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ORIGINAL COMMUNICATION

Efficacy and safety of leuprorelin acetate for subjects with spinal and bulbar muscular atrophy: pooled analyses of two randomized-controlled trials

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Abstract

Background Spinal and bulbar muscular atrophy (SBMA) is an adult-onset, hereditary neuromuscular disease characterized by muscle atrophy, weakness, contraction fasciculation, and bulbar involvement. Although the causative gene, *androgen receptor*, has been identified, the development of novel therapeutics for SBMA is incomplete. In this study, the efficacy and safety of leuprorelin acetate administration for patients with SBMA, using the pooled data of two randomized-controlled trials, was studied.

Methods Two randomized double-blinded studies (JASMITT-06DB and JASMITT-11DB) were done as multicentric, investigator-initiated clinical trials in Japan. In both studies, eligible patients were randomly assigned 1:1 to receive leuprorelin acetate administration once per 12 weeks for 48 weeks. The primary endpoint was the longitudinal change of pharyngeal barium residues from the baseline data measured with videofluorographic swallowing analyses. The pooled analysis plan was decided upon after the 06B study was finished and before the 11DB study began.

Results The primary endpoint difference between the leuprorelin group and the placebo group was pharyngeal barium residue after initial swallowing, −4.12% (95% CI, −8.40–0.15; $p=0.058$). The primary endpoint of this study does not reach significant results, although inter-group differences of pharyngeal barium residues after the initial swallowing indicated that leuprorelin acetate may be effective at each assessment point in both study groups.

Conclusions The efficacy of leuprorelin acetate for patients with SBMA was statistically similar in two randomized-controlled trials, and suggested that leuprorelin acetate may be effective and safe. Further investigations are needed to clarify the promising efficacy of the drug.

Keywords Spinal and bulbar muscular atrophy · Clinical trial · Leuprorelin acetate · Motor neuron disease · Disease-modifying therapy

Members of the group "JASMITT study group" are listed in Acknowledgements section.

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Introduction

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is an adult-onset, hereditary neuromuscular disease characterized by muscle atrophy, weakness, contraction fasciculation, and bulbar involvement [1–3]. The prevalence of this disease is estimated to be about 1–2 per 100,000 persons, worldwide [4]. The progression of neurological deficits is usually slow, with the average interval between the onset of weakness and death being approximately 20 years [5]. Following the identification of the causative gene, *androgen receptor*, studies using mouse models have clarified the ligand-dependent pathophysiology of this disease, which provided new opportunities to develop disease-modifying treatments with androgen deprivation [6–8]. Successful treatment of SBMA in mouse models with castration or administration of leuprorelin acetate, a luteinizing hormone-releasing hormone (LH-RH) agonist that reduces testosterone release from testes, supported the idea that testosterone blockade therapy could be beneficial and enabled subsequent human clinical trials [9]. So far, two randomized-controlled trials with the similar design, JASMITT-06DB and − 11DB, have been completed; however, it was difficult to demonstrate statistically significant efficacy of the administration of leuprorelin acetate for patients with SBMA in a single human clinical trial [10]. In addition to the JASMITT studies, a clinical trial, which aimed to demonstrate the efficacy of Dutasteride for patients with SBMA, also failed to confirm the drug efficacy [11]. One of the potential reasons why so many clinical trials for rare and slowly progressive neurodegenerative diseases have failed may be due to a lack of established clinical trial designs and methodologies for these diseases [12]. The clinical trial designs for slowly progressive neurodegenerative diseases such as SBMA are difficult regarding the selection of endpoints and sample sizes.

The results of the JASMITT-06DB study have been reported [10]. Here, we performed a pooled analysis of the two randomized-controlled trials to allow a more thorough evaluation of the treatment with leuprorelin acetate for SBMA: pooling data from identically designed studies provided greater statistical power for assessment. The protocol of the present pooled analysis was decided upon after the 06DB study was finished and before the 11DB study began. The protocol was predefined and submitted to Japan's regulatory agency, the Pharmaceutical and Medical Device Agency.

Materials and methods

Patients

The Japan SBMA Interventional Trial for TAP-144-SR (JASMITT) study was a randomized, double-blinded, placebo-controlled, parallel-grouped, multicenter trial. The first study was done at 14 hospitals in Japan (JASMITT-06DB study), and the second one was done at five hospitals in Japan (JASMITT-11DB study). The JASMITT 06DB study enrolled patients between August 2006 and March 2007 and ended in March 2008, and the JASMITT 11DB study enrolled patients between January 2012 and March 2013 and ended in February 2014. The principal inclusion criteria of these trials were as follows: a clinical diagnosis of SBMA with more than one motor symptom (muscle weakness, muscle atrophy, bulbar palsy, and hand tremor); confirmation of androgen receptor CAG repeat expansion (> 38 repeats); age from 30 to 70 years at the time of informed consent; no desire to father a child; serum aspartate aminotransferase less than four times the upper limit of normal; serum alanine aminotransferase less than four times the upper limit of normal; and ability to attend ambulatory hospital visits. The principal exclusion criteria were history of receiving treatment with LH-RH agonists, testosterone drugs, 5-alpha-reductase inhibitors, antiandrogen drugs, anabolic–androgenic steroids, progesterone, or estrogen drugs within 48 weeks before informed consent; the previous treatment with LH-RH agonists for more than 48 weeks; history of surgical androgen deprivation (e.g., orchiectomy); depression diagnosed with the M.I.N.I. international neuropsychiatric interview, Japanese version 5.0.0 major depression episode; coexisting severe disease besides SBMA; known allergy to leuprorelin, synthetic LH-RH, or LH-RH derivatives; and participation in other clinical trials within 12 weeks before informed consent. Patients provided written informed consent before enrollment. The protocols were approved by the institutional review boards at each participating center and by the Japan's regulatory authority (Pharmaceutical and Medical Devices Agency, Japan). These studies were done in accordance with the Declaration of Helsinki and good clinical practice.

Randomization and masking

In both studies, patients were randomly assigned (one treated: one control) to receive either leuprorelin or an identical placebo by an independent registration center (Clinical Trial Coordinating Center, Research Center for Clinical Pharmacology, The Kitasato Institute, Tokyo, Japan in the JASMITT-06DB study; or EPS Corporation in the JASMITT-11DB study). Dynamic random allocation was done with minimization on the basis of the patients' age (≤ 54 years or ≥ 55 years) and CAG repeat length (≤ 49 repeats or ≥ 50 repeats) in the JASMITT-06DB study, and on the basis of the CAG repeat length (≤ 49 repeats or ≥ 50 repeats), disease duration (< 10 years or ≥ 10 years), and pharyngeal barium residue after initial swallowing at baseline (< 5%, 5%≤, and < 20%, or ≥ 20%) in the

JASMITT-11DB study, respectively, to reduce bias. Patients were assigned to a computer-generated randomization list. Patients and investigators were masked to treatment allocation. An independent safety monitoring committee could request the unmasking of trial participants if necessary. The drug codes were broken and made available for data analyses when the study was completed and the data files had been verified.

Treatments

Leuprorelin acetate [The peptide sequence was: Pyr-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt (Pyr = L-pyroglutamyl)] or the placebo was subcutaneously injected at a dose of 11.25 mg every 12 weeks. The placebo was supplied as a vial-containing microcapsule without leuprorelin, which was suspended in the same solution as the active drug for injection.

Primary or secondary endpoints

The primary endpoint was the change from baseline data at the last visit of pharyngeal barium residue visualized by videofluoroscopic swallowing studies, according to a standardized method [13]. The pharyngeal barium residue was measured with two methods. The first method used to measure pharyngeal barium residue was that after initial swallowing, and the second was that after piecemeal deglutition: multiple repeated swallows to empty a bolus from the oral cavity. In the JASMITT-06DB or 11DB trials, the pharyngeal barium residue after the initial swallowing was adopted as a primary endpoint, and the pharyngeal barium residue after piecemeal deglutition was adopted an additional endpoint to confirm validity of this endpoint.

The secondary endpoints included the change of principle pathophysiological findings: frequency of anti-polyglutamine (1C2) antibody positive cells in scrotal skin biopsies, as well as the blood markers including serum testosterone or creatine kinase (CK) or the functional or quality-of-life (QOL) scores, such as the revised amyotrophic lateral sclerosis functional rating scale (ALSFRS-R), the modified quantitative myasthenia gravis (mQMG) score, and the amyotrophic lateral sclerosis assessment questionnaire 5 (ALSAQ-5).

The ALSFRS is a validated questionnaire-based scale that measures physical function in patients with amyotrophic lateral sclerosis performing activity of daily living [14]. The revised version of this scale, ALSFRS-R, was generated to improve the disproportion of weighting to the limbs and bulbar system, as compared to respiratory dysfunction. The ALSFRS-R was translated into Japanese and validated [15]. The ALSFRS-R was divided into five domains: bulbar-related (three items: speech; salivation; and swallowing), upper limb-related (two items: handwriting; cutting food; and handling utensils), trunk-related (two items: dressing and hygiene; turning in bed; and adjusting bed clothing), lower limb-related (two items: walking and climbing stairs), and respiration-related (three items: dyspnea; orthopnea; and respiratory insufficiency).

The QMG score is an objective measure to detect the weakness of enduring muscle power, which was originally designed for myasthenia gravis [16]. We used a part of the QMG score that measured the muscle power of the extremities and neck flexion as a modified QMG (mQMG) score. Therefore, the best possible score was 0 and the worst possible score was 15. Although this scale has not been previously validated in SBMA patients, the contents of the mQMG score were suitable for the evaluation of SBMA symptoms, and we, thus, considered them to be applicable to this disease [9].

The ALSAQ-5 is a subjective health measure that was designed to evaluate the quality-of-life factors in patients with ALS. This questionnaire was developed from the original version (ALSAQ-40) using item reduction [17, 18]. The validity of the Japanese version of the ALSAQ-40 has been confirmed [19].

Study population

The pooled intention-to-treat (ITT) population comprised all randomized populations in both clinical trials who underwent at least one administration of the study drug. The ITT population was utilized to evaluate patient demographics.

Adverse events

All adverse events at each visit were recorded. Adverse events were coded using the *Medical Dictionary for Regulatory Activities version 19.0* [20]. Serious adverse events were reported separately as follows: classification of adverse events as "serious" followed the judgement of the investigator. Summary statistics for adverse events were calculated for each study as well as pooled data of the 06DB and the 11DB studies.

Statistics

All patients who were randomized and who were assessed with videofluorography at least once were included in the analyses. Patients who discontinued treatment prematurely were encouraged to attend assessments, and the results of these assessments were used for efficacy analyses. We used the last observation carried forward method to impute values that were not available at 48 weeks. To confirm the robustness of the assessment of drug efficacy, the results

at last visit were added in addition to those at 48 weeks in the longitudinal analysis.

The primary endpoint was assessed by use of the two-sample *t* test. The analysis of covariance was used to adjust for baseline differences of the respective covariates. For the secondary endpoints, the two-*sample *t* test and the Wilcoxon rank sum test were used to calculate the differences between the groups. A $p < 0.05$ was considered to be statistically significant; however, the Japan's regulatory agency advised us that $p < 0.10$ could be considered to be statistically significant, because it was difficult to recruit a sufficient number of patients with rare, slowly progressive neurodegenerative diseases including SBMA.

As for the repeated-measures analyses for week 24 and week 48, changes in pharyngeal barium residue from baseline at week 24 and week 48 were analyzed to compare the leuprorelin acetate-treated group with the placebo-treated group using a mixed effect model for repeated measures. This model included the treatment group and time as fixed effects, as well as baseline value, disease duration (< 10 years, or ≥ 10 years), and participated trial as covariates. An unstructured variance–covariance matrix was used to model the inter-subject errors and sandwich estimates were provided for the variance of the coefficients. Point and interval estimates were provided for each fixed effect, each trial, the difference among treatment groups, and the difference among trials.

Adverse events and other safety information (i.e., laboratory tests and bone mineral density tests) were noted for safety analyses. The endpoints for adverse events were the numbers of patients with at least one event or an event under each recorded preferred term.

Statistical analyses were done using SAS (version 9.1.3). The JASMITT-06DB study was registered in the JMACCT clinical trials registry, number JMA-IIA00009 and the UMIN clinical trials registry, number UMIN000000465. The JASMITT-11DB study was registered in the JMACCT clinical trials registry, number JMA-IIA00080.

Results

Patients

The intention-to-treat (ITT) pooled population comprised 283 patients (leuprorelin acetate, $n = 142$; placebo, $n = 141$) (Fig. 1). Baseline demographic and clinical characteristics are shown in Table 1. There were no substantial differences in demographic and clinical characteristics between the groups in each study, although disease duration was shorter in both groups in the JASMITT-11DB trial.

Efficacy

Primary endpoint

Regarding the pharyngeal barium residue after the initial swallowing, the difference between the leuprorelin group and the placebo group did not reach statistically significance: −4.12% (95% CI, −8.40 to 0.15; $p = 0.058$; Table 2). The difference in pharyngeal barium residue after piecemeal deglutition was −2.36% (95% CI -4.90 to 0.19; $p = 0.069$). Chronological changes of inter-group differences of the pooled data were consistent among each assessment point, as were the point estimations of the inter-group differences (Table 5; Fig. 2). As shown in Table 3, the pharyngeal barium residue after the initial swallowing at baseline tended to be larger in the 11DB trial group, although there were no substantial differences of the other baseline characteristics.

Secondary endpoint

There were significant differences of the mean change between the groups in frequency of 1C2-positive cells ($p < 0.001$), in serum levels of testosterone ($p < 0.001$), and in serum creatine kinase levels ($p = 0.002$). These findings are evidence for a pharmacodynamic effect of the intervention. For the other secondary outcomes, no significant differences were seen. In patients who received leuprorelin acetate, serum testosterone levels were below sensitivity (castrated level) at month 12 (Table 2).

Comparison of the two clinical trials (06DB and 11DB groups)

The results of the primary and secondary endpoints in each clinical trial group are shown in Table 4 [7]. The results showed that the drug efficacy estimated by both the pharyngeal barium residue after the initial swallowing and after piecemeal deglutition was larger in the 06 DB study group compared with that of the 11DB study group. However, as the longitudinal analysis revealed that the baseline data of pharyngeal barium residue were intimately correlated with the drug effect size, we performed the repeated-measures analysis for week 24 and week 48 considering covariates that could influence the effect size, to reduce bias. The longitudinal changes of primary endpoints did not vary between the trials [inter-trial difference was 1.01 (−2.86 to 4.88) for pharyngeal barium residue after the initial swallowing, and 1.32 (−1.17 to 3.81) for pharyngeal barium residue after piecemeal deglutition]. Furthermore, the drug effect size was consistently seen [inter-group difference was −3.18 (−6.73 to 0.38) for the pharyngeal barium residue after the initial swallowing, and −2.82 (−5.15 to 0.50) for the pharyngeal barium residue after piecemeal deglutition]. These results

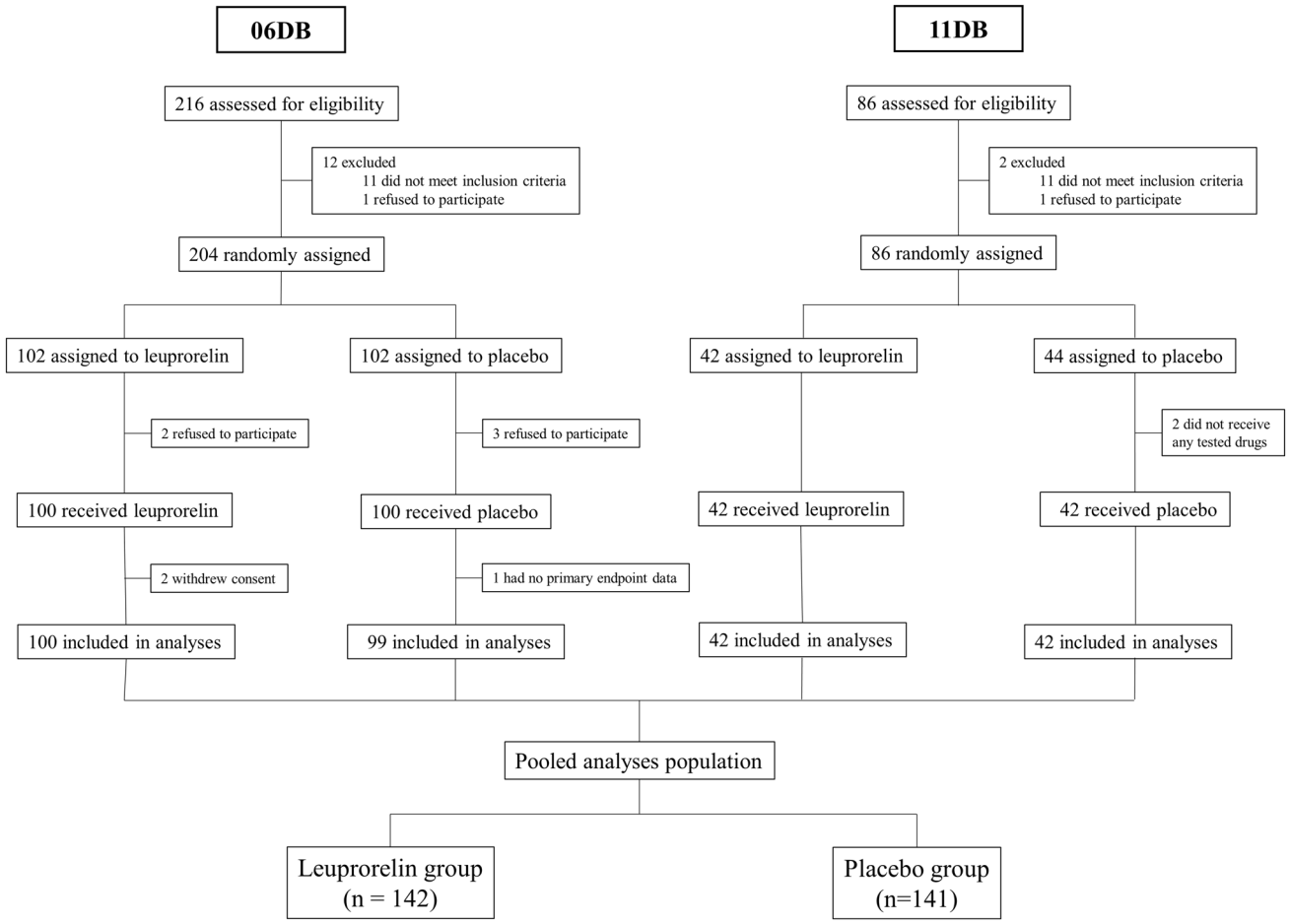

Fig. 1 Flow diagrams of subjects and progress. A total of 199 patients and 84 patients were included in the 06 DB and 11DB analyses, respectively. The present study analyzed a total of 283 patients

Table 1 Baseline characteristic of pooled data

Demographic characteristic	Pooled	
	TAP-144-SR (3M) ($N=142$) Mean ± SD (min. – max.)	Placebo ($N=141$) Mean ± SD (min. – max.)
Age (years)	52.9 ± 9.1 (31–69)	52.9 ± 9.1 (31–69)
Disease duration from the onset (years)	10.9 ± 7.8 (0.6–38.9)	11.7 ± 7.2 (0.3–33.0)
Serum creatinine (mg/dL)	0.46 ± 0.13 (0.23–0.88)	0.43 ± 0.12 (0.20–0.80)
Serum creatine kinase (IU/L)	754 ± 448 (55–2312)	799 ± 456 (63–2440)
Serum testosterone (ng/mL)	8.06 ± 2.98 (3.05–20.8)	7.73 ± 2.70 (2.66–14.7)
1C2 positive cells on scrotal skin	21.6 ± 14.6 (0.0–65.2)	23.1 ± 13.4 (0.0–61.5)
Pharyngeal barium residue after initial swallowing (%)	17.9 ± 24.4 (0.0–100)	16.5 ± 23.9 (0.0–100)
Pharyngeal barium residue after piecemeal deglutition (%)	10.1 ± 13.3 (0.0–75.0)	7.5 ± 9.4 (0.0–65.0)
ALSFRS-R	41.1 ± 3.5 (31–47)	41.0 ± 3.6 (31–48)
ALSAQ-5	10.0 ± 3.5 (5–19)	10.4 ± 3.7 (5–25)
mQMG score	6.6 ± 3.2 (0–14)	6.6 ± 2.8 (0–14)

ALSFRS-R revised amyotrophic lateral sclerosis functional rating scale, *ALSAQ-5* 5-item amyotrophic lateral sclerosis assessment questionnaire, *mQMG score* modified quantitative myasthenia gravis score, *SD* standard deviation

Table 2 Primary and secondary endpoints (pooled data)

	n	Baseline (95% CI)	Follow-up (95% CI)	Difference (95% CI)	Between-group difference (95% CI)	*P* value
Primary endpoint						
Pharyngeal barium residue after the initial swallowing (%)						
Leuprorelin acetate	139	17.46 (13.44 to 21.48)	13.03 (9.98 to 16.08)	−4.43 (−7.63 to −1.22)	−4.12 (−8.40 to 0.15)	0.058
Placebo	138	16.54 (12.51 to 20.58)	16.24 (12.44 to 20.03)	−0.30 (−3.16 to 2.55)		
Pharyngeal barium residue after piecemeal deglutition (%)						
Leuprorelin acetate	138	9.67 (7.64 to 11.71)	8.20 (6.49 to 9.90)	−1.48 (−3.49 to 0.53)	−2.36 (−4.90 to 0.19)	0.069
Placebo	138	7.45 (5.86 to 9.04)	8.33 (6.45 to 10.21)	0.88 (−0.70 to 2.46)		
Secondary endpoints						
ALSFRS-R score						
Leuprorelin acetate	141	41.2 (40.6 to 41.7)	40.4 (39.7 to 41.0)	−0.8 (−1.3 to −0.3)	−0.3 (−0.9 to 0.3)	0.350
Placebo	141	41.0 (40.4 to 41.6)	40.5 (39.9 to 41.1)	−0.5 (−0.9 to −0.1)		
Modified QMG score						
Leuprorelin acetate	141	6.6 (6.1 to 7.1)	6.7 (6.2 to 7.2)	0.1 (−0.2 to 0.5)	−0.1 (−0.6 to 0.3)	0.530
Placebo	141	6.6 (6.1 to 7.1)	6.9 (6.4 to 7.4)	0.3 (0.0 to 0.6)		
ALSAQ-5						
Leuprorelin acetate	141	9.9 (9.4 to 10.5)	11.1 (10.5 to 11.7)	1.2 (0.7 to 1.7)	0.7 (0.0 to 1.4)	0.052
Placebo	141	10.4 (9.7 to 11.0)	10.9 (10.3 to 11.5)	0.5 (0.0 to 1.0)		
1C2-positive cells (%)						
Leuprorelin acetate	140	21.40 (18.96 to 23.84)	9.46 (7.93 to 11.00)	−11.94 (−14.04 to −9.83)	−15.83 (−18.74 to −12.91)	<0.001
Placebo	140	23.00 (20.76 to 25.25)	24.33 (29.46 to 29.46)	3.89 (2.86 to 5.92)		
Serum creatine kinase (IU/L)						
Leuprorelin acetate	139	754.2 (563.0 to 697.9)	630.4 (563.0 to 697.9)	−123.8 (−163.4 to −84.2)	−94.8 (−156.9 to −32.7)	0.002
Placebo	140	800.0 (723.7 to 876.4)	771.0 (691.8 to 850.2)	−29.0 (−77.2 to 19.2)		
Serum testosterone (ng/mL)						
Leuprorelin acetate	141	8.061 (7.562 to 8.559)	0.553 (0.309 to 0.757)	−7.477 (−8.008 to −6.945)	−7.402 (−7.975 to −6.828)	<0.001
Placebo	141	7.729 (7.279 to 8.179)	7.650 (7.188 to 8.112)	−0.075 (−0.306 to 0.155)		

ALSFRS-R revised amyotrophic lateral sclerosis functional rating scale, *mQMG score* modified quantitative myasthenia gravis score, *ALSAQ-5* 5-item amyotrophic lateral sclerosis assessment questionnaire, *CI* confidence interval

indicated that there was no significant difference between the 06DB and 11DB study groups, although there was a meaningful and similar drug effect size between the treatment groups (Table 5). Namely, the results of these two clinical trials, the 06DB study and 11DB study, were equal for the drug effect size.

Safety

Drug treatment was well tolerated. The incidence of adverse events was similar between the groups: 81.7% (116/142 cases) in the leuprorelin-treated group and 80.1% (113/141 cases) in the placebo-treated group (Table 6). The incidences of drug-related adverse events were 62.7% (89/142 cases) in the leuprorelin-treated group and 53.9% (76/141 cases) in the placebo-treated group. The drug-related adverse events that emerged in more than 10% of the patients in the leuprorelin-treated group were as follows: hot flash, 11.3% (16 cases) in the leuprorelin-treated group, and 2.1% (3 cases) in the placebo-treated group; and injection site induration, 10.6% (15 cases) in the leuprorelin-treated group and 7.1% (10 cases) in the placebo-treated group. A total of 11 patients had severe adverse events (four in the leuprorelin-treated group and seven in the placebo-treated group), and a total of seven patients had drug-related severe adverse events (three in the leuprorelin-treated group: dyspnea, intervertebral disk protrusion, and diabetes mellitus; and four in the placebo-treated group: dyspnea, gastrointestinal neoplasms, foot fracture, and gastric cancer). We should note that some of these adverse events listed in the table such as abnormal hepatic function, weight gain, injection site reaction, decreased libido, erectile dysfunction, and hyperhydrosis emerged more frequently in the leuprorelin-treated group.

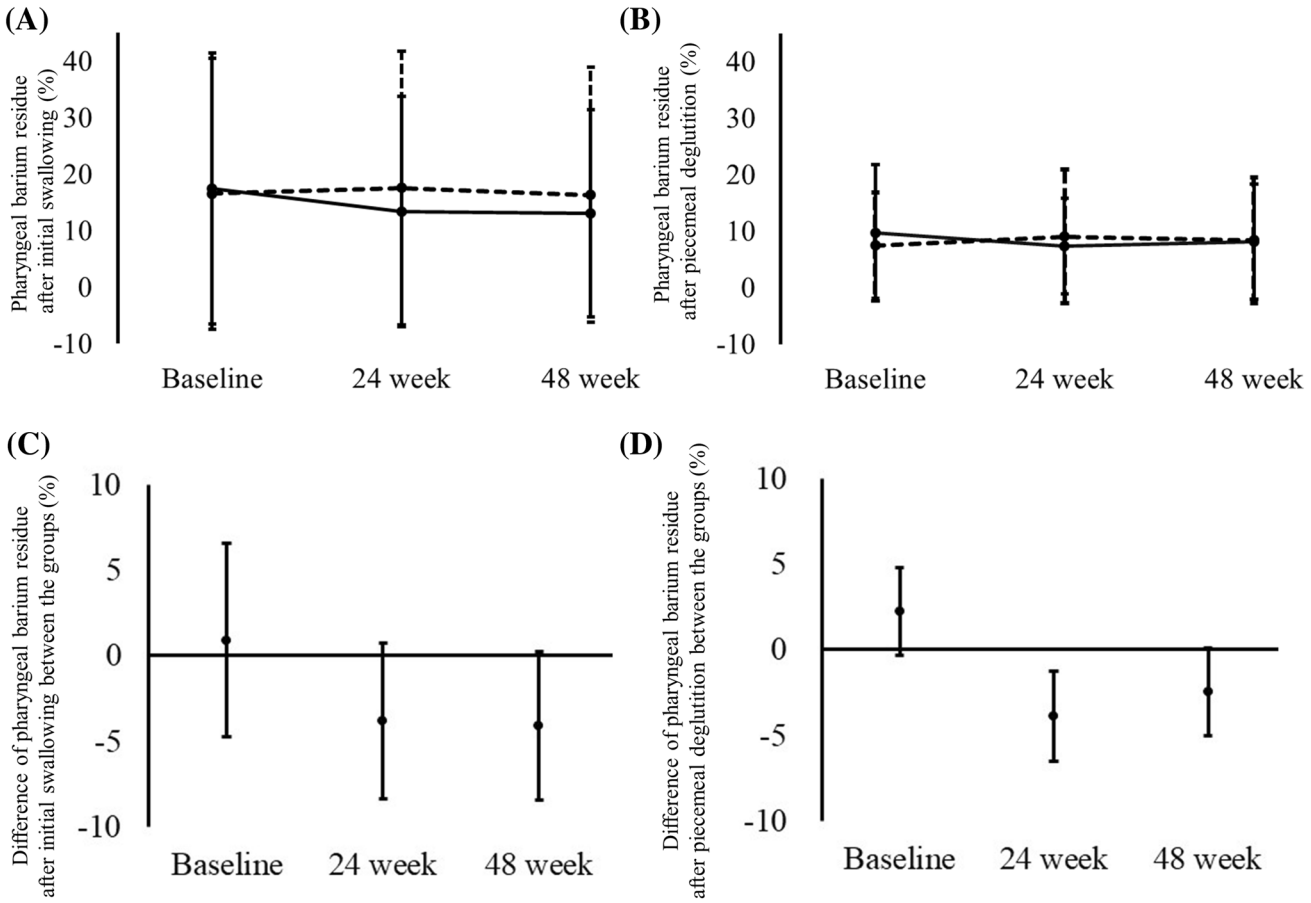

Fig. 2 Longitudinal changes of the pharyngeal barium residue. The longitudinal change of the pharyngeal barium residue after the initial swallowing (**a**) and that after piecemeal deglutition (**b**) in both groups. The point estimations of the inter-group differences at each time point were similar for both the pharyngeal barium residue after the initial swallowing (**c**) and that after piecemeal deglutition (**d**)

Table 3 Baseline characteristic of each clinical trial

Demographic characteristic	JASMITT-06DB		JASMITT-11DB	
	TAP-144-SR (3M) ($N=100$) mean ± SD (min.–max.)	Placebo ($N=99$) mean ± SD (min.–max.)	TAP-144-SR (3M) ($N=42$) mean ± SD (min.–max.)	Placebo ($N=42$) mean ± SD (min.–max.)
Age (years)	53.6 ± 9.2 (33–69)	54.2 ± 9.2 (31–69)	51.4 ± 8.9 (31–65)	49.8 ± 7.9 (33–66)
Disease duration from the onset (years)	12.7 ± 8.4 (1.5–38.9)	13.3 ± 7.3 (0.3–38.9)	6.5 ± 3.6 (0.6–15.7)	7.7 ± 5.1 (0.4–29.8)
Serum creatinine (mg/dL)	0.44 ± 0.13 (0.23–0.76)	0.43 ± 0.12 (0.20–0.80)	0.49 ± 0.13 (0.27–0.88)	0.43 ± 0.11 (0.27–0.69)
Serum creatine kinase (IU/L)	754 ± 443 (170–2312)	784 ± 480 (63–2440)	755 ± 464 (55–2037)	832 ± 397 (106–1564)
Serum testosterone (ng/mL)	7.84 ± 2.96 (3.05–20.8)	7.67 ± 2.57 (2.66–14.0)	8.58 ± 3.00 (3.91–19.5)	7.86 ± 3.02 (3.14–19.5)
1C2 positive cells on scrotal skin	20.6 ± 15.0 (0.0–65.2)	20.7 ± 14.0 (0.0–61.5)	24.0 ± 13.4 (1.3–49.8)	28.6 ± 10.2 (8.0–55.4)
Pharyngeal barium residue after initial swallowing (%)	20.3 ± 27.1 (0.0–100)	18.7 ± 26.6 (0.0–100)	12.2 ± 15.3 (1.0–75.0)	11.7 ± 15.4 (2.0–65.0)
Pharyngeal barium residue after piecemeal deglutition (%)	10.6 ± 13.5 (0.0–70.0)	6.72 ± 7.16 (0.0–35.0)	9.1 ± 12.8 (1.0–75.0)	9.2 ± 13.2 (2.0–65.0)
ALSFRS-R	40.8 ± 3.6 (31–47)	41.0 ± 3.7 (31–48)	41.9 ± 3.1 (33–47)	41.1 ± 3.2 (35–47)
ALSAQ-5	10.1 ± 3.4 (5–18)	10.7 ± 3.8 (5–25)	9.6 ± 3.5 (5–19)	9.5 ± 3.3 (5–18)
mQMG score	7.2 ± 3.0 (0–14)	6.8 ± 2.8 (0–14)	5.1 ± 3.1 (0–11)	6.1 ± 2.6 (0–11)

ALSFRS-R revised amyotrophic lateral sclerosis functional rating scale, *ALSAQ-5* 5-item amyotrophic lateral sclerosis assessment questionnaire, *mQMG score* modified quantitative myasthenia gravis score, *SD* standard deviation

Table 4 Primary and secondary endpoints (JASMITT-06DB and JASMITT-11DB)

JASMITT-06DB	*n*	Baseline (95% CI)	Follow-up (95% CI)	Difference (95% CI)	Between-group difference (95% CI)	*P* value
Primary endpoint						
Pharyngeal barium residue after the initial swallowing (%)						
Leuprorelin acetate	98	20.30 (14.87 to 25.73)	15.22 (11.14 to 19.31)	−5.08 (−8.28 to −0.87)	−5.26 (−10.82 to 0.30)	0.063
Placebo	98	18.65 (13.25 to 24.05)	18.83 (13.86 to 23.81)	0.18 (−3.50 to 3.86)		
Pharyngeal barium residue after piecemeal deglutition (%)						
Leuprorelin acetate	97	10.58 (7.86 to 13.31)	9.04 (6.76 to 11.31)	−1.55 (−4.15 to 1.06)	−3.21 (−6.41 to 0.00)	0.049
Placebo	96	6.68 (5.23 to 8.14)	8.34 (6.08 to 10.61)	1.66 (−0.23 to 3.55)		
Secondary endpoints						
ALSFRS-R score						
Leuprorelin acetate	100	40.8 (40.1 to 41.5)	40.5 (39.7 to 41.3)	−0.4 (−0.9 to −0.2)	−0.2 (−1.0 to 0.5)	0.537
Placebo	99	41.0 (40.2 to 41.7)	40.8 (40.2 to 41.5)	−0.1 (−0.6 to −0.3)		
Modified QMG score						
Leuprorelin acetate	100	7.2 (6.6 to 7.8)	7.1 (6.5 to 7.7)	−0.1 (−0.5 to 0.3)	−0.3 (−0.8 to 0.2)	0.204
Placebo	99	6.8 (6.3 to 7.4)	7.0 (6.5 to 7.6)	0.2 (−0.1 to 0.6)		
ALSAQ-5						
Leuprorelin acetate	100	10.1 (9.5 to 10.8)	11.1 (10.4 to 11.9)	1.0 (0.4 to 1.6)	0.9 (0.1 to 1.7)	0.033
Placebo	99	10.7 (10.0 to 11.5)	10.9 (10.1 to 11.6)	0.1 (−0.4 to 0.7)		
1C2-positive cells (%)						
Leuprorelin acetate	100	20.59 (17.61 to 23.57)	8.65 (7.00 to 10.30)	−11.94 (−14.04 to −9.83)	−14.68 (−18.20 to −11.17)	<0.001
Placebo	98	20.60 (17.80 to 23.41)	23.35 (20.47 to 26.22)	2.74 (0.33 to 5.16)		
Serum creatine kinase (IU/L)						
Leuprorelin acetate	100	754.2 (666.3 to 842.1)	632.7 (553.6 to 711.8)	−121.5 (−170.2 to −72.9)	−101.9 (−174.6 to −29.2)	0.006
Placebo	98	786.3 (689.7 to 882.9)	766.7 (667.4 to 865.9)	−19.6 (−74.4 to 35.1)		
Serum testosterone (ng/mL)						
Leuprorelin acetate	100	7.843 (7.225 to 8.431)	0.514 (0.260 to 0.767)	−7.282 (−7.907 to −6.656)	−7.238 (−7.911 to −6.565)	<0.001
Placebo	99	7.672 (7.159 to 8.185)	7.623 (7.084 to 8.161)	−0.043 (−0.303 to 0.217)		
JASMITT-11DB	*n*	Baseline (95% CI)	Follow-up (95% CI)	Difference (95% CI)	Between-group difference (95% CI)	*P* value
Primary endpoint						
Pharyngeal barium residue after the initial swallowing (%)						
Leuprorelin acetate	41	10.67 (6.93 to 14.41)	7.79 (4.70 to 10.89)	−2.88 (−7.25 to 1.50)	−1.46 (−7.55 to 4.63)	0.634
Placebo	42	11.73 (6.92 to 16.54)	10.31 (5.39 to 15.23)	−1.42 (−5.79 to 2.95)		
Pharyngeal barium residue after piecemeal deglutition (%)						
Leuprorelin acetate	41	7.52 (5.16 to 9.89)	6.21 (4.22 to 8.19)	−1.32 (−4.25 to 1.62)	−0.41 (−4.49 to 3.67)	0.841
Placebo	42	9.21 (5.11 to 13.32)	8.31 (4.79 to 11.83)	−0.90 (−3.83 to 2.02)		
Secondary endpoints						
ALSFRS-R score						
Leuprorelin acetate	41	42.0 (41.0 to 42.9)	40.1 (39.0 to 41.2)	−1.9 (−2.7 to −1.0)	−0.5 (−1.5 to 0.6)	0.365
Placebo	42	41.1 (40.1 to 42.1)	39.7 (38.7 to 40.7)	−1.4 (−2.1 to −0.7)		
Modified QMG score						
Leuprorelin acetate	41	5.0 (4.0 to 6.0)	5.8 (4.8 to 6.7)	0.8 (0.2 to 1.3)	0.3 (−0.5 to 1.1)	0.460
Placebo	42	6.1 (5.3 to 6.9)	6.5 (5.6 to 7.4)	0.5 (−0.1 to 1.0)		
ALSAQ-5						
Leuprorelin acetate	41	9.4 (8.3 to 10.5)	11.1 (10.4 to 11.9)	1.0 (0.4 to 1.6)	0.2 (−1.0 to 1.5)	0.719
Placebo	42	9.5 (8.5 to 10.5)	10.9 (9.8 to 12.0)	1.4 (0.6 to 2.2)		

Table 4 (continued)

JASMITT-11DB	*n*	Baseline (95% CI)	Follow-up (95% CI)	Difference (95% CI)	Between-group difference (95% CI)	*P* value
1C2-positive cells (%)						
Leuprorelin acetate	40	23.43 (19.13 to 27.73)	11.49 (8.02 to 14.96)	− 11.94 (− 15.67 to − 8.21)	− 18.50 (− 23.74 to − 13.26)	< 0.001
Placebo	42	28.61 (25..43 to 31.79)	35.17 (30.65 to 39.70)	6.56 (2.78 to 10.34)		
Serum creatine kinase (IU/L)						
Leuprorelin acetate	39	754.3 (601.5 to 907.1)	624.7 (489.7 to 759.7)	− 129.6 (− 198.9 to − 60.3)	− 78.7 (− 201.0 to − 43.6)	0.203
Placebo	42	832.1 (708.7 to 955.8)	781.2 (648.4 to 914.0)	− 50.9 (− 151.7 to 49.9)		
Serum testosterone (ng/mL)						
Leuprorelin acetate	41	8.592 (7.633 to 9.551)	0.580 (0.102 to 1.057)	− 7.955 (− 8.991 to − 6.918)	− 7.805 (− 8.916 to − 6.695)	< 0.001
Placebo	42	7.862 (6.922 to 8.802)	7.713 (6.786 to 8.640)	− 0.149 (− 0.638 to 0.340)		

ALSFRS-R revised amyotrophic lateral sclerosis functional rating scale, *mQMG score* modified quantitative myasthenia gravis score, *ALSAQ-5* 5-item amyotrophic lateral sclerosis assessment questionnaire, *CI* confidence interval

Table 5 Inter-trial or inter-treatment difference of the primary endpoints by repeated-measures analysis

	Inter-trial difference (95% CI)	Inter-group difference (95% CI)
Pharyngeal barium residue after the initial swallowing (%)	1.01 (− 2.86 to 4.88)	− 3.18 (− 6.73 to 0.38)
Pharyngeal barium residue after piecemeal deglutition (%)	1.32 (− 1.17 to 3.81)	− 2.82 (− 5.15 to − 0.50)

Inter-trial difference: the difference of longitudinal change between the trials (06DB and 11DB studies), Inter-group difference: The difference of estimated drug effects (leuprorelin group and placebo group)

CI confidence interval

Discussion

In the area of slowly progressive and rare neurodegenerative diseases, it is ideal to recruit a sufficient number of patients and to set up a trial period long enough to detect the drug efficacy, especially to confirm drug efficacy and safety. However, it is usually difficult to recruit a sufficient number of patients to satisfy sample size requirements based on the estimated effect size of the tested drugs. As the disease progression of the patients with SBMA is usually slow, a very long period, 10 years or more, would be necessary to demonstrate the drug efficacy when the events of death or pneumonia requiring hospitalization were selected as primary endpoints. Even when the quantitative clinical markers were adopted as primary endpoints instead of hard endpoints, such as death or pneumoniae requiring hospitalization, larger sample size would be necessary to confirm drug efficacy because of the slow progression of the disease. Therefore, the predefined pooled analysis of the some clinical trials that were done with the identical protocol would be useful for a comprehensive interpretation of the efficacy of the drug [21–25]. This approach has the advantage of enabling the inclusion of a large number of subjects, as well as comparing the effect of the tested drug on each outcome measure of the independent trial, thus allowing us to evaluate the efficacy of leuprorelin acetate several years earlier than would have been possible based on the individual trials alone, with higher statistical power. The present study was the first in the field of SBMA in which a pooled analysis of individual subject data from two independent randomized-controlled trials was planned before the second trial was initiated.

In this study, the pharyngeal barium residue and the number of anti-polyglutamine antibody, 1C2, positive cells in scrotal skin were selected as the primary and key second endpoints, respectively. As there was no established endpoint for clinical trials of SBMA, the pharyngeal barium residue was selected as the primary endpoint, because dysphagia and aspiration most strongly affect prognosis [9, 26, 27]. Bulbar palsy occurs in almost all patients with SBMA, and the incidence rate of aspiration pneumonia is high in advanced cases. In fact, a previous natural history study revealed that about half of the patients with SBMA-acquired fatal pneumoniae [4]. Therefore, the degree of bulbar palsy is an essential factor that defines the prognosis of patients

Table 6 Adverse events reported in ≥ 3% of patients in each group

Adverse event (SOC/PT)	Pooled			
	TAP-144-SR(3M) (*N* = 142)		Placebo (*N* = 141)	
Overall, *n* (%)				
Injection site induration	15	10.6	10	7.1
Hot flush	16	11.3	3	2.1
Back pain	8	5.6	8	5.7
Hepatic function abnormal	10	7.0	4	2.8
Weight increased	9	6.3	2	1.4
Headache	7	4.9	4	2.8
Constipation	6	4.2	5	3.5
Arthralgia	8	5.6	2	1.4
Hyperlipidemia	6	4.2	4	2.8
Myalgia	6	4.2	4	2.8
Injection site reaction	7	4.9	2	1.4
Libido decreased	8	5.6	0	0.0
Anemia	6	4.2	2	1.4
Blood phosphorus increased	7	4.9	0	0.0
Blood triglycerides increased	5	3.5	2	1.4
Erectile dysfunction	5	3.5	1	0.7
Hyperhydrosis	5	3.5	0	0.0

SOC/PT system organ class/preferred terms

with SBMA, because it directly causes life-threatening aspiration pneumoniae. To evaluate the degree of dysphagia, spatial analysis of videofluoroscopic swallowing is a useful tool. The most important finding of the videofluoroscopic swallowing in patients with SBMA is the presence of pharyngeal barium residue [14]. Therefore, pharyngeal barium residue was defined as the primary endpoint. As previously reported, the mutant androgen receptor proteins aggregate not only in the motor neuron nucleus but also in dividing cells such as the pancreas, prostate, or testis [28]. Our previous study showed that the extent of intra-nuclear accumulation of mutant androgen receptor protein in scrotal skin reflected diffuse accumulation of intra-nuclear accumulation of the spinal cord [29]. Furthermore, the amount of intra-nuclear accumulation of mutant androgen receptor correlated well with the severity of motor dysfunction. Therefore, the number of anti-polyglutamine antibody, 1C2, positive cells in scrotal skin was selected as the most important secondary endpoint [29].

The results from this pooled analysis did not reach significance as for the primary endpoint. However, the fact that similar results were obtained in each outcome at each time point consistently, as shown in Fig. 2, strengthened the conclusions concerning the efficacy of this drug. The absolute difference value of the primary endpoint, the pharyngeal barium residue after the initial swallowing or that after piecemeal deglutition, between the placebo and leuprorelin acetate groups was larger in the JASMITT-06DB than in the JASMITT-11DB study. However, the results of the analysis of covariance, which was performed to adjust the biased background data, indicated that the effect size was similar between these two trials, suggesting that drug efficacy was consistent between the two trials. As described in the 11DB protocol, the necessary sample size was calculated as approximately 200 subjects per arm to consider the detected difference of pharyngeal barium residue as statistically significant based on the 06DB study results. However, we could not design a clinical trial to enroll sufficient number of patients when considering the clinical trial feasibilities. This might be one of the reasons that we could not obtain statistically significant differences even in these pooled analyses. The American Statistical Association (ASA) released a statement regarding statistical significance and *p* values.[31] The ASA's statement indicated that scientific conclusions should not be based only on whether a *p* value passes a specific threshold, and alternative approaches including methods that emphasize estimation over testing, such as confidence, credibility, or prediction intervals, Bayesian methods, alternative measures of evidence, such as likelihood ratios or Bayes Factors, and other approaches such as decision-theoretic modeling and false discovery rates should be included [30]. These new approaches might resolve the difficulties in confirming new disease-modifying drug efficacy in the fields of slowly progressive and rare neurodegenerative diseases.

However, we should acknowledge some limitations of this study. First, the present analysis was not pre-specified before the 06DB trial, but was before the 11DB trial. Therefore, the findings are still exploratory. Second, the pooled data were derived from independent studies, which had some minor differences in the protocols. Therefore, the comparability of the two clinical trials was not guaranteed.

In conclusion, leuprorelin acetate may be safe and beneficial for improvement of swallowing dysfunction in the patients with SBMA, without increasing the number of serious side effects. Further investigations are needed to clarify the efficacy of this therapy for SBMA.

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Conflicts of interest MK and GS have received honoraria from Takeda Pharmaceuticals. All other authors have no conflicts of interest.

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