Safety and efficacy of deoxycytidine/deoxythymidine combination therapy in POLG-related disorders: 6-month interim results of an open-label, single arm, phase 2 trial

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eClinicalMedicine
2024;74: 102740
Published Online xxx
https://doi.org/10.1016/j.eclinm.2024.102740

Summary

Background DNA polymerase gamma (POLG)-related disorders are a group of rare neurodegenerative mitochondrial diseases caused by pathogenic variants in *POLG*, the gene encoding POLG. Patients may experience a range of signs and symptoms, including seizures, vision loss, myopathy, neuropathy, developmental impairment or regression, and liver failure. The diseases follow a progressive, degenerative course, with most affected individuals dying within 3 months–12 years of diagnosis. At present, there are no effective treatments for POLG-related disorders.

Methods In this study we report the interim 6-month data from a long term open-label, single arm phase 2 trial, in which we assessed the safety and efficacy of combination therapy with deoxycytidine and deoxythymidine (dC/dT) in children with POLG-related disorders. dC/dT was given enterally in powder form, dissolved in water. The primary outcome measures included Newcastle Mitochondrial Disease Scale (NMDS) score, serum growth differentiation factor 15 (GDF-15; a biomarker of mitochondrial dysfunction), electroencephalography (EEG), seizure diary, and blood and urine tests to assess end organ and mitochondrial function. Secondary outcome measures included recording of all adverse events to evaluate the safety of the intervention. The trial is registered with ClinicalTrials.gov, NCT04802707 (https://clinicaltrials.gov/ct2/show/NCT04802707). Data were collected from 14 October, 2021 to 13 December, 2023.

Findings We present 6-month interim data from the first ten people with POLG-related disorders enrolled in the trial, six with Alpers-Huttenlocher syndrome, two with ataxia-neuropathy spectrum, and two who do not fit into a classical POLG-related phenotype. During the 6 months of treatment, NMDS score improved from a mean of 27.3 at baseline to 20.7 at 6 months (estimated difference 6.0; 95% CI 2.5–∞). GDF-15 values remained stable or decreased in all patients; the mean decreased from 1031 pg/ml to 729 pg/ml (estimated difference 200; 95% CI 12–∞). 8/10 patients had abnormal baseline EEG; improvement in EEG was seen in 5 of these 8. There were no significant changes in other blood and urine testing. Regarding adverse events, two patients experienced diarrhea that spontaneously resolved.

Interpretation dC/dT is a promising treatment option for people with POLG-related disorders. Further research is needed to assess the long-term safety and efficacy in POLG-related disorders, as well as safety and efficacy in other mitochondrial DNA depletion disorders.

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Funding This study was primarily funded by the Liam Foundation, with additional funding from the Savoy Foundation, Grand Défi Pierre Lavoie Foundation, and Fonds de Recherche du Québec - Santé.

Keywords: POLG; Deoxynucleosides; Deoxycytidine; Deoxythymidine; Thymidine

Research in context

Evidence before this study

We searched PubMed for the term "deoxynucleosides" in combination with "POLG" or "mitochondrial DNA depletion" for any publications describing the effect of deoxynucleoside treatment, *in vivo* or *in vitro*, related to pathogenic variants in *POLG* or other genes associated with mitochondrial DNA depletion from inception to 14 October, 2021. Blázquez-Bermejo et al. (2019) reported that supplementation with deoxyribonucleosides in combination with erythro-9-(2-hydroxy-3-nonyl) adenine (an inhibitor of deoxyadenosine degradation) partially rescued ethidium bromide-forced mitochondrial DNA depletion in human fibroblasts carrying *POLG* pathogenic variants. Pyrimidine deoxynucleoside therapy has been used in a different mitochondrial DNA depletion disorder, TK2 deficiency; reports from both *in vitro* and *in vivo* human studies have demonstrated effectiveness of supplementation with a combination of deoxycytidine (dC) and deoxythymidine (dT).

Added value of this study

This study presents the first human data regarding the safety and efficacy of dC/dT in a mitochondrial DNA depletion disorder other than TK2 deficiency. Our findings represent an important step forward in understanding the therapeutic potential of deoxynucleoside therapy in mitochondrial diseases.

Implications of all the available evidence

Our findings, in combination with previously published data, demonstrate that dC/dT may have broader therapeutic potential for a range of different mitochondrial disorders. The data provide a rationale for follow-up studies of dC/dT as a treatment for POLG-related disorders, including longer term open-label studies and randomized controlled trials.

Introduction

POLG (OMIM 174763) encodes DNA polymerase gamma (POLG) an enzyme that is essential for mitochondrial DNA (mtDNA) replication and repair.[1–3] POLG dysfunction results in depletion of mtDNA and/or accumulation of deletions in mtDNA. Pathogenic variants in *POLG* result in POLG-related disorders, a group of conditions which may involve a broad range of phenotypes including seizures, cortical vision loss, myopathy, neuropathy, developmental impairment or regression, and liver failure.[4] POLG-related disorders are rare, with an incidence estimated at 0.3 in 100,000.[5] Affected individuals usually present with one of six clinical phenotypes, including Alpers-Huttenlocher Syndrome, a devastating condition involving infantile onset seizures, hepatic failure, developmental regression, and early death.[6] The other POLG-related disorder phenotypes include childhood myocerebrohepatopathy spectrum, myoclonic epilepsy myopathy sensory ataxia, ataxia neuropathy spectrum, autosomal recessive progressive external ophthalmoplegia, and autosomal dominant progressive external ophthalmoplegia.[3] POLG-related disorders share the common features of involving progressive decline and having no effective treatment.

One potential therapeutic avenue for POLG-related disorders and other mtDNA depletion disorders is supplementation with deoxynucleosides. There are data from *in vitro* and *in vivo* human studies suggesting effectiveness of supplementation with deoxycytidine (dC) and deoxythymidine (dT) in a different mtDNA depletion disorder, TK2 deficiency.[7–9] *In vitro* studies have shown that mtDNA depletion can be rescued in POLG-deficient fibroblasts and neural stem cells via deoxyribonucleoside supplementation[10,11]; however, there are no published data regarding the effects of similar interventions in humans with POLG-related disorders. In this study, we investigate the safety and efficacy of dC and dT combination therapy in the treatment of POLG-related disorders.

Methods

Study design and participants

This is a single-centre open-label phase II single arm trial to assess safety, tolerability, and efficacy of a 50:50 mixture of dC/dT, administered enterally, for people with POLG-related disorders. The study is based at Montreal Children's Hospital. A data safety monitoring board reviews data at approximately 6-month intervals. The trial is registered with ClinicalTrials.gov, NCT04802707 (https://clinicaltrials.gov/ct2/show/NCT04802707). All data are securely stored on

REDCap, hosted by the Research Institute of the McGill University Health Centre. Inclusion criteria for the study are age 3 months to 60 years at time of enrolment (* the upper age limit was initially 18 years, later increased to 60 years in a protocol amendment), clinical diagnosis of a POLG-related disorder, and having biallelic pathogenic or likely pathogenic variants in *POLG*. This is a sub-study of a broader trial exploring the safety and efficacy of dC/dT for mtDNA depletion disorders of various genetic causes, including pathogenic variants in any of *POLG*, *RRM2B*, *C10Orf2*, *MPV17*, *SUCLA2*, *SUCLG1*, or *FBXL4*. Exclusion criteria are known hypersensitivity to dC or dT, inability to receive the product enterally (i.e., orally or via nasogastric or gastrostomy tube), and inability to provide informed consent (from patient or legal representative). Here, we present an interim analysis of data from the initially planned 6-month timepoint; data were collected from 14 October, 2021 to 13 December, 2023.

Ethics

The study was approved by the McGill University Health Centre Research Ethics Board (2021–7654), with a no objection letter from Health Canada. Informed consent was obtained from patients or parents prior to enrollment.

Procedures

All participants receive the same drug intervention. dC and dT are obtained from Biosynth (United Kingdom) or Alfa Chemistry (United States) in powder form, dissolved in water and distributed in three oral portions over the day. Dosing is 100 mg/kg/day (50 mg/kg dC and 50 mg/kg dT) in week #1, 200 mg/kg/day in week #2, 300 mg/kg/day in week #3, and then 400 mg/kg/day for the remaining period. This dose is at the low end of what has been used previously in studies for TK2 deficiency[7,8]; a lower dose was chosen due to the uncertain safety of the intervention in this patient population. The initial protocol was for a 6-month treatment period, subsequently amended to 24 months after some participants had significant positive responses and wished to continue on the therapy.

The Newcastle Mitochondrial Disease Scale (NMDS) is a validated clinical scale used to assess the progression of mitochondrial disease.[12–15] There are four versions based on age: 0–24 months, 2–11 years, 12–18 years, and >18 years. There are four sections to the NMDS: Section I—current function, Section II—system specific involvement, Section III—current clinical assessment, and Section IV—quality of life. This latter section is a questionnaire completed by the patient or caregivers; in our analysis, we considered Section IV separately, as is recommended in the test manual.[16] Patients are scored on the NMDS at baseline, 1-month, 3-month, 6-month, and every subsequent 6-month visit by a clinical medical geneticist.

Growth differentiation factor 15 (GDF-15) is a blood serum test validated as a quantitative biomarker of mitochondrial dysfunction. It has been used as a biomarker of treatment response in many studies of mitochondrial disease, including some involving TK2 deficiency.[17] Serum GDF-15 levels are collected at baseline and at every subsequent trial visit, with analysis performed at Mayo Clinic Laboratories (Rochester, MN).

Blood and urine samples are collected at baseline, and all subsequent visits, to monitor for any concerning deteriorations in end organ function (i.e., liver, kidney, muscle) and overall mitochondrial function. Biochemical evaluations include complete blood count (CBC), serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), bilirubin, albumin, creatinine, urea, electrolytes, creatine kinase (CK), capillary/venous blood gas, lactate, plasma amino acids, plasma acylcarnitine profile, urine amino acids, urine purines and pyrimidines. Routine electroencephalography (EEG) studies are performed to assess cerebral activity at baseline, 3 months, and 6 months; a qualified epileptologist (KAM) evaluates whether there has been a significant change in frequency of epileptiform discharges or background activity abnormalities (slowing and organization). All patient-reported adverse events are recorded.

Outcomes

The primary outcome measures include the NMDS score, serum GDF-15 level, biochemical tests, seizure diary, and EEG. Secondary outcome measures include patient-reported adverse events.

Statistics

As this trial was primarily aimed at determining the safety of dC/dT for POLG-related disorders, we did not initially perform a formal sample size calculation. Our

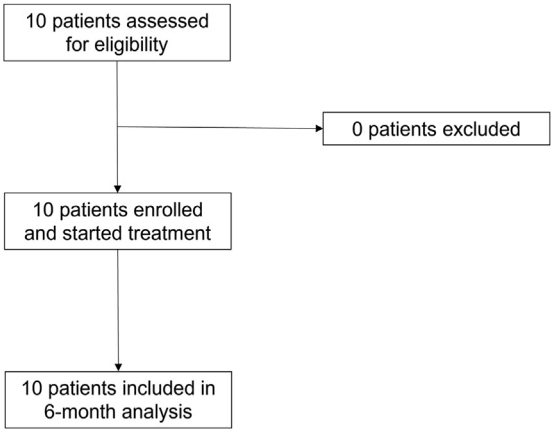

Fig. 1: CONSORT flow chart of participants.

initial recruitment goal was five patients, given the rarity of POLG-related disorders. A paired one-tailed Wilcoxon signed rank test was used to assess changes in NMDS scores and GDF-15 values from baseline to each timepoint, as we hypothesized dC/dT would elicit clinical benefit. Fisher's exact test was used to compare the proportion of patients with abnormal GDF-15 at baseline to subsequent timepoints. A paired two-tailed Wilcoxon signed rank test was used to assess for changes in all other biochemical tests as these were primarily followed for safety reasons; we did not know if there would be baseline abnormalities, and there was no hypothesis as to what change, if any, would be expected. p values given are descriptive, given the exploratory nature of this study.

Role of funding source

The funding sources for this study were the Liam Foundation, Savoy Foundation, Grand Défi Pierre Lavoie Foundation, and Fonds de Recherche du Québec—Santé. None of these entities had any role in the writing of the manuscript or the decision to submit it for publication. We have not been paid to write this article by a pharmaceutical company or other agency.

Patient #	Age at treatment initiation (y)	Sex	*POLG* variant(s)	Clinical phenotype	Concurrent medications at treatment initiation	Nationality (Ethnic background)
1	8	M	c.2554C > T, p.(Arg852Cys); c.1399G > A, p.(Ala467Thr) (NM_002693.2)	Alpers-Huttenlocher syndrome: Seizures (including epilepsia partialis continua), developmental impairment, ataxia, movement disorder, autism spectrum disorder.	Topiramate, carbamazepine, levetiracetam, clobazam, memantine, carnitine, leucovorin, creatine, alpha lipoic acid, liposomal ubiquinol, melatonin, vitamin D, B100 complex	Canadian (Caucasian)
2	2	M	c.1399G > A, p.(Ala467Thr); c.2740A > C, p.(Thr914Pro) (NM_002693.2)	Alpers-Huttenlocher syndrome: Seizures, stroke-like events, developmental regression.	Levetiracetam, clobazam, perampanel, leucovorin, carnitine, ubiquinol, creatine, methylated B12, multivitamin, budesonide, albuterol	American (Caucasian)
3	8	F	c.1399G > A, p.(Ala467Thr); c.1874C > T, p.(Pro625Leu) (NM_002693.2)	No defined syndrome: Seizure onset at age 6 y, mild developmental impairment, liver failure requiring transplant (after treatment with valproic acid).	Phenobarbital, levetiracetam, sertraline, leucovorin, levocarnitine, sirolimus, aspirin, ursodiol, arginine, coenzyme Q10, multivitamin	American (Caucasian)
4	6	F	c.2740A > C, p.(Thr914Pro); c.1399G > A, p.(Ala467Thr) (NM_002693.2)	Alpers-Huttenlocher syndrome: Seizures, developmental regression.	Midazolam infusion, phenobarbital, topiramate, lacosamide, levetiracetam, coenzyme Q10, riboflavin, thiamine, pyridoxine, leucovorin, alpha-lipoic acid, carnitine	Canadian (Caucasian)
5	3	F	c.911T > G, p.(Leu304Arg) (homozygous) (NM_002693.2)	No defined syndrome: Photophobia, constipation, mild language delay (* patient was only mildly affected at time of enrolment; diagnosed because her older brother has POLG-related disorder and is severely affected).	None	Canadian (Pakistani)
6	17	F	c.2243G > C, p.(Trp748Ser); c.1399G > A, p.(Ala467Thr) (NM_002693.2)	Ataxia neuropathy spectrum: Depression, motor regression, fatigability, gait instability, seizures, ptosis.	Topiramate, lamotrigine, levetiracetam, phenytoin, memantine, clobazam, sertraline, omeprazole, coenzyme Q10, folic acid, B complex, riboflavin, other vitamins	Brazilian (Brazilian)
7	11	F	c1399G > A, p.(Ala467Thr) (homozygous) (NM_002693.2)	Alpers-Huttenlocher syndrome: Seizures, stroke-like episodes, global regression.	Levetiracetam, lamotrigine, lacosamide, brivaracetam, clobazam, gabapentin, clonidine, baclofen, famotidine, spironolactone, levocarnitine, melatonin, ferrous sulfate, lorazepam, diazepam, phenobarbital	American (Caucasian)
8	10	M	c.911T > G, p.(Leu304Arg) (homozygous) (NM_002693.2)	Ataxia neuropathy spectrum: Ptosis, progressive external ophthalmoplegia, progressive muscle weakness, ataxia, areflexia	None	American (Pakistani)
9	2	M	c.911T > G, p.(Leu304Arg) (homozygous) (NM_002693.2)	Alpers-Huttenlocher syndrome: Seizures, developmental regression.	Clobazam, lacosamide, phenobarbital, brivaracetam, topiramate, coenzyme Q10, riboflavin, carnitine	Indian (Indian)
10	2	M	c.2243G > C, p.Trp748Ser; c.2440_2442del, p.Val814del (NM_002693.2)	Alpers-Huttenlocher syndrome: Seizures, developmental regression.	Cannabidiol, brivaracetam, lacosamide, clonazepam, lorazepam, gabapentin, leucovorin, ubiquinone, omeprazole, fluticasone, carnitine, vitamin C, melatonin, vitamin D, ferrous sulfate	Canadian (Caucasian)

Table 1: **Baseline demographic table.**

Results

We report the first 10 patients (five males (50%), five females (50%)) with POLG-related disorders enrolled, all of whom were under age 18 years at enrolment (mean age at enrolment 6.9 y, standard deviation 4.9 y) and have received at least 6 months of treatment with dC/dT (Fig. 1, phenotypic details in Table 1). Of these ten patients, four live in Canada, four in the United States, one in India, and one in Brazil; in all cases, patients self-referred or were referred by their treating neurologist or geneticist, having learned about the study from the trial registration website or patient advocacy groups. The enrolment period was from October 2021 to June 2023. Age at treatment initiation ranged from 29 months to 17 years. Six patients have Alpers-Huttenlocher syndrome and two ataxia-neuropathy spectrum; the remaining two patients do not fit into a classical POLG-related phenotype. There were no withdrawals during the first 6 months of treatment.

All participants were scored on the same NMDS version at baseline and subsequent timepoints. Mean NMDS sections I-III score decreased from 27.3 at baseline to 23.6 at 1 month (estimated difference 4.0; 95% confidence interval (CI) 1.0–∞; p = 0.0069), 21.0 at 3 months (estimated difference 6.5; 95% CI 4.0–∞; p = 0.0045), and to 20.7 at 6 months (estimated difference 6.0; 95% CI 2.5–∞; p = 0.0046) (Table 2). The ∞ and −∞ symbols are used when the statistical analysis algorithm does not allow calculation of a maximum or minimum for the 95% confidence interval range. NMDS section IV score also decreased, though not always with statistical significance, from 14.6 at baseline to 13.9 at 1 month (estimated difference 1.2; 95% CI −2.9 to ∞; p = 0.28), 12.1 at 3 months (estimated difference 3.2; 95% CI 0.3–∞; p = 0.039), and 12.6 at 6 months (estimated difference 2.2; 95% CI −0.4 to ∞; p = 0.15). In all cases, patients or caregivers reported being stable or improved in terms of clinical status (Table 3). The most commonly reported changes were improvements in energy level, motor function, cognitive status, and communication.

Serum GDF-15 values are given in Table 4. Overall, the mean GDF-15 level decreased from 1031 pg/ml at baseline to 729 pg/ml at six months (estimated difference 200; 95% CI 12–∞; p = 0.048). Of note, when comparing the GDF-15 decreases at 1-month, 2-month, and 3-month timepoints to baseline, p was also <0.05 in all cases. At baseline, 5/10 (50%) of patients had abnormally elevated GDF-15, which decreased to 2/10 (20%) at six months (not statistically significant). Of note, GDF-15 for patient 8 was reported as "greater than 6000 pg/ml" (higher than the laboratory's quantitation limit) at all time points, so it was not possible to assess if there was a change from baseline. For the remaining four patients with elevated baseline GDF-15 levels, the levels decreased in all cases, falling to within the normal range at 6 months in 3/4 (Fig. 2). For patients with normal GDF-15 levels at baseline, values remained normal in all cases.

The data for CBC, electrolytes, renal/liver function tests, CK, and lactate are given in Table 5, along with selected values from urine purine/pyrimidine testing. Baseline values tended to be normal for most tests, with the exception of baseline elevations in AST, ALT, and GGT. White blood cell count was increased at 6 months compared to baseline (p = 0.010), but this did not appear

Patient #	Baseline		1-month		3-month		6-month	
Section(s)	I-III	IV	I-III	IV	I-III	IV	I-III	IV
1	20	13.3	19	14.6	17	14.6	16	12
2	38	18	30	18	28	15.8	26	15
3	18	16	16	16	13	10	14	14.5
4	33	15	29	15	23	13.75	22	17
5	10	7.5	9	7.5	9	7.5	9[a]	7.5[a]
6	33	19.6	19	15.8	15	11.25	14	9.2
7	33	15.4	32	15	31	12	31	7.1
8	21	7.5	21	13.75	14	9.2	15	10.4
9	29	16.6	23	8	22	10	21	13.75
10	38	17.2	38	15.6	38	17.2	39	19.3
Mean ± SD	27.3 ± 9.5	14.6 ± 4.1	23.6 ± 8.6	13.9 ± 3.4	21.0 ± 9.1	12.1 ± 3.1	20.7 ± 9.1	12.6 ± 4.1
p value			0.0069	0.28	0.0045	0.039	0.0046	0.15
Estimated difference from baseline (95% CI)			−4.0 (−1.0 to −∞)	−1.2 (+2.9 to −∞)	−6.5 (−4.0 to −∞)	−3.2 (−0.3 to −∞)	−6.0 (−2.5 to −∞)	−2.2 (+0.4 to −∞)

A paired one-tailed Wilcoxon signed rank test was used to compare each timepoint to baseline. Abbreviations: CI, confidence interval, SD, standard deviation. The ∞ and −∞ symbols are used when the statistical analysis algorithm does not allow calculation of a maximum or minimum for the 95% confidence interval range. [a]6-month values for patient #5 are imputed—the patient could not attend the scheduled visit with the medical geneticist, but family reported no significant clinical change since the 3-month visit and neurological examination was unchanged.

Table 2: **Newcastle Mitochondrial Disease Scale scores.**

Patient #	EEG baseline	EEG at 6 months	Summary of parental observation at 6 month follow up
1	Moderate background slowing and frequent multifocal spike-wave discharges.	Moderate background slowing and abundant multifocal spike-wave discharges (mainly right hemisphere). No clear improvement from baseline.	- More alert, interactive, trying to communicate more, repeating more words and more spontaneous words.
2	Background that is disorganized and slow, with superimposed diffuse fast activity. Intermittent focal slowing over right posterior region. Occasional focal spikes over right posterior temporal region.	Improved in terms background organization. Only occasional slowing seen. No definitive epileptiform discharges seen.	- More alert, aware and interactive, improved eye contact, laughing more. - Wants to play with toys. - Improved motor function - using standing frame, started with gait trainer. Can get from supine to sitting independently. Improved right arm and leg strength, and fine motor skills.
3	Abundant-to-continuous multifocal and bisynchronous spike-wave discharges, sometimes occurring in rhythmic runs at ~3 Hz. Background disorganized and diffusely slow.	Abundant-to-continuous multifocal and bisynchronous spike-wave discharges. Some background slowing and disorganization. Not clearly improved from baseline.	- Increased energy level. - Memory appears to be improving.
4	Continuous multifocal and bisynchronous spike-wave discharges. Occasional bursts of bilateral PFA. Background activity is slow and disorganized.	Abundant multifocal and bisynchronous spike-wave discharges but greater percentage of background without epileptiform discharges. No PFA seen. Slightly improved compared to baseline.	- Energy level improved. Now going to school in afternoons for 2 h. Learning new things. - More clear speech, energy to speak full sentences. - Chewing and swallowing improved. No more choking episodes.
5	Normal	No data.	- Stable
6	Normal except for diffuse fast activity (likely medication related).	Normal except for diffuse fast activity (likely medication related). No change from baseline.	- Improvement in energy, cognitive function and gait stability.
7	Continuous focal spikes, maximal over the left central region. Questionable focal electrographic seizure from the left central region. Continuous jerks, mainly involving the face, but these are not clearly correlated with epileptiform discharges, and appear to be more likely non-epileptic.	Slow and disorganized background and abundant multifocal sharp waves and spikes. Still improved from baseline, due to no seizures, reduction in frequency of interictal epileptiform discharges, and reduction in non-epileptic jerks.	- Improved eye contact, increased energy. - More spontaneous movement which seem purposeful.
8	Intermittent focal slowing, sometimes over left posterior region, other times over right hemisphere. Occasional spikes over right frontal-central region.	Intermittent slowing over posterior regions. Improved in comparison to baseline due to absence of epileptiform discharges and improvement in the intermittent slowing.	- Stabilization in evolution, regression stopped.
9	Diffuse background slowing. Frequent multifocal spikes. Patient in epilepsia partialis continua with continuous rhythmic jerking of the right arm.	Diffuse background slowing. Frequent multifocal spikes. EEG improved from baseline due to absence of epilepsia partialis continua.	More active and alert. Motor function much improved. Epilepsia partialis continua resolved. Only possible seizures are several jerks that occurred in context of a febrile illness.
10	Diffuse background slowing and disorganization. Continuous multifocal spikes and sharp waves. Patient in epilepsia partialis continua with continuous rhythmic jerking of the right arm.	Diffuse background slowing and disorganization. Continuous multifocal spikes and sharp waves. Patient in epilepsia partialis continua with continuous rhythmic jerking of the right arm. No improvement from baseline.	Overall, at best very minor clinical improvement. Is more vocal and moves more. Seizure frequency variable, but not clearly improved. Still in constant EPC. No deterioration from baseline.

Abbreviations: EEG, electroencephalography; PFA, paroxysmal fast activity.

Table 3: **EEG changes and parental observations at 6-month follow-up.**

to be clinically relevant as the majority of values were still in the normal range. There were no significant changes in the profiles for plasma amino acids, urine amino acids, or acylcarnitine profile. Urine purine/pyrimidine profiles were essentially normal at baseline, whereas at all timepoints after the start of treatment the profiles showed elevated thymine and uracil, as well as elevations of their metabolites, dihydrothymine, dihydrouracil, β-ureidoisobutyric, β-ureidopropionic, and 5-hydroxymethyluracil; these post-treatment profiles were consistent with a normal process of degradation of excess deoxycytidine and deoxythymidine by known metabolic pathways, followed by disposal of the degradation products by excretion.[18]

8/10 patients reported no seizures in the two months prior to enrolment. Patients 2 and 6 had temporary recurrence of seizures in the context of attempted weaning of pre-existing anti-seizure medications. Patient 4 also had temporary recurrence of seizures in the context of a febrile illness. Patients 9 and 10 had epilepsia partialis continua at the time of enrolment. Patient 9's focal jerks progressively decreased in frequency; at the 6-month timepoint, he was having a few jerks at a time, noted only every few weeks in the context of fever. Patient 10's epilepsia partialis continua was not significantly changed at the 6-month timepoint.

EEG findings are summarized in Table 3. Two patients had normal EEG at baseline. Of the eight with

Patient #	Baseline	1-month	2-month	3-month	6-month
1	631	632	602	735	493
2	**2340**	**1242**	**812**	**839**	715
3	528	ND	398	348	324
4	**925**	**808**	567	565	493
5	579	552	511	493	605
6	**792**	490	466	**830**	737
7	680	661	616	602	712
8	**>6000**[a]	**>6000**[a]	**>6000**[a]	**>6000**[a]	**>6000**[a]
9	**2354**	**1301**	**961**	**935**	**1866**
10	447	389	ND	351	619
Mean ± SD	1031 ± 759	759 ± 340	617 ± 186	633 ± 215	729 ± 447
p value		0.0078	0.0039	0.027	0.048
Estimated difference from baseline (95% CI)		−172 (−27 to −∞)	−285 (−68 to −∞)	−180 (−29 to −∞)	−200 (−12 to −∞)

All values are in pg/ml. The normal range is < 750 pg/ml; abnormal results are in bold. A paired one-tailed Wilcoxon signed rank test was used to compare each timepoint to baseline. Abbreviations: ND, no data due to sample processing issue; SD, standard deviation. The ∞ and −∞ symbols are used when the statistical analysis algorithm does not allow calculation of a maximum or minimum for the 95% confidence interval range. [a]Serum GDF-15 level was above laboratory's upper limit of quantification, reported as ">6000 pg/ml".

Table 4: **Serum GDF-15 levels.**

abnormal EEG at baseline, five showed some improvement at six months, with reduction in seizures, interictal epileptiform discharges, or background abnormalities.

Two patients experienced mild diarrhea in the first weeks of treatment, which spontaneously resolved. Patient 3, who had a previous liver transplant, required admission to hospital on one occasion with increased serum liver enzymes and fever not thought to be related to dC/dT treatment. She was ultimately diagnosed with bile duct and small intestine obstruction requiring surgical drain placement. Patient 5 who had intermittent constipation prior to starting the trial, was admitted to hospital for one day with episodes of vomiting and constipation. Patient 8 had a 4-day bout of diarrhea attributed to a viral infection, which did not require hospitalization. Blood and urine testing did not find any concerning changes in end organ function in any patients, other than patient 3. There were no significant adverse events attributed to the therapy. No patients died during the first 6 months of treatment.

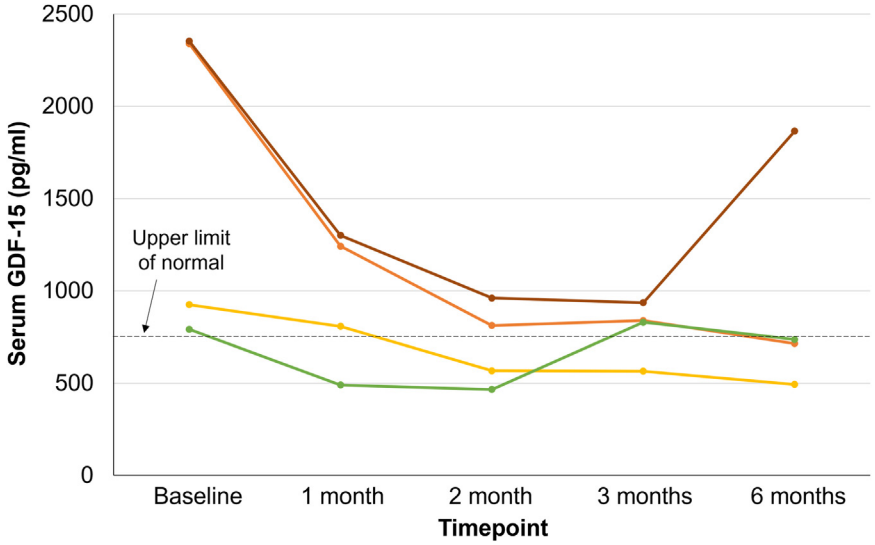

Fig. 2: Serum growth differentiation factor 15 (GDF-15) over time in patients with elevated baseline levels. Orange line, patient #2; yellow line, patient #4; green line, patient #6; red line, patient #9.

Outcome, units (n)	Baseline		6 months		p	Estimated difference (95% CI)
	Mean ± SD	Abnormal values	Mean ± SD	Abnormal values		
Hemoglobin (blood), 10^{12}/L (10)	125.7 ± 10.4	1 ↓	124.2 ± 13.2	2 ↓	0.67	−1.5 (−12.0 to 7.0)
WBC (blood), 10^9/L (10)	6.9 ± 2.3	2 ↓	8.8 ± 4.7	1 ↑	0.010	+1.4 (+0.4 to +4.6)
Platelets (blood), 10^9/L (10)	281.6 ± 65.8	1 ↓	306.4 ± 85.9	1 ↓	0.082	+21.0 (−7.0 to +49.0)
AST (blood), U/L (9)	43.3 ± 17.5	4 ↑	35.6 ± 14.9	3 ↑	0.10	−8.5 (−20.0 to +3.0)
ALT (blood), U/L (10)	30.3 ± 15.3	8 ↑	26.2 ± 10.8	6 ↑	0.81	−0.5 (−19.5 to +5.0)
GGT (blood), U/L (8)	76.3 ± 75.8	4 ↑	40.3 ± 21.3	5 ↑	0.44	−47.5 (−113.5 to +17.0)
Total bilirubin (blood), µmol/L (9)	5.1 ± 0.9	–	5.0 ± 1.6	1 ↑	0.91	−0.2 (−1.2 to +1.1)
Albumin (blood), g/L (10)	42.8 ± 3.9	2 ↓, 1 ↑	41.2 ± 4.8	1 ↓	0.10	−1.5 (−4.0 to +1.0)
Creatinine (blood), µmol/L (10)	30.4 ± 11.5	4 ↓	30.5 ± 11.2	2 ↓	0.95	−1.0 (−5.0 to +5.0)
Urea (blood), mmol/L (6)	3.6 ± 1.0	–	3.7 ± 1.0	–	0.88	+0.1 (−0.8 to +1.2)
Sodium (blood), mmol/L (10)	139.4 ± 2.8	1 ↑	138.9 ± 2.0	–	0.63	−1.0 (−3.5 to +2.0)
Potassium (blood), mmol/L (10)	4.3 ± 0.3	–	4.2 ± 0.2	–	0.56	−0.1 (−0.2 to +0.2)
Chloride (blood), mmol/L (10)	103.8 ± 4.4	1 ↓	102.7 ± 4.3	2 ↓	0.20	−1.5 (−4.0 to +1.0)
CK (blood), U/L (10)	114.1 ± 138.8	5 ↓, 1 ↑	120.1 ± 93.2	1 ↓, 2 ↑	0.91	+10.0 (−92.0 to +118.5)
Lactate (blood), mmol/L (10)	1.9 ± 0.7	1 ↑	2.0 ± 1.1	1 ↑	0.69	+0.1 (−0.5 to +0.6)
Uracil (urine), mmol/mol-Cr (8)	7.1 ± 5.6	–	533.1 ± 716.2	7 ↑	0.0078	+283.0 (+37.0 to +1131.5)
Thymine (urine), mmol/mol-Cr (8)	0.1 ± 0.4	–	154.5 ± 178.8	7 ↑	0.016	+179.8 (+6.5 to +391.0)
DHU (urine), mmol/mol-Cr (8)	6.4 ± 4.4	1 ↑	27.4 ± 4.5	7 ↑	0.014	+13.0 (+6.0 to +39.0)
DHT (urine), mmol/mol-Cr (8)	1.1 ± 0.4	–	16.5 ± 14.4	7 ↑	0.018	+15.5 (+4.5 to +36.0)
β-ureidoisobutyric (urine), mmol/mol-Cr (8)	0.3 ± 0.5	1 ↑	10.1 ± 12.1	7 ↑	0.022	+10.0 (+2.0 to +19.0)
β-ureidopropionic (urine), mmol/mol-Cr (8)	3.5 ± 4.0	1 ↑	46.5 ± 51.6	7 ↑	0.014	+22.0 (+10.5 to +78.0)
5-hydroxymethyluracil (urine), mmol/mol-Cr (8)	1.1 ± 0.6	2 ↑	8.1 ± 9.0	7 ↑	0.022	+6.0 (+2.0 to +16.0)

Legend: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; CK, creatine kinase; Cr, urine creatinine; DHT, dihydrothymine; DHU, dihydrouracil; GGT, gamma glutamyl transferase; WBC, white blood cells; ↓, below lower limit of laboratory age-related reference range; ↑, above upper limit of laboratory age-related reference range.

Table 5: **Biochemical testing.**

Discussion

Our results support dC/dT as a promising therapeutic option for people with POLG-related disorders. dC/dT was well tolerated amongst the ten participants over six months of treatment, supporting the safety of the intervention. The few reported serious adverse events were not attributable to dC/dT and did not lead to discontinuation of the treatment. As well, biochemical parameters remained generally stable during the treatment period, indicating the intervention is also safe at the metabolic level. After six months of treatment, NMDS scores were lower, suggesting therapeutic efficacy, which is further supported by the decrease in serum GDF-15 levels.

As well, when compared to available natural history data, the clinical status of the patients in our cohort is much better than would be expected. Hikmat et al. (2017) studied 27 patients with biallelic *POLG* pathogenic variants and found that the median time from symptom onset to death was 4.9 months. Based on these data, we would have expected at least half of our cohort to die during the initial 6-month period of treatment; however, we had zero mortality.

The results should still be considered with some caution given that this is an open-label trial. We aimed to reduce bias by choosing an objective, quantitative clinical measure as primary outcome (sections I-III of the NMDS). NMDS scores, however, are still susceptible to bias, and can be impacted by medical decisions and daily life events outside of the trial. For example, two patients (Patients 2 and 6), attempted a wean of their antiseizure medication, resulting in recurrence of seizures which added points to section II (system-specific involvement) for their NMDS at 6-month follow up.

Serum GDF-15 levels were used as a surrogate of mitochondrial dysfunction and its decrease an indication of improvement of mitochondrial function. While this value is a useful biomarker for mitochondrial disease, it has limitations. First, the measure has a reported sensitivity of only 0.83,[19] so that even symptomatic patients with mitochondrial disease may have normal levels (5/10 patients in our study had normal values at baseline). Also important for our trial is that there is an upper threshold level for GDF-15 quantification that was surpassed by multiple samples. Patient 8's GDF-15 level was reported as "> 6000 pg/ml" over the 6-month period and therefore we could not assess if there was an increase or decrease.

The mechanism of action of dC/dT in POLG-related disorders is not completely clear. We initially hypothesized that dC/dT supplementation increases the circulating pool of these deoxynucleosides, which causes a shift in the equilibrium of the relevant enzymatic

reactions, resulting in increased production of mtDNA. However, there are alternate possible mechanisms that may at least contribute. *In vitro* supplementation with dT was recently shown to stimulate telomere elongation and stabilization[20]; such an effect may stabilize cellular function and contribute to clinical benefit, particularly given that premature telomere shortening has been reported in a POLG mouse model.[21] Future investigations to elucidate the mechanism(s) of action may have implications for dosing regimen and/or determining candidates for treatment. As well, supplementation with all four deoxynucleosides could theoretically provide a greater effect; however, the other purine deoxynucleosides, deoxyguanosine and deoxyadensosine, are unstable and would likely be broken down before eliciting a therapeutic benefit.[22,23]

In conclusion, our study demonstrates that enteral dC/dT is a safe and potentially effective treatment for POLG-related disorders. Longer term studies with larger cohorts are necessary to determine what impact this intervention has on the natural history of these conditions, as well as to assess the long-term safety. Further research is also needed to assess whether dC/dT could be an effective treatment option for other mitochondrial DNA depletion disorders.

Contributors

Dr. Pekeles: Literature search, figures, data collection, data analysis, data interpretation, writing—original draft.

Dr. Berrahmoune: Literature search, study design, data collection, methodology, project administration.

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Dr. Eberhard: Data collection, writing—review and editing.

Dr. Buhas: Study design, data collection, data interpretation, writing—review and editing.

Dr. Myers: Literature search, figures, study design, data collection, data analysis, data interpretation, writing—review and editing, conceptualization, funding acquisition, methodology, supervision.

HP and KAM have verified the underlying data.

All authors read and approved the final version of the manuscript.

Data sharing statement

Individual participant data that underlie the results reported in this article, after de-identification will be made available for any reasonable request from any qualified researcher who provide a methodologically sound proposal. Study protocol will also be made available. Data will be available beginning 3 months and ending 5 years following article publication. Proposals should be directed to kenneth.myers@mcgill.ca; to gain access, data requestors will need to sign a data access agreement.

Declaration of interests

KAM reports a consulting or advisory role with Jazz Pharmaceuticals and research grants paid to institution from Liam Foundation, Savoy Foundation, Fonds de Recherche du Québec—Santé, Grand Défi Pierre Lavoie Foundation, Pediatric Research Foundation, Epilepsy Canada, Ultragenyx and LivaNova. PJW reports a leadership role with the Garrod Association. HP, SB, CD, ACTC, TG, RE, DB report no competing interests.

Acknowledgements

We thank the patients and their families. We also thank the Liam Foundation for providing the majority of funding for the study, as well as the Savoy Foundation, Grand Défi Pierre Lavoie Foundation, and Fonds de Recherche du Québec—Santé who provided important financial support as well.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102740.

References

1 Basel D. Mitochondrial DNA depletion syndromes. *Clin Perinatol*. 2020;47:123–141.
2 Copeland WC. Defects of mitochondrial DNA replication. *J Child Neurol*. 2014;29:1216–1224.
3 Rahman S, Copeland WC. POLG-related disorders and their neurological manifestations. *Nat Rev Neurol*. 2019;15:40–52.
4 Cohen BH. Mitochondrial and metabolic myopathies. *Continuum*. 2019;25:1732–1766.
5 Gorman GS, Schaefer AM, Ng Y, et al. Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. *Ann Neurol*. 2015;77:753–759.
6 Huttenlocher PR, Solitare GB, Adams G. Infantile diffuse cerebral degeneration with hepatic cirrhosis. *Arch Neurol*. 1976;33:186–192.
7 Lopez-Gomez C, Levy RJ, Sanchez-Quintero MJ, et al. Deoxycytidine and deoxythymidine treatment for thymidine kinase 2 deficiency. *Ann Neurol*. 2017;81:641–652.
8 Dominguez-Gonzalez C, Madruga-Garrido M, Mavillard F, et al. Deoxynucleoside therapy for thymidine kinase 2-deficient myopathy. *Ann Neurol*. 2019;86:293–303.
9 Garone C, Garcia-Diaz B, Emmanuele V, et al. Deoxypyrimidine monophosphate bypass therapy for thymidine kinase 2 deficiency. *EMBO Mol Med*. 2014;6:1016–1027.
10 Blázquez-Bermejo C, Carreño-Gago L, Molina-Granada D, et al. Increased dNTP pools rescue mtDNA depletion in human POLG-deficient fibroblasts. *FASEB J*. 2019;33:7168–7179.
11 Kristiansen CK, Furriol J, Chen A, Sullivan GJ, Bindoff LA, Liang KX. Deoxyribonucleoside treatment rescues EtBr-induced mtDNA depletion in iPSC-derived neural stem cells with POLG mutations. *FASEB J*. 2023;37:e23139.
12 Phoenix C, Schaefer AM, Elson JL, et al. A scale to monitor progression and treatment of mitochondrial disease in children. *Neuromuscul Disord*. 2006;16:814–820.
13 Schaefer AM, Phoenix C, Elson JL, McFarland R, Chinnery PF, Turnbull DM. Mitochondrial disease in adults: a scale to monitor progression and treatment. *Neurology*. 2006;66:1932–1934.
14 Lim AZ, Ng YS, Blain A, et al. Natural history of Leigh syndrome: a study of disease burden and progression. *Ann Neurol*. 2022;91:117–130.
15 Wang J, Liu Z, Xu M, et al. Cinical, metabolic, and genetic analysis and follow-up of eight patients with HIBCH mutations presenting with leigh/leigh-like syndrome. *Front Pharmacol*. 2021;12:605803.
16 Wellcome Centre for Mitochondrial Research. *Manual for Newcastle mitochondrial disease adult scale (NMDAS)*. 2016.
17 Dominguez-Gonzalez C, Badosa C, Madruga-Garrido M, et al. Growth Differentiation Factor 15 is a potential biomarker of therapeutic response for TK2 deficient myopathy. *Sci Rep*. 2020;10:10111.
18 Cremonesi A, Meili D, Rassi A, et al. Improved diagnostics of purine and pyrimidine metabolism disorders using LC-MS/MS and its clinical application. *Clin Chem Lab Med*. 2023;61:1792–1801.
19 Lin Y, Ji K, Ma X, et al. Accuracy of FGF-21 and GDF-15 for the diagnosis of mitochondrial disorders: a meta-analysis. *Ann Clin Transl Neurol*. 2020;7:1204–1213.
20 Mannherz W, Agarwal S. Thymidine nucleotide metabolism controls human telomere length. *Nat Genet*. 2023;55:568–580.
21 Yu T, Slone J, Liu W, et al. Premature aging is associated with higher levels of 8-oxoguanine and increased DNA damage in the Polg mutator mouse. *Aging Cell*. 2022;21:e13669.
22 Ramón J, Vila-Julià F, Molina-Granada D, et al. Therapy prospects for mitochondrial DNA maintenance disorders. *Int J Mol Sci*. 2021;22:6447.
23 Camara Y, Gonzalez-Vioque E, Scarpelli M, et al. Administration of deoxyribonucleosides or inhibition of their catabolism as a pharmacological approach for mitochondrial DNA depletion syndrome. *Hum Mol Genet*. 2014;23:2459–2467.