Annals of Internal Medicine ORIGINAL RESEARCH

B-Lymphocyte Depletion in Patients With Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

A Randomized, Double-Blind, Placebo-Controlled Trial

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Background: Previous phase 2 trials indicated benefit from B-lymphocyte depletion in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS).

Objective: To evaluate the effect of the monoclonal anti-CD20 antibody rituximab versus placebo in patients with ME/CFS.

Design: Randomized, placebo-controlled, double-blind, multicenter trial. (ClinicalTrials.gov: NCT02229942)

Setting: 4 university hospitals and 1 general hospital in Norway.

Patients: 151 patients aged 18 to 65 years who had ME/CFS according to Canadian consensus criteria and had had the disease for 2 to 15 years.

Intervention: Treatment induction with 2 infusions of rituximab, 500 mg/m^2 of body surface area, 2 weeks apart, followed by 4 maintenance infusions with a fixed dose of 500 mg at 3, 6, 9, and 12 months (n = 77), or placebo (n = 74).

Measurements: Primary outcomes were overall response rate (fatigue score ≥4.5 for ≥8 consecutive weeks) and repeated measurements of fatigue score over 24 months. Secondary outcomes included repeated measurements of self-reported function over 24 months, components of the Short Form-36 Health Survey and Fatigue Severity Scale over 24 months, and changes from baseline to 18 months in these measures and physical activity level. Between-group differences in outcome measures over time were assessed by general linear models for repeated measures.

Results: Overall response rates were 35.1% in the placebo group and 26.0% in the rituximab group (difference, 9.2 percentage points [95% CI, −5.5 to 23.3 percentage points]; P = 0.22). The treatment groups did not differ in fatigue score over 24 months (difference in average score, 0.02 [CI, −0.27 to 0.31]; P = 0.80) or any of the secondary end points. Twenty patients (26.0%) in the rituximab group and 14 (18.9%) in the placebo group had serious adverse events.

Limitation: Self-reported primary outcome measures and possible recall bias.

Conclusion: B-cell depletion using several infusions of rituximab over 12 months was not associated with clinical improvement in patients with ME/CFS.

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Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a disease of unknown cause that affects approximately 0.2% of the population according to Canadian consensus criteria (1). Characteristic symptoms include postexertional malaise, fatigue, sleep disturbances, cognitive symptoms, sensory hypersensitivity (including pain), and other symptoms related to autonomic or immune function. The disease is associated with greatly reduced quality of life (2, 3). The socioeconomic costs are high, and elucidation of disease mechanisms, improvement in diagnostic approaches, and rational treatment are urgently needed (4).

In our cancer ward, we have seen 7 patients since 2004 with long-standing ME/CFS who developed malignant disease (Hodgkin lymphoma, diffuse large B-cell lymphoma, or breast cancer). They independently reported alleviation of their ME/CFS symptoms after chemotherapy with cyclophosphamide or ifosfamide, which are cytotoxic drugs, or after receiving the combination of chemotherapy and rituximab, an anti-CD20 monoclonal antibody. In separate clinical trials, we have explored the effect of specific B-cell depletion using rituximab and immune modulation using cyclophosphamide intravenous infusions.

After an initial case series of 3 patients with ME/CFS who were treated with a single infusion of rituximab (5), we did a small, randomized, placebo-controlled, phase 2 trial where 30 patients with ME/CFS received 2 infusions (rituximab or placebo) 2 weeks apart with 12 months of follow-up (6). The groups did not differ in the primary end point of self-reported fatigue score at 4 months of follow-up, whereas the secondary end points favored the rituximab group at 6 to 12 months. To explore dose–response relationships and gain experience for a larger randomized trial, we then did an open-label trial using rituximab maintenance (6 infusions over 15

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months) with 3 years of follow-up (7). Half of patients reported considerably improved physical function, as assessed by the Short Form-36 Health Survey (SF-36) 15 to 30 months after treatment initiation (7). We interpreted these data to reflect clinical improvements possibly related to the B-cell depletion intervention. We hypothesized that ME/CFS in a subgroup of patients could be caused by immunologic dysfunction with a role for B lymphocytes. We here report a randomized, phase 3 trial (RituxME) of rituximab or placebo for treatment of ME/CFS.

METHODS

Design Overview

RituxME was a national, multicenter, randomized, parallel-group, double-blind, placebo-controlled, phase 3 trial. Its objective was to verify or disprove the association between B-cell depletion and clinical responses in patients with ME/CFS. Recruitment lasted from September 2014 until September 2015, and follow-up was completed in September 2017. Induction treatment with rituximab or placebo was given as 2 infusions, 2 weeks apart, and was followed by maintenance infusions at 3, 6, 9, and 12 months; follow-up lasted 24 months (ClinicalTrials.gov: NCT02229942; EudraCT: 2014-000795-25). The study was approved by the Regional Committees for Medical and Health Research Ethics in Norway (2014/365) and by the Norwegian Medicines Agency. The approved study protocol is in **Supplement 1** (available at Annals.org).

Setting and Participants

Five national trial centers (4 university hospitals and 1 general hospital) enrolled patients. After publishing the small, randomized study that used rituximab (or placebo) for ME/CFS in 2011 (6), we received referrals from physicians or direct requests from patients or their relatives to be evaluated for future clinical trials. After approval of the protocol, we screened these referrals and, on the basis of the available information, selected patients who seemed to fulfill the inclusion criteria. Because we received more referrals than the study needed, each center randomly selected patients to receive more information on the trial. After signing informed consent, patients were screened for eligibility.

The study included patients who had ME/CFS according to Canadian consensus criteria (1), were aged 18 to 65 years, had had the disease for at least 2 years (or ≥5 years if disease severity was mild) but less than 15 years, and had signed informed consent. Patients with very severe disease were not included. The exclusion criteria are shown in the protocol.

Randomization and Interventions

Smerud Medical Research International (Oslo, Norway) randomly assigned patients 1:1 to receive either rituximab or placebo (Methods section of the **Appendix**, available at Annals.org). Treatment allocation was concealed from trial participants and study personnel. Only Smerud and the involved pharmacies had access to the allocation code.

Patients received induction treatment with 2 infusions, 2 weeks apart, of either rituximab (MabThera, Roche), 500 mg/m^2 of body surface area (maximum of 1000 mg), or an equal volume of saline with added human albumin (Flexbumin [Baxalta] or Albunorm [Octapharma]), 0.4 mg/mL, to ensure no visible difference from the active comparator. In the maintenance phase, patients received a 500-mg fixed dose of rituximab or an equal volume of saline with human albumin at 3, 6, 9, and 12 months. One hour before infusions, all patients received premedication with 1 g of oral acetaminophen, 10 mg of cetirizine, and 8 mg of dexamethasone (**Supplement 1**). Six weeks after the first intervention, patients registered whether they believed that they had been given rituximab or placebo.

Outcomes and Follow-up

At baseline, patients registered self-reported symptoms using the SF-36, version 1.2 (8, 9); the Hospital Anxiety and Depression Scale (10); the Fatigue Severity Scale (11, 12), and a modified DePaul Symptom Questionnaire (13–15) (**Supplement 1**). Patients recorded baseline scores of ME/CFS symptoms (postexertional malaise, fatigue, pain, cognitive symptoms, and other symptoms) using a scale of 1 to 10 and function level using a percentage, where 100% denoted a completely healthy state (**Supplement 2**, available at Annals.org). Each patient used an electronic SenseWear armband at home continuously for 5 to 7 days to record baseline level of physical activity (number of steps) (16, 17).

Every 3 months during follow-up, patients had visits at the trial centers for clinical assessments and collection of self-reported forms. Patients retained copies of their baseline symptom scores and function level as a reminder of their baseline status but did not have access to baseline scores for other self-reported forms or objective measurements. Every second week, patients recorded their current function level (percentage) and changes from baseline in ME/CFS symptoms (**Supplement 2**). The scale for symptom change was adapted from a Clinical Global Impression scale previously used in CFS (18). The relative scale for each symptom was 0 to 6, in which 3 denoted no change from baseline; 4, 5, and 6 slight, moderate, and major improvement, respectively; and 2, 1, and 0 slight, moderate, and major worsening, respectively. The primary variable, fatigue score (scale, 0 to 6), was calculated as the mean of the following 4 items, which correspond to the 4 fatigue-related symptoms: "fatigue," "postexertional exhaustion," "need for rest," and "daily functioning." Patients completed SF-36 forms every 3 months and the Fatigue Severity Scale every 6 months. Recording of physical activity with SenseWear armband registration was repeated between 17 and 21 months.

The trial had 2 primary end points based on fatigue score: difference between treatment groups for repeated measurements of fatigue score (mean of 4-month intervals) through 24 months, and overall rate of response, defined as a score of at least 4.5 for at least 8 consecutive weeks. Secondary outcome measures were between-

group differences in repeated measurements of the SF-36 physical function subscale (SF-36-PF), SF-36 physical component summary (SF-36-PCS), and Fatigue Severity Scale over 24 months and between-group differences in changes in these variables and mean number of steps per 24 hours from baseline to 18 months (Methods section of the Appendix).

Adverse events were registered continuously; summarized according to Common Terminology Criteria for Adverse Events, version 4.03; and assessed by the investigators for relationships with the intervention. The Viedoc electronic case report form system (PCG Solutions) was used for data collection and management. The trial was externally monitored by the sections for monitoring at the Departments of Research and Development of Haukeland University Hospital and St. Olavs Hospital and at the Clinical Trial Unit of Oslo University Hospital. After the last included patient had completed 24 months of follow-up, trial staff did final monitoring, blinded review, and database locking.

Statistical Analysis

We estimated that we needed a sample size of 152 patients, using presumed overall response rates (as defined in Outcomes and Follow-up) of 50% in the rituximab group and 25% in the placebo group (19); an expected distribution of a variable with 7 categories corresponding to the fatigue score with a power of 0.80 and a 2-sided α level of 0.05; and allowance for 5% withdrawal.

We used descriptive methods to characterize the sample. Primary and secondary outcome measures were analyzed according to treatment group allocation by the intention-to-treat principle; patients who were randomly allocated and received at least 1 dose of rituximab or placebo were included. Missing data were

Figure 1. Flow diagram of inclusion and randomization procedures, treatment, and follow-up.

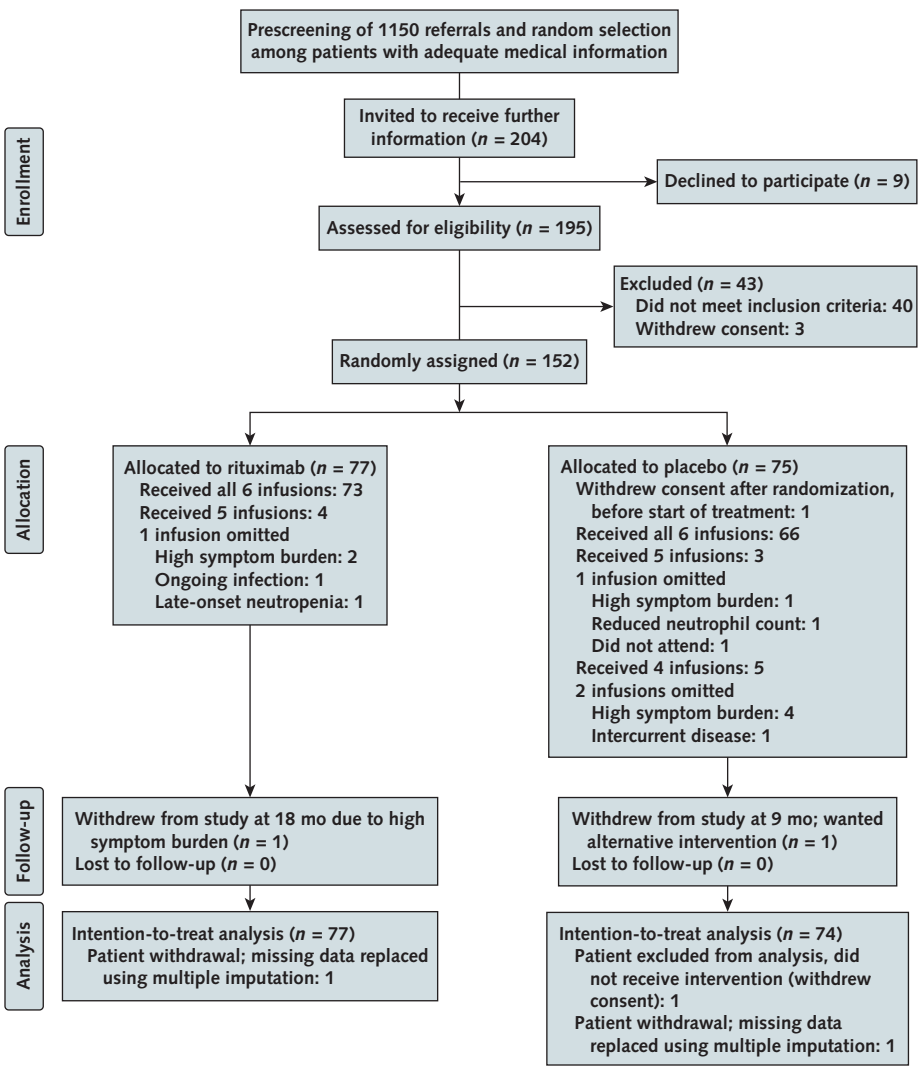


Table 1. Baseline Characteristics of the Study Population, by Intervention Group

Characteristic	Rituximab (*n* = 77)	Placebo (*n* = 74)
Mean age (SD), *y*	37.8 (11.4)	35.5 (11.2)
Female sex, *n (%)*	64 (83.1)	60 (81.1)
Mean body mass index (SD), kg/m^2		
Women	24.8 (5.5)	25.2 (5.0)
Men	26.3 (4.1)	24.5 (2.6)
ME/CFS severity, *n (%)**		
Mild or mild-to-moderate	30 (39.0)	30 (40.5)
Moderate	22 (28.6)	23 (31.1)
Moderate-to-severe or severe	24 (31.2)	21 (28.4)
Mean ME/CFS disease duration (SD), *y*	8.4 (3.1)	7.6 (2.9)
ME/CFS duration, *n (%)*		
2–<5 y	11 (14.3)	18 (24.3)
5–<10 y	45 (58.4)	44 (59.5)
10–15 y	21 (27.3)	12 (16.2)
Infection before ME/CFS, *n (%)*	59 (76.6)	56 (75.7)
Mean steps per 24 h (SD), *n*†	3297 (2047)	3213 (2099)
Mean maximum steps per 24 h (SD), *n*†	5307 (3661)	5190 (3416)
Mean function level (SD), %‡	20.1 (11.5)	18.4 (8.8)
Mean SF-36-PF score (SD)§	35.2 (21.9)	32.5 (19.1)
Mean SF-36-PCS score (SD)‖	24.8 (7.0)	23.6 (6.2)
Mean Fatigue Severity Scale score (SD)¶	59.1 (6.7)	59.9 (3.3)

ME/CFS = myalgic encephalomyelitis/chronic fatigue syndrome; SF-36-PCS = Short Form-36 Health Survey physical component summary; SF-36-PF = SF-36 physical function subscale.
* One patient had missing data for ME/CFS severity.
† Measured continuously by SenseWear armbands for 5–7 d. One patient had missing data.
‡ Self-reported; range, 0%–100% (**Supplement 2**).
§ Raw score; range, 0–100.
‖ Norm-based score with a population mean of 50.
¶ Range, 9–63; higher scores indicate worse symptoms.

replaced by multiple imputation (20, 21) (Methods section of the **Appendix**). The difference in overall response rates between the treatment groups was assessed by the Mantel–Haenszel test with stratification by study center. The Breslow–Day test was used to test homogeneity of the odds ratios across study centers (Methods section of the **Appendix**). For the primary outcome (fatigue score) and secondary outcomes, between-group differences in repeated measurements over time were assessed by the general linear model for repeated measures. Time, intervention (treatment group), and their 2-way interactions were included as predictors, and study center was included as a covariate (Methods section of the **Appendix**). Greenhouse–Geisser corrections were used for all general linear model analyses, with multiple levels of the dependent variable due to violations of the sphericity assumption that variances of the differences between all combinations of related levels are equal. The differences between groups in the outcome measures over time were assessed by the time-by-treatment interaction. The effect sizes are the differences between treatment groups for the averaged outcome measures over time. Simple contrasts in time domain were used to compare treatment groups at each time point. The general linear model for repeated measures was also used to assess between-group differences in changes from baseline to 18 months for secondary outcomes (Methods section of the **Appendix**). All tests were 2-sided, and the significance level was set to 0.05. For the 2 primary end points, we used a Bonferroni adjustment–that is, a marginal level of 0.025. All analyses were done using IBM SPSS Statistics, version 25 (IBM), and GraphPad Prism, version 7 (GraphPad Software).

Role of the Funding Source

The research group for ME/CFS at Haukeland University Hospital receives funding from the Kavli Trust. The RituxME trial received funding from the Norwegian Research Council, the Norwegian Regional Health Trusts, the MEandYou Foundation, the Norwegian ME Association, and the legacy of Torstein Hereid. Funders had no role in trial design, data collection, analysis, or preparation of the manuscript.

RESULTS

Study Population

Approximately 1150 referrals were received, and available medical information was prescreened to determine whether these patients could fulfill the inclusion criteria. From 491 referrals with adequate medical information, each center randomly selected patients: 204 were invited to receive information and (subject to consent) to undergo assessment at the trial centers, 40 were not eligible, and 12 decided not to participate or withdrew consent before treatment started. The last consent was withdrawn after randomization when enrollment had already closed. Thus, 152 trial participants were randomly assigned and 151 commenced treatment: 77 in the rituximab group and 74 in the placebo group. These 151 patients constitute the efficacy and safety analysis samples (**Figure 1**).

Baseline characteristics were similar between the rituximab and placebo groups (Table 1). One patient in the placebo group withdrew after 44 weeks because of patient preferences, and 1 in the rituximab group withdrew after 82 weeks because of high symptom burden and poor adherence. Except for 1 patient in the placebo group who received cognitive therapy and 1 in the rituximab group who participated in a rehabilitation program, none received alternative interventions aimed at ME/CFS during the trial. Appendix Tables 1 to 3 (available at Annals.org) summarize medical history and concomitant diseases at baseline, previous treatments for ME/CFS, and concomitant medication, respectively. Of note, 93 patients (61.6%) had previously tried some form of cognitive therapy at least once (Appendix Table 2).

In total, 139 patients received all 6 planned infusions, 7 received 5 infusions, and 5 received 4 infusions. The reasons for omitting infusions were high symptom burden or intercurrent disease (Figure 1).

Patients' Suspicion of Randomization Group Allocation

Of 75 patients in the rituximab group who responded to a question about suspected treatment allocation after 6 weeks of follow-up, 17 (22.7%) believed that they had received rituximab, 9 (12.0%) believed that they had received placebo, and 49 (65.3%) were not sure. Among 74 patients in the placebo group, 8 (10.8%) believed that they had received rituximab, 2 (2.7%) believed that they had received placebo, and 64 (86.5%) were not sure. In total, 19 of 151 patients (12.6%) correctly guessed their allocation status. Of 25 patients who believed that they had received rituximab after 6 weeks of follow-up, 12 later achieved a clinical response: 6 of 17 in the rituximab group and 6 of 8 in the placebo group.

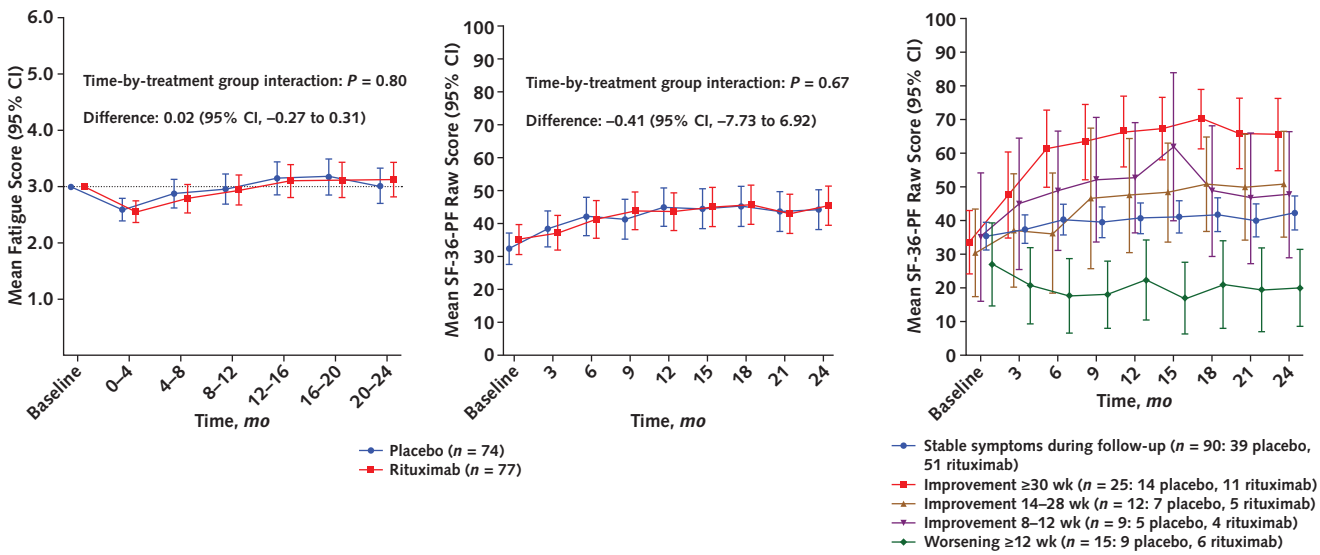
Missing Data

Between 0.4% and 1.3% of data for the outcome measures were missing (Results section of the Appendix).

Primary Outcome

Repeated measurements of fatigue score did not differ significantly between the treatment groups during follow-up (P = 0.80). The difference in average fatigue score between placebo and rituximab groups was 0.02 (95% CI, −0.27 to 0.31) (Figure 2, *left*). Overall response rates did not differ significantly between the treatment groups when study center was taken into account. In total, 46 patients fulfilled the predefined criterion for overall response: 26 (35.1% [CI, 25.2% to 46.5%]) in the placebo group and 20 (26.0% [CI, 17.5% to 36.7%]) in the rituximab group. The difference between treatment groups was 9.2 percentage points (CI, −5.5 to 23.3 percentage points). The Breslow–Day test showed homogeneity of the odds ratio across study centers (P = 0.54), and the Mantel–Haenszel common odds ratio estimate was 0.65 (CI, 0.32 to 1.30; P = 0.22).

Figure 2. Fatigue score (*left*) and SF-36-PF score (*middle* and *right*) throughout the study.



P values are from the general linear model for repeated measures for time-by-treatment interaction, adjusted for study center. Differences (95% CIs) between groups (placebo vs. rituximab) are for the averaged outcome variable over time. Missing data were replaced by multiple imputation. Two patients had incomplete follow-up: 1 in the rituximab group lacked self-reported data from week 82 to the end of the study, and 1 in the placebo group (withdrawal) lacked self-reported data from week 44 to the end of the study. In total, 4 data points were missing (0.4%) for fatigue score and 7 (0.5%) for SF-36-PF score. SF-36-PF = Short Form-36 Health Survey physical function subscale. **Left.** Mean fatigue scores (primary end point) with 95% CIs at time points through 24 mo of follow-up, by treatment group, from self-reported symptom scores every second week, with mean data for 4-mo intervals. The scale is 0–6, where 3 indicates no change from baseline (*dotted line*) and higher scores indicate less fatigue. **Middle.** Mean SF-36-PF scores with 95% CIs at time points through 24 mo of follow-up, by treatment group. The scale is 0–100, where higher scores indicate better function. **Right.** SF-36-PF scores, by clinical course during follow-up: stable clinical course, improvement of duration ≥30 wk, improvement of duration 14–28 wk, improvement of duration 8–12 wk, or worsening lasting ≥12 wk consecutively, with number of patients allocated to rituximab and placebo groups indicated.

Table 2. Effects of B-Cell Depletion Using Rituximab, Compared With Placebo, in the Intention-to-Treat Population

Secondary Outcome Measure*	Mean in Rituximab Group (n = 77)	Mean in Placebo Group (n = 74)	Mean Difference (95% CI)	P Value for Interaction†
Fatigue score‡				
Baseline	3.0	3.0		
16–20 mo	3.12	3.18	−0.06 (−0.51 to 0.39)	0.79
Function level, %§				
Baseline	20.14	18.37	1.77 (−1.50 to 5.05)	
16–20 mo	25.25	25.93	−0.68 (−5.90 to 4.54)	0.31
SF-36-PF score‖				
Baseline	35.24	32.45	2.79 (−3.79 to 9.36)	
18 mo	45.67	45.25	0.42 (−8.12 to 8.96)	0.52
SF-36-PCS score¶				
Baseline	24.80	23.61	1.19 (−0.92 to 3.32)	
18 mo	28.79	29.00	−0.21 (−3.18 to 2.77)	0.27
Fatigue Severity Scale score**				
Baseline	59.10	59.88	−0.78 (−2.47 to 0.92)	
18 mo	55.98	56.05	−0.07 (−3.21 to 3.08)	0.68
Mean steps per 24 h, n††				
Baseline	3297	3233	64 (−599 to 727)	
17–21 mo	3777	3904	−127 (−1004 to 749)	0.58

SF-36-PCS = Short Form-36 Health Survey physical component summary; SF-36-PF = SF-36 physical function subscale.
* For changes from baseline to 18-mo follow-up. Values are from self-reported registrations and questionnaires. Missing data were replaced by multiple imputation.
† P values from the general linear model for repeated measures for time-by-treatment interaction (18 mo vs. baseline); study center was included as a covariate in the model (Methods section of the Appendix).
‡ Compared with baseline; range, 0–6 (3 indicates no change from baseline). Missing 1 data item at 18 mo.
§ Range, 0%–100%. Missing 1 data item at 18 mo.
‖ Raw score; range, 0–100. Missing 2 data items at 18 mo.
¶ Norm-based score with a population mean of 50. Missing 2 data items at 18 mo.
** Range, 9–63; higher scores indicate worse symptoms. Missing 1 data item at 18 mo.
†† Measured continuously by SenseWear armbands for 5–7 d. Missing 1 data item at baseline and 2 at 18 mo.

Within the 5 study centers, the response rates were as follows: Oslo University Hospital, 4 of 16 patients in the placebo group (25.0% [CI, 10.2% to 49.5%]) and 6 of 16 in the rituximab group (37.5% [CI, 18.5% to 61.4%]); Notodden Hospital, 5 of 15 in the placebo group (33.3% [CI, 15.2% to 58.3%]) and 3 of 17 in the rituximab group (17.6% [CI, 6.2% to 41.0%]); Haukeland University Hospital, 8 of 20 in the placebo group (40.0% [CI, 21.9% to 61.3%]) and 4 of 20 in the rituximab group (20.0% [CI, 8.1% to 41.6%]); St. Olavs Hospital, 3 of 12 in the placebo group (25.0% [CI, 8.9% to 53.2%]) and 3 of 12 in the rituximab group (25.0% [CI, 8.9% to 53.2%]); and University Hospital of Northern Norway, 6 of 11 in the placebo group (54.5% [CI, 28.0% to 78.7%]) and 4 of 12 in the rituximab group (33.3% [CI, 13.8% to 60.9%]).

Secondary Outcomes

The treatment groups did not differ significantly in repeated measurements of the secondary outcome measures SF-36-PF (difference in average score between placebo and rituximab groups, −0.41 [CI, −7.73 to 6.92]; P = 0.67) (**Figure 2**, *middle*), function level (difference, −0.21% [CI, −4.18% to 3.76%]; P = 0.48), SF-36-PCS (difference, −0.44 [CI, −2.90 to 2.02]; P = 0.63) (**Appendix Figure 1**, available at Annals.org), or Fatigue Severity Scale (difference, −0.25 [CI, −2.44 to 1.95]; P = 0.61). Further, the same outcome measures did not change significantly from baseline to 18 months of follow-up, nor did mean number of steps per 24 hours between baseline and repeated assessment in the time interval 17 to 21 months (**Table 2**). In general, during 24 months of follow-up, both treatment groups had slight but significant improvements over time in mean values for all self-reported outcome variables and for physical activity level (**Figure 2** [*left* and *middle*] and **Appendix Figure 1**).

Appendix Figure 2 (available at Annals.org) shows the repeated measurements of the outcome variables fatigue score, SF-36-PF, and steps (mean per 24 hours) over time, by study center. The median time to first clinical response (that is, fatigue score ≥4.5 for ≥8 consecutive weeks) was 41 weeks among 46 patients who fulfilled the response criteria for the fatigue measure, and time lags were comparable for responders in both treatment groups. First clinical response was recorded before 18 weeks in 25% of responders and after 12 months in 25%.

During blinded review, we identified clinical courses in patients from both treatment groups. Ninety patients (51 in the rituximab group and 39 in the placebo group) reported stable symptoms over time. Sustained worsening of ME/CFS symptoms for at least 12 weeks was found in 15 patients: 6 (7.8%) in the rituximab group and 9 (12.2%) in the placebo group. **Figure 2** (*right*) shows the

repeated measurements of SF-36-PF, sorted by duration of clinical response; **Appendix Table 4** (available at Annals.org) summarizes clinical characteristics.

Adverse Events

Adverse events were seen in 63 patients in the rituximab group (81.8%) and 48 in the placebo group (64.9%). We report all adverse events with a severity grade of at least 2 according to the Common Terminology Criteria for Adverse Events (at least moderate symptoms where minimal, local, or noninvasive intervention is indicated). **Table 3** summarizes adverse events of grade 2 or higher, serious adverse events, and suspected unexpected serious adverse reactions, by treatment group. Twenty-six patients in the rituximab group (33.8%) and 12 in the placebo group (16.2%) had adverse events that were considered by the investigators to be possibly or probably related to the intervention. The 31 serious adverse events in 20 patients (26.0%) in the rituximab group all required hospitalization. In the placebo group, 16 serious adverse events in 14 patients (18.9%) included 1 unintended pregnancy and 15 hospital admissions (Results section of the **Appendix** and **Appendix Table 5**, available at Annals.org).

Three suspected unexpected serious adverse reactions were seen: 1 metrorrhagia with hysterectomy and 1 suspected but unconfirmed coronary artery disease in the rituximab group and 1 transient paresis of the left extremities in the placebo group.

Discussion

This randomized, double-blind, placebo-controlled trial did not detect a benefit from rituximab treatment in patients with ME/CFS during 24 months of follow-up. Mean values of outcome variables improved slightly through follow-up but did not differ between the rituximab and placebo groups. Regardless of treatment group, 30% of patients reported various degrees of improvement and fulfilled the response criteria for the fatigue measure. Sustained worsening for at least 3 months was reported by 10% of patients. Neither clinical response nor clinical worsening was associated with the rituximab intervention. Few patients had a major and sustained alleviation of ME/CFS symptoms. Intervention tolerance was generally good, and few serious adverse events had a suspected or probable relation to the study drug. However, the relatively large number of unrelated adverse events probably reflects the low tolerance for physical and cognitive strain in patients with ME/CFS.

Previously, we did a small randomized trial (6) that had negative results for the primary end point but suggested a benefit of rituximab in secondary end points after 6 months of follow-up. The open-label trial with rituximab maintenance therapy (7) showed improvements lasting several years in half of included patients but was limited by lack of a placebo group and small sample size. This discrepancy between results of RituxME and previous trials has several possible explanations. First, placebo mechanisms are operative and possibly amplified by high expectations, as shown in trials of analgesics (22). In a review and meta-analysis of placebo responses in CFS intervention studies, the pooled placebo response was 19.6% (19). In our study with long observation, the response rate in the placebo group was 35.1%. The patients' expectations of group allocation after 6 weeks of follow-up in the present trial showed that only 12.6% of included patients correctly guessed their intervention; thus, the predictive value of patients' expectations is uncertain. Second, knowledge of the natural symptom variation over time for patients with ME/CFS is limited. Slight improvements in mean values for fatigue-related symptoms in both active and placebo groups were also reported in a randomized study that assessed cytokine inhibition by the interleukin-1 receptor antagonist anakinra over 6 months (23). Third, unintended patient selection effects may have contributed to the positive results, especially in our open-label rituximab

Table 3. Adverse Events of CTCAE Grade ≥2, SAEs, and Suspected Unexpected Serious Adverse Reactions During 24 Months of Follow-up, by Intervention Group*

Outcome	Rituximab (*n* = 77)	Placebo (*n* = 74)
Any adverse event of CTCAE grade ≥2	63 (81.8)	48 (64.9)
Any adverse event of CTCAE grade ≥2 with possible or probable relation to intervention	26 (33.8)	12 (16.2)
Any SAE†	20 (26.0)	14 (18.9)
Any SAE with possible or probable relation to intervention	8 (10.4)	0
Suspected unexpected serious adverse reaction‡	2 (2.6)	1 (1.4)
Withdrawal due to adverse event	0	0
Neutropenia§	5 (6.5)	3 (4.1)
Infection	33 (42.9)	30 (40.5)
Gastrointestinal event	20 (26.0)	12 (16.2)
Nervous system event	14 (18.2)	5 (6.8)
Infusion-related reaction	10 (13.0)	0
Death	0	0

CTCAE = Common Terminology Criteria for Adverse Events; SAE = serious adverse event.
* Values are numbers (percentages) of patients who had the outcome.
† 20 patients had 31 SAEs in the rituximab group, and 14 patients had 16 SAEs in the placebo group (including 1 unintended pregnancy). All SAEs except the pregnancy were hospital admissions.
‡ 1 metrorrhagia with hysterectomy and 1 suspected but unconfirmed coronary disease in the rituximab group and 1 transient paresis in left extremities in the placebo group.
§ 2 patients with late-onset neutropenia in the rituximab group were hospitalized for intravenous antibiotics. Three in each group had slight neutropenia.

maintenance study (7). In the RituxME trial, the rituximab maintenance doses were 50% to 60% lower than those in the previous maintenance study. However, rituximab dose reductions are not a plausible main cause for the lack of clinical efficacy because early responses should nevertheless have been more frequent in the rituximab group than in the placebo group.

We hypothesized that ME/CFS in a subgroup of patients could involve a variant of an autoimmune disease mechanism with a role for B lymphocytes. This hypothesis was partly built on the observed pattern of improvements and relapses after B-cell depletion intervention. Also, the modest but highly significant increase in risk for B-cell lymphoma observed among elderly patients with ME/CFS (24) indicates an activated B-cell system in long-standing ME/CFS. The lack of clinical effect of B-cell depletion in this trial weakens the case for an important role of B lymphocytes in ME/CFS but does not exclude an immunologic basis. Several randomized and placebo-controlled trials with negative results have evaluated rituximab in established autoimmune diseases (25–27). In line with conclusions from these trials, the negative outcome of RituxME should spur research to assess patient subgroups and further elucidate disease mechanisms, of which recently disclosed impairment of energy metabolism may be important (28–30). Limitations of the present study are self-referral and use of self-reported primary outcome measures with possible recall bias. Although strict criteria were used for inclusion, the unknown cause and lack of specific biomarkers for ME/CFS could introduce unintended heterogeneity of the patient sample.

In future research, smaller studies are justified initially to explore new therapeutic principles and investigate possible signs of clinical activity. This study highlights the importance of randomized and blinded clinical trials with a placebo group, especially in diseases that lack specific and sensitive biomarkers, have limited possibilities for objective end points, and rely mainly on self-reported symptom scores. This trial did not show benefit from rituximab treatment in patients with ME/CFS.

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Reproducible Research Statement: *Study protocol:* See **Supplement 1** (available at Annals.org). *Statistical code:* Example analysis codes are in the Methods section of the **Appendix** (available at Annals.org). *Data set:* Available to approved individuals through written agreements with the author and research sponsor.

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Appendix: Additional Methods and Results

Methods

Randomization and Interventions

Patients were randomly assigned 1:1 to receive either rituximab or placebo intervention. The randomization schedule was generated by Smerud Medical Research International (Oslo, Norway) using SAS, version 9.3 (SAS Institute), and was stratified by center using blocks of 8. Patients were enrolled by investigators at each study site and were allocated consecutive randomization numbers by the national trial coordinator, who had no knowledge of the allocation sequence. The pharmacy at each study site received the site allocation sequence directly from Smerud and prepared the appropriate infusion bag according to randomization number. Treatment allocation was concealed from trial participants and all study personnel.

Outcomes and Follow-up

On the basis of experiences from the previous phase 2 trials (6, 7) and the observed kinetics for responses and relapses, we expected that the best time to capture any differences in symptom severity between intervention groups would be at approximately 18 months, 6 months after the last infusion. For several secondary end points, we therefore assessed the between-group difference in change of the outcome variable from baseline to 18 months.

Additional secondary end points defined in the study protocol were differences between the treatment groups in repeated measurements of SF-36-mean5 (mean of raw scores for physical function, bodily pain, vitality, social function, and general health) through 24 months of follow-up and changes from baseline to 18 months in maximum number of steps per 24 hours and mean and maximum duration of physical activity of at least 3.5 metabolic equivalents (METs) per 24 hours. The longest duration of lasting clinical response was another secondary end point.

Statistical Analyses and Missing Data

Missing data were replaced by multiple imputation (20, 21) with 5 imputations using SPSS, version 25. One model was used to obtain the imputed values for all of the variables with missing data. The model included the following variables: treatment group; center; age; sex; baseline and follow-up scores (to 24 months) for fatigue score, function level (percentage), SF-36-PF, SF-36-PCS, SF-36mean5, and Fatigue Severity Scale; ME/CFS disease duration (2 to 5 years, 5 to 10 years, or 10 to 15 years) (Duration_3cat); ME/CFS disease severity (mild or mild-to-moderate, moderate, or moderate-to-severe or severe) (Severity_3cat); steps (mean and maximum) per 24 hours at baseline and 18 months; and duration of physical activity exceeding 3.5 METs (mean and maximum) at baseline and 18 months. The output file with 5 imputed data sets was used for analyses of primary and secondary outcome measures, using pooled estimates for means and CIs.

The SPSS analysis code for multiple imputation is as follows:

```
MULTIPLE IMPUTATION Center treatment
_group Duration3 Severity_3cat
FS_baseline FSmean0_4m FSmean4_8m
FSmean8_12m FSmean12_16m
FSmean16_20m FSmean20_24m
Function_baseline Function0_4m Function4
_8m Function8_12m Function12_16m
Function16_20m Function20_24m
SF36PF_baseline SF36PF_3m SF36PF_6m
SF36PF_9m SF36PF_12m SF36PF_15m
SF36PF_18m SF36PF_21m SF36PF_24m
SF36PCS_baseline SF36PCS_3m SF36PCS_6m
SF36PCS_9m SF36PCS_12m SF36PCS_15m
SF36PCS_18m SF36PCS_21m SF36PCS_24m
SF36mean5_baseline SF36mean5_3m SF36
mean5_6m SF36mean5_9m SF36mean5_12m
SF36mean5_15m SF36mean5_18m SF36
mean5_21m SF36mean5_24m
FSS_baseline FSS_6m FSS_12m FSS_18m
FSS_24m
Steps_meanbaseline Steps_maxbaseline
Stepsmean_18m Stepsmax_18m
Physactivedur_meanbaseline Physactivedur
_maxbaseline
Physactivedur_mean18m
Physactivedur_max18m
Age_inclusion Sex
/IMPUTE METHOD=AUTO NIMPUTATIONS =
5 MAXPCTMISSING=NONE
/MISSINGSUMMARIES NONE
/IMPUTATIONSUMMARIES MODELS
DESCRIPTIVES
/OUTFILE IMPUTATIONS=RituxME_imputation
_011218c
```

Statistical Analyses and Outcome Measures

For the overall response rates in the rituximab and placebo groups and for the difference in response rates between groups, we obtained 95% CIs for proportions (http://vassarstats.net/prop1.html). The between-group differences in overall response rates were assessed by the Mantel–Haenszel test, stratified by study center, and the Mantel–Haenszel common odds ratio estimate was used to summarize the effect size pooled across study centers. The Breslow–Day test was used to test homogeneity of the odds ratios across study centers.

The SPSS analysis code for the Mantel–Haenzel test to assess the difference in overall response rates between treatment groups, stratified by study center, is as follows:

```
CROSSTABS
/TABLES=response BY treatment_group BY
Center
/FORMAT=AVALUE TABLES
/STATISTICS=CHISQ CMH(1)
/CELLS=COUNT ROW COLUMN TOTAL
/COUNT ROUND CELL.
```

Differences between the treatment groups in course over time for outcomes with repeated measurements of the dependent variable were assessed by the general linear model for repeated measures. The analyses were done on the imputation data set with 5 parallel sets of results. The general linear model repeated measures analyses included time as a within-subject factor (2, 5, 7, or 9 levels for the different outcome measures). Treatment group was a between-subject factor (rituximab vs. placebo), and study center was included as a covariate (5 centers). The model used type III sum of squares. The time-by-treatment interaction was included as a predictor. For all general linear model analyses with 5, 7, or 9 repeated measures, Mauchly tests were significant ($P < 0.001$), indicating violations of the sphericity assumption, and Greenhouse–Geisser corrections were used.

Estimated marginal means were calculated for treatment group, time, and the time-by-treatment interaction. The plots of outcomes by treatment group versus time (**Figure 2** [*left* and *middle*] and **Appendix Figure 1**) and by study center versus time (**Appendix Figure 2**) included 95% CIs. Output included descriptive statistics, parameter estimates, and estimates of effect sizes, including the contrasts between treatment groups–that is, between-group differences (with 95% CIs) for the averaged outcome measures over time.

Simple contrasts were used in the time domain for the time-by-treatment interaction to assess the difference between treatment groups in change of the outcome measure at each time point compared with baseline. An example is shown in the Results section of the **Appendix** for the outcome measure SF-36-PF.

The general linear model repeated measures approach was used to estimate the primary outcome (fatigue score) and the following secondary outcomes with repeated measurements: SF-36 scores (SF-36-PF, SF-36-PCS, and SF-36mean5), function level, and Fatigue Severity Scale.

Several secondary end points aimed to assess between-group differences in change of outcome measures from baseline to 18 months; **Table 2** shows the results. To assess the course of the outcome measure over time by study center, the general linear model analysis included study center as a between-subjects factor (for **Appendix Figure 2**). All analyses were done using IBM SPSS Statistics, version 25 (IBM). Figures were made in GraphPad Prism, version 7 (GraphPad Software).

For general linear model repeated measures analyses, the means and CIs in the text and figures are the pooled estimates from the multiple imputation data sets. Similarly, differences between the treatment groups for

the averaged outcome measures over time are the mean values (with 95% CIs) of analyses from the 5 imputation data sets. The *P* values for the time-by-treatment interaction are shown as the mean of analyses from 5 imputation data sets. Because of a low frequency of missing data, the 5 imputation data sets produced similar results.

The SPSS analysis code for general linear model repeated measures, for the example SF-36-PF, is as follows:

```
GLM SF36PF_baseline SF36PF_3m SF36PF_6m
SF36PF_9m SF36PF_12m SF36PF_15m
SF36PF_18m SF36PF_21m SF36PF_24m
BY treatment_group WITH Center
/WSFACTOR=time 9 Simple(1)
/MEASURE=SF36PF
/CONTRAST(treatment_group)=Simple(1)
/METHOD=SSTYPE(3)
/PLOT=PROFILE(time*treatment_group)
TYPE=LINE ERRORBAR=CI
MEANREFERENCE=NO YAXIS=AUTO
/EMMEANS=TABLES(OVERALL)
WITH(Center=MEAN)
/EMMEANS=TABLES(treatment_group)
WITH(Center=MEAN)COMPARE ADJ(LSD)
/EMMEANS=TABLES(time)
WITH(Center=MEAN)COMPARE ADJ(LSD)
/EMMEANS=TABLES(treatment_group*time)
WITH(Center=MEAN)
/PRINT=DESCRIPTIVE ETASQ PARAMETER
/CRITERIA=ALPHA(.05)
/WSDESIGN=time
/DESIGN=Center treatment_group.
```

This analysis code was used to estimate the *P* value for the time-by-treatment interaction for the between-group difference in the course of the outcome measure through 24 months of follow-up, taken from the table tests for within-subjects effects (using Greenhouse–Geisser corrections). The effect size was calculated as the difference between treatment groups for the averaged SF-36-PF score over time through follow-up (with 95% CI).

The same analysis code was used to estimate the *P* value for the time-by-treatment interaction for the between-group difference in change of the outcome measure from baseline to 18 months. This was taken from the simple contrast in the time domain, from the table tests for within-subjects contrasts, level 7 (18 months) versus level 1 (baseline). The effect size was calculated from the parameter estimates for baseline and for 18 months as the difference between treatment groups (with 95% CI).

All tests were 2-sided, and the general significance level was set to 0.05. For the 2 primary end points, we used a Bonferroni adjustment—that is, a marginal level of 0.025.

Results

Missing Data

For fatigue score raw data, 25 of 7852 data points (0.3%) were missing. For fatigue score (mean of 4-month intervals, used as input for statistical analyses), 4 of 906 data points (0.4%) were missing. These 4 missing data points were from 1 patient who withdrew from the study after 9 months and 1 who withdrew after 18 months. Thus, for fatigue scores between 12 and 16 months (FS12_16m) and between 16 and 20 months (FS16_20m), each variable had 1 missing data item, and the variable fatigue score at 24 months (FS20_24m) had 2 missing data points. Similarly, 4 of 906 data points (0.4%) were missing for function level (mean of 4-month intervals) at the same time intervals (Function12_16m, Function 16_20m, and Function 20_24m) for the same 2 patients who withdrew from the study.

For SF-36 data included in outcome measures (SF-36-PF, SF-36-PCS, and SF-36-mean5), 7 of 1359 data points (0.5%) were missing for each outcome measure, for the same 2 patients who withdrew from study. Thus, SF-36 forms recorded at 12, 15, and 18 months were missing 1 data item for each variable, and SF-36 forms recorded at 21 and 24 months were missing 2 data points for each variable. For Fatigue Severity Scale, recorded by patients with a 6-month interval, 4 of 755 data points (0.5%) were missing.

For physical activity measurements using SenseWear armbands for 5 to 7 consecutive days, data were missing for 1 of 151 patients (0.7%) at baseline and for 2 patients (1.3%) at 18 months. The variables based on measures of physical activity used to analyze secondary outcome measures (that is, changes from baseline to 18 months) were mean steps per 24 hours at baseline (Steps_meanbaseline), maximum steps per 24 hours at baseline (Steps_maxbaseline), mean duration of activity of at least 3.5 METs per 24 hours at baseline (Physactivedur_meanbaseline), maximum duration of activity of at least 3.5 METs per 24 hours at baseline (Physactivedur_maxbaseline), and the corresponding variables at 18 months (Stepsmean_18m, Stepsmax_18m, Physactivedur_mean18m, and Physactivedur_max18m).

For ME/CFS disease severity recorded at baseline (Severity_3cat), 1 of 151 patients (0.6%) was missing an assessment.

Outcomes

Beyond the outcome measures described in the article, none of the following secondary end points defined in the study protocol differed significantly between intervention groups: repeated measurements of SF-36-mean5, changes from baseline to 18-month follow-up in maximum number of steps per 24 hours, mean and maximum duration of physical activity of at

least 3.5 METs per 24 hours, or longest duration of lasting clinical response (data not shown).

Beyond the results from general linear model repeated measures in the article, Appendix Table 6 shows an example of SF-36-PF (scale, 0 to 100) with mean scores in the rituximab and placebo groups and differences between groups (with 95% CIs) for each of the 9 time points during follow-up. *P* values are for the interaction term (time by treatment group) for the simple contrasts in the time domain—that is, to assess between-group differences in change of the outcome measure at specific time points compared with baseline.

Characteristics of Patients With Response, Stable Symptoms, or Worsening During Follow-up

During blinded review of data before the intervention code was unlocked, we identified clinical courses in patients from both treatment groups combined. A group of 15 patients had sustained moderate to major worsening of ME/CFS symptoms, defined as fatigue score less than 1.0 for at least 12 consecutive weeks. Of these 15 patients (9.9% of the study population) with clinical worsening, 9 were in the placebo group and 6 in the rituximab group. A "stable" disease course characterized 90 patients (59.6% of the study population), of whom 39 were in the placebo group and 51 in the rituximab group. Clinical response was reported in 46 patients (30.5% of the study population): 26 in the placebo group and 20 in the rituximab group.

Figure 2 (*right*) shows the course of the SF-36-PF score for patients who had clinical response of long duration (>30 weeks; *n* = 25), moderate duration (14 to 28 weeks; *n* = 12), or short duration (8 to 12 weeks; *n* = 9); stable symptoms (*n* = 90); or clinical worsening (*n* = 15).

Appendix Table 4 shows the characteristics of 46 patients who fulfilled response criteria, 90 who had a stable symptom course through follow-up, and 15 whose symptoms worsened for at least 12 consecutive weeks.

Adverse Events: Serious Adverse Events and Hospital Admissions

The 31 serious adverse events in 20 patients (26.0%) in the rituximab group all involved hospitalization. Twelve hospital admissions in 8 individual patients had a possible or probable relation to the intervention. Of these, 4 admissions in 2 patients were due to febrile neutropenia, 2 admissions in 1 patient were due to dizziness and nausea, and 1 admission in 1 patient was due to headache and gastroenteritis. Two patients had infusion-related reactions, 1 of whom was also admitted for tests because of noncardiac chest pain. One patient was admitted for examination for involuntary movements, and another for a transient facial paresis. In the placebo group, 16 serious adverse events in 14 patients (18.9%) included 1 unintended pregnancy and 15 hospital admissions, all of which were considered to have no or unlikely relation to the intervention. Appendix Table 5 lists all serious adverse events, by treatment group and relation to the intervention.

Appendix Table 1. Medical History and Concomitant Diseases Reported by Trial Participants at Baseline, by Intervention Group and System Organ Class

System Organ Class	CTCAE Term	Rituximab (*n* = 77), *n (%)*	Placebo (*n* = 74), *n (%)*
Endocrine disorder	Hypothyroidism	4 (5.2)	4 (5.4)
Immune system disorder	Allergy	31 (40.3)	31 (41.9)
Musculoskeletal and connective tissue disorder	Fibromyalgia	6 (7.8)	5 (6.8)
Psychiatric disorder	Depression	7 (9.1)	6 (8.1)
Psychiatric disorder	Anxiety	9 (11.7)	8 (10.8)
Other (unspecified)	Other	21 (27.3)	17 (23.0)

CTCAE = Common Terminology Criteria for Adverse Events.

Appendix Table 2. Previous Treatments of ME/CFS, by Treatment Group, Reported by Trial Participants at Baseline

Type of Treatment	Rituximab (*n* = 77), *n* (%)	Placebo (*n* = 74), *n* (%)
Cognitive therapy	49 (63.6)	44 (59.5)
"Lightning Process"	19 (24.7)	22 (29.7)
Mindfulness	18 (23.4)	11 (14.9)
Other cognitive therapy	34 (44.2)	32 (43.2)
Physical therapy	32 (41.6)	38 (51.4)
Graded exercise therapy	14 (18.2)	22 (29.7)
Other physical therapy	27 (35.1)	30 (40.5)
Activity management (adaptive pacing)	38 (49.4)	34 (45.9)
Medical treatments		
Vitamin B_{12} injections	32 (41.6)	32 (43.2)
Long-term antibiotics	14 (18.2)	15 (20.3)
Low-dose naltrexone	16 (20.8)	26 (35.1)
Other medical treatment	13 (16.9)	5 (6.8)
Did not receive any of these treatments	16 (20.8)	14 (18.9)

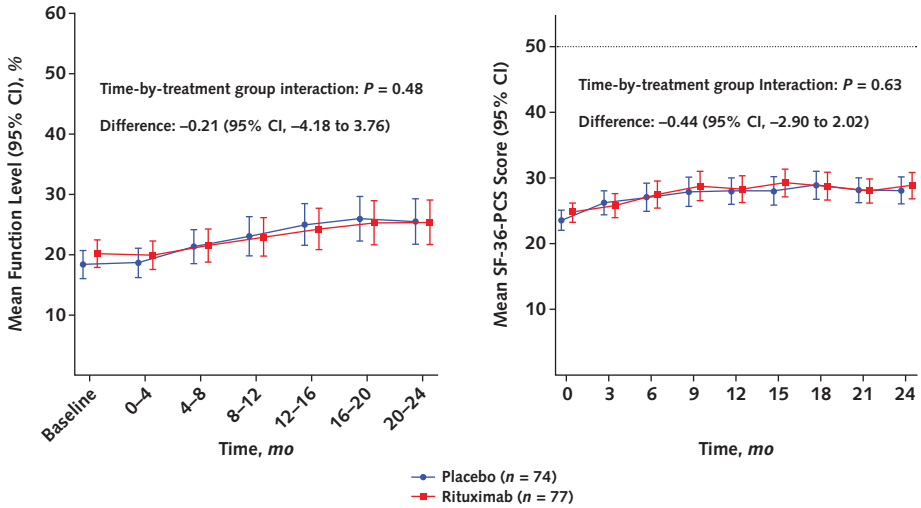
ME/CFS = myalgic encephalomyelitis/chronic fatigue syndrome.

Appendix Table 3. Concomitant Medication During 24 Months of Follow-up, by Treatment Group and ATC Code, for Trial Participants

Medication	ATC Code	Rituximab (*n* = 77), *n (%)*	Placebo (*n* = 74), *n (%)*
Antacids	A02	25 (32.5)	16 (21.6)
Antiemetics	A03, A04	10 (13.0)	6 (8.1)
Laxantia	A06	4 (5.2)	5 (6.8)
Antithrombotic agents	B01	1 (1.3)	2 (2.7)
Vitamin B_{12} supplements	B03A, B03BB	8 (10.4)	11 (14.9)
β-Blockers	C07	4 (5.2)	6 (8.1)
Antihypertensive agents	C08, C09	3 (3.9)	1 (1.4)
Statins	C10	6 (7.8)	4 (5.4)
Antibiotics	G01, J01-05	31 (40.3)	31 (41.9)
Contraceptives (systemic)	G03A	15 (19.5)	18 (24.3)
Thyroid hormone replacement	H03	4 (5.2)	8 (10.8)
NSAIDs	M01A	31 (40.3)	37 (50.0)
Opioids	N02A	22 (28.6)	21 (28.4)
Acetaminophen	N02B	33 (42.9)	39 (52.7)
Antimigraine agents	N02C	10 (13.0)	10 (13.5)
Antiepileptic agents	N03A	8 (10.4)	2 (2.7)
Anxiolytics	N05B	10 (13.0)	8 (10.8)
Hypnotics and sedatives	N05C	36 (46.8)	35 (47.3)
Antidepressants	N06	17 (22.1)	15 (20.3)
Allergy and asthma medications	R01, R03, R06A, S01G	45 (58.4)	38 (51.4)
Other medications	–	52 (67.5)	55 (74.3)
Dietary supplements (non-ATC)	–	32 (41.6)	31 (41.9)

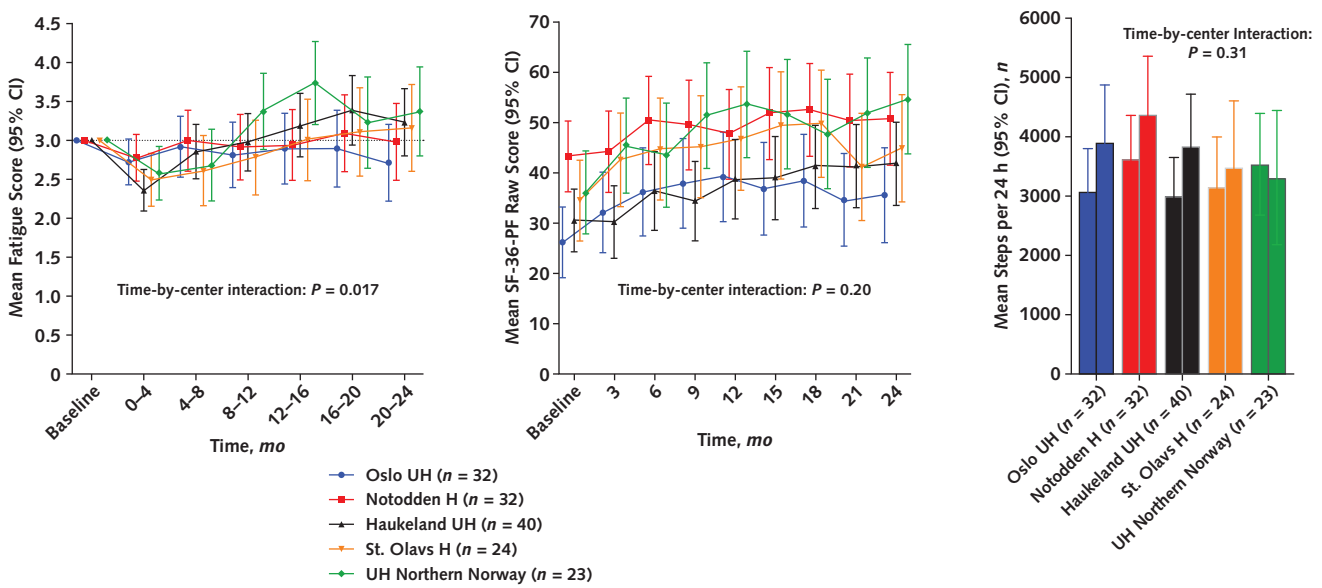
ATC = Anatomic Therapeutic Chemical; NSAID = nonsteroidal anti-inflammatory drug.

Appendix Figure 1. Function level (*left*) and SF-36-PCS score (*right*), by treatment group.



Means with 95% CIs are presented for time points through 24 mo of follow-up. *P* values are from the general linear model for repeated measures for time-by-treatment interaction, adjusted for study center. Differences (with 95% CIs) between groups (placebo vs. rituximab) are for the averaged outcome variable over time. Missing data were replaced by multiple imputation. Two patients had incomplete follow-up: 1 in the rituximab group lacked self-reported data from week 82 to the end of the study, and 1 in the placebo group (withdrawal) lacked self-reported data from week 44 to the end of the study. In total, 4 data points were missing (0.4%) for function level and 7 (0.5%) for SF-36-PCS score. SF-36-PCS = Short Form-36 Health Survey physical component summary. **Left.** Function level (percentage) is from self-reported symptom scores every second week, with mean data for 4-mo intervals. **Right.** The SF-36-PCS score is norm-based, with a population mean of 50 (*dotted line*).

Appendix Figure 2. Fatigue score (*left*), SF-36-PF score (*middle*), and mean steps per 24 h (*right*), by study center.



Means with 95% CIs are presented for time points through 24 mo of follow-up. *P* values are from the general linear model for repeated measures for time-by-center interaction. Missing data were replaced by multiple imputation. Two patients had incomplete follow-up: 1 in the rituximab group lacked self-reported data from week 82 to the end of the study, and 1 in the placebo group (withdrawal) lacked self-reported data from week 44 to the end of the study. In total, 4 data points were missing (0.4%) for fatigue score and 7 (0.5%) for SF-36-PF score. For physical activity measurements (mean steps per 24 h), 1 data point was missing at baseline and 2 at 18 mo. H = Hospital; SF-36-PF = Short Form-36 Health Survey physical function subscale; UH = University Hospital. **Left.** Fatigue score from self-reported symptom scores every second week, with mean data for 4-mo intervals. The scale is 0–6, where 3 indicates no change from baseline (*dotted line*) and higher scores indicate less fatigue. **Middle.** SF-36-PF score. The scale is 0–100, where higher scores indicate better function. **Right.** Steps, mean per 24 h, at baseline and in the time interval 17–21 mo.

Appendix Table 4. Baseline Characteristics of Patients Who Fulfilled Response Criteria, Patients With a Stable Symptom Course, and Patients Experiencing Clinical Worsening During Follow-up*

Characteristic	Clinical Improvement (*n* = 46)	Stable Symptoms (*n* = 90)	Clinical Worsening (*n* = 15)
Mean age (SD), *y*	38.8 (11.4)	36.3 (11.3)	32.3 (10.3)
Female sex, *n (%)*	42 (91.3)	68 (75.6)	14 (93.3)
ME/CFS severity, *n (%)*†			
Mild or mild-to-moderate	17 (37.0)	38 (42.2)	5 (33.3)
Moderate	15 (32.6)	25 (27.8)	5 (33.3)
Moderate-to-severe or severe	13 (28.3)	27 (30.0)	5 (33.3)
Mean ME/CFS disease duration (SD), *y*	7.7 (3.3)	8.3 (3.0)	7.1 (2.3)
ME/CFS duration, *n (%)*			
2–5 y	12 (26.1)	12 (13.3)	5 (33.3)
5–10 y	25 (54.3)	56 (62.2)	8 (53.3)
10–15 y	9 (19.6)	22 (24.4)	2 (13.3)
Infection before ME/CFS, *n (%)*	34 (73.9)	69 (76.7)	12 (80.0)
Mean steps per 24 h (SD), *n*‡	3210 (2227)	3438 (2008)	2456 (1813)
Mean function level (SD), %§	18.0 (10.0)	20.5 (10.6)	15.7 (8.4)
Mean SF-36-PF score (SD)‖	33.1 (22.2)	35.4 (19.3)	27.0 (22.4)
Treatment group, *n (%)*			
Rituximab	20 (43.5)	51 (56.7)	6 (40%)
Placebo	26 (56.5)	39 (43.3)	9 (60%)

ME/CFS = myalgic encephalomyelitis/chronic fatigue syndrome; SF-36-PF = Short Form-36 Health Survey physical function subscale.
* Clinical courses during follow-up (regardless of treatment group) were identified during blinded review of data before the intervention code was unlocked. The group of 15 patients with sustained clinical worsening was defined as having fatigue score <1.0 for ≥12 wk consecutively.
† One patient had missing data for ME/CFS severity.
‡ Measured continuously by SenseWear armbands for 5–7 d. 1 patient had missing data.
§ Self-reported; range, 0%–100% (**Supplement 2**).
‖ Raw score; range, 0–100.

Appendix Table 5. SAEs During 24 Months of Follow-up, by Intervention Group, System Organ Class, CTCAE Term, SAE Category, and Relation to Treatment

System Organ Class and CTCAE Term	SAE Category	Relation to Treatment*
Placebo group		
Cardiac disorders		
Acute coronary syndrome	Hospitalization	Unlikely
Gastrointestinal disorders		
Stomach pain	Hospitalization	Unlikely
Gastroenteritis	Hospitalization	Unlikely
Immune system disorders		
Allergic reaction with tachycardia	Hospitalization	Unlikely
Injury, poisoning, and procedural complications		
Other: readmission after FNH resection	Hospitalization	Unlikely
Other: stomach pain after gastric bypass	Hospitalization	No relation
Fracture	Hospitalization	No relation
Musculoskeletal and connective tissue disorders		
Chest wall pain	Hospitalization	Unlikely
Chest wall pain	Hospitalization	Unlikely
Prolapsed disc; discectomy	Hospitalization	No relation
Pregnancy, puerperium, and perinatal conditions		
Unintended pregnancy	Other	No relation
Psychiatric disorders		
Suicidal attempt	Hospitalization	Unlikely
Respiratory, thoracic, and inflammatory disorders		
Epistaxis	Hospitalization	Unlikely
Surgical and medical procedures		
FNH resection†	Hospitalization	Unlikely
Gastric bypass†	Hospitalization	Unlikely
Laparoscopic oophorectomy†	Hospitalization	Unlikely
Rituximab group		
Blood and lymphatic system disorders		
Febrile neutropenia‡	Hospitalization	Probable
Febrile neutropenia	Hospitalization	Probable
Cardiac disorders		
Supraventricular tachycardia	Hospitalization	Unlikely
Gastrointestinal disorders		
Gastroenteritis and strong headache	Hospitalization	Possible
Gastroenteritis	Hospitalization	Unlikely
Gastritis†	Hospitalization	Unlikely
General disorders and administration site conditions		
Facial pain	Hospitalization	Unlikely
Infusion-related reaction	Hospitalization	Probable
Infusion-related reaction	Hospitalization	Probable
Noncardiac chest pain	Hospitalization	Possible
Noncardiac chest pain	Hospitalization	Unlikely
Other (reduced general condition)	Hospitalization	Unlikely
Hepatobiliary disorders		
Cholecystitis	Hospitalization	Unlikely
Immune system disorders		
Autoimmune disorder†	Hospitalization	No relation
Infections and infestations		
Lung infection	Hospitalization	Unlikely
Appendicitis	Hospitalization	Unlikely
Injury, poisoning, and procedural complications		
Fracture	Hospitalization	No relation
Metabolism and nutrition disorders		
Anorexia†	Hospitalization	No relation
Nervous system disorders		
Headache	Hospitalization	Unlikely
Facial nerve disorder	Hospitalization	Possible
Headache	Hospitalization	Unlikely
Dizziness§	Hospitalization	Possible
Involuntary movements†	Hospitalization	Possible
Cerebrovascular ischemia§	Hospitalization	Unlikely
Psychiatric disorders		
Anxiety and depression†	Hospitalization	Unlikely
Reproductive system and breast disorders		
Uterine hemorrhage†	Hospitalization	Unlikely
Surgical and medical procedures		
Other (admission for study-related procedures)†	Hospitalization	Not related

CTCAE = Common Terminology Criteria for Adverse Events; FNH = focal nodular hyperplasia in liver; SAE = serious adverse event.
* Assessed by the investigators at each study center during the trial.
† Elective hospitalizations/procedures.
‡ 3 hospitalizations for 1 study patient.
§ 2 hospitalizations for 1 study patient.

Appendix Table 6. Differences Between Rituximab and Placebo Groups in SF-36-PF at Each Time Point During Follow-up*

Time Point	Mean SF-36-PF Score in Rituximab Group	Mean SF-36-PF Score in Placebo Group	Mean Difference (95% CI)	*P* Value for Interaction†
Baseline	35.24	32.45	2.79 (−3.79 to 9.36)	
3 mo	37.31	38.43	−1.12 (−8.74 to 6.50)	0.142
6 mo	41.36	42.17	−0.81 (−8.97 to 7.36)	0.23
9 mo	43.90	41.37	2.53 (−5.90 to 10.95)	0.94
12 mo	43.69	45.01	−1.32 (−9.47 to 6.84)	0.22
15 mo	45.13	44.60	0.53 (−7.99 to 9.05)	0.53
18 mo	45.67	45.25	0.42 (−8.12 to 8.96)	0.52
21 mo	43.13	43.74	−0.61 (−9.10 to 7.88)	0.34
24 mo	45.52	44.28	1.24 (−7.38 to 9.86)	0.68

SF-36-PF = Short Form-36 Health Survey physical function subscale.
* SF-36-PF (scale, 0–100) with mean scores in the rituximab and placebo groups, and differences between groups (with 95% CIs), for each of the 9 time points during follow-up.
† From the time-by-treatment interaction, for the simple contrasts in the time domain—i.e., to assess differences between treatment groups in change of the outcome measure at specific time points compared with baseline.