Safety and tolerability of arimoclomol in patients with sporadic inclusion body myositis: a randomised, double-blind, placebo-controlled, proof-of-concept trial

Sporadic inclusion body myositis (IBM) is the commonest idiopathic inflammatory myopathy (IIM) occurring in patients over the age of 50 years.[1-4] The prevalence of IBM differs between different populations and ethnic groups, with estimates between 4.9-70.6 per million population.[5-10] IBM is distinguished from the other IIM by early asymmetric finger flexor and knee extensor weakness (leading to loss of hand function and propensity to fall) and resistance to immunosuppressive therapy. Any skeletal muscle can be affected, including oesophageal and pharyngeal muscles. Late-stage disease can be characterized by very significant morbidity including disability and loss of quality of life. Death in IBM is related to malnutrition, cachexia, aspiration, respiratory infection and respiratory failure, as a consequence of dysphagia, severe global weakness and weakness of the respiratory muscles.[1-4] In a Dutch cohort, end-of-life care interventions were used by 13% of patients with IBM, highlighting the morbidity experienced by these patients and the need of supportive and palliative care in this disease.[2]

The aetiopathogenesis of IBM remains uncertain. The varied pathological findings observed have driven a number of theories including viral infection, accumulation of toxic proteins, autoimmune attack, myonuclear degeneration, endoplasmic reticulum stress and impairment of autophagy and proteasomal proteolysis.[11-15] Muscle biopsies from patients with IBM typically show several different pathological features broadly described as inflammatory or degenerative. Inflammatory features include endomysial infiltration by mononuclear cells (predominantly CD8+ T-cells), which surround and invade otherwise normal appearing muscle fibres, and overexpression of major histocompatibility complex (MHC) class I, which is not constitutively expressed by skeletal muscle. The degenerative features include the formation of rimmed vacuoles and aggregation of proteins, namely β-amyloid precursor protein (β-APP), phosphorylated tau (p-Tau), p62 and cytoplasm translocated TAR DNA-binding protein 43 (TDP-43).[16-19] Cytochrome *c* oxidase (COX) deficient fibres and ragged-red fibres reflect mitochondrial impairment.[20]

Previous clinical trials have only involved agents directed purely at the inflammatory component of IBM pathology. All were ineffective.[21] Whether the degenerative aspect of IBM is primary to the pathogenesis or not, it clearly plays a role in the deleterious effects of the disease in muscle fibres. Therefore modulating the protein handling mechanisms which may be disrupted in IBM muscle cells may be a particularly effective treatment strategy for IBM.

Protein homeostasis (proteostasis) is essential for normal cellular functions.[22] In the event of cell stress, proteins can become unfolded or misfolded leading to aggregation.[23] Protein misfolding is usually managed by endogenous chaperone proteins,[24] which ensure that proteostasis is maintained either by preventing the protein from interacting with others or by encouraging folding into it native structure. The main family of chaperone proteins are the heat shock proteins (HSP), which are up-regulated following activation of the heat shock response (HSR),[25] an endogenous, ubiquitous and protective cell mechanism. Therefore up-regulating the HSR in disorders with protein mishandling features, such as IBM, may be an effective therapeutic strategy.

Arimoclomol is a pharmacological agent that can up-regulate the HSR[26] and co-induce the synthesis of HSP.[27] Arimoclomol acts by prolonging the activity of the transcription factor, heat shock factor-1 (HSF-1), amplifying HSP gene expression[27] and further elevating the HSP levels already induced by cellular stresses, a response which appears to be attenuated with advanced age.[26-29] When administered in the absence of a stressor, these agents do not induce the HSR,[30] therefore potentially reducing the side effects of activation of the HSR. HSP have been shown to attenuate protein misfolding and aggregation promoting cellular defences against such processes[31]. Via inhibition of the proinflammatory transcription factor NFkB, they have also been shown to dampen inflammatory response. Notably, in an in vitro model of IBM (primary rat muscle cells transfected with β-APP in order to model the protein mishandling features of IBM), arimoclomol ameliorated several key pathological features of IBM-like pathology.[32,33]

Arimoclomol is generally non-toxic in animal models and healthy individuals[26,27,34] and has been indicated to have a variety of therapeutic benefits in several disorders including diabetic peripheral neuropathy and retinopathy (in animal model),[35] peripheral nerve injury (in animal model)[28,29] and amyotrophic lateral sclerosis (ALS) (in animal model and humans).[26,36] A phase II/III study with arimoclomol in familial ALS caused by mutations in the superoxide dismutase (SOD1) gene in ongoing (ClinicalTrials.gov identifier: NCT00706147).

Herein we report the results of the first randomized, double-blind, placebo-controlled, proof-of-concept trial of arimoclomol versus placebo for the treatment of IBM. Our aim was to evaluate the safety and tolerability of arimoclomol and to gather exploratory efficacy data of this orally administered amplifier of HSP expression in IBM.

Material and methods

Study design and patient population

In this investigator-initiated, double-blind, placebo-controlled study, 24 patients with IBM were randomised to arimoclomol 100mg or placebo (2:1 ratio), three time a day, over 4 months (as mandated by the Food and Drug Administration), followed by an 8-month follow up period. The study was conducted from August 2008 to May 2012, at two centres in two countries (12 patients in each Centre): University of Kansas Medical Centre (KUMC), Kansas, USA, and Medical Research Council (MRC) Centre for Neuromuscular Diseases, London, UK.

Patients fulfilling the following criteria were eligible for enrolment: 1) meet the Griggs diagnostic criteria for definite or probable IBM,[37] 2) muscle function adequate for quantitative muscle testing, with at least 8 of the following 16 muscle groups having a manual muscle testing (MMT) muscle grade ≥3- on the modified MRC scale – neck flexors, neck extensors, shoulder abductors, elbow flexors, elbow extensors, wrist flexors, knee extensors, knee flexors and ankle dorsiflexors, 3) age >50 years and 4) post-menopausal (no menses in >12 months) or status post-hysterectomy for females.

The presence of any of the following were exclusion criteria: 1) medical conditions: diabetes mellitus or patients taking anti-diabetic medications, chronic infection, chronic renal insufficiency, cancer other than skin cancer less than 5 years previously, multiple sclerosis or prior episode of central nervous system demyelination, or other chronic serious medical illnesses, 2) laboratory abnormalities: white blood cell count <3000/cm3, platelets <100,000/cm3, haematocrit <30%, urea >10mmol/L, creatinine >150µmol/L, symptomatic liver disease with serum albumin <30g/L, prothrombin time or activated partial thromboplastin time greater than the upper range of control values, 3) currently taking riluzole, 4) women who were pregnant or lactating, 5) history of non-compliance with other therapies, 6) coexistence of other neuromuscular disease, 7) drug or alcohol abuse within last 3 months, 8) inability to give informed consent, 9) known bleeding disorder (eg. haemophilia, Von Willebrand's Disease), 10) use of potentially nephrotoxic drugs and 11) prior difficulties with local anaesthetic.

The study was conducted according to the ethical principles of the Declaration of Helsinki and approved by the Independent Ethics Committee or Institutional Review Board for each centre. Informed consent was obtained from each patient before randomisation. The study is registered with ClinicalTrials.gov (NCT00769860) and with International Standard Randomised Controlled Trial Number Register (ISRCTN80057573).

Randomisation procedure and visit schedule

Randomisation was performed centrally for both study sites, at KUMC, by a General Clinical Research Centre (GCRC) statistician, who sent the randomisation codes to the respective research pharmacies. Codes were created using a using a random number generator table. Nobody involved in the conduct of the trial had access to the identity of the treatment assignments except for the unblinded statistician and the pharmacist at each site who labeled the study medication using codes provided by the unblinded statistician. The appearance of the placebo was identical to that of arimoclomol.

The treatment phase lasted 4 months. The remaining 8 months of the trial constituted a blinded clinical assessment phase. Visits were fortnightly during the first 4 months and monthly after that.

Primary outcome

An analysis of the safety and tolerability of arimoclomol as compared with placebo was the primary outcome. At every study visit participants were seen for assessment of adverse events. Unscheduled visits to evaluate potential adverse events could occur at any time. All serious adverse events (SAE) were reported to the sponsor and regulatory authorities according to standard operating procedures. The trial was monitored by an Independent Safety Monitoring Committee.

During the first 4 months (treatment phase) all participants completed a study medication diary. Pill bottles were brought to each visit for a count by a research team member to check on whether participants were taking the study medication in the appropriate dosages.

At screening and months 1, 2, 3, 4 and 12 the study participants had full safety laboratory studies including: full blood count with differential, prothrombin time, activated partial thromboplastin time, urea, creatinine and electrolytes, glucose, phosphate and calcium, alanine transaminase, aspartate transaminase, total bilirubin, albumin, full urine analysis and 24-hour urine protein content and creatinine clearance. At months 0.5, 1.5, 2.5, and 3.5 participants had partial safety laboratory studies including: serum creatinine, urea, electrolytes, glucose, phosphate, calcium and a full urine analysis. An electrocardiogram was performed at screening and months 1 and 3. An ophthalmic examination was performed at screening and month 10. The electrocardiogram and ophthalmic examination were introduced as safety measures owed to animal model safety concerns

of potentially accelerated cataract formation and apparent risk of sudden unexplained death with arimoclomol at very high doses and with arimoclomol used in conjunction with riluzole.

Physical function, muscle strength and fat-free mass

Physical function was measured using the IBM functional rating scale (IBMFRS). The IBMFRS is a functional rating scale that is intended only for patients with IBM. It includes 10 items (swallowing, handwriting, cutting food and handling utensils, fine motor tasks, dressing, hygiene, turning in bed and adjusting covers, changing position from sitting to standing, walking, and climbing stairs), graded on a Likert scale from 0 (being unable to perform) to 4 (normal). The sum of the 10 items gives a value between 0 and 40, with a higher score representing less functional limitation. The IBMFRS is a sensitive and reliable tool for assessing activities of daily living function in patients with IBM and it is quickly administered.[4,38-40]

Muscle strength was assessed by MMT and maximum voluntary isometric contraction testing (MVICT) using the Quantitative Muscle Assessment (QMA) system designed by Computer Source, Atlanta, Georgia, USA.[39,40] MMT was performed on 26 muscle groups: neck flexors and extensors, shoulder abductors, elbow flexors and extensors, wrist flexors and extensors, hip flexors, extensors and abductors, knee flexors and extensors, and ankle dorsiflexors and plantar flexors.[39,40] Muscle strength of each muscle group was graded utilizing a modified MRC score that was converted to a 13-point scale (grade 0 = 0, 1 = 1, 2- = 1.67, 2 = 2, 2+ = 2.33, 3- = 2.67, 3 = 3, 3+ = 3.33, 4- = 3.67, 4 = 4, 4+ = 4.33, 5- = 4.67, 5 = 5), and the average MMT scores for each patient were calculated. QMA was performed to measure MVICT on 12 muscle groups: shoulder abductors, elbow flexors and extensors, knee flexors and extensors, and hand grip. Each muscle was tested twice and the maximum force generated by the patient from the two trials was recorded for each muscle group. The total summed score of strength in kilograms was computed for each patient. MVICT has been shown to be reliable and valid in several neuromuscular disorders, including IBM.[39-41]

Body composition was obtained using a standard dual-energy X-ray absorptiometry (DEXA) whole body scan to assess total body fat-free mass. DEXA has been used to measure lean body mass in previous neuromuscular diseases' studies, including IBM.[39,40]

HSP70 levels in muscle tissue

A muscle biopsy was performed at baseline and end of the treatment period (month 4). Muscle biopsy tissue from the USA was shipped to the UK and muscle tissue analyses were centralized and performed simultaneously, with all staff blinded for treatment allocation.

Sandwich ELISA assay kits for determination of total human myosin content (USCN Life Science Inc E86098Hu) and HSP70 (Enzo ADI-EKS-700B) were used. Prior to ELISA assays, all muscle biopsies were stored at -80C and all samples were processed together. Samples were first homogenized in Extraction Reagent supplied with the HSP70 ELISA kit supplemented with protease inhibitors at an initial concentration of 0.5 g/ml using a hand held electronic homogenizer. Samples were then centrifuged at 21 000g for 10 minutes at 4 C in a refrigerated centrifuge. Supernatants were transferred into new tubes.

Protein concentration of each sample was determined using a BioRad DC Protein Assay that uses the Lowry method for the determination of protein content. Samples were then diluted to 0.5 mg/ml using Sample diluent supplied with the HSP70 ELISA kit. Following this step, another protein assay was carried out to get an accurate reading of actual protein concentrations in each sample. Samples were then aliquoted and stored until ELISA assay was carried out.

HSP70 and Myosin assays were carried out using the same aliquots of each sample. For the HSP70 assay, samples were diluted 1 in 8 in sample diluent and 100 µl of this solution was loaded onto the ELISA plates in duplicates, alongside HSP70 standard wells (also in duplicates, ranging from 0.78 ng/ml to 50 ng/ml). For the determination of total myosin content, samples were diluted 1 in 32 using sample diluent supplied with the Myosin ELISA kit and also 100 µl samples were loaded onto ELISA plates in duplicates, alongside Myosin standard (also in duplicates, concentration ranging from 0.156 ng/ml to 10 ng/ml). Both assays were then carried out according to manufacturer's description. Once absorbance in each plate was measured, HSP70 and myosin content in each sample was determined using the standard curves in each assay and expressed as ng/ml.

Raw HSP70 and Myosin values for each assay were first normalized to take into account the dilution factors. HSP70 content in each sample was then normalized for myosin content, so that for each sample HSP70 content was expressed in ng/100ng myosin.

Statistical methods

This was an exploratory study conducted without prior knowledge of effect size of arimoclomol in IBM. The sample size was chosen based on feasibility. Data management and statistical analysis were performed by GCRC informatics staff and a GCRC statistician. Data was entered blindly to a GCRC password protected electronic database and analyses were performed after database lock.

Descriptive statistics were used to summarize subject disposition and adverse events by treatment group. In order to reduce measurement error, baselines scores were computed by calculating the average of visit 1 (screening visit) and visit 2 (baseline visit), which had to be less that 21 days apart. Categorical variables were compared between treatment groups using Chi-square or Fisher´s exact test, as appropriate. Continuous variables were compared between treatment groups using the Mann-Whitney U test. Treatment groups were compared at baseline as well as regarding changes in the several outcome measures at 4 months (IBMFRS, MMT, MVICT, DEXA and muscle tissue analyses), 8 months (IBMFRS, MMT and MVICT) and 12 months (IBMFRS, MMT, MVICT and DEXA). Statistical analyses were performed using STATA v10 and in all analyses we took statistical significance at the two-sided 5% level.

Results

Patient disposition and baseline characteristics

Sixteen subjects were randomized to receive arimoclomol and eight to receive placebo. At 4 months (end of the treatment phase) all patients were still participating in the trial. At 8 months two patients had discontinued the study owed to travel difficulties, but one of these patients returned for the final assessment at 12 months (Figure 1). Mean age of the population was 67, the majority of patients being male (71%) and Caucasian (92%). Baseline clinical and demographic characteristics were similar between groups (Table 1). There was only one patient taking immunosuppressive medication; this patient was randomized to the arimoclomol group and was on a stable low dose of steroids for more than 3 months before starting the trial and throughout the entire trial period.

Figure 1. Patient disposition in the clinical trial

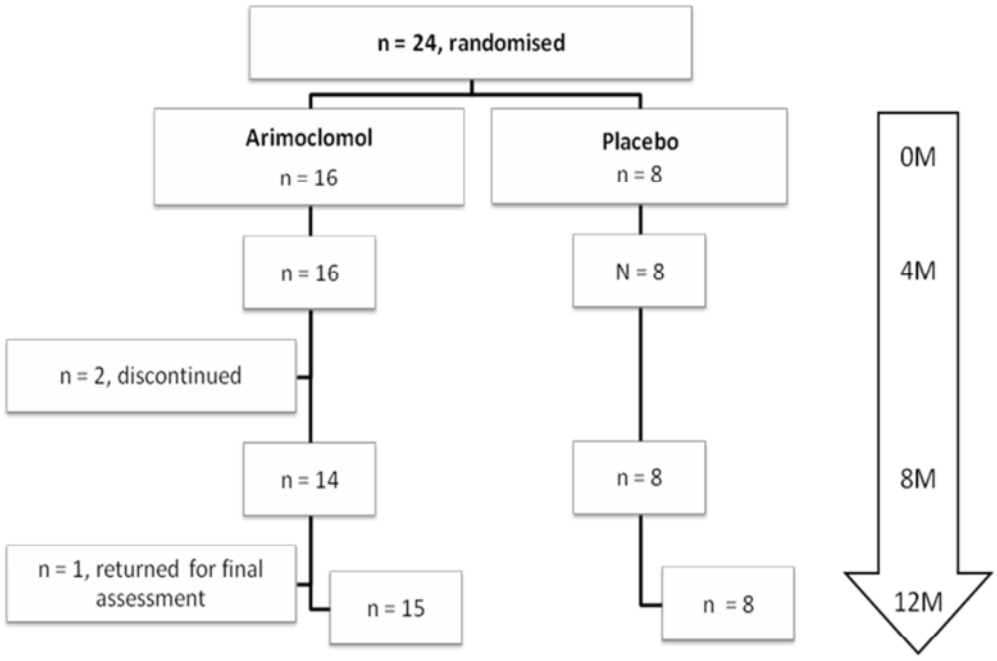


Table 1 Baseline characteristics of the study population

	All population (n=24)	**Arimoclomol (n=16)**	**Placebo (n=8)**	**p-value***
Sex (male), n (%)	17 (70.8)	12 (75.0)	5 (62.5)	0.647
Age, mean ± SD	66.84 ± 7.49	65.85 ± 7.86	68.83 ±6.70	0.426
Ethnicity, n (%)				1.000
- White, not of Hispanic origin	22 (91.7)	14 (87.5)	8 (100)	
- Black, not of Hispanic origin	1 (4.2)	1 (6.3)	0	
- Native American	1 (4.2)	1 (6.3)	0	
Disease duration, mean ± SD	8.4 ± 4.3	7.7 ± 4.5	9.8 ± 3.5	0.298
Initial symptoms/signs, n (%)				1.000
- Swallowing problems	1 (4.2)	1 (6.3)	0	
- Proximal upper extremity involvement	1 (4.2)	1 (6.3)	0	
- Distal upper extremity involvement	4 (16.7)	3 (18.8)	1 (12.5)	
- Proximal lower extremity involvement	18 (75.0)	11 (68.8)	7 (87.5)	
- Distal lower extremity involvement	1 (4.2)	1 (6.3)	0	
IBM diagnostic criteria, n (%)				1.000
- Definite IBM	10 (41.7)	7 (43.8)	3 (37.5)	
- Probable IBM	14 (58.3)	9 (56.3)	5 (62.5)	
IBMFRS score (0-40), mean ± SD	26.6 ± 6.4	27.5 ± 7.0	24.6 ± 5.1	0.375
MMT average score (0-5), mean ± SD	4.2 ± 0.4	4.2 ± 0.5	3.6 ± 1.5	0.540
MVICT sum score, mean ± SD	119.4 ± 63.4	130.4 ± 70.4	94.4 ± 39.8	0.198
Right quadriceps femoris MVICT score, mean ± SD	7.7 ± 8.2	8.8 ± 9.2	5.4 ± 5.4	0.240
Left quadriceps femoris MVICT score, mean ± SD	6.8 ± 6.8	7.3 ± 7.2	6.0 ± 6.2	0.462
Right hand grip MVICT score, mean ± SD	13.6 ± 11.8	15.2 ± 13.6	10.4 ± 6.3	0.373
Left hand grip MVICT score, mean ± SD	11.6 ± 11.5	13.5 ± 13.2	7.8 ± 5.9	0.111
DEXA total body fat free mass (Kg), mean ± SD	47.1 ± 11.3	49.3 ± 9.7	42.6 ± 13.6	0.221

*Arimoclomol versus placebo. DEXA, Dual-energy X-ray absorptiometry; IBM, sporadic Inclusion Body Myositis; IBMFRS, Inclusion Body Myositis Functional Rating Scale; MMT, manual muscle testing; MVICT, maximum voluntary isometric contraction testing; SD, standard deviation.

Safety and tolerability

There were no significant differences between treatment groups regarding the rate, type and severity of adverse events (Table 3). In the arimoclomol group, one serious adverse was reported: a study subject requiring prolonged hospitalization after the first trial muscle biopsy owed to persistent high blood pressure. This patient had known poorly controlled high blood pressure and the muscle biopsy was identified as a stressful event that raised the patient´s blood pressure. Blood pressure normalized after adjustment of the patient´s anti-hypertensive medication and kept within normal range throughout the trial. High blood pressure episodes were also observed in two placebo patients, under similar circumstances (same day as the muscle biopsy and previously known poorly controlled high blood pressure), but in these cases prolonged hospitalization was not required. Two cases of hyponatremia and one case of high thyroid count were observed in the arimoclomol group; however these changes were transient, asymptomatic and did not require specific treatment. The episode of hematuria in a patient belonging to the arimoclomol group was also auto-limited and did

not require specific treatment. All cases of infection resolved with standard treatments, with or without antibiotics, and did not require hospitalization. Ocular toxicity and arrhythmia were not observed in any study subjects.

Table 2 Adverse events over the course of 1 year.*

MedDRA System Organ Class	**Arimoclomol (16 patients)**	**Placebo (8 patients)**
Blood and lymphatic system disorders		
Cardiac disorders	Palpitations (n=1)	
Congenital, familial and genetic disorders		
Ear and labyrinth disorders	Tinnitus (n=2)	
Endocrine disorders		
Eye disorders	Conjunctivitis (n=1), eye pain (n=1)	Dry eyes (n=1)
Gastrointestinal disorders	Constipation (n=5), throat irritation (n=4), diarrhea (n=2), nausea (n=2), dry mouth (n=2), epigastralgia (n=1), gas pain (n=1), pyrosis (n=1), vomiting (n=1), geographic tongue (n=1)	Constipation (n=4), diarrhea (n=4), painful parotids (n=2)
General disorders and administration site conditions	Weight loss (n=1), dizziness (n=1), loss of consciousness (n=1)	Fatigue (n=1)
Hepatobiliary disorders		
Immune system disorders		
Infections and infestations	Sinus infection (n=2), upper respiratory tract infection (n=7), lower respiratory tract infection (n=2), erysipelas (n=1), tooth infection (n=1)	Tooth infection (n=4), upper respiratory tract infection (n=3), cellulitis (n=1), leg ulcer infection (n=1)
Injury, poisoning and procedural complications	Fall/contusion (n=23), post-biopsy pain (n=3), post-biopsy fatigue (n=1)	Fall/contusion (n=9), post-biopsy pain (n=1), pruritus in biopsy scar (n=1), finger cut (n=1)
Investigations	Hyponatremia (n=2), high thyroid count (n=1)	Spinal stenosis (n=1), herniated disk (n=1)
Metabolism and nutrition disorders		
Musculoskeletal and connective tissue disorders	Musculoskeletal pain (n=10), cramps (n=1), rheumatoid arthritis flare (n=1), heat and soreness of proximal lower limbs (n=1)	Musculoskeletal pain (n=2)
Neoplasms benign, malignant and unspecified (including cysts and polyps)		
Nervous system disorders	Headache (n=7), worsening of restless leg syndrome (n=1), paresthesia (n=1)	Headache (n=3), paresthesia (n=1), stroke (n=1)
Pregnancy, puerperium and perinatal conditions		
Psychiatric disorders		
Renal and urinary disorders	Hematuria (n=1)	
Reproductive system and breast disorders		Decreased libido (n=1)
Respiratory, thoracic and mediastinal disorders	Cough (n=2)	Cough (n=1),
Skin and subcutaneous tissue disorders	Rash (n=2), rosacea (n=1), insect bite with erythema (n=1), cold sores (n=1)	Rash (n=1)
Social circumstances		
Surgical and medical procedures	Tooth extraction (n=1), sinus surgery (n=1), solar lentigines removal (n=1)	Tooth extraction (n=1)
Vascular disorders	Hypertension (n=3), edema (n=2)	Hypertension (n=3), edema (n=3)
Average number of adverse events (AEs) per patient	**AEs = 6.8/patient**	**AEs = 6.5/patient**

Efficacy measures and muscle tissue analyses

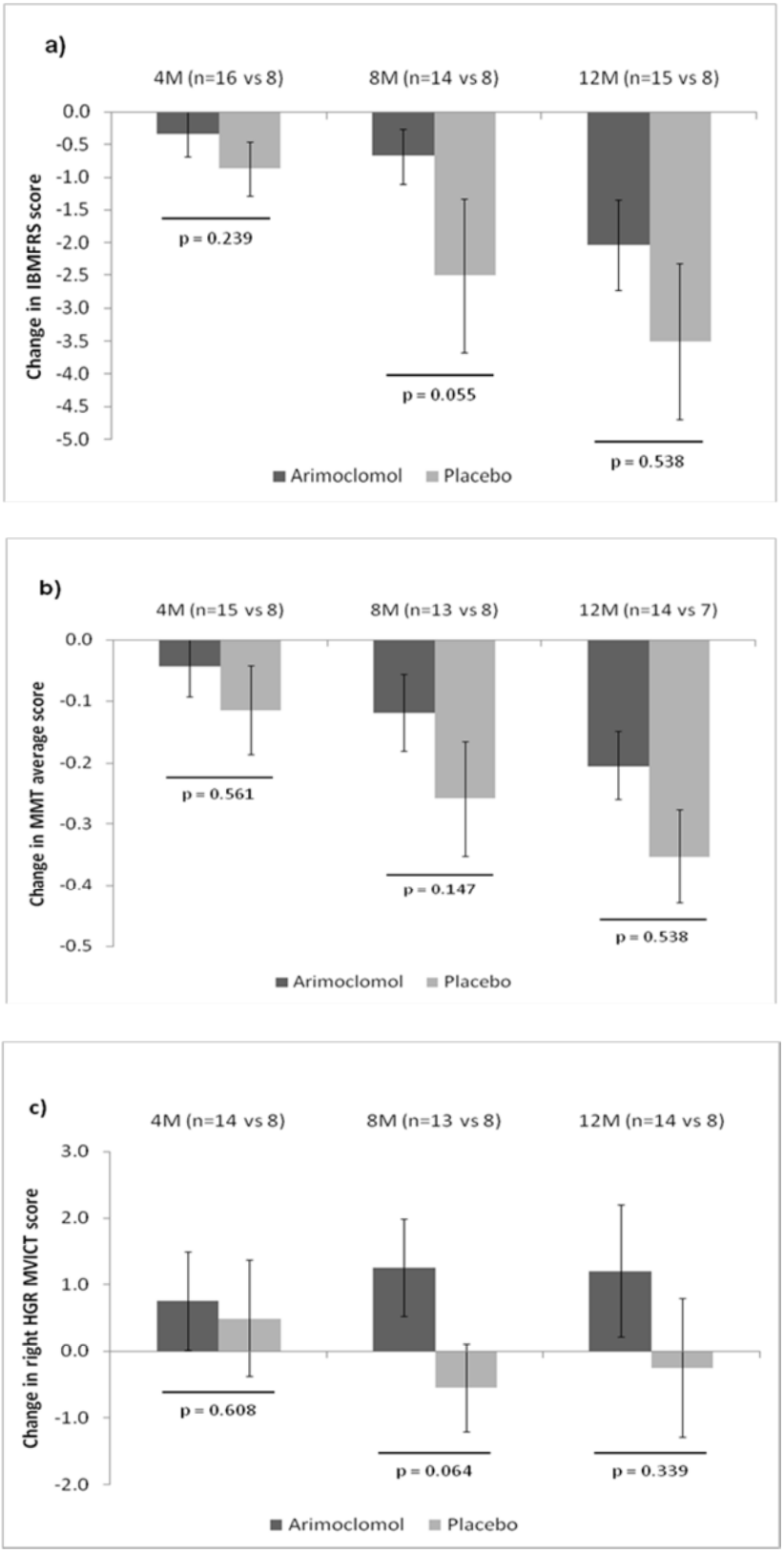
Physical function and muscle strength decline rates over time were numerically higher in the placebo group compared to the arimoclomol group (Table 3 and Figure 2). At 8 months, differences were almost statistically significant, with a p-value of 0.055 for the IBMFRS score, 0.147 for the average MMT score, and 0.064 for the right grip MVICT score, but no differences were seen for changes in the other MVICT scores (left hand grip, left and right quadriceps, and total sum score), changes in DEXA fat free mass percentage and changes in the myosin-adjusted HSP70 levels in the muscle tissue (Table 3).

Table 3 Mean changes (±standard deviation) throughout the study period

Outcome Variable	**Arimoclomol change**	**Placebo change**	**p-value**
IBMFRS score			
4M (n=16+8)	-0.34±1.38	-0.88±1.16	0.239
8M (n=14+8)	-0.68±1.58	-2.50±3.31	0.055
12M (n=15+8)	-2.03±2.68	-3.50±3.35	0.538
Average MMT score			
4M (n=15+8)	-0.04±0.19	-0.12±0.20	0.561
8M (n=13+8)	-0.12±0.22	-0.26±0.27	0.147
12M (n=14+7)	-0.21±0.21	-0.35±0.20	0.232
MVICT sum score			
4M (n=14+8)	0.46±12.11	-0.30±14.49	0.633
8M (n=13+8)	7.20±19.65	-1.71±17.80	0.347
12M (n=14+8)	-1.21±20.76	0.52±17.98	0.946
Right quadriceps femoris MVICT score			
4M (n=14+8)	0.33±2.37	0.33±1.49	0.495
8M (n=13+8)	0.21±1.65	-0.09±2.14	0.942
12M (n=14+8)	-0.44±2.18	-0.00±1.89	0.453
Right hand grip MVICT score			
4M (n=14+8)	0.76±2.74	0.50±2.46	0.608
8M (n=13+8)	1.26±2.63	-0.54±1.86	0.064
12M (n=14+8)	1.21±3.70	-0.24±2.94	0.339
Left quadriceps femoris MVICT score			
4M (n=15+8)	0.65±1.26	-0.20±1.22	0.302
8M (n=13+8)	0.31±1.77	-0. 19±1.41	0.664
12M (n=14+8)	-0.44±1.28	-0.07±2.35	0.707
Left hand grip MVICT score			
4M (n=14+8)	0.92±2.44	1.201±2.47	0.946
8M (n=13+8)	1.09±2.43	1.43±3.38	0.638
12M (n=14+8)	1.04±3.10	0.52±2.22	0.891
DEXA total body fat free mass percentage, mean ± SD			
4M (n=15+8)	1.3±1.3	1.9±2.8	0.949
12M (n=14+8)	-2.0±3.8	-1.0±2.0	0.339
HSP70 levels (ng/100ng myosin), mean ± SD			
4M (n=15+8)	-110.72±757.40	-34.70±336.35	0.466

DEXA, Dual-energy X-ray absorptiometry; HSP, heat shock protein; IBM, sporadic Inclusion Body Myositis; IBMFRS, Inclusion Body Myositis Functional Rating Scale; MMT, manual muscle testing; MVICT, maximum isometric voluntary contraction testing; SD, standard deviation.

Figure 2 Bar charts showing change from baseline to endpoint (mean ± SEM) in a) IBMFRS score, b) MMT average score, and c) right hand grip MVICT score.



Discussion

This is the first study to examine the effects of amplification of HSP expression in a cohort of patients with IBM. Arimoclomol was safe and well tolerated in our study. A trend of slower decline in muscle strength (measured by MMT and right hand grip MVICT) and physical function (measured by the IBMFRS) was observed in the arimoclomol group compared with placebo.

The frequency and type of AEs was similar between the arimoclomol and the placebo groups and the arimoclomol safety profile was consistent with previous studies in healthy volunteers and in ALS.[34,36] The observation of consistent numerical differences favoring arimoclomol compared to placebo in physician reported (MMT and right hand grip MVICT) and patient reported outcomes (IBMFRS) support the view that arimoclomol might exert biologically relevant effects is IBM and might be able to at least slow down the disease process. It is curious that the main effect was at 8 months only, not 4 or 12 months. One explanation is that this could be a spurious finding in this 4-month treatment trial. Another possibility is that muscle regeneration in this slow disease may be delayed leading to the 8 month observation of a trend following a 4-month treatment epoch but that this effect may wane at 12 months. On the other hand, the fact that muscle strength and physical function decline rates were always numerically higher in the placebo group compared to the arimoclomol group suggests that the inability to demonstrate a statistical significant difference between groups at multiple timepoints may simply be related to the fact that the study was not powered for efficacy.

After 4 months of treatment with arimoclomol, we did not observe differences between groups regarding changes in HSP70 levels in the muscle tissue. This may be related to the fact that opposite sides of the body (albeit the same muscle) was biopsied at baseline and after treatment. HSP70 levels are sensitive to physiological stress, such as exercise-induced stress, which may have influenced the results. Factors such as individual muscles' disease stage, the level of physical activity prior to the muscle biopsy and the level of manipulation of the muscle tissue during the biopsy procedure itself may have influenced the HSP70 results, questioning the validity of this read-out system.[42-44] Additional limitations of our study include the small sample size and the small duration of treatment (4 months, as mandated by the Food and Drug Administration, based on the available human safety data at the time the study was conducted). Furthermore, this study was underpowered to achieve statistical significance for secondary outcomes.

Arimoclomol has previously been shown to be generally safe in animal models and humans[26,27,34] and has been indicated to have a variety of therapeutic benefits in several disorders including diabetic peripheral neuropathy and retinopathy, where up-regulation of HSP appears to be

beneficial.[35] The effects of arimoclomol have been assessed in adult rats following sciatic nerve injury and showed morphological improvement in sensory neuron markers and restored functional activity of sensory fibres[29] and in motoneurons.[28] Arimoclomol has also been found to have major therapeutic effects on mouse models of ALS where up-regulation of HSP in mutant SOD1G93A mice has proved beneficial.[26] In arimoclomol treated SOD1G93A mice there was a reduction in muscle atrophy and an improvement in muscle tone, contractile characteristics and motoneuron survival; arimoclomol delayed disease progression and increased mice lifespan.

Arimoclomol has been through seven phase I clinical trials in healthy volunteers to assess safety and tolerability as well as the pharmacokinetic properties of the compound. In addition a small-scale phase II trial on ALS patients has also been completed. The results from these trials have shown arimoclomol to be safe and well tolerated in both healthy individuals and ALS patients.[34] Further parameters were investigated subsequently in a phase IIa dose-ranging ALS trial. This multi-centre, double-blind, placebo-controlled study assessed safety, tolerability and cerebrospinal fluid penetration as well as disease outcome measures using the ALS functional rating scale and physical examination. Up to 100mg three times daily was given to patients and this dosage was found to be safe and well tolerated.[36] At present a phase II/III double-blind, randomized, placebo-controlled trial is underway to evaluate the safety and efficacy of arimoclomol in familial ALS patients with mutations in SOD1 (ClinicalTrials.gov identifier: NCT00706147). The hypothesis of this study is that arimoclomol, at 200mg three times a day, will be safe, well tolerated, and reduce disease progression by at least a rate of 30%.

The results of our IBM trial are supported by recent in vitro data using a model in which primary rat muscle cells in vitro were transfected with β-APP in order to model the protein mishandling features of the disease.[32,33] Over-expression of human β-APP in primary rat muscle cells recapitulated several of the key pathological characteristics of IBM, including the formation of intracellular inclusion bodies which were immunoreactive for β-APP and ubiquitin as well as Aβ-42, TDP-43, p-Tau, caspase-3, HSP70 and p62. In addition, β-APP transfection resulted in activation of the NFkB cascade as well as endoplasmic reticulum (ER) stress. Using this model, the effects of treatment with arimoclomol on these IBM-like pathological characteristics were assessed. Following treatment with arimoclomol, there was a significant increase cell survival, an increase in HSP70 expression and a significant reduction in the formation of ubiquitinated inclusions in β-APP transfected myotubes. In addition, p62 inclusions and cytoplasmic mislocalisation of TDP-53 were dramatically reduced in arimoclomol-treated cultures. Furthermore, the proportion of β-APP transfected myotubes in which the NFkB cascade was activated was also reduced by treatment with arimoclomol. Finally, examination of ER calcium handling and markers of ER stress revealed that β-

APP transfection resulted in a significant reduction in ER calcium concentration (an indicator of ER stress), a deficit that was completely prevented by arimoclomol. This dramatic and beneficial effect of arimoclomol on ER stress was reflected in a reduction in the expression of the ER stress markers CCAAT/enhancer-binding protein (C/EBP)-homologous protein (CHOP) and binding immunoglobulin protein (BiP) in arimoclomol-treated β-APP transfected myotubes, compared to untreated cultures. Together these *in vitro* results indicate that arimoclomol ameliorates several key pathological features of IBM-like pathology, providing supportive information in the context of the human trial we know report.

In conclusion, arimoclomol was safe and well tolerated and demonstrated a preliminary signal for potential therapeutic benefit in patients with IBM. These data support further research of arimoclomol in IBM.

References:

1. Dimachkie, M.M. & Barohn, R.J. Inclusion body myositis. *Curr Neurol Neurosci Rep* **13**, 321 (2013).
2. Cox, F.M. *et al.* A 12-year follow-up in sporadic inclusion body myositis: an end stage with major disabilities. *Brain* **134**, 3167-75 (2011).
3. Benveniste, O. *et al.* Long-term observational study of sporadic inclusion body myositis. *Brain* **134**, 3176-84 (2011).
4. Cortese, A. *et al.* Longitudinal observational study of sporadic Inclusion Body Myositis: implications for clinical trials. *Neuromuscular Disorders* (2013).
5. Badrising, U.A. *et al.* Epidemiology of inclusion body myositis in the Netherlands: a nationwide study. *Neurology* **55**, 1385-7 (2000).
6. Phillips, B.A., Zilko, P.J. & Mastaglia, F.L. Prevalence of sporadic inclusion body myositis in Western Australia. *Muscle Nerve* **23**, 970-2 (2000).
7. Felice, K.J. & North, W.A. Inclusion body myositis in Connecticut: observations in 35 patients during an 8-year period. *Medicine (Baltimore)* **80**, 320-7 (2001).
8. Wilson, F.C., Ytterberg, S.R., St Sauver, J.L. & Reed, A.M. Epidemiology of sporadic inclusion body myositis and polymyositis in Olmsted County, Minnesota. *J Rheumatol* **35**, 445-7 (2008).
9. Needham, M. *et al.* Prevalence of sporadic inclusion body myositis and factors contributing to delayed diagnosis. *J Clin Neurosci* **15**, 1350-3 (2008).
10. Shamim, E.A. *et al.* Differences in idiopathic inflammatory myopathy phenotypes and genotypes between Mesoamerican Mestizos and North American Caucasians: ethnogeographic influences in the genetics and clinical expression of myositis. *Arthritis Rheum* **46**, 1885-93 (2002).
11. Greenberg, S.A. Theories of the pathogenesis of inclusion body myositis. *Curr Rheumatol Rep* **12**, 221-8 (2010).
12. Dalakas, M.C. Pathogenesis and therapies of immune-mediated myopathies. *Autoimmun Rev* **11**, 203-6 (2012).
13. Greenberg, S.A. Inclusion body myositis. *Curr Opin Rheumatol* **23**, 574-8 (2011).
14. Askanas, V. & Engel, W.K. Inclusion-body myositis, a multifactorial muscle disease associated with aging: current concepts of pathogenesis. *Curr Opin Rheumatol* **19**, 550-9 (2007).
15. Needham, M. & Mastaglia, F.L. Inclusion body myositis: current pathogenetic concepts and diagnostic and therapeutic approaches. *Lancet Neurol* **6**, 620-31 (2007).
16. Salajegheh, M. *et al.* Sarcoplasmic redistribution of nuclear TDP-43 in inclusion body myositis. *Muscle Nerve* **40**, 19-31 (2009).
17. Weihl, C.C. *et al.* TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. *J Neurol Neurosurg Psychiatry* **79**, 1186-9 (2008).
18. Askanas, V. & Engel, W.K. Inclusion-body myositis: a myodegenerative conformational disorder associated with Abeta, protein misfolding, and proteasome inhibition. *Neurology* **66**, S39-48 (2006).
19. Nogalska, A., Terracciano, C., D'Agostino, C., King Engel, W. & Askanas, V. p62/SQSTM1 is overexpressed and prominently accumulated in inclusions of sporadic inclusion-body myositis muscle fibers, and can help differentiating it from polymyositis and dermatomyositis. *Acta Neuropathol* **118**, 407-13 (2009).
20. Oldfors, A. *et al.* Mitochondrial abnormalities in inclusion-body myositis. *Neurology* **66**, S49-55 (2006).
21. Breithaupt, M. & Schmidt, J. Update on treatment of inclusion body myositis. *Curr Rheumatol Rep* **15**, 329 (2013).
22. Douglas, P.M. & Cyr, D.M. Interplay between protein homeostasis networks in protein aggregation and proteotoxicity. *Biopolymers* **93**, 229-36 (2010).

23. Kopito, R.R. Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol* **10**, 524-30 (2000).
24. Brown, I.R. Heat shock proteins and protection of the nervous system. *Ann N Y Acad Sci* **1113**, 147-58 (2007).
25. Kalmar, B. & Greensmith, L. Activation of the heat shock response in a primary cellular model of motoneuron neurodegeneration-evidence for neuroprotective and neurotoxic effects. *Cell Mol Biol Lett* **14**, 319-35 (2009).
26. Kieran, D. *et al.* Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. *Nat Med* **10**, 402-5 (2004).
27. Hargitai, J. *et al.* Bimoclomol, a heat shock protein co-inducer, acts by the prolonged activation of heat shock factor-1. *Biochem Biophys Res Commun* **307**, 689-95 (2003).
28. Kalmar, B. *et al.* Upregulation of heat shock proteins rescues motoneurones from axotomy-induced cell death in neonatal rats. *Exp Neurol* **176**, 87-97 (2002).
29. Kalmar, B. *et al.* The effect of treatment with BRX-220, a co-inducer of heat shock proteins, on sensory fibers of the rat following peripheral nerve injury. *Exp Neurol* **184**, 636-47 (2003).
30. Vigh, L. *et al.* Bimoclomol: a nontoxic, hydroxylamine derivative with stress protein-inducing activity and cytoprotective effects. *Nat Med* **3**, 1150-4 (1997).
31. Muchowski, P.J. & Wacker, J.L. Modulation of neurodegeneration by molecular chaperones. *Nat Rev Neurosci* **6**, 11-22 (2005).
32. Ahmed, M., Miller, A., Hanna, M.G. & Greensmith, L. Investigating the effects of pharmacological up-regulation of the heat shock response in an in-vitro model of sporadic inclusion body myositis. *Neuromuscular Disorders* **22**, S26-S26 (2012).
33. Miller, A., Ahmed, M., Hanna, M.G. & Greensmith, L. Developing New Therapeutic Strategies for Inclusion Body Myositis. *Journal of Neurology Neurosurgery and Psychiatry* **83**(2012).
34. Lanka, V., Wieland, S., Barber, J. & Cudkowicz, M. Arimoclomol: a potential therapy under development for ALS. *Expert Opin Investig Drugs* **18**, 1907-18 (2009).
35. Kurthy, M. *et al.* Effect of BRX-220 against peripheral neuropathy and insulin resistance in diabetic rat models. *Ann N Y Acad Sci* **967**, 482-9 (2002).
36. Cudkowicz, M.E. *et al.* Arimoclomol at dosages up to 300 mg/day is well tolerated and safe in amyotrophic lateral sclerosis. *Muscle Nerve* **38**, 837-44 (2008).
37. Tawil, R. & Griggs, R.C. Inclusion body myositis. *Curr Opin Rheumatol* **14**, 653-7 (2002).
38. Jackson, C.E., Barohn, R.J., Gronseth, G., Pandya, S. & Herbelin, L. Inclusion body myositis functional rating scale: a reliable and valid measure of disease severity. *Muscle Nerve* **37**, 473-6 (2008).
39. The Muscle Study Group. Randomized pilot trial of betaINF1a (Avonex) in patients with inclusion body myositis. *Neurology* **57**, 1566-70 (2001).
40. The Muscle Study Group. Randomized pilot trial of high-dose betaINF-1a in patients with inclusion body myositis. *Neurology* **63**, 718-20 (2004).
41. Rose, M.R. *et al.* A prospective natural history study of inclusion body myositis: implications for clinical trials. *Neurology* **57**, 548-50 (2001).
42. Njemini, R. *et al.* Circulating heat shock protein 70 in health, aging and disease. *BMC Immunol* **12**, 24 (2011).
43. Henstridge, D.C. *et al.* The relationship between heat shock protein 72 expression in skeletal muscle and insulin sensitivity is dependent on adiposity. *Metabolism* **59**, 1556-61 (2010).
44. Morton, J.P., Kayani, A.C., McArdle, A. & Drust, B. The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. *Sports Med* **39**, 643-62 (2009).