Erythromycin for myotonic dystrophy type 1: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial

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Summary

Background Myotonic dystrophy type 1 (DM1) is a devastating multisystemic disorder caused by a CTG repeat expansion in the *DMPK* gene, which subsequently triggers toxic RNA expression and dysregulated splicing. In a preclinical study, we demonstrated that erythromycin reduces the toxicity of abnormal RNA and ameliorates the aberrant splicing and motor phenotype in DM1 model mice.

Methods This multicentre, randomised, double-blind, placebo-controlled, phase 2 trial was conducted at three centres in Japan to translate preclinical findings into practical applications in patients with DM1 by evaluating the safety and efficacy of erythromycin. Between Nov 29, 2019, and Jan 20, 2022, a total of 30 adult patients with DM1 were enrolled and randomly assigned in a 1:2:2 ratio to receive either placebo or erythromycin at two daily doses (500 mg or 800 mg) for 24 weeks. The primary outcome included the safety and tolerability of erythromycin. The secondary efficacy measures included splicing biomarkers, 6-min walk test results, muscle strength, and serum creatinine kinase (CK) values. This trial is registered with the Japan Registry of Clinical Trials, jRCT2051190069.

Findings Treatment-related gastrointestinal symptoms occurred more frequently in the erythromycin group, but all adverse events were mild to moderate and resolved spontaneously. No serious safety concerns were identified. The CK levels from baseline to week 24 decreased in the overall erythromycin group compared with the placebo group (mean change of −6.4 U/L [SD 149] vs +182.8 [SD 228]), although this difference was not statistically significant ($p = 0.070$). Statistically significant improvements in the overall erythromycin treated groups compared to placebo were seen for two of the eleven splicing biomarkers that were each evaluated in half of the trial sample. These were *MBNL1* ($p = 0.048$) and *CACNA1S* ($p = 0.042$).

Interpretation Erythromycin demonstrated favourable safety and tolerability profiles in patients with DM1. A well-powered phase 3 trial is needed to evaluate efficacy, building on the preliminary findings from this study.

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Abbreviations: CGI, Clinical global impression; CK, Creatinine kinase; COPD, Chronic obstructive pulmonary disease; DM1, Myotonic dystrophy type 1; ECG, Electrocardiogram; EMG, Electromyography; INQoL, Individualised neuromuscular quality of life questionnaire; LEC score, Lower extremity composite score; MBNL, Muscleblind-like; 6MWT, 6-Minute walk test; UEC score, Upper extremity composite score

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Keywords: Erythromycin; Myotonic dystrophy; RNA toxicity; Splicing

Research in context

Evidence before this study

We conducted a literature search on PubMed using the search terms "myotonic dystrophy" AND "erythromycin" OR "antibiotics" OR "macrolide," while filtering for articles categorised as "Randomised Controlled Trials" and published in English up to August 31, 2023. Our search did not yield any trials assessing the safety and efficacy of erythromycin in patients with DM1. Furthermore, despite many clinical trials exploring various therapeutic interventions for DM1, including metformin, mexiletine, tideglusib, baliforsen, aerobic exercise, and cognitive behavioral therapy, none of these studies demonstrated significant improvements in splicing abnormalities in DM1.

Added value of this study

This multicentre, randomised, double-blind, placebo-controlled, phase 2 trial was conducted to translate preclinical findings into practical applications in patients with DM1 by evaluating the safety and efficacy of erythromycin. The results demonstrated that erythromycin was well-tolerated, with no serious safety concerns observed. Furthermore, erythromycin exhibited promising improvements in splicing biomarkers and serum levels of creatine kinase. Notably, this paper stands as the sole evidence demonstrating substantial splicing improvement in patients with DM1 through therapeutic intervention.

Implications of all the available evidence

This phase 2 trial provides valuable insights into the safety and potential efficacy of erythromycin in treating DM1. It introduces splicing biomarkers as a novel way to assess treatment effects and paves the way for further research and clinical trials in the pursuit of effective therapies for DM1, which currently lacks fundamental treatment options.

Introduction

Myotonic dystrophy type 1 (DM1) is the most common type of muscular dystrophy in adults, with a prevalence of 1 in every 2100 births.[1] This devastating disease presents multiple systemic symptoms, including myotonia, progressive muscle weakness, insulin resistance, cardiac conduction defects, and cognitive dysfunction.[2] DM1 is caused by the expansion of a CTG repeat in the 3' untranslated region of *DMPK* gene, giving rise to toxic RNAs containing expanded CUG repeats.[3] These mutant RNAs are confined within the nucleus, forming ribonuclear inclusions that sequester muscleblind-like (MBNL) family-member proteins. Given that MBNL proteins serve a regulatory role in alternative splicing, their sequestration to the toxic RNAs leads to the misregulation of alternative splicing of many genes, consequently resulting in diverse multisystemic symptoms. For example, aberrant splicing of muscle-specific chloride channel (*CLCN1*) and insulin receptor causes myotonia and glucose intolerance in DM1, respectively.[4–6] Moreover, numerous distinct splicing misregulation has likewise been documented to precipitate the symptomatic manifestations of DM1.[7–11]

At present, there is no effective fundamental treatment for DM1. We have endeavoured to ascertain compounds that mitigate the deleterious effects of toxic RNA in DM1. Through drug repositioning screening, we have identified that erythromycin, a widely used antibiotic, can inhibit MBNL protein sequestration caused by abnormal RNA in DM1.[12] Furthermore, we have demonstrated that erythromycin suppresses RNA toxicity in DM1 model cells and improves splicing abnormalities and myotonia in DM1 model mice.[12] Erythromycin has been utilised for many years and is also prescribed for long-term low-dose oral therapy in chronic obstructive pulmonary disease (COPD).[13] Our preclinical studies have shown that therapeutic effects can be achieved in DM1 mice at doses equivalent to those used in COPD treatment, leading us to anticipate that erythromycin may be efficacious in patients with DM1. Based on these findings, we conducted an investigator-initiated phase 2, double-blind, placebo-controlled, randomised clinical trial to investigate the safety, efficacy, and tolerability of erythromycin (MYD-0124) in patients with DM1.

Methods

Study design and participants

This study was a phase 2, randomised, double-blind, placebo-controlled clinical trial conducted at three centres in Japan. The eligible participants were ambulatory men and women aged 20–55 years at the time of informed consent, with a clinical diagnosis of myotonic dystrophy type 1 (DM1), without an exclusive restriction to adult-onset cases, and at least 100 CTG repeats in the *DMPK* gene. Patients were excluded if they had an implantable cardiac device; a serious cardiovascular risk based on electrocardiogram (ECG) results during the screening visit; clinically significant liver or renal disease; or a history of malignancy. The full list of entry and exclusion criteria is provided in

Supplementary Table S1. Participants were recruited with the corporation of the Japanese National Registry of Muscular Dystrophy (REMUDY). All participants provided written informed consent before screening or any study-specific procedures. The protocol and all documentation received ethics approval from institutional review boards at each study site. The study adhered to all applicable local regulations, was done following Good Clinical Practice, as outlined by the International Conference on Harmonization, and complied with ethical standards described in the Declaration of Helsinki. This trial is registered with the Japan Registry of Clinical Trials, jRCT2051190069.

Randomisation and masking

After the screening, eligible participants with DM1 were randomly assigned in a 1:2:2 ratio to receive either placebo or erythromycin (MYD-0124) at doses of either 500 mg or 800 mg using the EDC system (DATATRAK ONE); block randomisation scheme. Then the participants in each group were randomly assigned to undergo or not to undergo muscle biopsy in a 1:1 ratio. Randomisation was done using a block randomisation process by the centres, with random block sizes of ten to ensure a balance between the presence or absence of muscle biopsy in each group. The investigators and participants were unaware of assigned treatments during the study treatment period. The study investigators did the blinded clinical assessments. The placebo was indistinguishable in appearance and taste from erythromycin.

Procedures

The study comprised a 6-week screening period and a 24-week double-blind treatment period (including visits for safety and efficacy measurements at weeks 0, 4, 8, 16, and 24). Participants received erythromycin or placebo orally (twice daily) for 24 weeks. Patient compliance was checked by the clinical research coordinator at each visit (amount of treatment returned used or unused and doses administered). Safety measures were also monitored. Blood and urine analyses and ECGs were performed at all visits, while Holter ECG was done before the treatment and at week 24.

For splicing biomarker analysis, the tibialis anterior muscles were collected from the same leg via needle biopsy using a 16-gauge BARD MONOPTY Biopsy Needle (BARD) before the first dose (day −1, baseline) and at week 24. Samples were immediately immersed in a tissue storage reagent (RNAlater, Thermo Fisher Scientific) and stored at −20 °C for batch analysis. Total RNA extraction from biopsied muscles and cDNA synthesis were performed as described previously.[14] Reverse transcriptase (RT)-PCR analysis of alternative splicing of *CLCN1* exon 7a, *ATP2A1* exon 22, *DTNA1* exons 11a and 12, *DTNA2* exons 11a and 12, *BIN1* exon 11, and *DMD* exon 78 was performed using gene-specific primers (Supplementary Table S2), as previously described.[15] The percentage of normal splicing (% [normal splicing isoform]/[normal + aberrant splicing isoforms]) in each splicing event was calculated for the value of the splicing biomarker. Splicing misregulations in *CACNA1S* exon 29, *MBNL1* exon 7, *CLASP1* exon 19, *PDLIM3* exon 5, and *CAPZB* exon 8 were evaluated for additional splicing biomarkers as a post-hoc study.

Muscle strength was measured using a hand-held dynamometer (ErgoFET, Hogan Health Industries) at baseline and treatment weeks 8, 16, and 24. A 6-min walk test (6MWT) was performed at baseline and treatment weeks 8 and 24. Electromyography (EMG) was performed at baseline and week 24 on the opposite side of the tibialis anterior muscle where the muscle biopsy was performed. The Individualised Neuromuscular Quality of Life questionnaire (INQoL) was assessed at baseline and weeks 8, 16, and 24. Meanwhile, Clinical Global Impression of Severity (CGI-S) and Clinical Global Impression of Improvement (CGI-I) were assessed at baseline and week 24. Serum CK levels were evaluated at baseline and weeks 8, 16, and 24.

Blood samples for pharmacokinetic analysis of erythromycin were obtained from participants undergoing muscle biopsy before the first dose, at 40 min, and 90 min after the first dose.

Outcomes

The primary outcome measure was safety, including assessments of adverse events reported by the patients and assessed by physical examinations, vital signs, clinical laboratory tests, and standard and Holter ECG. The Medical Dictionary for Regulatory Activities version 24.1 was used to code adverse events.

Secondary efficacy outcome measures were changes from baseline in 1) splicing biomarkers (percentage of normal splicing isoform), 2) distance walked during the 6MWT, 3) upper extremity composite (UEC) score comprising the sum of bilateral average of raw strength values for gross grip, elbow flexors, and elbow extensors; lower extremity composite (LEC) score comprising the sum of bilateral average of raw strength values in hip flexors, hip adductors, hip abductors, and knee extensors; and muscle strength of ankle dorsiflexion, 4) 4-point myotonia score evaluated in 20 needle insertions by EMG: 0, no myotonia; 1, occasional myotonic discharge in ≤50% insertions; 2, myotonic discharge in >50% of insertions; and 3, myotonic discharge with nearly all (19 or 20) insertions, 5) INQoL overall score, 6) CGI-I scale, and 7) serum CK values.

Statistical analysis

The sample size was determined based on the feasibility of enrolment and assessment of biopsied muscles, as there was insufficient a priori information to set it statistically when the study was planned. The safety analysis set included all patients who received their assigned drug. The efficacy analysis set included all

randomly assigned patients with a valid baseline and at least one post-baseline assessment.

Patient demographic data were calculated as summary statistics in each treatment. For the primary safety analysis, the number and frequency of drug-related adverse events and serious adverse events were tabulated. The secondary endpoints (changes from baseline to week 24) were compared between the placebo treatment and each dose of erythromycin using the Wilcoxon rank-sum test for continuous variables because of the limited sample size and the inability to establish the assumption of normality necessary for parametric analyses. Numerical data were presented as means and standard deviations (SDs) due to the challenges associated with summarising them using medians and interquartile ranges (IQRs) in the context of such small sample sizes. All reported p-values are two-sided, with a nominal significance level of 0.05. No adjustments for multiple testing were made as this is an exploratory study. All statistical analyses were made using SAS version 9.4 (SAS Institute Inc.).

Role of the funding source

This study's funder had no role in study design; data collection, analysis, and interpretation; or report writing. MN, TS, and EH had access to dataset and MN had the final responsibility for the decision to submit for publication.

Results

Between Nov 29, 2019, and Jan 20, 2022, we enrolled thirty patients with myotonic dystrophy type 1 recruited in three centres: Osaka University Hospital (n = 10), NHO Aomori National Hospital (n = 10), and NHO Osaka Toneyama Medical Centre (n = 10). Twelve patients were randomly assigned to receive high-dose erythromycin (800 mg), twelve patients to receive low-dose erythromycin (500 mg), and six patients to receive a placebo (Fig. 1). All participants completed the study without premature discontinuation.

Demographics and clinical characteristics were similar between treatment groups except for a slight trend toward decreased tibialis anterior muscle strength in the treatment group (Table 1). In addition, the INQoL overall score, which indicates the patient's disease burden, was higher in the treatment group (Supplementary Table S3). Seventeen participants (57%) were females. Participants' age ranged between 23 and 53 years (mean age 38.1 [SD 8.9]). Years from the onset were less than 5 years in two (7%) participants, 5 to less than 10 years in eight (27%), and 10 years or more in twenty (67%).

Concerning safety endpoints, the incidence of adverse events was as follows: nine participants (75%) in erythromycin 800 mg group, six participants (50%) in erythromycin 500 mg group, and three participants (50%) in the placebo group (Table 2). One serious adverse event (pneumonia) was reported in the placebo group. Concerning adverse events possibly related to the drug, five participants (42%) in erythromycin 800 mg group, three participants (25%) in erythromycin 500 mg group, and one participant (17%) in the placebo group reported such events. Among the total erythromycin group (n = 24), gastrointestinal events were the most frequently reported treatment-emergent adverse events, with six participants (25%) experiencing them. In contrast, no participants in the placebo group (n = 6) reported the same. All adverse events were mild or moderate in severity and resolved during the study period. None of the treatment-emergent adverse events must discontinue the treatment or the trial, and there were no reported deaths throughout the trial. No

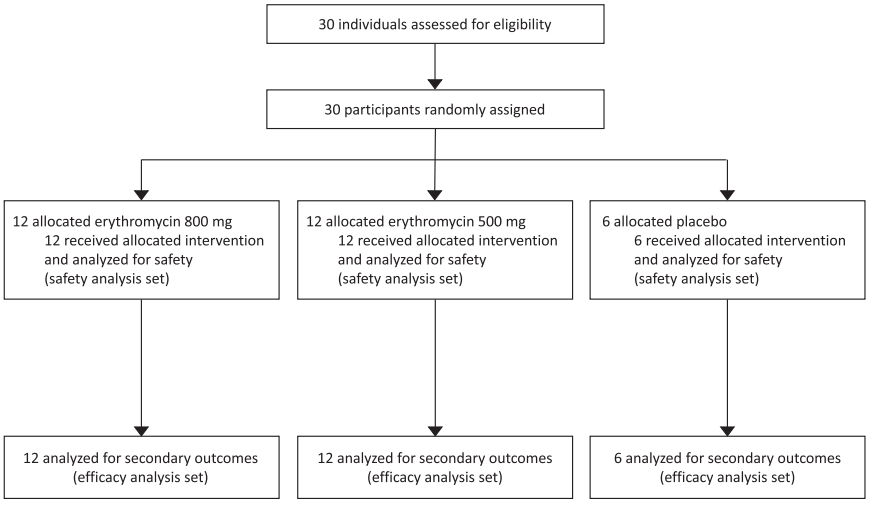

Fig. 1: **Trial profile**. The safety population comprised 30 participants who received at least one dose of the study drug. The efficacy population comprised 30 participants who received at least one dose of study treatment and had at least one post-treatment assessment.

	Erythromycin 800 mg (n = 12)	Erythromycin 500 mg (n = 12)	Combined erythromycin-treated group (n = 24)	Placebo (n = 6)	Total (n = 30)
Sex					
Male	6 (50%)	5 (42%)	11 (46%)	2 (33%)	13 (43%)
Female	6 (50%)	7 (58%)	13 (54%)	4 (67%)	17 (57%)
Age, years					
Mean (SD)	40.0 (8.6)	38.0 (9.4)	39.0 (8.9)	34.7 (9.2)	38.1 (8.9)
Body weight, kg					
Mean (SD)	56.0 (9.6)	56.8 (7.2)	56.4 (8.3)	59.6 (9.8)	57.0 (8.6)
Strength of ADF (MRC score)					
Left					
2	1 (8%)	2 (17%)	3 (13%)	0 (0%)	3 (10%)
3	2 (17%)	0 (0%)	2 (8%)	0 (0%)	2 (7%)
4	8 (67%)	10 (83%)	18 (75%)	4 (67%)	22 (73%)
5	1 (8%)	0 (0%)	1 (4%)	2 (33%)	3 (10%)
Right					
2	1 (8%)	2 (17%)	3 (13%)	0 (0%)	3 (10%)
3	2 (17%)	1 (8%)	3 (13%)	0 (0%)	3 (10%)
4	8 (67%)	9 (75%)	17 (71%)	5 (83%)	22 (73%)
5	1 (8%)	0 (0%)	1 (4%)	1 (17%)	2 (7%)
Years from onset					
Less than 5 years	2 (17%)	0 (0%)	2 (8%)	0 (0%)	2 (7%)
5 to less than 10 years	2 (17%)	5 (42%)	7 (29%)	1 (17%)	8 (27%)
10 years or more	8 (67%)	7 (58%)	15 (63%)	5 (83%)	20 (67%)

ADF = ankle dorsiflexion, MRC score = Medical Research Council score.

Table 1: **Demographic data and baseline characteristics (safety analysis set).**

clinically meaningful differences were observed in laboratory parameters, vital signs, weight, or ECG parameters including PR, QRS, and QTc intervals between the placebo and erythromycin groups (Supplementary Figure S1). New-onset arrhythmias were not observed.

As a key secondary efficacy outcome, we evaluated splicing biomarkers (percentage of normal splicing isoform) of *CLCN1*, *ATP2A1*, *DTNA1*, *DTNA2*, *BIN1*, and *DMD* in biopsied TA muscles, which have been reported to exhibit a compelling correlation with the severity of

	Erythromycin 800 mg (n = 12)	Erythromycin 500 mg (n = 12)	Combined erythromycin-treated group (n = 24)	Placebo (n = 6)
Patients with ≥1 adverse event	9 (75%)	6 (50%)	15 (63%)	3 (50%)
Patients with ≥1 adverse event related to treatment	5 (42%)	3 (25%)	8 (33%)	1 (17%)
Patients with ≥1 serious adverse event	0 (0%)	0 (0%)	0 (0%)	1[a] (17%)
Adverse events leading to discontinuation	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Deaths	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Adverse events				
Gastrointestinal disorders	4 (33%)	2 (17%)	6 (25%)	0 (0%)
Injury and procedural complications	3 (25%)	2 (17%)	5 (21%)	0 (0%)
General disorders and administration site conditions	1 (8%)	2 (17%)	3 (13%)	0 (0%)
Infections and infestations	1 (8%)	0 (0%)	1 (4%)	3 (50%)
Metabolism and nutrition disorders	1 (8%)	1 (8%)	2 (8%)	0 (0%)
Musculoskeletal and connective-tissue disorders	1 (8%)	0 (0%)	1 (4%)	1 (17%)
Blood and lymphatic-system disorders	0 (0%)	1 (8%)	1 (4%)	0 (0%)
Eye disorders	0 (0%)	1 (8%)	1 (4%)	0 (0%)
Nervous system disorders	1 (8%)	0 (0%)	1 (4%)	0 (0%)
Investigations	3 (25%)	1 (8%)	4 (17%)	1 (17%)

[a]One patient in the placebo group was hospitalised to be treated for pneumonia.

Table 2: **Adverse events.**

disease.[15] The comparison between groups regarding the changes in splicing biomarkers from baseline to week 24 did not reveal any significant effects of erythromycin in any of the dose groups (Table 3, Fig. 2, and Supplementary Figure S2). However, within the treatment group, a notable improvement in the splicing biomarker of *CLCN1* was observed in several patients in both erythromycin 500 mg and 800 mg groups, possibly in a dose-dependent manner (Fig. 2). Further post-hoc analyses were done to evaluate splicing biomarkers of

Splicing biomarker (%)	Erythromycin 800 mg (n = 6)		Erythromycin 500 mg (n = 6)		Combined erythromycin-treated group (n = 12)		Placebo (n = 3)
		p value vs placebo		p value vs placebo		p value vs placebo	
CLCN1							
Baseline	13.5 (17.9)		20.3 (19.3)		16.9 (18.1)		34.4 (18.1)
Week 24	16.4 (17.8)		23.1 (17.1)		19.8 (17.0)		35.6 (17.5)
Change from BL	2.9 (3.9)	1.00	2.9 (5.9)	0.52	2.9 (4.7)	0.72	1.3 (0.6)
ATP2A1							
Baseline	5.6 (6.1)		26.9 (33.7)		16.3 (25.7)		45.5 (39.7)
Week 24	11.4 (12.0)		33.6 (39.2)		22.5 (30.0)		54.1 (40.2)
Change from BL	5.8 (12.4)	0.70	6.6 (10.3)	0.90	6.2 (10.9)	0.94	8.5 (14.6)
DTNA1							
Baseline	17.4 (12.6)		30.1 (27.2)		23.7 (21.3)		54.9 (7.8)
Week 24	19.5 (13.1)		30.9 (24.8)		25.2 (19.9)		67.5 (14.0)
Change from BL	2.1 (1.4)	0.52	0.8 (4.3)	0.37	1.5 (3.2)	0.35	12.6 (18.1)
DTNA2							
Baseline	24.0 (18.1)		33.2 (24.4)		28.6 (21.0)		47.9 (31.3)
Week 24	25.3 (12.6)		35.6 (23.4)		30.5 (18.7)		50.2 (28.2)
Change from BL	1.3 (6.8)	0.90	2.4 (5.0)	0.70	1.9 (5.7)	0.94	2.4 (3.2)
BIN1							
Baseline	51.8 (26.7)		72.4 (20.5)		62.1 (25.1)		68.1 (22.6)
Week 24	58.9 (26.4)		88.8 (4.5)		73.8 (23.9)		76.9 (2.8)
Change from BL	7.1 (15.7)	0.90	16.3 (23.3)	0.52	11.7 (19.6)	0.61	8.8 (22.9)
DMD							
Baseline	31.6 (15.0)		17.5 (15.7)		24.6 (16.4)		35.5 (35.2)
Week 24	34.1 (23.1)		27.8 (20.1)		31.0 (20.9)		35.5 (34.7)
Change from BL	2.5 (12.9)	0.52	10.3 (13.5)	0.52	6.4 (13.2)	1.00	0.05 (0.7)
CACNA1S							
Baseline	19.6 (22.2)		45.4 (24.0)		32.5 (26.5)		77.1 (32.4)
Week 24	24.3 (20.4)		56.2 (14.5)		40.2 (23.9)		72.1 (30.3)
Change from BL	4.7 (10.8)	0.19	10.8 (14.1)	**0.024**	7.7 (13.0)	**0.042**	−5.0 (6.2)
MBNL1							
Baseline	57.5 (24.6)		44.0 (13.5)		50.7 (21.0)		53.4 (10.5)
Week 24	62.2 (28.9)		49.0 (20.6)		55.6 (26.0)		44.5 (16.9)
Change from BL	4.7 (6.1)	**0.048**	5.0 (7.5)	0.17	4.9 (6.8)	**0.048**	−9.0 (6.6)
CLASP1							
Baseline	0 (0)		15.9 (23.7)		8.0 (18.5)		0 (0)
Week 24	2.2 (5.0)		9.9 (15.4)		6.1 (12.1)		4.3 (6.0)
Change from BL	2.2 (5.0)	0.72	−6.0 (8.6)	0.13	−1.9 (8.1)	0.27	4.3 (6.0)
PDLIM3							
Baseline	81.4 (11.8)		62.0 (15.1)		71.7 (16.7)		45.4 (17.3)
Week 24	85.9 (11.3)		59.5 (22.5)		72.7 (22.2)		50.1 (12.6)
Change from BL	4.5 (6.4)	0.91	−2.5 (10.5)	0.55	1.0 (9.4)	0.63	4.7 (10.8)
CAPZB							
Baseline	13.6 (17.5)		13.0 (12.7)		13.3 (15.3)		19.4 (27.4)
Week 24	7.5 (16.7)		5.4 (7.6)		6.4 (13.0)		25.9 (28.1)
Change from BL	−6.2 (12.0)	0.080	−7.6 (16.1)	0.15	−6.9 (14.2)	0.078	6.5 (5.2)

Data are mean (SD). The bolded numbers signify results that have achieved statistical significance.

Table 3: **Key secondary outcomes (change from BL to week 24, efficacy analysis set).**

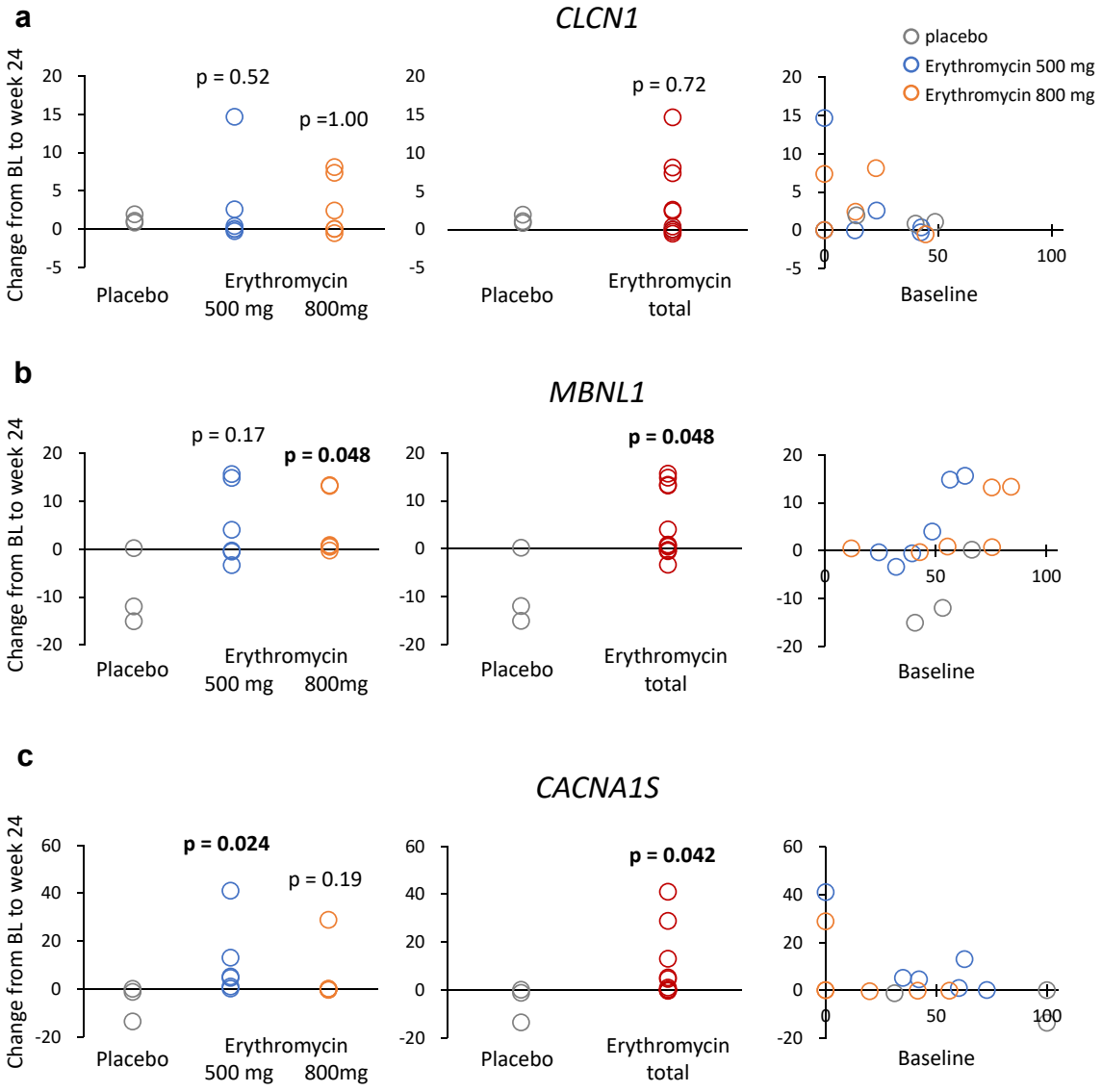

Fig. 2: **Change from baseline to week 24 in splicing biomarkers in the efficacy analysis set**. **a**. Left: a scatterplot showing changes in *CLCN1* splicing biomarker in placebo, erythromycin 500 mg, and erythromycin 800 mg groups. Middle: a scatter plot showing changes in *CLCN1* splicing biomarker in placebo and total erythromycin treated groups. Right: A scatterplot depicting changes in the *CLCN1* splicing biomarker (y-axis) in relation to the baseline values (x-axis), with distinct colour-coding to represent the placebo, erythromycin 500 mg, and 800 mg groups. **b.** Left: a scatter plot showing changes in *MBNL1* splicing biomarker in each group. Middle: a scatter plot showing changes in *MBNL1* splicing biomarker in placebo and total erythromycin treated groups. Right: A scatterplot depicting changes in the *MBNL1* splicing biomarker (y-axis) in relation to the baseline values (x-axis), with distinct colour-coding to represent each group. **c.** Left: a scatter plot showing changes in *CACNA1S* splicing biomarker in each group. Middle: a scatter plot showing changes in *CACNA1S* splicing biomarker in placebo and total erythromycin treated groups. Right: A scatterplot depicting changes in the *CACNA1S* splicing biomarker (y-axis) in relation to the baseline values (x-axis), with distinct colour-coding to represent each group. The p values represent comparisons to the placebo group.

CACNA1S, *MBNL1*, *CAPZB*, *CLASP1*, and *PDLIM3* in biopsied TA muscles. These specific biomarkers are regarded as robust indicators relying on MBNL function.[16] As a result, the splicing biomarkers of *MBNL1* and *CACNA1S* exhibited significant improvements in the overall erythromycin group (p = 0.048 and 0.042, respectively, Table 3 and Fig. 2). Despite the limited number of participants within each group, the impact of individual doses (500 mg and 800 mg of erythromycin) also displayed significant effects (p = 0.024 for *CACNA1S* and p = 0.048 for *MBNL1*, respectively).

The serum CK levels, serving as a marker for muscular injury, either remained comparable or showed an increase after 24 weeks of treatment in the placebo group (Fig. 3).

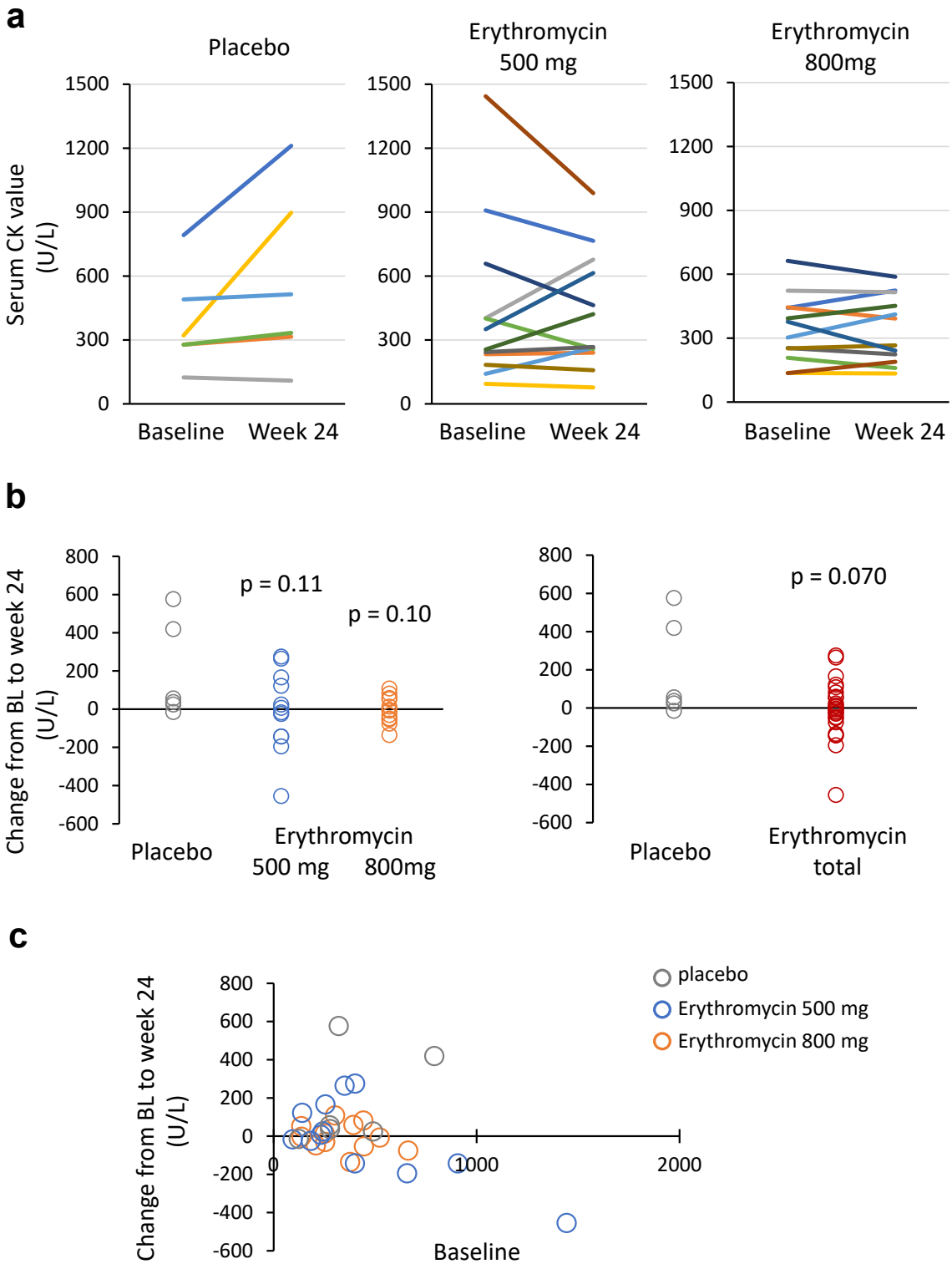

***Fig. 3*: Change from baseline to week 24 in serum CK values in the efficacy analysis set. a.** Serum CK levels at baseline and week 24 in each patient. **b.** Scatter plots showing a change in CK values in placebo, erythromycin 500 mg and erythromycin 800 mg groups (left), and placebo and total erythromycin treated groups (right). **c.** A scatter plot depicting changes in CK values (y-axis) in relation to the baseline values (x-axis), with distinct colour-coding to represent the placebo, erythromycin 500 mg, and 800 mg groups.

Conversely, a reduction in CK levels was observed in numerous instances among individuals in the total erythromycin group. The mean change in CK levels from baseline to week 24, another secondary outcome, exhibited a decrease in the total erythromycin group compared with the increase observed in the placebo group (mean −6.4 [SD 149] U/L in erythromycin vs mean + 182.8 [SD 228] U/L in placebo), although the difference was not significant ($p = 0.070$, Fig. 3 and Supplementary Table S3).

The other secondary outcomes, changes from baseline to week 24 in 6MWT, UEC score, LEC score, muscle strength of ankle dorsiflexion, myotonia score, INQoL overall score, and CGI-I scale, did not show any clear differences between the placebo and erythromycin groups (Supplementary Table S3 and Supplementary Figure S3). The only exception was the lower mean change in the UEC score at week 16 in the treatment group in contrast to the placebo ($p = 0.038$).

Assessments of MYD-0124 pharmacokinetic parameters are reported in Supplementary Figure S4.

Discussion

Therapeutic advancements for addressing abnormal RNA activity in DM1 are undergoing development. A phase 1/2a trial employing antisense oligonucleotides (ASOs) designed to degrade abnormal RNA in DM1 showed a broad level of tolerability, yet failed to attain the desired drug concentrations within the skeletal muscle tissue.[17] Ongoing clinical trials are investigating ASOs linked to antigen-binding fragments for a transferrin receptor showing enhanced delivery to muscle tissue (ClinicalTrials.gov Identifier: NCT05027269 and NCT05481879). Alongside these ASOs, there is an ongoing pursuit of therapeutic advancements involving small molecules that specifically target the aberrant RNA associated with DM1.[18] In our previous study, we used a drug repositioning screening approach that successfully identified erythromycin as a compound inhibiting MBNL sequestration and mitigating RNA toxicity in DM1.[12] Its effectiveness was further confirmed through preclinical studies, which exhibit improvements of splicing abnormalities and myotonia in DM1 model mice.[12] In this investigation, we initiated a phase 2 clinical trial to affirm the safety and tolerability of erythromycin in patients with DM1. To our knowledge, this is the first trial of a small molecule directly impacting the aberrant RNA toxicity evident in DM1.

Erythromycin is a well-established and widely used antibiotic with a good safety profile. It has been administered in short-term high doses as antibiotics and long-term oral therapy at lower doses for COPD management.[19] Notably, our preclinical study showed that administrating erythromycin orally for 21 days at doses equivalent to those prescribed to patients with COPD was effective in DM1 model mice.[12] In this exploratory study, patients with DM1 received erythromycin (MYD-0124) at two different doses: 500 mg/day, equivalent to the dose used in COPD patients, and 800 mg/day, a historical dosage previously used to address gastrointestinal symptoms in patients with DM1.[20]

Overall, erythromycin was well-tolerated without reporting major safety issues. Gastrointestinal adverse events were reported more frequently in the overall erythromycin group, as erythromycin is known for stimulating gastrointestinal motor activity via effects on motilin receptors.[21] However, all observed events were mild to moderate intensity and spontaneously resolved. Additionally, concerns regarding the safety of erythromycin in DM1 were raised due to its capacity to prolong the QTc interval.[22] However, our present study revealed no prolongation of the PR interval, QRS interval, or QTc interval, and no proarrhythmic effects were detected through Holter ECG evaluation in erythromycin groups at both doses. Furthermore, no significant disparities were observed between erythromycin and placebo in terms of laboratory parameters, vital signs, and weight.

Aberrant mRNA splicing is the distinctive feature of DM1.[15] The extent of splicing abnormality indicates RNA toxicity and can be used as a splicing biomarker of DM1. The therapeutic responsiveness of splicing biomarkers has also been shown in animal models of DM1.[15] The recent clinical trial investigating ASOs for DM1 utilised a composite score of 22 splicing events as an efficacy measure.[17] In that particular study, a discernible improvement in the composite splicing index was observed in several individuals within the high-dose ASOs-treated cohort. In this study, six independent splicing biomarkers were utilised as key secondary efficacy endpoints. Although no significant differences in the change from baseline were observed between the groups, we did see a possible dose-dependent improvement in the *CLCN1* splicing biomarker among several patients. Furthermore, the post-hoc analysis revealed significant improvements in the splicing biomarkers of *MBNL1* and *CACNA1S* within the overall erythromycin group. The improvement of these biomarkers demonstrated statistical significance within one dosage group, while failing to do so within the other. The dosage required to induce splicing alterations may vary among distinct genes. Moreover, the limited number of participants in each group, coupled with the variability of splicing biomarkers across individual time points,[17] may have hindered further clarification or the manifestation of significant differences between treated patients and those receiving a placebo. Nonetheless, these findings present, for the first time, substantial and significant splicing improvements among patients with DM1 through therapeutic intervention, thereby emphasising the potential value of splicing biomarkers in evaluating treatment efficacy. However, certain splicing biomarkers, such as *BIN1*, *DTNA1*, *PDLIM3*, and *CAPZB*, showed excessive variability and were therefore unsuitable for use as biomarkers. The reason

why only specific participants in the recent DM1 trial with ASOs demonstrated improvement in the splicing index and little correlation was found with skeletal muscle drug concentrations,[17] might be attributed to the utilisation of a composite index incorporating diverse splicing biomarkers. Moreover, a certain level of test–retest variability was inevitably observed in the composite splicing index. Consequently, selecting appropriate splicing biomarkers for future clinical trials becomes imperative.

In this study, additional secondary endpoints showed no statistically significant difference, yet demonstrated a favourable improvement in the erythromycin-treated group, specifically regarding serum levels of CK. Moreover, the 6MWT demonstrated a slight upward trajectory in walking distance within the high-dose erythromycin group. Due to the limited number of participants in this study and the substantial variability observed in the changes in CK values and 6MWT, it is necessary to validate these findings through a larger-scale study. Conversely, the evaluation of muscular strength of ankle dorsiflexion, which stands as a salient symptom of DM1, demonstrated significant variability, possibly attributed to challenges in achieving precise evaluation, thus impeding its suitability for a reliable assessment. Additionally, the UEC score and myotonia score exhibited a tendency to improve in the placebo group, indicative of the placebo effect. Moreover, there is a widespread consensus regarding the challenge of identifying a reliable and feasible outcome measure that can offer comprehensive information on the progressive decline of patients with DM1, as well as their response to therapeutic interventions.[23] Hence, establishing an appropriate efficacy endpoint for clinical symptoms in the future will hold significant importance.

Several clinical trials utilising small molecules have been conducted in individuals with DM1. Mexiletine, an anti-arrhythmic medicine that blocks sodium channels responsible for muscle contraction and relaxation,[24] was assessed in a phase 2 study.[25] The results indicated a favourable impact on objectively measured hand grip myotonia. Another phase 2 study examined metformin, a biguanide anti-diabetic medicine believed to act on the pathogenesis of DM1 by possibly regulating general metabolic pathways, including AMPK, autophagy, and insulin sensitivity.[23] In this investigation, metformin demonstrated significant improvements in the distance walked during the 6MWT in the per protocol population. Additionally, tideglusib, a GSK3β inhibitor, was evaluated in a phase 2 single-blinded proof-of-concept study in patients with congenital and childhood-onset DM1.[26] Considering the multi-systemic nature of DM1, small molecules hold promise as therapeutic agents, particularly those that possess the ability to diffuse into various organs without the need for carriers.

This study is subject to several limitations. First, the placebo group had a relatively small number of patients, a deliberate measure to minimise the requirement for invasive muscle biopsies within the placebo group. Given the limited sample size, the statistical power of the Wilcoxon rank-sum test employed in this study is very low for detecting statistically significant differences, especially for muscle biopsy data. Second, due to constraints in statistical power for firmly establishing effects and our primary objective of validating trends, we refrained from implementing adjustments for multiplicity. It's crucial to strategise the forthcoming study on a scale that enables a thorough examination of the validity of the statistically significant differences observed in this study. Third, the treatment duration was short; longer treatment could have resulted in greater improvements in clinical outcomes. Fourth, the disease severity, as indicated by the INQoL score, was greater in the overall erythromycin group. This could have made detecting significant improvements in erythromycin group even more difficult. Furthermore, the matter of erythromycin absorption presents a notable consideration. Erythromycin's absorption capacity diminishes when consumed following a meal,[27,28] warranting its optimal administration in an inter-prandial fashion. However, in our study, erythromycin was administered after meals to address the concern of patients inadvertently omitting doses due to decreased attentiveness associated with DM1. Indeed, the serum concentration of erythromycin observed in our study after oral administration following meals was comparatively lower than that in the previous study when administered between meals.[29] Therefore, inter-prandial administration is preferable to achieve higher systemic concentrations and will be prioritised in the upcoming trial.

Overall, this phase 2 trial provides promising evidence of erythromycin's safety, tolerability, and potential efficacy in treating patients with DM1. Based on the results observed, it is prudent to proceed with a subsequent phase 2b trial that meticulously defines the choice of pertinent splicing biomarkers and clinical endpoints. Additionally, a well-powered phase 3 trial with extended treatment durations is warranted to further substantiate the potential benefits of erythromycin in treating DM1. If successful, erythromycin may present a novel therapeutic option for patients with DM1, offering hope for improving their quality of life and addressing the unmet medical need in this devastating disease.

Contributors

M.N., D.N. H.N., M.P.T., E.H., H.K., T.M., H.T., and H.M. contributed to the conception and design of the study; M.N., D.N., T.S., Y.H., S.K., T.S., E.H., T.M., and H.T. contributed to the acquisition and analysis of data; M.N., T.S., and E.H accessed and verified the underlying data; M.N., T.S., and E.H. contributed to drafting the text or preparing the figures. All authors critically reviewed drafts of the manuscript and approved the final manuscript.

Data sharing statement

This study's data supporting the results and the trial protocol are available by contacting the corresponding author upon reasonable request.

Declaration of interests

M.N. and H.M. are coinventors on a patent (no. JP6460552B2, US10500223B2, EP3323419B1) for MYD-0124 owned by Osaka University. EH belongs to a joint research chair with Shionogi Pharma Co., Ltd.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102390.

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