database is considered adequate for regulatory decision-making, particularly for FOLR1-CFTD given the rarity of the condition, the severity of untreated disease, and the established safety profile of leucovorin in approved indications.

8.4.3. Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality
The safety data were derived from published literature rather than direct submission of clinical study reports, thus the Agency was unable to verify the reported safety data.

8.4.4. Safety Results

Deaths
No deaths were reported as related to leucovorin treatment in any of the reviewed studies. The case report literature did not systematically report mortality data.

Serious Adverse Events (SAEs)
The case report literature provided limited systematic reporting of SAEs. No treatment-related SAEs were explicitly reported across the 46 subjects.

Treatment Emergent Adverse Events (TEAEs)
TEAE reporting was provided in only 3 cases from the literature review:

- Two subjects reported no side effects from oral leucovorin treatment
- One patient experienced mild leukopenia assessed as possibly related to IV treatment

Laboratory Findings

Limited laboratory monitoring data were available from the reviewed studies. The case report literature rarely provided detailed laboratory monitoring results.

8.4.5. Analysis of Submission-Specific Safety Issues

No submission-specific safety issues were identified.

8.4.6. Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability

N/A

8.4.7. Safety Analyses by Demographic Subgroups

No safety analyses were available by demographic subgroups.

8.4.8. Specific Safety Studies/Clinical Trials

No dedicated safety studies were conducted specifically for leucovorin in the FOLR1-CFTD population. Safety data were derived from efficacy studies and case reports as described above.

8.4.9. Additional Safety Explorations

Human Carcinogenicity or Tumor Development

N/A

Human Reproduction and Pregnancy

N/A

Pediatrics and Assessment of Effects on Growth

N/A

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

N/A

8.4.10. Safety in the Postmarket Setting

In the context of more than 70 years of marketing, the Division of Pharmacovigilance (DPV) manually reviewed over 1,500 FAERS reports to identify cases that reported leucovorin use outside of the oncology setting and did not identify any new safety signals. DPV identified 13 deduplicated cases that reported leucovorin use for various indications and/or in subjects with various underlying conditions (some with rare genetic syndromes), included a broad age range, and noted varying leucovorin regimens (doses, frequency, duration of use) making it difficult to establish any association between reported adverse events or product quality issues and leucovorin. In 11 cases, leucovorin was used in the setting of ASD (n=3) and folate deficiency (n=8), but no pattern of reported adverse events was evident. The paucity of cases and reported adverse events with leucovorin in ASD and folate deficiency does not allow us to reach any conclusions with respect to safety and leucovorin use in this setting. Based on the available information in this review, DPV did not identify compelling evidence of new safety concerns associated with leucovorin use outside of the oncology setting that could be considered for inclusion in the updated proposed labeling for leucovorin with the new indication for CFD. In conclusion, no new safety signals were identified that warrant inclusion in the Product Information.

Epidemiology: Review of Literature

The Division of Epidemiology I (DEPI-I) conducted a review of published medical literature regarding the safety of leucovorin calcium (NDA 018342) to support labeling, specifically to Section 5 (Warnings and Precautions) and Section 6 (Adverse Reactions), for a new indication for the treatment of cerebral folate deficiency (CFD) in adult and pediatric subjects. DEPI-I conducted comprehensive PubMed literature searches using a phased approach, with focus on pediatric and adult non-oncology publications. Because it is not feasible to determine what, if any, role leucovorin may contribute when adverse drug reactions (ADRs) are observed in a setting of concomitant toxic therapy, a decision was made to exclude publications in which leucovorin was administered in an oncology setting. We identified a total of 225 publications for full text review. Of these, the majority (134/225, 60%) did not include any mention of ADRs attributed to leucovorin, and 13 were identified with potential ADRs of interest. Of the 134 publications in which we found no mention of ADRs attributed to leucovorin, 69% were case reports/case series, 17% were randomized controlled trials, and 14% were other clinical studies.

The most common indications for leucovorin use in these 134 publications included: Cerebral folate deficiency (22%), congenital folate malabsorption (8%), Cobalamin C disease (7%), endstage renal disease (ESRD)/homocystinuria (7%), seizures/epilepsy (7%), healthy volunteers in pharmacokinetic studies (5%), autism spectrum disorder (4%), dihydropteridine reductase (DHPR) deficiency (4%), methylenetetrahydrofolate reductase (MTHFR) deficiency (4%), and Rett Syndrome (4%). Most (83%) of the 134 publications with no mention of ADRs attributed to

leucovorin described subjects who were treated with leucovorin for ≥4 weeks, comprising approximately 1200 subjects. Of these, at least 140 subjects were treated with leucovorin for at least one year, reflecting long-term exposure without mention of any ADRs.

Thirteen publications were identified for in-depth assessment of potential ADRs with leucovorin. Study types included randomized controlled trials (n=4), other clinical studies (n=4), and case reports/case series (n=5). Of these, 10 publications described approximately 275 subjects who were treated with leucovorin for ≥4 weeks, of which approximately 135 subjects were treated with leucovorin for at least one year. Hypersensitivity reactions were described in three subjects. No reports of serious ADRs attributed to oral administration of leucovorin were identified in this review. As planned, a Warning and Precaution (Section 5.1) for hypersensitivity reactions remains appropriate. Limitations of this review include: 1) the potential for missing data if authors did not describe the occurrence of adverse events or ADRs in their publication, 2) the frequent presence of confounding factors in published studies and case reports that prohibited isolation of the effects of leucovorin and resulted in study exclusions, and 3) the varied indications for leucovorin use, dosage, and duration of therapy in published studies. Although DEPI-I did not identify any new ADRs that would necessitate regulatory action (e.g., labeling), we recommend continued surveillance.

8.4.11. Integrated Assessment of Safety

The safety profile observed in the FOLR1-CFTD population across the descriptive analysis of the 46 genetically conformed cases appears favorable, with no deaths reported as related to leucovorin treatment and no treatment-related serious adverse events explicitly documented across the reviewed studies. Notably, only three published cases provided systematic adverse event reporting, with two subjects reporting no side effects and one experiencing mild leukopenia possibly related to intravenous treatment. Laboratory monitoring data were limited but revealed no clinically significant abnormalities.

Post-marketing surveillance over more than 70 years of leucovorin use identified no new safety signals relevant to the CFTD indication. The Division of Pharmacovigilance reviewed over 1,500 FAERS reports and identified 13 cases of leucovorin use outside oncology settings, including 11 cases in autism spectrum disorder and folate deficiency contexts, but found no pattern of adverse events that would suggest new safety concerns. Additionally, a comprehensive literature review by the Division of Epidemiology identified 225 publications for evaluation, with the majority (60%) containing no mention of adverse drug reactions attributed to leucovorin. Of particular relevance, approximately 1,200 subjects were treated with leucovorin for at least four weeks without reported adverse reactions, and at least 140 subjects received treatment for one year or longer, demonstrating acceptable tolerability with extended exposure. The few adverse reactions identified were primarily hypersensitivity reactions in three subjects, supporting the retention of existing warnings and precautions in product labeling.

Based on this comprehensive safety evaluation, leucovorin demonstrates an acceptable safety profile for the treatment of FOLR1-CFTD, consistent with its established safety profile in approved indications. The absence of new safety signals, combined with the severity of untreated FOLR1-CFTD and the rarity of the condition, supports a favorable risk-benefit assessment for this indication. Continued post-marketing surveillance remains appropriate to monitor for any emerging safety concerns as clinical experience expands in this population.

8.5. Statistical Issues

N/A

8.6. Conclusions and Recommendations

The literature-based analysis of 46 subjects with FOLR1-CFTD across 26 independent studies provides substantial evidence of effectiveness for leucovorin treatment based on an adequate and well-controlled study in this context plus confirmatory evidence of a mechanistic effect of leucovorin on the underlying pathophysiology of the disease.

Clinical symptom domains were evaluated as a change from pre-treatment baseline and were compared to an estimate of no change/decline in the trajectory of these clinical domains that was based on the natural history of FOLR1-CFTD in the absence of treatment with leucovorin.

The clinical efficacy data demonstrate an 87% overall response rate with 65% of subjects achieving substantial improvement or complete recovery.

A clear pattern exists with treatment timing, as complete recovery was achieved only in subjects treated within 6 months of symptom onset. The consistent therapeutic benefit observed across 26 studies spanning 15 years and 20 countries demonstrates reproducible effects that extend beyond investigator bias or chance.

Eighty-four percent of subjects with available CSF samples had severely reduced baseline CSF 5-MTHF levels below 10 nmol/L, and 80% of subjects with follow-up data achieved CSF 5-MTHF normalization. This provides direct strong mechanistic evidence that leucovorin restores central nervous system folate availability, confirming target engagement and substantiating the treatment effects observed clinically.

Given the rarity of FOLR1-CFTD with a prevalence of less than 1 in 1,000,000 individuals, combined with the severity and progressive nature of the condition, controlled trials are not feasible. The literature-based baseline-controlled approach represents an appropriate methodology consistent with regulatory precedent for other rare metabolic disorders.

No treatment-related deaths or serious adverse events were reported across the reviewed studies. Only one documented treatment-related adverse event, mild leukopenia, occurred among the 46 subjects treated. The observed safety profile remains consistent with established leucovorin safety in approved indications. Approval is recommended.

9 Advisory Committee Meeting and Other External Consultations

An Advisory Committee was not conveyed for this application.

10 Pediatrics

This application triggers PREA as a new indication for an approved product. The application was discussed at the Pediatric Review Committee (PeRC) meeting on January 13, 2026, where the Committee reviewed the pediatric assessment approach. The Applicant utilized literature to support this application, with 46 cases reviewed for which age information was described for 41 subjects, and 38 were pediatric subjects with an age range of 0.2 years (2.4 months) to 17 years. The review Division stated that the analysis of the case reports meets criteria for an adequate and well-controlled study based on a clearly stated objective to evaluate the evidence for the efficacy of leucovorin in FOLR1-CFTD in pediatric and adult subjects, description of methods for appropriate selection of subjects, assessment of clinical outcomes, and descriptive analytical approaches, and design that allows a valid comparison to historic control. The PeRC agreed with the review Division that this application has been fully assessed in the pediatric patients birth to 17 years of age, and the labeling will be reflected accordingly.

11 Labeling Recommendations

11.1. Prescription Drug Labeling

Information highlighted below are significant changes made to the Prescribing Information (PI) from the previously approved Wellcovorin PI, which adhered to the labeling regulations under 21 CFR 201.80. According to 21 CFR 201.56(c)(1), because S-015 is an efficacy supplement, the Wellcovorin PI to be approved with S-015 must comply with the content and format requirements under the Physician Labeling Rule (PLR), which are noted in 21 CFR 201.56 and 201.57. Changes to the PI specifically related to the conversion to PLR are not outlined below, but have been incorporated into the agreed upon labeling. High level changes are noted below for additions related to the new indication; see the respective portions of this review for supporting evidence.

Prescribing information

Section	Summary of Labeling Changes
1 INDICATIONS AND USAGE	1.2 Cerebral Folate Transport Deficiency with Folate Receptor 1 Genetic Variant • The new indication for the treatment of cerebral folate transport deficiency in adults and pediatric patients with a confirmed variant in the folate receptor 1 gene has been added. • A Limitations of Use (LOU) has been added for patients with a deficiency in the primary enzyme for leucovorin metabolism (MTHFS) because of the issue of converting to the active metabolite. 1.3 Limitations of Use • The information regarding pernicious anemia or other megaloblastic anemias was relocated from the CONTRAINDICATIONS section in old format labeling to a Limitations of Use subsection in the I&U section because we generally do not contraindicate uses. This information has been added under a separate subsection because it is not specifically related to any of the approved indications.
2 DOSAGE AND ADMINISTRATION	2.1 Important Administration Instructions • Information has been added regarding literature reports of crushing leucovorin tablets and mixing with food or liquid. 2.2 Recommended Dosage to Reduce the Toxicity of

	Methotrexate in Patients with Impaired Methotrexate Elimination or to Reduce the Toxicity of Folic Acid Antagonists or Dihydrofolate Reductase Inhibitors Following Overdose • Edits have been made for consistency with oncology labeling, for clarity, readability, and presentation. Routes of administration in this section have been limited to the dosing formulations for Wellcovorin (i.e., oral) and information regarding other dosage forms that are not included in this PI have been removed. 2.3 Recommended Dosage for Cerebral Folate Transport Deficiency with Folate Receptor 1 Genetic Variant • The recommended dosage for FOLR1-CFTD has been added, which is derived from the cases that support the effectiveness of Wellcovorin for this indication.
4 CONTRAINDICATIONS	A contraindication for hypersensitivity was added based on postmarketing reports of hypersensitivity reactions with leucovorin that included anaphylactic reactions and urticaria. GSK proposed to contraindicate with a history of any hypersensitivity reaction for patients with FOLR1-CFTD; however, GSK recommended a contraindication with a history of severe hypersensitivity reaction for patients being treated for folic acid antagonist or DHFR inhibitor toxicity. GSK's rationale is that these patients would be treated for a limited duration and are usually receiving treatment in the hospital setting, which would have experienced healthcare professionals and proper medication and equipment required to treat hypersensitivity reactions. The Agency agrees with this rationale.
5 WARNINGS AND PRECAUTIONS	Information that was previously under the WARNINGS section of the old format labeling has been relocated to more appropriate sections in PLR format. The hypersensitivity information, which could be potentially serious or otherwise clinically significant [see 21 CFR 201.57(c)(6)(i)] that was previously under the ADVERSE REACTIONS section of the old format labeling has been relocated to the WARNINGS AND PRECAUTIONS section [see also CONTRAINDICATIONS above].
6 ADVERSE REACTIONS	Adverse reactions have been added consistent with postmarketing cases and approved NDA labeling of the class (i.e., levoleucovorin).
7 DRUG REACTIONS	A subsection for the effect of other drugs on leucovorin has been added based on interactions with antiepileptic drugs.

8 USE IN SPECIFIC POPULATIONS	8.1 Pregnancy • Because of the differences in administration duration and available data for the respective Wellcovorin indications, risk summary statements have been added that are specific and appropriate for each indication. • A statement regarding the background rate has been added as required per 21 CFR 201.57(c)(9)(i)(B). 8.2 Lactation • The risk and benefit statement for systemically absorbed drugs has been added as per 21 CFR 201.57(c)(9)(ii)(A)(3). • A statement regarding the effects on the breastfed infant and on milk production has been added as required per 21 CFR 201.57(c)(9)(ii)(A)(2)(ii) and (iii), respectively. 8.4 Pediatric Use • Pediatric use statements have been added for each indication per 21 CFR 201.57(c)(9)(iv). 8.5 Geriatric Use • A geriatric use statement has been added per 21 CFR 201.57(c)(9)(v).
10 OVERDOSAGE	The Poison Help Line phone number has been added for reference in the event of an overdosage.
11 DESCRIPTION	The equivalency statement has been added as recommended in the Guidance for Industry, Naming of Drug Products Containing Salt Drug Substances.
12 CLINICAL PHARMACOLOGY	The CLINICAL PHARMACOLOGY section has been divided into subsections, which incorporates recommendations from the Guidance for Industry, Clinical Pharmacology Section of Labeling for Human Prescription Drug and Biological Products – Content and Format (December 2016). 12.1 Mechanism of Action • Although the mechanism of action is not required in "old" format labeling, it is required for PLR format labeling per 21CFR 201.57(c)(13)(i)(A). Therefore, this has been added for each indication. 12.2 Pharmacodynamics • A short summary regarding levoleucovorin and its metabolites and "one carbon" metabolism has been included.

	12.3 Pharmacokinetics • Information has been added under the following headings: Absorption (e.g., information in the literature regarding crushing leucovorin tablets), Distribution, Eliminination (e.g., plasma elimination half-life), and Specific Populations (e.g., data regarding patients with renal or hepatic impairment); see the clinical pharmacology portion of this integrated review for supporting evidence.
14 CLINICAL STUDIES	Information regarding the cases in the literature that support the efficacy for FOLR1-CFTD have been added. Specific details regarding the 27 patients who received leucovorin via the oral route only (e.g., age, dosing, and clinical improvements, and available CSF 5-MTHF measurements) have been included; see the clinical portion of this integrated review for supporting evidence.
17 PATIENT COUNSELING INFORMATION	This section has been included, as recommended in the Guidance for Industry, Patient Counseling Information Section of Labeling for Human Prescription Drug and Biological Products — Content and Format (December 2014).

12 Risk Evaluation and Mitigation Strategies (REMS)

A REMS was not requested for this application.

13 Postmarketing Requirements and Commitment

No Postmarketing Requirements or Commitments are deemed necessary for this supplemental NDA.

14 Division Director (DHOT) Comments

Appears this way on original

15 Division Director (OCP) Comments

16 Division Director (OB) Comments

17 Division Director (Clinical) Comments

The content of this Unireview reflects the issues discussed in the marketing application assessment and regulatory decisions and actions taken. My feedback and edits have been incorporated above. I agree with the findings as documented by the primary review team.

18 Office Director (or designated signatory authority) Comments

19 Appendices

Appears this way on original

19.1. Additional Assessment Analyses

Table 16 Key Clinical and Biomarker Characteristics of 46 Subjects with FOLR1-CFTD

Patient Details (Citation)	Clinical Manifestations at Pre-Treatment Baseline	Leucovorin Dosage & Formulation	Treatment Delay (Years)	CSF 5-MTHF (Pre/Post-treatment)	Specific Outcome Details
M/8y, Chinese (Wang et al. 2024)	Progressive ataxia, hypotonia, daily myoclonic seizures, developmental regression	3-5 mg/kg/d oral calcium levofolinate	5y	23.40 nmol/L / N/A	Decreased seizure frequency, some language improvement
F/5y 8mo, Chinese (Wang et al. 2024)	Ataxia, hypotonia, daily myoclonic seizures, developmental regression, autistic features	3-5 mg/kg/d oral calcium levofolinate	2.7y	11.08 nmol/L / N/A	Decreased seizure frequency, some language improvement
M/15y, UAE (Almahmoud et al. 2023)	Spastic quadriplegia, refractory GTCS, developmental regression	1.5-3 mg/kg/d oral leucovorin	8y	<10 nmol/L / 26 nmol/L	Reduced seizures, slight spasticity improvement, remains bedridden
F/13y, UAE (Almahmoud et al. 2023)	Spastic quadriplegia, absence and GTCS, developmental regression	1 mg/kg/d oral leucovorin	4y	<10 nmol/L / 51 nmol/L	Better seizure control, reduced spasticity, can say <5 words
F/8y 9mo, Egyptian (Girgis et al. 2024)	Hypotonia, hyperreflexia, ataxia, intention tremors, intractable	1 mg/kg/d IM folinate	1.8y	N/A / N/A	Better seizure control, improved gait and social interaction

Patient Details (Citation)	Clinical Manifestations at Pre-Treatment Baseline	Leucovorin Dosage & Formulation	Treatment Delay (Years)	CSF 5-MTHF (Pre/Post-treatment)	Specific Outcome Details
	myoclonic-atonic seizures (20-50/day), developmental regression, autistic features				
F/12y, Egyptian (Girgis et al. 2024)	Hypotonia, ataxia, intention tremors, myoclonic clusters (20-30/day), developmental regression	2 mg/kg/d IM folinate	6y	N/A / N/A	Reduction in seizures
F/4y, Lebanese (Jaafar & Obeid 2022)	Ataxia, weekly tonic seizures, developmental regression, autistic features (poor eye contact, poor communication, stereotypic movements)	5-8 mg/kg/d oral + 20 mg/kg monthly IV leucovorin	2y	N/A / 87 nmol/L (normal on oral)	Worsening seizures on oral with loss of ambulation and verbal communication. After IV: increased eye contact and verbal interactions, improved gait, complete seizure resolution
F/2y, Lebanese (Jaafar & Obeid 2022)	Mild autistic features	5 mg/kg/d oral + IV (dose not specified) leucovorin	No delay	N/A / N/A	Remained stable with only mild autism features
M/17y, Indian (Gowda et al. 2021)	Spastic quadriparesis, cerebellar signs, multiple episodes of seizures, developmental regression	Oral leucovorin (dose not specified)	N/A	N/A / N/A	Seizure control, but persistent ataxia and cognitive decline

Patient Details (Citation)	Clinical Manifestations at Pre-Treatment Baseline	Leucovorin Dosage & Formulation	Treatment Delay (Years)	CSF 5-MTHF (Pre/Post-treatment)	Specific Outcome Details
M/6y, Indian (Gowda et al. 2021)	Spastic quadriparesis, ataxia, epileptic spasms, developmental regression	Oral leucovorin (dose not specified)	N/A	N/A / N/A	Significant decrease in seizure frequency
F/18y, Algerian (Brunetti et al. 2021)	Progressive ataxia, spasticity, dystonia, refractory tonic-myoclonic seizures, developmental regression	4.5 mg/kg/d oral + 300 mg/month IV leucovorin	15.8y	N/A / N/A	No appreciable improvement after >15 years delay
F/16y, Algerian (Brunetti et al. 2021)	Progressive ataxia, spasticity, dystonia, tonic and myoclonic seizures, developmental regression	4.5 mg/kg/d oral + 300 mg/month IV leucovorin	14.2y	N/A / N/A	No appreciable improvement after >14 years delay
M/15y, Algerian (Brunetti et al. 2021)	Progressive ataxia, spasticity, dystonia, refractory tonic-myoclonic seizures, developmental regression	4.5 mg/kg/d oral + 300 mg/month IV leucovorin	13.3y	9 nmol/L / N/A	No appreciable improvement after >13 years delay
F/3.5y, Greek (Papadopoulou et al. 2021)	Hypotonia, ataxia, epileptic spasms, developmental	6 mg/kg/d oral + twice weekly IV 10 mg/kg leucovorin	1.6y	19 nmol/L / 52 nmol/L	Seizure reduction, regained motor skills, some speech recovery

Patient Details (Citation)	Clinical Manifestations at Pre-Treatment Baseline	Leucovorin Dosage & Formulation	Treatment Delay (Years)	CSF 5-MTHF (Pre/Post-treatment)	Specific Outcome Details
	regression, autistic features				
M/2y, Greek (Papadopoulou et al. 2021)	Hypotonia, dyskinesia, developmental stagnation	6 mg/kg/d oral leucovorin	No delay	49 nmol/L / 71 nmol/L	Good developmental progress: walks independently, uses words, normal communication
F/6y, Turkish (Kanmaz et al. 2023)	Generalized hypotonia, ataxia, dysmetria, refractory myoclonic-atonic seizures, developmental regression, autistic features	0.5-9 mg/kg/d oral + 24 mg/kg/month IV for 6 mo, then 6 mg/kg/week IV leucovorin	5.9y	<1.6 nmol/L / Normal	Slight decrease in seizures, improved eye contact, improved gait
M/7y 8mo, Saudi (Al-Baradie et al. 2014)	Ataxia, generalized hypotonia, hyperreflexia, drop attacks and myoclonic jerks, global developmental delay, autistic features	0.75-1.7 mg/kg/d oral leucovorin	3.7y	7 nmol/L / N/A	Seizure-free, marked improved motor skills and social interaction
F/6y, Saudi (Al-Baradie et al. 2014)	Ataxia, generalized hypotonia, hyperreflexia, drop attacks	0.75-2 mg/kg/d oral leucovorin	2y	11 nmol/L / N/A	Seizure-free, neurological improvement

Patient Details (Citation)	Clinical Manifestations at Pre-Treatment Baseline	Leucovorin Dosage & Formulation	Treatment Delay (Years)	CSF 5-MTHF (Pre/Post-treatment)	Specific Outcome Details
M/7y 7mo, Chinese (Zhang et al. 2020)	Ataxia, tonic-clonic status epilepticus, global developmental delay, autistic features	2 mg/kg/d IV for 1 week, then up to 11 mg/kg/d oral calcium folinate	4.9y	1.4 nmol/L / 79 nmol/L	Improved seizure control, gait more steady, able to hold objects more readily
M/7y 7mo, German (Steinfeld et al. 2009)	Ataxia, athetosis, tremor, daily myoclonic-astatic seizures, developmental regression, autistic features (no social contact, loss of speech, undirected looking)	5 mg/kg/d oral leucovorin	2y	1.4 nmol/L / 41 nmol/L	Reduced seizure frequency, walking with support
F/5y 3mo, German (Steinfeld et al. 2009)	Ataxia	5 mg/kg/d oral + 100 mg/week IV leucovorin	No delay	2 nmol/L / 53 nmol/L	Complete clinical recovery, no neurological symptoms
F/8y, Italian (Steinfeld et al. 2009)	Ataxia, tremor, spasticity, daily myoclonic astatic seizures, severe developmental regression, autistic features (little social interaction, no verbal communication)	Dose not specified	3y	<5 nmol/L / N/A	Slow improvement in clinical condition
F/22y, Turkish (Karin et al. 2017)	Dystonia, ataxia, parkinsonism, well-controlled partial	3-5 mg/kg/d oral leucovorin	18y	Below detection, <2 nmol/L / 61 nmol/L	Improvement: reduced sleepiness, stabilized gait and improved ability to climb stairs

Patient Details (Citation)	Clinical Manifestations at Pre-Treatment Baseline	Leucovorin Dosage & Formulation	Treatment Delay (Years)	CSF 5-MTHF (Pre/Post-treatment)	Specific Outcome Details
	seizures, developmental regression				
F/7y, Belgian (Delmelle et al. 2016)	Progressive dysmetria, walking difficulties, severe axial hypotonia, chorea, refractory myoclonic seizures, severe developmental regression, autistic features (irritable, poor eye contact, several stereotypies)	2-7 mg/kg/d oral + 20-25 mg/kg IV monthly leucovorin	1.8y	<1 nmol/L / 51 nmol/L after oral, up to 181 nmol/L after IV	No improvement on oral; improvement on IV with reduced seizures, stabilized condition
F/5y, Belgian (Delmelle et al. 2016)	Ataxic gait, myoclonic and tonic seizures, moderate developmental regression	2-7 mg/kg/d oral + 20-25 mg/kg IV monthly leucovorin	0.5y	<1 nmol/L / N/A	Worsening on oral; significant improvement on IV with walking independently, language recovery, seizure-free
F/33y, Italian (Ferreira et al. 2016)	Progressive ataxia, wheelchair-dependent by 7y, myoclonic then GTCS, severe developmental regression	2 mg/kg/d oral leucovorin	31y	N/A / N/A	50% seizure reduction, improved vocalizations and motor skills
F/28y, Italian (Ferreira et al. 2016)	Progressive ataxia, wheelchair-dependent by 5y, myoclonic then GTCS, severe	2 mg/kg/d oral leucovorin	26y	<10 nmol/L / N/A	50% seizure reduction, improved alertness

Patient Details (Citation)	Clinical Manifestations at Pre-Treatment Baseline	Leucovorin Dosage & Formulation	Treatment Delay (Years)	CSF 5-MTHF (Pre/Post-treatment)	Specific Outcome Details
	developmental regression				
F/11y, French (Mafi et al. 2020)	Dyskinesia, ataxia, febrile then myoclonic seizures	8.9 mg/kg/d oral + 500 mg/week IV calcium folinate	9.5y	1 nmol/L / N/A	>50% seizure reduction, improved motor coordination and social interactions
M/9y 8mo, Gambian (Pérez-Dueñas et al. 2010)	Progressive ataxia, chorea, daily tonic and myoclonic seizures, severe developmental regression, autistic features (no social smile or language communication)	30 mg/d oral initially, then 4 mg/kg/d oral leucovorin	5y	2 nmol/L / N/A	Complete seizure control initially after trial at 26 months, then relapse and progression when stopped; then seizure-free and modestly improved psychomotor development when restarted at 7y
M/5y, Ghanaian (Toelle et al. 2014)	Ataxia, chorea, myoclonic-tonic stimulus-sensitive drop attacks, severe developmental regression	4.5-5.6 mg/kg/d oral and 3 months trial with additional 120 mg/week IV leucovorin	2.5y	Below detection, <0.0 nmol/L / 33 nmol/L	50-75% reduction in drop attacks, persistent ataxia and myoclonic jerks
F/8y 7mo, Russian (Kobayashi et al. 2017)	Progressive ataxia, hypotonia, mild spasticity, refractory epileptic spasms, severe developmental regression	2-2.5 mg/kg/d oral + 6 mo trial 4 mg/kg/d IM + 1 mo trial 4 mg/kg/d IV leucovorin	6.5y	0.5 nmol/L / 78.6 nmol/L	Reduced seizures, able to hold head and speak words

Patient Details (Citation)	Clinical Manifestations at Pre-Treatment Baseline	Leucovorin Dosage & Formulation	Treatment Delay (Years)	CSF 5-MTHF (Pre/Post-treatment)	Specific Outcome Details
F/9.5y, Saudi (Tabassum et al. 2019)	Intention tremor, mild hypotonia, developmental regression	25-50 mg/d oral leucovorin	6y	N/A / N/A	Stable condition with no improvement, continues to be seizure-free
F/unknown age, Finnish (Grapp et al. 2012)	Hypotonia, ataxia, athetosis, daily myoclonic and tonic seizures, atypical absence seizures, recurrent status epilepticus, severe developmental regression	Oral (dose not specified)	N/A	5 nmol/L / N/A	Reduced frequency of epileptic seizures, improved motor skills
M/unknown age, Azerbaijani (Grapp et al. 2012)	Hypotonia, ataxia, daily myoclonic seizures, severe developmental regression, autistic features	Oral (dose not specified)	N/A	<5 nmol/L / N/A	Reduced frequency of epileptic seizures, improved motor skills
F/unknown age, Finnish (Grapp et al. 2012)	Congenital microcephaly, ataxia, hypotonia, daily partial, myoclonic, tonic, and tonic-clonic seizures, severe developmental regression, autistic features (aggressive behavior, very little verbal)	Oral (dose not specified)	14.8y	3 nmol/L / N/A	Reduced frequency of epileptic seizures, improved motor skills

Patient Details (Citation)	Clinical Manifestations at Pre-Treatment Baseline	Leucovorin Dosage & Formulation	Treatment Delay (Years)	CSF 5-MTHF (Pre/Post-treatment)	Specific Outcome Details
F/unknown age, Finnish (Grapp et al. 2012)	Ataxia, tremor, hypotonia, 2 episodes of status epilepticus, moderate developmental regression	Oral (dose not specified)	11y	5 nmol/L / N/A	Improved motor skills
F/unknown age, Finnish (Grapp et al. 2012)	Ataxia, daily myoclonic and few tonic-clonic seizures, moderate developmental regression, autistic features (aggressive behavior)	Oral (dose not specified)	N/A	<5 nmol/L / N/A	Seizure-free, improved motor skills
M/unknown age, Finnish (Grapp et al. 2012)	Hypotonia, ataxia, athetosis, daily myoclonic, tonic, and atonic seizures, severe developmental regression	Oral (dose not specified)	N/A	5 nmol/L / N/A	No improvement (patient had POLG1 mutation in addition to FOLR1-CFTD)
F/unknown age, Turkish (Grapp et al. 2012)	Ataxia, tremor, slight hypotonia, 1 episode of status epilepticus, 2 GTCS, sometimes short myoclonic seizures, moderate developmental regression, autistic features	Oral (dose not specified)	N/A	<5 nmol/L / N/A	Reduced frequency of epileptic seizures, improved motor skills

Patient Details (Citation)	Clinical Manifestations at Pre-Treatment Baseline	Leucovorin Dosage & Formulation	Treatment Delay (Years)	CSF 5-MTHF (Pre/Post-treatment)	Specific Outcome Details
F/12y, Serbian (Potic et al. 2023)	Ataxia, mild spasticity, bulbar symptoms, daily refractory focal tonic seizures with impaired awareness and atonic and tonic-clonic seizures, developmental regression, autistic features (outbursts of anger and poor social contact)	2-8 mg/kg/d leucovorin	6.5y	N/A / N/A	Resolved bulbar and pyramidal signs, improved ataxia, drastic seizure reduction (dozens daily to 0-3 brief seizures)
M/6y, Serbian (Potic et al. 2023)	Truncal ataxia, intention tremor	2-7 mg/kg/d leucovorin	0.2y	N/A / N/A	Complete clinical recovery
M/8y, Turkish (Dill et al. 2011)	Hypotonia, ataxia, dysmetria, intractable epilepsy with myoclonus and drop attacks, developmental regression	3 mg/kg/d oral leucovorin + 20 mg/kg/d pyridoxal 5'-phosphate (PLP)	7.8y	<3 nmol/L (undetectable) / 32-37 nmol/L	Patient has CFD + LAMM: Seizure-free with leucovorin + PLP, regained consciousness, walks with support
F/8y, German (Dreha-Kulaczewski et al. 2024)	No symptoms	2.7-8.5 mg/kg oral leucovorin	Started treated before symptoms at 2m	<10 nmol/L / 50.5-89 nmol/L	Complete normal development, no symptoms throughout course
F/8y 7m, Turkish (Dreha-Kulaczewski et al. 2024)	Ataxia, poor fine motor skills, intractable daily astatic seizures, developmental	4-6 mg/kg oral + 50-200 mg/week IV + 2-5 mg bi-monthly intrathecal leucovorin	2.5y	<5 nmol/L / 62-127 nmol/L	At last follow up: No change in seizures. Normal motor skills, reduced vocabulary, mild autistic features, severe cognitive impairment

Patient Details (Citation)	Clinical Manifestations at Pre-Treatment Baseline	Leucovorin Dosage & Formulation	Treatment Delay (Years)	CSF 5-MTHF (Pre/Post-treatment)	Specific Outcome Details
	regression, mild autistic features				
F/13y 7m, Turkish (Dreha-Kulaczewski et al. 2024)	Tremor, ataxia, dysmetria, mild cognitive impairment	90 mg/d to 1.9 mg/kg oral + 200 mg/week IV leucovorin	6y	N/A / N/A	At last follow up: No seizures, mild cognitive impairment, dysmetric movements
F/16y 6m, Turkish (Dreha-Kulaczewski et al. 2024)	Tremor, dysmetria, pyramidal signs, weekly generalized seizures, severe developmental regression, autistic features (reduced communication)	2.4-4.5 mg/kg oral + 50-200 mg/week IV + 4-8 mg monthly intrathecal leucovorin	7.4y	N/A / 51 nmol/L	At last follow up: Walking with support, slurred speech, frequent seizures

Data collated from published case reports by clinical reviewer.
Abbreviations: CFD, cerebral folate deficiency; CSF, cerebrospinal fluid; 5-MTHF, 5-methyltetrahydrofolate; WM, white matter; GTCS, generalized tonic-clonic seizures; IM, intramuscular; IV, intravenous; IT, intrathecal; LAMM, labyrinthine aplasia, microtia and microdontia; LTFU, lost to follow-up; N/A, not available; PLP, pyridoxal 5'-phosphate; SE, status epilepticus

19.2. Cerebral Folate Deficiency with folate receptor alpha autoantibodies (CFD-FRAA)

CFD-FRAA represents a potentially distinct etiology of primary cerebral folate deficiency (CFD) caused by antibodies to the folate receptor alpha that may manifest with heterogeneous neuropsychiatric symptoms and may have a higher prevalence than FOLR1-CFTD. For this reason, we also searched for randomized controlled clinical trials of leucovorin enrolling subjects with neuropsychiatric manifestations and detectable folate receptor alpha autoantibodies (FRAA). Open-label studies or case series were not deemed sufficient for regulatory decision-making due to the potentially high prevalence of the condition. Our search revealed two controlled trials of leucovorin in subjects with autism spectrum disorder who had suspected CFD-FRAA (Frye, 2018; Panda, 2024); however, Panda et al. 2024 was retracted due to concerns with the statistical analyses performed (Retraction note: Panda, 2026). Given the limited controlled trial evidence, studies of leucovorin for the treatment of CFD-FRAA did not form the basis for the efficacy determination in this review.

19.2.1. Table of Clinical Studies

Table 17 Table of Clinical Studies in Subjects with CFD- FRAA

Trial Identity	NCT no.	Trial Design	Regimen/ schedule/ route	Study Endpoints	Treatment Duration/ Follow Up	No. of subjects enrolled
Frye et al. 2018	NCT01602016	Randomized (1:1), double-blind, placebo-controlled	d,l-leucovorin 2 mg/kg/day (max 50 mg/day) or placebo	Primary: ability-appropriate language assessment: Clinical Evaluation of Language Fundamentals (CELF)-4, CELF-preschool-2, or Preschool Language Scale-5 (PLS-5) Secondary: Ohio Autism Clinical Impression Scale (OACIS), Vineland Adaptive Behavior Scale 2nd Edition (VABS), Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS), Behavioral Assessment System for Children 2nd Edition (BASC), Autism Symptoms Questionnaire (ASQ), Autism Impact Measure (AIM) Biomarkers: serum FRAA titers, plasma free reduced-to-oxidized glutathione redox ratio, serum levels of folate and vitamin B_{12}	12 weeks double-blind	48 subjects

19.2.2. Review Strategy

CFD-FRAA Literature Search:
A comprehensive literature search was conducted in partnership with the National Institutes of Health (NIH) to identify all published double-blind, placebo-controlled trials of leucovorin enrolling subjects with neuropsychiatric manifestations due to CFD-FRAA. The search strategy included:

Databases Searched:
- PubMed/MEDLINE
- Cochrane Library
- ClinicalTrials.gov

Search Terms:
- ("leucovorin" OR "leucovorin" OR "5-formyltetrahydrofolate" OR "calcium folinate" OR "calcium levofolinate")
- AND ("cerebral folate deficiency" OR "CFD" OR "autism spectrum disorder" OR "ASD" OR "developmental regression" OR "language impairment" OR "communication disorders" OR "schizophrenia" OR "depression" OR "behavioral problems")

Additional Search Strategy:
- Hand-searching of reference lists from identified studies

Inclusion Criteria:
- Study Design: Randomized controlled trials
- Population: Subjects with confirmed or suspected CFD due to FRAA
- Intervention: Leucovorin treatment (any dose, route, duration)
- Outcomes: Clinical efficacy measures, FRAA levels
- Publication: Peer-reviewed journals, English language
- Time Period: 2000-2025

Exclusion Criteria:
- Case reports/series or uncontrolled clinical trials
- Studies without FRAA assessment
- Genetic forms of CFD (FOLR1 mutations)
- Studies without leucovorin treatment
- Duplicate publications of the same patient cohorts
- Studies lacking clinical outcome data

Results of Literature Search

The clinical manifestations of CFD-FRAA are markedly heterogeneous and studies enrolled relatively clinically homogeneous populations such as subjects with ASD or schizophrenia who were noted to have positive serum FRAA.

Our literature search revealed 5 randomized controlled trials investigating the safety and efficacy of leucovorin for the treatment of ASD in general. Three studies were excluded because they did not include an assessment of FRAA (Renard, 2020; Zhang, 2025; Batebi, 2021). No randomized controlled trials of leucovorin were identified that enrolled subjects with CFD and other neuropsychiatric manifestations.

Two randomized controlled studies were found to enroll subjects with ASD with suspected CFD FRAA (Frye, 2018; Panda, 2024); however, Panda et al. 2024 was retracted due to concerns with the statistical analyses performed (Retraction note: Panda, 2026). The study selected for review by Frye et al. 2018 is described in Table 17. Of note, in this trial CFD was suspected clinically in subjects with ASD, and FRAA were tested after enrollment, but the presence of FRAA was not amongst the eligibility criteria. Similarly, levels of 5-MTHF in the CSF were not confirmed for enrollment or monitored during treatment. A prospective study enrolling only subjects with established CFD-FRAA prior to randomization was not found.

Frye et al. 2018 conducted a Phase 2, single-site, randomized, double-blind, placebo-controlled trial to evaluate the efficacy of leucovorin in children with ASD and language impairment. The study enrolled 48 children 3 to 13 years old based on a primary diagnosis of “non-syndromic ASD and language impairment” into a 12-week, single-site, randomized, placebo-controlled trial (Frye, 2018). The primary endpoint was verbal communication at 12 weeks assessed by ability-appropriate standardized instruments (CELF-preschool-2, CELF-4, or PLS-5) as a summary score.

The primary objective was to determine whether leucovorin improves verbal communication in children with non-syndromic ASD and language impairment, with a secondary objective to evaluate whether FRAA could predict treatment response. For certain analyses, subjects were stratified based on biomarker status, including FRAA positive or negative or glutathione ratio high or low, although these biomarkers were collected during the study and this stratification was not conducted prior to randomization.

The intervention consisted of DL-leucovorin calcium salt (leucovorin calcium) as a compounded product from Lee Silsby Compounding Pharmacy (Cleveland Heights, OH, USA). The target dose was 2 mg/kg/day (maximum 50 mg/day) administered in two equally divided doses. Dosing was escalated over 2 weeks, with half the target dose given during the first 2 weeks, followed by the full target dose if well tolerated.

Subjects were enrolled in the study who had an ASD diagnosis with language impairment, between 3 and 14 years of age and must have unchanged complementary, traditional, and/or behavioral intervention for approximately 8 weeks prior to study entry, when possible

Key exclusion criteria included:

- Currently taking antipsychotic medication
- Vitamin or supplementation intake that exceeds Institute of Medicine tolerable upper intake levels
- Drugs known to affect folate metabolism (e.g. methotrexate)
- Genetic defect or mutation related to a disease that affecs folate, methylation, or glutathione metabolism
- Metabolic diseases such as mitochondrial disease, urea cycle disorders, etc.
- Other single-gene diseases or pathogenic copy number variants
- Congenital brain malformations, acquired brain insults, microcephaly, or CNS infection

Frye et al. measured verbal communication as the primary endpoint using ability-appropriate standardized instruments (CELF-preschool-2, CELF-4, or Preschool Language Scale-5) selected through a structured algorithm based on each child's developmental level, with assessments conducted at baseline and week 12 by research staff trained by a multidisciplinary team of two licensed psychologists and a speech therapist. Secondary efficacy endpoints included several measure behavior, autism symptoms, clinical impression, and social behavior. All secondary outcomes were recorded at baseline and end of study, and questionnaires were also requested 6 weeks after randomization.

FDA reviewed the statistical analysis plan (SAP) published with the Frye et al. 2018 study; however, because the protocol was published alongside the article and not prior to publication, whether the SAP was prespecified is unclear. The publication does not discuss whether the trial experienced any departures from prospectively planned analyses.

Biomarker stratification analyses were conducted using separate mixed-model regressions on biomarker-defined subgroups. FRAA status was dichotomized as positive versus negative, and glutathione redox ratio was dichotomized as high (above median 8.30) versus low (below median 8.30). Since the study was not powered for biomarker interaction testing, subgroups were analyzed separately. A responder analysis was performed using logistic regression with backward elimination ($p \leq 0.05$ to stay in model), with response defined as ≥5 standardized point increase in verbal communication representing a clinically meaningful change. Potential covariates included age, baseline language scores, and baseline adaptive behavior composite scores. A logistic regression was used to evaluate association between treatment group and each of biomarkers.

Secondary endpoints were analyzed using mixed-model regression similar to the primary endpoint analysis, with false discovery rate correction applied due to the multiple secondary outcomes.

Study Results

No datasets or patient level data were available to conduct this review, and therefore it was not possible to verify the reported analyses. Below is a summary of the published study results.

Forty-eight (48) subjects were randomized: 23 to leucovorin and 25 to placebo.
In the placebo group, 1 subject was withdrawn at week 3 due to unblinding for a potential serious adverse effect. Twenty-three (23) subjects in the leucovorin group and 25 in the placebo group were included in intention-to-treat (ITT) analyses.

Demographic characteristics were similar between the treatment groups, although race and ethnicity were not reported. Subjects in the placebo group with a negative FRAA status had a lower standardized language score than subjects in the leucovorin group with a negative FRAA status (see Error! Reference source not found. placebo FRAA-negative: 48.0 (45.5, 50.5) versus leucovorin FRAA-negative: 57.9 (50.6, 65.2)), which may indicate lower functioning in this subgroup. Other baseline standard scores for language measures and adaptive behavior were similar between treatment groups.

There were slightly more subjects in the placebo group who were positive for FRAA (72% in placebo versus 57% in leucovorin group), with similar levels of blocking and binding titers. It is unclear what effect a higher portion of subjects in placebo being FRAA positive would have on outcome measures, although it is possible that antibody positive subjects may benefit more than antibody negative subjects from oral leucovorin. Other biomarker assessments, including glutathione redox ratio, were similar between treatment groups.

Efficacy Results – Primary Endpoint

Mixed Model Analysis

As reported by the Authors, the primary efficacy analysis demonstrated a statistically significant treatment effect favoring leucovorin over placebo for verbal communication improvement, with subjects in the leucovorin group showing a mean change of 7.3 standardized points compared to 1.7 points in the placebo group from baseline to week 12. The between-group difference yielded an estimated treatment effect of 5.7 points (95% CI: 1.0, 10.4; p=0.02), which exceeded the pre-defined clinically meaningful threshold of 5 points and corresponded to a medium-to-large standardized effect size (Cohen's d = 0.70).

Subjects were stratified by FRAA status and glutathione ratio. The study was not powered for biomarker interaction testing, so subgroups were analyzed separately.

Among subjects positive for FRAA (n=31), leucovorin treatment produced a significantly greater improvement with an estimated effect of 7.3 standardized points (95% CI: 1.4, 13.2; p=0.02) and a large effect size (Cohen's d = 0.91). In contrast, FRAA-negative subjects (n=17) showed no significant benefit from leucovorin treatment, with an estimated effect of 2.5 points (95% CI: -5.9, 10.9; p=0.58) and a small effect size (Cohen's d = 0.30).

Subjects with low glutathione redox ratios (below the median of 8.30) demonstrated significant improvement with leucovorin, showing an estimated effect of 9.1 points (95% CI: 0.9, 17.3; p=0.04) and a large effect size (Cohen's d = 0.95). Subjects with high glutathione redox ratios showed no significant treatment benefit, with an estimated effect of 3.0 points (95% CI: -2.5, 8.5; p=0.30) and a small-to-medium effect size (Cohen's d = 0.46).

Responder Analysis

The responder analysis is shown in Table 181818 and defined treatment response as a clinically meaningful improvement of ≥5 standardized points on the primary verbal communication outcome measure. Overall, the leucovorin group demonstrated significantly higher response rates compared to placebo, with 65% of leucovorin subjects (15/23) achieving response versus 24% of placebo subjects (6/25), representing a 41% difference in response rates (p=0.01).

Among FRAA-positive subjects, 77% (10/13) in the leucovorin group achieved response compared to 22% (4/18) in the placebo group, yielding a 55% difference in response rates and an adjusted odds ratio of 67.4 (95% CI: 5.6, 999.9; p=0.01). In contrast, FRAA-negative subjects showed no significant difference between treatment groups, with response rates of 50% (5/10) for leucovorin versus 29% (2/7) for placebo (p=0.45).

Subjects with low glutathione redox ratios showed response rates of 67% (6/9) with leucovorin versus 20% (3/15) with placebo, resulting in a 47% difference and an adjusted odds ratio of 10.2 (95% CI: 1.4, 140.6; p=0.04). Subjects with high glutathione ratios also showed benefit, with 64% (9/14) responding to leucovorin versus 30% (3/10) to placebo, though the unadjusted analysis was not significant (p=0.11) while the adjusted analysis reached significance (p=0.04).

Table 1818 Statistical Analysis of Primary Outcome Measure of Verbal Communication Responder Analysisa, ITT Population

	Leucovorin treatment[b,c]	Placebo treatment[b,d]	% Difference responders	Unadjusted odds ratio and P-value	Adjusted odds ratio and P-value	Number needed to treat
Overall	15 65% (46%, 84%)	6 24% (7%, 41%)	41% (13%, 63%)	5.9 (1.7, 20.9) 0.01	14.9 (2.1, 116.9) 0.003	2.4 (1.6, 7.7)

	Leucovorin treatment[b,c]	Placebo treatment[b,d]	% Difference responders	Unadjusted odds ratio and P-value	Adjusted odds ratio and P-value	Number needed to treat
Antibody status						
Negative	5 50% (19%, 81%)	2 29% (-4%, 62%)	21% (-27%, 60%)	2.5 (0.3, 19.5) 0.32	3.6 (0.1, 95.6) 0.45	4.7 (1.5, -4.1)
Positive	10 77% (54%, 99%)	4 22% (3%, 41%)	55% (19%, 78%)	11.7 (2.1, 64.0) 0.005	67.4 (5.6, 999.9) 0.01	1.8 (1.3, 5.2)
Glutathione ratio						
High	9 64% (39%, 89%)	3 30% (2%, 58%)	34% (-4%, 72%)	4.2 (0.73, 23.9) 0.11	21.9 (1.9, 956.9) 0.04	2.9 (1.4, -27.6)
Low	6 67% (36%, 98%)	3 20% (0%, 40%)	47% (10%, 84%)	8.0 (1.2, 52.2) 0.03	10.2 (1.4, 140.6) 0.04	2.1 (1.2, 10.2)

From Table 2B, Fryte et al. 2018
a Response was defined as an increase in five standardized points on the primary outcome, which was measured using the Preschool Language Scales-5, the Clinical Evaluation of Language Fundamentals-preschool-2 or Clinical Evaluation of Language Fundamentals 4.
b Number of responders %responders (95% confidence interval).
c Overall N = 23; antibody negative = 10; antibody positive = 13; glutathione high = 14; glutathione low = 9.
d Overall N = 25; antibody negative = 7; antibody positive = 18; glutathione high = 10; glutathione low = 15.

Efficacy Results – Secondary Endpoints

The study demonstrated statistically significant improvements favoring leucovorin on several secondary measures after correction for multiple comparisons using false discovery rate. However, while statistically significant, many of the observed effect sizes were small and may not represent clinically meaningful changes. Additionally, multiple secondary endpoints failed to demonstrate significant treatment effects. Overall, the inconsistent pattern of findings across related measures and concerns about clinical significance of effect sizes limit the strength of evidence for broad therapeutic benefits beyond the primary verbal communication endpoint.

FDA statistical reviewers identified several methodological concerns which limit the interpretability and generalizability of the findings. The degree to which the statistical analysis plan was prespecified is unclear, and there is no information about protocol violations or deviations. From a statistical perspective, the handling of missing data is insufficiently described, with no information on how various factors leading to missing data (incomplete assessments, lost to follow-up, treatment discontinuation) were defined or managed, which may lead to bias in treatment effect estimates. Additionally, details on the analytical approach are lacking, including the missing data mechanism assumed for multiple imputation, inclusion of baseline outcome as a covariate, and the covariance matrix used.

The composite primary endpoint utilizing different language assessment instruments introduces measurement heterogeneity that complicates result interpretation. Authors reported that subjects randomized to leucovorin showed greater improvement in standardized summary scores of verbal communication than placebo; however, no datasets or patient-level data were available to verify these analyses, the verbal communication endpoint comprised multiple screening tests not considered fit-for-purpose for regulatory-grade ASD trials, and the clinical meaningfulness of the 5.7-point improvement is uncertain.
The secondary endpoint results present a mixed pattern, with several outcome measures showing significant improvements while others examining similar domains showed no treatment effects, and many significant findings had small effect sizes that may not represent clinically meaningful changes.

A critical limitation is the highly selected study population requiring both ASD diagnosis and documented language impairment without baseline stratification by FRAA status, as FRAA assays were conducted during the study rather than prior to enrollment or at randomization. An encouraging finding was that among FRAA-positive subjects, leucovorin treatment produced greater improvement, representing a hypothesis that should be investigated in a larger, adequately controlled trial with baseline stratification.

The biomarker stratification analyses represent subgroup analyses not powered for interaction testing and analyzed separately rather than through formal statistical interaction models. Moreover, the single-site design limits generalizability, and the 12-week treatment duration provides no information about long-term efficacy or durability of response.

Of note, the authors also disclosed that JM Sequeira and EV Quadros are inventors in a patent for detecting the autoantibodies described in the study (US patent 7,846,672 B2) issued to the Research Foundation of the State University of New York. Authors RE Frye and EV Quadros are members of the Scientific Advisory Board to Illiad Neurosciences, Inc. The remaining authors declared no conflict of interest. Funding was provided by Lee Silsby Compounding Pharmacy (to RE Frye), Autism Speaks (#8202 to EV Quadros), Brenen Hornstein Autism Research and Education Foundation (to RE Frye), Fraternal Order of Eagles (to RE Frye), the Autism Research Institute (to RE Frye), and the Jane Botsford Johnson Foundation (to S Jil James).

No deaths occurred in the Frye et al. 2018 study, and no SAEs were reported in the leucovorin group. One subject in the placebo group was unblinded and removed from the study due to a potential SAE. Routine laboratory monitoring was not systematically reported in Frye et al. 2018, though no clinically significant laboratory abnormalities were noted. In conclusion, no new safety signals were identified in Frye et al. 2018.

In conclusion, while the study provides preliminary evidence of a treatment effect of leucovorin in children with ASD and language impairment, particularly those who are FRAA-positive, the absence of a prospective study specifically enrolling and stratifying subjects based on confirmed CFD

due to FRAA, combined with significant methodological limitations preclude a conclusion of substantial evidence of effectiveness for this population.

Key limitations include a single-site design that limits generalizability across diverse autism spectrum disorder populations, uncertain clinical meaningfulness of observed treatment effects on primary endpoint, lack of standardized and validated outcome measures appropriate for regulatory decision-making, highly selected study population that may not represent broader ASD populations, and insufficient treatment duration to assess long-term efficacy or durability of response. The post-hoc nature of biomarker subgroup analyses, small sample sizes, and absence of prospective biomarker stratification limit the strength of these findings and require validation in larger, adequately powered studies before establishing leucovorin as an evidence-based intervention for this indication.

19.2.3. Conclusions and Recommendations

Although the Frye et al. 2018 study provides preliminary evidence of a potential treatment effect of leucovorin in subjects with ASD, particularly those who are FRAA-positive, several critical limitations preclude a conclusion of effectiveness as outlind above.

The clinical meaningfulness of observed treatment effects on primary endpoints remains uncertain, and the study lacked standardized, validated outcome measures appropriate for regulatory decision-making. The treatment duration was insufficient to assess long-term efficacy or durability of response.

Population specificity concerns further limit the applicability of these findings. The highly selected study population may not represent broader ASD populations, and geographic and healthcare system differences raise questions about generalizability. Non-validated laboratory methods for FRAA assessment prevent reliable biomarker interpretation, and the lack of prospective biomarker stratification limits conclusions about predictive utility.

Substantial evidence gaps remain that would need to be addressed to support an indication for CFD-FRAA. These include the need for larger multi-center trials with diverse populations, prospective FRAA assessment with standardized methodologies with established cut-points, validated outcome measures with established minimal clinically important differences, longer treatment duration with assessment of durability, and correlation of FRAA status with CSF 5-MTHF levels and clinical outcomes.

Although no concerning safety signals were identified, the evidence does not support approval based on lack of substantial evidence of effectiveness, though the controlled trial provides hypothesis-generating data warranting further investigation.

19.3. Clinical Pharmacology

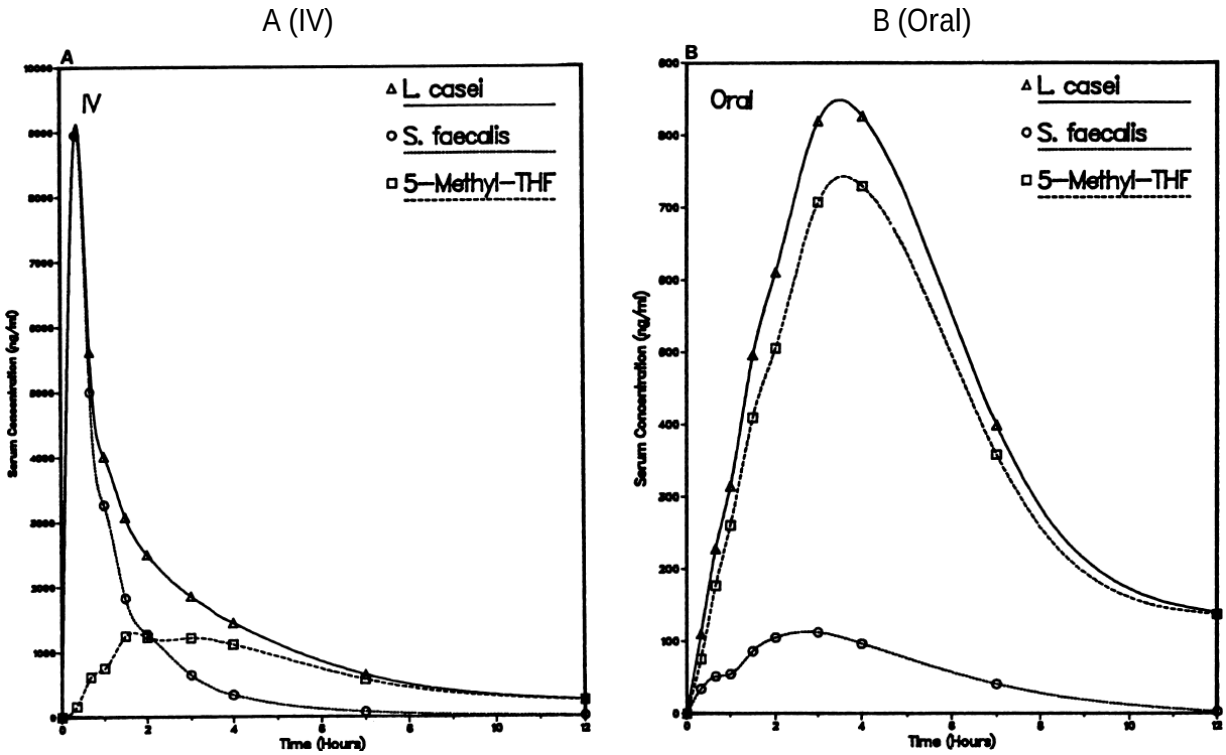
De Vito et al (1989) conducted a single-dose, crossover pharmacokinetic (PK) study comparing relative bioavailability between Lederle leucovorin (leucovorin oral tablet from Lederle Labs which is associated with Pfizer) and Wellcovorin after a single dose of 25 mg (5 x 5 mg) in 36 healthy adult volunteers. Blood samples were collected at predose (0), 20, 40 min, 1, 1.5, 2, 3, 4, 7, 12, and 24 hours. Microbiological folate assay used the growth response of two folate-dependent microprganisms Lactobacillus casei (L. casei) ATCC 7469 to measure total l-tetrahydrofolates, and Streptococcus faecalis (S. faecalis) ATCC 8043 to measure total l-tetrahydrofolates except 5-MTHF. The assays have a limit of sensitivity of approximately 0.3 ng folate/mL serum (1.0×10^{-9}M), the CV was 5.4% for L. casei assay and 7.6 % for the S. faecalis assay. The results showed superimposible PK profiles for total serum folates (Figure 1) and comparable key PK parameters for l-leucovorin, 5-MTHF, and total serum folates (Table 2) between Lederle and Wellcovorin oral tablet formulations.

Duan et al. (2002) conducted a single-dose, crossover PK study to compare the relative bioavaialiblity between test oral capsule (75 mg; 3 x 25 mg capsules), test oral tablet (75 mg; 5 x 15 mg) and reference tablet (a generic drug, Antrex, for Lederle leucovorin oral tablet, 75 mg; 5 x 15 mg) in 12 healthy male adult volunteers. Blood samples were collected at predose, 0.5, 1, 1.5, 2, 2.5, 3, 5, 7, 9, 12 and 24 h after drug administration. A High-Performance Liquid Chromatography-Ultraviolet (HPLC-UV) method was used to determine the PK parameters of leucovorin and 5-MTHF. The sample was analyzed by HPLC with UV detection at 286 nm for levoleucovorin. The limit of detection was 10 ng/mL for leucovorin with lienar range of 50-1500 ng/mL. The relative standard deviation (RSD) of intra-day and inter-day assays was 2.8–6.1% and 2.4–5.3%, respectively. The extraction recoveries of leucovorin in plasma were over 90%. The results showed that mean plasma concentration–time curves are similar (Figure 2 and Table 3), and mean PK parameters of leucovorin after oral test tablet, capsule and reference tablet are comparable, demonstrating similar bioavailability between capsule and tablet formulations.

Mehta et al. (1978) conducted a crossover study to determine the serum distribution of both leucovorin and 5-MTHF following oral solution, oral tablet and intramuscular (IM) administration of leucovorin calcium at 15 mg (Lederle Labs) in 15 healthy adult subjects. Each subject received a single 15 mg oral solution dose, a single 15 mg oral tablet, and 15 mg IM injection, with a washout period of 1 week between each treatment. Serial blood samples were collected at times 30 min, 1, 2, 4, and 6 hours. Systemic exposure of leucovorin was determined by the microbiologic disc-assay method described by Mehta et al. 1975 using methotrexate (MTX) resistant Saccharomyces cerevisiae (P. cerevisiae) as the assay organism. Systemic exposure of 5-MTHF was determined by a differential microbiologic disc-assay procedure (Metha et al. 1977) using MTX-resistant strains of Lactobacillus casei (L. casei) and Streptococcus faecalis (S. faecium). The study showed no significant difference in the serum levels of 5-MTHF following oral solution, oral tablet or IM administration of leucovorin calcium.

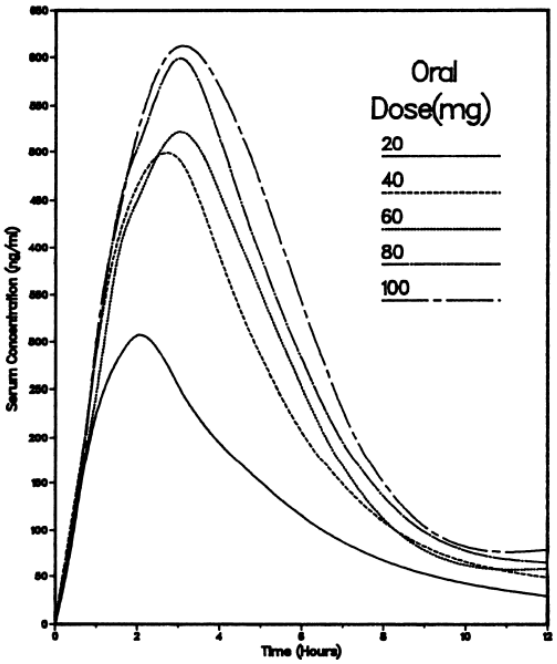
McGuire et al. 1987 conducted a randomized, single-dose study to evaluate the dose proportionality of oral leucovorin (Lederle Labs) at dose levels from 20 to 200 mg and characterize the PK of leucovorin in 30 healthy male subjects and compared with intravenous (IV) leucovorin at 200 mg. Serial blood samples were drawn at times 0 (predose), 20, 40, 60 minutes , 1.5, 2, 3, 4, 7, 12, and 24 hours. Microbiological folate assays were used to determine serum concentrations of folates. Folates were measured by means of differential microbiological assays utilizing the growth response of the 2 folate-dependent organisms L. casei and S. faecalis. Using L. casei activity to measure total serum folates, AUC_{inf} was compared between different oral doses and 200 mg i.v. administration (Figure 6). The sensitivity of the microbiological assays for both L. casei and S. faecalis extends below 0.5 ng folate/ml serum (1.0×10^{-9}M). Reproducibility was measured by determining the standard deviation and coefficient of variation (CV) of 250-ng/mL standard extracts each day of assay. The CV for L. casei was 4.8% and for S. faecalis was 6.9 %. At 20 mg, l-leucovorin is almost completely absorbed. Exposures of total folates increased as the oral dose level escalated (Figure 7Figure 7); however, the increase was less than dose proportional. The absolute oral bioavailability of leucovorin was decreased from 98% at 20 mg to 31% at 200 mg (see Table 1919Table 7).

Figure 6. Concentration-time Profiles by Differential Microbiological Assay Following 200 mg Leucovorin Administered as IV (A) and Orally (B)



L. casei activity or total folates; S. faecalis activity or folates other than 5-methyl-THF; 5-Methyl-THF: the difference between L casei and S faecalis actities. Note the 10-fold difference in scale between panels A and B.
Source: Figure 5, McGuire et al. 1987.

Figure 7. Concentration-time Profiles of Total Folates after Oral Leucovorin 20 to 100 mg.



Source: Figure 1, McGuire et al. 1987.

Schilsky et al. 1990 conducted a crossover study comparing high-dose oral leucovorin with i.v. administration in 5 healthy subjects. Leucovorin 1000 mg doses were administered as a 2-hour IV infusion or divided as 100-mg doses for oral Wellcovorin given over 24 hours (100 mg orally every hour for 5 doses, then every 4 hours for remaining 5 doses). PK samples were collected at predose, 30, 60, and 90 minutes during the infusion; at the end of infusion; and at 10, 20, 30, 60, 90, and 120 minutes and 4, 6, 8, 12, and 24 hours after the end of the infusion. A series of PK samples for oral leucovorin were collected for up to 48 hours postdose. Chiral HPLC was used to separate leucovoron setroisomers and 5-MTHF, UV detection was accomplished at 282 nM. The assay calibration range was 2.5-100 µg/mL for levoleucovorin, 0.5 to 10 µg/mL for 5-MTHF. The intra-assay CV% for levoleucovorin and 5-MTHF were approximately 5% and 10%, respectively. Systemic exposures of l-leucovorin, d-leucovorin, 5-MTHF, and total reduced folates metabolized from l-leucovorin are listed in the table below. An average absolute bioavailability of 37.3% (range 17.6-41.5%) was obtained.

Table 1919. Comparison of Metabolite AUC and Absolute Bioavailability Following Intravenous and Oral Administration of 1,000 mg of Wellcovorin.

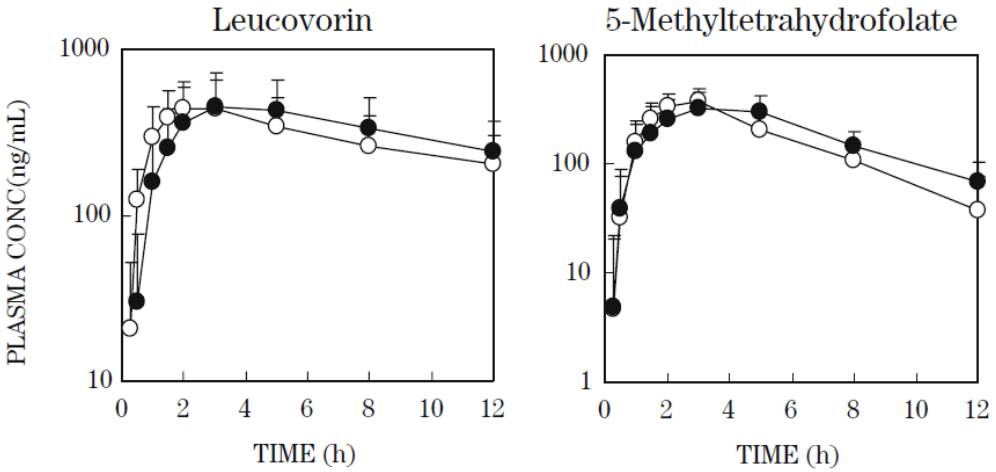
Subject No.	$AUC_{0\text{-}50hr}$ **(µg×hr/mL)**		Absolute Bioavailability (%)
	IV	Oral	
l-Leucovorin			

1	101.6	ND	—
2	64.3	ND	—
3	47.4	ND	—
4	93.8	1.4	1.5
5	70.5	0.8	1.1
Mean ± SD	75.5 ± 22.0	1.1 ± 0.5	1.3 ± 0.3
d-Leucovorin			
1	595.9	38.0	6.4
2	487.6	15.2	3.1
3	334.3	18.0	5.4
4	831.1	23.1	2.8
5	502.6	39.8	7.9
Mean ± SD	550.4 ± 182.6	26.9 ± 11.3	5.1 ± 2.2
5-MTHF			
1	67.7	55.1	—
2	68.5	28.8	—
3	23.6	26.5	—
4	44.5	64.6	—
5	130.3	96.3	—
Mean ± SD	67.0 ± 39.9	54.2 ± 28.6	—
Reduced folates			
1	—	—	32.6
2	—	—	21.5
3	—	—	37.2
4	—	—	41.5
5	—	—	17.6
Mean ± SD	—	—	37.3 ± 11

Source: Table 1, Schilsky et al. 1990.

Furuhata et al. 2009 conducted a single-dose, crossover, PK study to evaluate the impact of a low-fat meal on the oral bioavailabilty of UFT (tegafur/uracil=1/4) and leucovorin (25 mg tablet) in 12 patients with colorectal cancer. Blood samples were collected at predose, 15 and 30 min, and at 1, 1.5, 2, 3, 5, 8, and 12 h post-dose. Exposures of leucovorin after oral leucovorin were determined using HPLC-UV at 310 nm, and 5-MTHF was detected with a fluorescence detector at 295 nm excitation and 355 nm emission. The assay range were linear over the concentration range of 50-1000 ng/ml; the accuracy of the assays for levoleucvoroin and 5-MTHF was less than ±3.8% and less than ±1.1%, respectively, and the precision was less than 5.3% and less than 3.7%, respectively. The results from the study showed that when oral leucovorin tablet is administered after a standard Japanese breakfast with a total calorie of 641 kcal, Cmax and AUC0-t for leucovorin were not affected, and a similar trend was observed for 5-MTHF (Figure 8). The Tmax values of both leucovorin and 5-MTHF were delayed by food (Table 2020).

Figure 8. Mean Plasma Concentration-time Profiles for Leucovorin and 5-MTHF in Cancer Patients after Oral Administration of a 200-mg dose of UFT and 25 mg of Leucovorin under Fasting Conditions (Open Circle) and after a Low-fat Meal (Black Circle)



Source: Figure 2, Furuhata et al. 2009.

Table 2020. Geometric Mean Ratio and 90% Confidence Intervals (CIs) for Leucovorin and 5-MTHF after Oral Administration of a 200-mg Dose of UFT and 25 mg of Leucovorin under Fasting Conditions (Reference Meal) and after a Low-fat Meal (Test Meal)

Analyte	PK Parameters	Test	Ref	GeoMean Ratio (Test/ref)	90%CI
Leucovorin	C_{max} (ng/mL)	424	423	0.999	0.715, 1.395
	AUC_{0-t} (ng*hr/mL)	3168	3437	1.085	0.772, 1.525
	T_{max} (hr)	2.1	3.8	-	-
5-MTHF	C_{max} (ng/mL)	370	360	0.973	0.804, 1.178
	AUC_{0-t} (ng*hr/mL)	1781	2095	1.176	0.974, 1.419
	T_{max} (hr)	2.6	3.8	-	-

UFT: a mixture of tegafur and uracil at a ratio of 1:4; Cmax: maximum plasma concentration; AUC: area under the plasma concnetation-time curve; Tmax: time to reach peak concentration
Source: Adapted from Table 3, Furuhata et al. 2009.

Straw et al. (1984) conducted a PK study to compare the PK of leucovorin after IV and oral administration in 12 healthy adult volunteers. All subjects received leucovorin IV at a dose of 50 mg, and orally at doses of 50 and 100 m. Six of the above subjects also received leucovorin IV at doses of 25 and 100 mg and 25 mg orally every 8 hr for a total of 9 doses, respectively. Leucovorin provided by Lederle Labs. Blood samples were collected at predose, 1, 2, 3, 4, 6, 8, and 10 hours post oral doses. For the multiple-dose studies, blood samples were collected at 24, 48 and 64 hrs after the first dose and, after the last dose. For IV administration, blood samples were collected at predose, 30 min, 1, 2, 4, 6, 8 10, and 24 hours post IV infusion. Non-

chiral HPLC was used to separate 5-MTHF from d,l-leucovorin, UV detection was accomplished at 300 nM; levoleucovorin was meansured using the method described by Mehta et al 1975. The results from the study showed that absorption of d,l-leucovorin after oral administration was stereoselective in that absorption of the l-isomer was approximately 5 times that of the d-isomer. The apparent bioavailability of oral leucovorin is gradually reduced from 97% to 75% to 37% when the oral dose was increased from 25 mg to 50 mg to 100 mg (Table 2121).

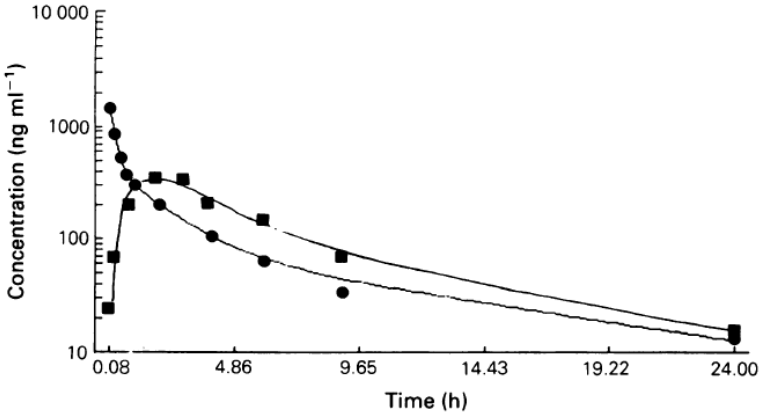
Table 2121. Bioavailability of Oral Leucovorin Tablet Compared to IV Leucovorin

Compound	Dose (mg)	N	AUC_{0-t} of 5-MTHF		Mean Bioavailability
			Oral Tablet (mg*min/L)	IV[c] (mg*min/L)	
l-Leucovorin	25[a]	6	226	129	0.97 ± 0.16[d]
	50[b]	11	240	310	0.75 ± 0.10
	100[b]	10	234	480	0.37 ± 0.04

[a]Oral leucovorin tablets was administered at 25 mg every 8 hours for 9 doses. Apparent bioavailability of 25 mg dose was determined during the last dosing interval.
[b]Oral leucovorin tablet was administered as single doses at 50 mg and 100 mg.
[c]IV leucovorin injection was administered as single doses of 50 mg, or at 25 and 100 mg every 8 hours for 9 doses.
[d]Mean ± S.E.
Source: Adapted from Table 7, Straw et al. 1984, using 5-mg oral tablet from Lederle Labs. Concentrations were determined by HPLC assays.

Greiner et al. 1989 conducted a pharmacokinetic study comparing the exposures of l-leucovorin and total folates after IV and oral capsule administration of the racemic form of leucovorin at 25 mg in 12 healthy subjects. PK samples were collected at 0, 0.08, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 9.0, and 24.0 h for IV administration and 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 9.0 and 24.0 h for the oral administration. Systemic exposures of l-leucovorin, total folates and metabolites were measured using a microbiological assay: Pediococcus cerevisiae for measurement of l-leucovorin and L. casei for total folates, metabolites of l-leucovorin were measured in plasma as the difference between total folates and l-leucovorin. Each calibration point and sample was assayed in quadruplicate. The CV for intra- and inter-assay variability were less than 10%. The detection limit of the assay was 0.05 ng/ml for levoleucovorin and 0.1 ng/ml for total reduced folates. AUC_{0-24h} values for total folates were similar after both formulations, indicating that the absolute bioavailability of leucovorin is high after oral administration and leucovorin is a well-absorbed drug (Figure 9 and Table 5).

Figure 9. Mean Plasma Concentration of Total Folates vs Time after Oral Capsule and Intravenous Administration of 25 mg Leucovorin



Source: Figure 1, Greiner et al. 1989

McGuire et al. 1988 conducted a randomized, crossover study comparing the PK of leucovorin after intravenous (IV), intramuscular (IM) and oral administration in 37 healthy men at a single dose 25-mg dose. Serial blood samples were drawn at times 0 (predose), 10, 20, 30, 45 minutes, 1, 1.5, 2, 2.5, 3, 5, 8, 12, and 24 hours postdose. Plasma concentrations of total folates, leucovorin and 5-MTHF were evaluated using differential microbiological assays with L. casei and S. faecalis. The sensitivity of the microbiological assays for both L. casei and S. faecalis extends below 0.3 ng folate/ml serum (6.0×10^{-10} M). Reproducibility was measured by determining the standard deviation and coefficient of variation (CV) of 250-ng/mL standard extracts each day of assay. The CV for L. casei was 4.9% and for S. faecalis was 5.6 %. The results from the study showed that the systemic exposures of total folates and 5-MTHF were comparable after IV, IM and oral administration of leucovorin (see Table 6Table 7). After oral administration, systemic exposures of l-leucovorin were lower than those of IV administration while IM administration produced systemic exposures similar to IV administration (Table 2222). The lower exposures of l-leucovorin after oral administation could be possibly due to the first-pass metabolism.

Table 2222. Mean Pharmacokinetic Parameters for l-Leucovorin after IV, IM and Oral Administration of 25 mg Leucovorin

	Intravenous	Intramuscluar	Oral
C_{max} (ng/mL)	1206 ± 211	360 ± 114	51 ± 50
AUC_{inf} (ng×hr/mL)	792 ± 162	759 ± 212	140 ± 81

Source: Adopted from Table 1, McGuire et al. 1988

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19.5 Financial Disclosure

N/A

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

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03/09/2026 04:23:42 PM