Short-Term Administration of Mycophenolate Is Well-Tolerated in CLN3 Disease (Juvenile Neuronal Ceroid Lipofuscinosis)

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Abstract Mycophenolate, an immunosuppressant, is commonly used off-label for autoimmune neurological conditions. In CLN3 disease, a neurodegenerative disorder of childhood, preclinical and clinical data suggest secondary autoimmunity and inflammation throughout the central nervous system are key components of pathogenesis. We tested the short-term tolerability of mycophenolate in individuals with CLN3 disease, in preparation for possible long-term efficacy trials of this drug. We conducted a randomized, double-blind, placebo-controlled, crossover

study of mycophenolate in 19 ambulatory individuals with CLN3 disease to determine the safety and tolerability of short-term administration (NCT01399047). The study included two 8-week treatment periods with a 4-week intervening washout. Mycophenolate was well tolerated. 89.5% of participants completed the mycophenolate arm, on the assigned study dose (95% CI: 66.9–98.7%), and there were no significant differences in tolerability rates between mycophenolate and placebo arms (10.5%; 95% CI: –3.3–24.3%, $p = 0.21$). All reported adverse events were mild in severity; the most common adverse events on mycophenolate were vomiting (31.6%; 95% CI: 12.6–56.6%), diarrhea (15.8%; 95% CI: 3.4–39.6%), and cough (15.8%; 95% CI: 3.4–39.6%). These did not occur at a significantly increased frequency above placebo. There were no definite effects on measured autoimmunity or clinical outcomes in the setting of short-term administration. Study of long-term exposure is needed to test the impact of mycophenolate on key clinical features and CLN3 disease trajectory.

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Introduction

Juvenile neuronal ceroid lipofuscinosis (JNCL, CLN3 disease) is a rare, fatal, inherited lysosomal storage disorder caused by mutations in the *CLN3* on chromosome 16 (<http://omim.org/entry/204200#> (Retrieved: October 14, 2016); Lerner et al. 1995). At 4–6 years of age, affected individuals develop blindness, followed by developmental regression that includes cognitive and motor decline along with epilepsy, and eventual premature death in the third

decade (Adams and Mink 2013; Augustine et al. 2012; Cialone et al. 2012; Kwon et al. 2011; Marshall et al. 2005). Symptomatic treatments exist but are only partially effective at best (Augustine and Mink 2016). Preclinical and clinical data suggest secondary autoimmunity, and an inflammatory response in the central nervous system is a key component of disease pathogenesis (Castaneda and Pearce 2008; Chattopadhyay et al. 2002a, b; Drack et al. 2012; Lim et al. 2006, 2007; Pearce et al. 2004; Ramirez-Montealegre et al. 2005). Genetic and pharmacological interference with the ability to mount an immune response in the *Cln3*^{-/-} mouse model resulted in reduced serum immunoreactivity, absence of brain IgG deposition, decreased neuroinflammation, and better motor performance (rotarod testing) compared to untreated mutant animals (Seehafer et al. 2011). Given these preclinical data, we sought to test whether treating individuals with CLN3 disease with an immunosuppressive agent is a strategy appropriate for clinical use.

Mycophenolate is an immunosuppressive agent approved by the United States Food and Drug Administration for prophylaxis against organ transplant rejection in adult and pediatric populations. It is commonly used as an immunosuppressant off-label for immune-mediated neurological conditions (Downing et al. 2013; Montcuquet et al. 2017; Vermersch et al. 2005). However, it has not been used frequently in primary monogenic conditions. In light of growing evidence for non-CNS involvement in CLN3 disease (Ostergaard et al. 2011; Staropoli et al. 2012), and considering the anticipated prolonged trial duration needed to evaluate efficacy in this slowly progressive disease,

evaluation of the safety of short-term mycophenolate exposure in this population was warranted before determining whether to move forward with a longer-term efficacy study. In this trial, we evaluated the safety and tolerability of short-term mycophenolate administration in individuals with CLN3 disease.

Methods

Drug Dosing

The trial was a 22-week, randomized, double-blind, crossover study of mycophenolate versus placebo. Study participants were randomized to receive 8 weeks of mycophenolate followed by 8 weeks of placebo with an intervening 4-week washout or vice versa, in a blinded manner. Participants received liquid mycophenolate or matching placebo at standard pediatric dosing of 600 mg/m²/dose twice daily up to a maximum of 1,000 mg twice daily.

Eligibility, Recruitment, Infrastructure, and Study Conduct

Ambulatory individuals between ages 6 and 25 years with genetically confirmed CLN3 disease and manifest clinical features were eligible to participate. Prior exposure to mycophenolate was exclusionary. Full inclusion and exclusion criteria are listed in Table 1. Potential participants were recruited directly, through an existing IRB-approved contact registry held at the University of Rochester Batten Center (URBC) (de Blieck et al. 2013), in conjunction with

Table 1 Inclusion and exclusion criteria

Inclusion criteria

- Diagnosis of CLN3 disease as determined by a characteristic clinical presentation and confirmatory genetic evidence of two *CLN3* mutations
- Able to walk 10 ft without assistance beyond that required due to vision impairment
- Between 6 years and 25 years of age
- Local treating clinician willing to conduct the trial as a co-investigator
- Parent/legal guardian willing to accompany subject to all study visits and oversee study drug compliance
- Stable dose of allowed concomitant medications for at least 30 days prior to study enrollment
- Negative PPD within 6 months of the baseline visit

Exclusion criteria

- Inability to tolerate oral administration of medications
- Concomitant medical condition that would place the child at greater than acceptable risk from travel or exposure to mycophenolate
- Use of disallowed concomitant medications
- Administration of immunosuppressive medications
- History of any prior exposure or hypersensitivity to mycophenolate mofetil
- History of frank gastrointestinal hemorrhage, ulceration, or melena
- White blood cell count <3,000/μL, absolute neutrophil count <1,500/μL, hemoglobin <10 g/dL, or thrombocytopenia <100,000/μL
- Abnormal liver function (aspartate aminotransferase, alanine aminotransferase, or bilirubin greater than three times the upper limit of normal)
- Immunizations not up to date for age according to Centers for Disease Control and Prevention guidelines

the Batten Disease Support and Research Association (BDSRA), BDSRA-designated Centers of Excellence, and through web-based announcements to professional organizations involved in the care of patients with Batten diseases.

We developed a novel, hybrid trial infrastructure that combined single (efficacy)- and multicenter (safety) study activities. The central site (URBC) partnered with a local site investigator for each participant to provide close monitoring of safety in between visits at the University of Rochester. Four study visits were conducted in person at the URBC: at screening/baseline, and at weeks 8, 12, and 20, i.e., at the start and end of each study arm (period 1 and period 2, mycophenolate or placebo). Participants completed four scheduled safety monitoring visits with their local site investigator at weeks 2, 4, 14, 16, and any unscheduled or early termination visits. Telephone contacts between the URBC and the participant's caregiver occurred at weeks 10 and 22 for additional safety oversight.

Outcome Measures

The primary study outcome was tolerability, defined as the proportion of subjects able to complete 8 weeks on the assigned dose of mycophenolate without interruption, dose reduction, or suspension, versus those able to complete 8 weeks on placebo. Secondary tolerability and safety measures included the number of drug suspensions and adverse events. Safety and tolerability assessments were conducted at every visit. Secondary measures also included measurement of known autoantibodies occurring in CLN3 disease [anti-glutamic acid decarboxylase 65 (GAD65), anti-alpha fetoprotein (AFP), and anti-collapsin mediator protein 2 (CRMP2)] (Castaneda and Pearce 2008; Chattopadhyay et al. 2002a, b; Lim et al. 2006; Ramirez-Montealegre et al. 2005), and the Unified Batten Disease Rating Scale (UBDRS) which is a disease-specific clinical assessment (de Blicke et al. 2013; Marshall et al. 2005). The UBDRS is a clinician-reported outcome that includes four subscales and clinical global impression: motor subscale (20 items, score range 0–112), seizure subscale (12 items, score range 0–54), behavior subscale (10 items, score range 0–55), and a capability assessment (5 items, score range 0–14). Clinical global impression for each subscale and for the overall clinical picture is also assigned (score range 0–5, each). For each domain, higher scores are worse, except for the capability subscale, in which lower scores are worse. Participants were also assessed with a cognitive battery that included assessments of estimated verbal IQ (Wechsler Intelligence Scale for Children 4th edition – WISC-IV Vocabulary and Vocabulary Multiple Choice subtests), attention (WISC-IV Digit

Span Forward), working memory (WISC-IV Digit Span Backward), verbal memory (Wide Range Assessment of Memory and Learning – 2nd edition – WRAML-2 Story Memory [immediate learning, delayed recall, recognition], WRAML-2 Sentence Recall), and verbal fluency (Controlled Oral Word Association Test). All tasks were presented verbally and required only oral responses, to accommodate participants' anticipated vision loss based on the CLN3 disease diagnosis. Both raw scores and age-corrected scaled scores were recorded; the former provided information on within-subject change over time, and the latter provided information on cognitive test performance in relation to healthy (non-CLN3 disease) same-age peers from each test's respective normative sample. For all cognitive tests, higher scores indicate better performance on the test. The details of the cognitive battery and its established use in individuals with CLN3 disease have been described previously (Adams and Mink 2013).

Autoantibody ELISAs

The presence of autoantibodies was determined using a previously established protocol (Sato et al. 2016). Briefly, recombinant human GAD65, AFP, and CRMP2 were incubated on separate Maxisorp 96-well plates (Nunc) overnight at 4°C in 100 uL sterile PBS (Hyclone) at a final concentration of 1 ug/mL. Plates were then blocked with 200 uL of 1% BSA in PBS for 1 h at room temp. Reference controls were generated from commercial antibodies against the target protein (anti-GAD65-cell signaling, anti-AFP-R&D Systems, anti-CRMP2-cell signaling). Participants' serum samples were then loaded in triplicates at 1:1,000 diluted in 100 uL PBS and incubated overnight at 4°C. Plates were washed five times with PBS 0.05% Tween for 10 min intervals. Samples were then incubated with 1:5,000 HRP-conjugated secondary antibodies in 100 uL sterile PBS for 2 h at room temp. Plates were washed again five times with PBS 0.05% Tween for 10 min intervals. 100 uL of TMB substrate (cell signaling) was incubated in the wells for 1.5 min (anti-AFP) or 4 min (anti-CRMP2 and anti-GAD65), and the reaction was terminated with 50 uL of 1N hydrochloric acid. Plates were read at OD 450 and data given as U/mL in reference to the respective standard.

Sample Size

The target sample size was 30 subjects. This sample size was chosen to provide at least 80% power to detect a difference in tolerability rates between the treatment groups in the range of 25–51% at the two-sided 5% level of significance.

Statistical Analysis

Tolerability was compared between the treatment groups using Prescott's test (1981). Exact 95% confidence intervals (CIs) were computed for tolerability rates on mycophenolate and placebo, and a 95% Wald CI was calculated for the difference in tolerability rates. Secondary tolerability and safety measures were analyzed similarly.

The effect of mycophenolate on each efficacy assessment was evaluated using a mixed effects heteroscedastic analysis of covariance model for the changes in outcomes from baseline (week 0) to the end of treatment periods 1 and 2 (weeks 8 and 20), with fixed effects for treatment group, period, and baseline outcome, and a random effect for subject (Senn 2002). Each model was used to test for significance of treatment, period, and treatment-by-period interaction effects. Model assumptions were examined using numerical and graphical techniques; when violations were detected, the robustness of the analysis was assessed using remedial measures (Byron Jones 2003).

Ethics Approval and Safety Monitoring

Prior to recruitment of study participants, the trial was reviewed and approved by the University of Rochester Research Subjects Review Board (RSRB #33940) and was registered at [Clinicaltrials.gov](https://clinicaltrials.gov) (NCT01399047). Parent permission was obtained for the enrollment of each study participant. A local site was established prior to the formal enrollment of each participant, thus a staged consent process was conducted. The initial consent was for prescreening and establishment of a local site for safety monitoring. The subsequent consent process covered final screening for eligibility and participation in the study. Subject safety was monitored by the study investigators in conjunction with a medical monitor and a data and safety monitoring committee.

Results

A total of 19 affected subjects were enrolled into the study. After completion of 13 subjects in the study, an interim set of conditional power analyses were conducted under the following conditions: (1) performance trends to date in discordance and tolerability, (2) extreme assumptions of discordance and tolerability, and (3) an intermediate set of conditions between 1 and 2 above. Analyses for each set of assumptions above were conducted for a sample size of $n = 20$ and $n = 30$. In each case, except for the most extreme assumptions with a sample size of $n = 30$, conditional power was poor ($\leq 31\%$) for detecting a difference in tolerability between mycophenolate and placebo. In addition, even with accrual of the planned total

of 30 subjects, the threshold at which mycophenolate was a priori deemed intolerable (tolerated $< 50\%$ of the time) would not be reached. On the basis of these conditional power estimations and in discussion with the Data Safety Monitoring Committee, we truncated enrollment to 20 participants. The decision to terminate the trial before full enrollment was based on low conditional power estimates for the primary analysis comparing tolerability rates. The final identified participant decided not to proceed with study enrollment, and thus the study was closed after enrollment of 19 subjects.

The mean participant age was 12.53 years (range 6–20 years; Table 2). Eighteen participants were non-Hispanic Caucasian in ethnicity/race, which was expected based on the known demographics of CLN3 disease. Fourteen subjects were homozygotes for the common *CLN3* 1 kb deletion; the remaining participants were compound heterozygotes for the common deletion and another disease-causing mutation.

Mycophenolate was tolerated in 17/19 subjects (89.5%; 95% CI: 66.9–98.7%), while placebo was tolerated in 19/19 study participants (100%; 95% CI: 82.4–100%). There was no significant difference in tolerability rates (10.5%; 95% CI: -3.3 – 24.3% , $p = 0.21$). One participant withdrew from the trial prematurely due to factors unrelated to study drug. The most common adverse events (AEs) in participants while taking mycophenolate were vomiting (31.6%; 95% CI: 12.6–56.6%), diarrhea (15.8%; 95% CI: 3.4–39.6%), and cough (15.8%; 95% CI: 3.4–39.6%). Each of these occurred in excess of the frequency observed during the placebo arm (10.5%, 95% CI: 1.3–33.1%; 10.5%, 95% CI: 1.3–33.1%; 5.3%, 95% CI: 0.1–26.0%; respectively), but differences were not significant ($p = 0.22, 1.00, 0.63$, respectively). All AEs were mild in severity. The mean number of AEs per subject was 2.4 (range 0–6) while assigned to mycophenolate and 3.0 (range 0–10) while assigned to placebo ($p = 0.62$, Wilcoxon signed-rank test). There was one serious adverse event during the course of the study. While taking mycophenolate, one participant experienced hyperkalemia associated with nausea and headache, resulting in hospitalization. The hyperkalemia resolved without sequelae and was considered possibly, but not definitely, related to study drug. Study drug was discontinued in week 19, and the subject completed the remainder of study visits off study drug without any additional adverse events. Unblinding occurred after study closure.

Treatment effect estimates appear in Table 3. Significant treatment-by-period interactions were identified for some of the cognitive outcomes (both raw and scaled scores for the WISC-IV Vocabulary and Digit Span Backward subtests). However, the magnitude of these interaction effects was not clinically significant; therefore, the overall treatment effect

Table 2 Participant demographics and baseline assessments

	Mycophenolate to placebo (<i>n</i> = 10)	Placebo to mycophenolate (<i>n</i> = 9)	Total (<i>n</i> = 19)
Age – years	10.5 (6)	14.8 (2.4)	12.5 (5)
Male, <i>n</i> (%)	8 (80)	7 (77.8)	15 (79)
Caucasian race, <i>n</i> (%)	9 (90)	8 (88.9)	17 (89.4)
Common del homozygotes, <i>n</i> (%)	7 (70)	7 (77.8)	14 (73.7)
UBDRS			
Motor subscale	20.3 (14.7)	24.9 (10.6)	22.5 (12.8)
Seizure subscale	2.3 (3.4)	6.2 (4.6)	4.3 (4.4)
Behavior subscale	13.4 (6.6)	13.0 (4.9)	13.2 (5.7)
Capability subscale	5.4 (3.5)	6.8 (2.2)	6.1 (3.0)
CGI seizure	2.0 (1.2)	2.7 (1.0)	2.3 (1.1)
CGI cognitive	3.0 (1.2)	4.0 (0.7)	3.5 (1.1)
CGI behavior	3.2 (0.8)	3.1 (1.1)	3.2 (0.9)
CGI mood	2.8 (0.6)	2.8 (1.0)	2.8 (0.8)
CGI motor	2.7 (1.2)	3.4 (0.7)	3.1 (1.0)
CGI overall	3.6 (0.7)	4.0 (0.7)	3.8 (0.7)
Subject status			
Completed study, <i>n</i> (%)	10 (100)	7 (77.8)	17 (89.5)
Premature withdrawal, <i>n</i> (%)	0 (0)	1 (11.1)	1 (5.3)
Study drug discontinuation, <i>n</i> (%)	0 (0)	1 (11.1)	1 (5.3)
Study drug compliance (%)	97.3	98.8	

Data presented as mean (SD) unless otherwise noted

CGI clinical global impression

is reported in Table 3. Treatment-by-period interactions were not identified for UBDRS subscales, UBDRS clinical global impression scores, or antibody data (anti-AFP, anti-GAD, anti-CRMP2). Sensitivity analyses assessing the impact of deviations from the underlying model assumptions were consistent with the analyses presented in Table 3. Taking into account multiple comparisons, no significant treatment effects were observed in any clinical measures.

Discussion

This was one of the first prospective, blinded, randomized studies in CLN3 disease (Hatonen et al. 1999; Zweije-Hofman et al. 1982) and one of the few controlled clinical trials targeting disease modification in this specific population in the past 40 years (Santavuori et al. 1985, 1989; Santavuori and Moren 1977). Through this study, we have introduced new knowledge about the tolerability of an immunosuppressant medication in a vulnerable population with a genetic disorder.

This study focused on an initial assessment of safety for a novel application of mycophenolate in a genetic condition, targeting secondary autoimmunity. We employed a

blinded, crossover design to increase the validity of the study results through use of internal controls and to maximize the power of the requisite small sample in this rare disease. Our novel infrastructure model was a feasible method to implement and was effective in expanding the recruitment reach of potential study participants.

Mycophenolate was well tolerated in the short term. As expected, gastrointestinal adverse events were the most common side effects reported in participants while taking mycophenolate. Overall, adverse events were mild in severity.

There were no definite effects on autoantibody levels or on clinical outcomes in the setting of short-term administration. It is notable that antibody levels varied widely across participants, and further characterization of this variability is necessary to better understand expected change over time and correlation, if any, with disease state or severity. With respect to knockdown of autoantibodies during mycophenolate exposure, it is possible that greater duration of exposure or higher doses would be required. Impact on clinical outcomes was not anticipated, given the short-term exposure compared to the very long course of the disease (Cialone et al. 2012). Long-term exposure will be needed to test the impact of mycophenolate on

Table 3 Change in secondary outcomes expressed as treatment effect size

	Treatment effect ^a	95% CI	<i>p</i> -value
<i>UBDRS</i>			
Motor subscale	−0.69	−4.67–3.28	0.72
Seizures subscale	−0.17	−1.45–1.12	0.78
Mood and behavior subscale	0.04	−3.47–3.55	0.98
Capability subscale	−0.38	−1.08–0.32	0.26
CGI change since last assessment	0.37	−0.25–0.98	0.22
CGI seizure	0.17	−0.15–0.50	0.27
CGI cognitive	−0.06	−0.35–0.23	0.67
CGI behavior	−0.12	−0.62–0.37	0.60
CGI mood	0.04	−0.48–0.57	0.86
CGI motor	0.07	−0.29–0.44	0.67
CGI overall	0.05	−0.28–0.38	0.75
<i>Cognitive battery</i>			
WRAML-2 story memory (raw scores)			
Immediate recall	0.77	−1.18–2.72	0.39
Delayed recall	−0.98	−3.23–1.27	0.35
Recognition	1.37	−2.85–5.60	0.46
WRAML-2 sentence memory raw score	−0.10	−1.32–1.13	0.87
<i>WISC-IV (raw scores)</i>			
Vocabulary	−0.24	−2.07–1.59	0.78
Digit span forward	−0.53	−0.99–0.07	0.03
Digit span backward	0.63	−0.43–1.68	0.20
Longest digit span forward	−0.17	−0.47–0.13	0.24
Longest digit span backward	0.29	−0.49–1.08	0.42
Controlled oral word association (FAS): total words	−0.88	−5.00–3.25	0.65
NEPSY-II word generation – semantic: total words	0.88	−2.03–3.79	0.52
<i>Autoantibodies</i>			
AFP (U/L)	1.28	−33.87–36.43	0.94
CRMP2 (U/L)	−406.46	−2,786.72–1,973.80	0.72
GAD65 (U/L)	−684.30	−1,908.49–539.88	0.25

AFP alpha-fetoprotein, CGI clinical global impression, CRMP2 collapsin response mediator protein 2, GAD65 glutamic acid decarboxylase 65, UBDRS Unified Batten Disease Rating Scale

^a Mean difference (mycophenolate minus placebo) in outcome, adjusted for baseline value and period effects

disease-relevant clinical features and disease trajectory such as motor and cognitive decline, epilepsy, mood and behavior, and lifespan.

As is typical in rare disorders, this study was limited in part by small sample size (Augustine et al. 2013; Hee et al. 2017). This limits the ability of standard randomization strategies to balance multiple factors across groups. Although randomized, the participants who received placebo followed by mycophenolate were older and had more advanced disease as measured by the UBDRS. Study of a single, standard pediatric dose was another limitation. It is possible that the effective dose for CLN3 disease may differ from that of other previously described disease indications for mycophenolate or that individual titration to a specific

mycophenolate level is warranted. While beneficial for studying short-term risk related to adverse events, and for “go-no go” decisions regarding proceeding to future long-term studies, the brief duration of exposure did not allow clinical insights into potential efficacy.

Long-term administration of mycophenolate in individuals with CLN3 disease is needed to determine whether there is a disease-modifying clinical benefit and whether it is safe for long-term use in this fatal neurodegenerative condition.

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Synopsis

Short-term administration of mycophenolate was well tolerated in an ambulatory sample of individuals with juvenile neuronal ceroid lipofuscinosis, a monogenic disorder with secondary autoimmunity.

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Compliance with Ethics Guidelines

Conflict of Interest

EF Augustine, HR Adams, and JW Mink have received research support from Abeona Therapeutics. JW Mink has received consulting fees from Sumitomo, Inc. CA Beck, S Defendorf, A Vierhile, D Timm, JM Weimer, and FJ Marshall declare that they have no conflicts of interest.

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Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. The trial was reviewed and approved by the University of Rochester Research Subjects Review Board (#33940) and was registered at [Clinicaltrials.gov](https://clinicaltrials.gov) (NCT01399047). Parent permission was obtained for the enrollment of each study participant.

Contributions of Individual Authors

EF Augustine	Conceptualization of the study, conducting the study, interpretation of the data, drafting and revising the manuscript
CA Beck	Conceptualization of the study, analysis and interpretation of the data, revising the manuscript
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JM Weimer	Specimen analysis, revising the manuscript
JW Mink	Conceptualization of the study, conducting the study, interpretation of the data, revising the manuscript
FJ Marshall	Conceptualization of the study, conducting the study, interpretation of the data, revising the manuscript

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