

# Therapeutic efficacy and safety of chaperonin 10 in patients with rheumatoid arthritis: a double-blind randomised trial

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## Summary

### Background

Chaperonin 10 (heat shock protein 10, XToll™) has anti-inflammatory properties related to the inhibition of Toll-like receptor signalling pathways. Our aim was to establish whether chaperonin 10 is safe and effective in the treatment of rheumatoid arthritis.

### Methods

In this randomised, double-blind, multicentre study, 23 patients with moderate to severe active rheumatoid arthritis receiving disease modifying antirheumatic drugs were randomly allocated to three treatment groups receiving intravenous chaperonin 10 twice weekly for 12 weeks at doses of 5 mg (n=8), 7.5 mg (8), or 10 mg (7). The primary outcomes were change in disease activity score (DAS28) and improvement of core disease measures (American College of Rheumatology response score) from baseline to week 12. All analyses were done by intention to treat. This study is registered with the Australian Clinical Trials Registry, number ACTRNO12606000041550.

### Findings

Primary endpoint measures improved from day 14 in all groups and continued to improve to day 84. By end of study, a 20% improvement of core disease measures was seen in six (86%, 95% CI 43–100), a 50% improvement in four (57%, 14–86), and a 70% improvement in two (29%, 0–57) patients given the highest dose of chaperonin 10. Clinical remission (as defined by a DAS28 <2.6) was achieved in three (13%) of 23 patients. Three individuals dropped out during the study: one in the 5 mg group (rheumatoid arthritis not controlled), one in the 7.5 mg group (adverse event), and one in the 10 mg group (lost to follow-up). The most common adverse events were exacerbation of rheumatoid arthritis (both during and after the study) and upper respiratory tract infection. Only one adverse event was judged to be of severe intensity.

### Interpretation

Chaperonin 10 seems to be well tolerated and efficacious in treatment of the symptoms of rheumatoid arthritis, at least in the short term.

## Introduction

Chaperonin 10, which is also called heat shock protein 10, is a 70 kDa mitochondrial protein (heptamer) known mainly for its role in intracellular protein folding, in concert with heat shock protein 60.<sup>1</sup> Extracellular chaperonin 10 might have a role in the modulation of the innate immune response. The molecule has anti-inflammatory and immunomodulatory properties via inhibition of downstream events in Toll-like receptor (TLR) activated pathways.<sup>2</sup> Due to their roles in the regulation and linking of the immune and inflammatory responses, TLRs and their exogenous and endogenous ligands, which propagate inflammation, are the subject of intense research.<sup>3–5</sup> TLRs are highly expressed in synovial tissue<sup>3,6,7</sup> and their activation contributes to the pathological processes of autoimmune and chronic inflammatory diseases such as rheumatoid arthritis. We have shown that chaperonin 10 inhibits both the TLR4-mediated induction of nuclear factor kappa B (NFκB) activation by lipopolysaccharide and the production of tumour necrosis factor (TNF) α and interleukin 6 in human peripheral blood mononuclear cells (PBMC) from healthy volunteers and patients with multiple sclerosis.<sup>2,8</sup>

Although the initial trigger of rheumatoid arthritis is not defined, many cell populations seem to be implicated. Inflammatory cells—including monocytes, macrophages, dendritic cells, T cells, and B cells—infiltrate the synovial membrane, where together with chondrocytes, osteoclasts, and synovial fibroblasts they secrete cytokines, chemokines, and matrix metalloproteinases, inducing osteoclastogenic activity and resulting in the erosion of cartilage and bone.<sup>9–12</sup> Many pro-inflammatory cytokines (including TNFα, interleukin 1, and interleukin 6) and anti-inflammatory cytokines (interleukin 4, interleukin 10, tumour growth factor β) are released. The inflammatory response in rheumatoid arthritis is thought to be a result of the balance of these mediators shifting in favour of the pro-inflammatory cytokines, thus driving the arthritic process. TNFα is a critical mediator in the inflammatory response cascade<sup>9–12</sup> and is the target for several of the latest registered treatments for rheumatoid arthritis, including two monoclonal antibodies (infliximab and adalimumab)<sup>11,13</sup> and a recombinant TNF decoy receptor (etanercept).<sup>14,15</sup> The TNF inhibitors are more specific in their activity than

are disease-modifying antirheumatic drugs (DMARDs; eg, methotrexate), which often become ineffective over time and can produce serious adverse events.<sup>16</sup>

The primary objective of this study was to determine the clinical efficacy and safety of chaperonin 10 in patients with moderate-to-severe active rheumatoid arthritis, even with concurrent treatment with standard DMARDs.

## Methods

### Participants

Otherwise healthy non-pregnant patients aged 18–75 years with disease duration of at least 6 months, onset of rheumatoid arthritis after 18 years of age (with one exception), and classified as American College of Rheumatology (ACR) functional class I–III<sup>17,18</sup> were eligible for enrolment. Enrolment criteria included active disease at screening, which was defined as eight or more tender and swollen joints, and either an ESR of 28 mm/h or more, a C-reactive protein (CRP) concentration of 10 mg/L or greater, or morning stiffness for 45 min or more, plus a disease activity score (DAS28) of more than 3.2. Patients taking DMARDs were eligible for inclusion if they were on a stable dose for at least 3 months before screening and remained on this regimen throughout the study. Patients on stable doses of one non-steroidal anti-inflammatory drug (NSAID) plus up to 10 mg daily prednisone or equivalent were also included. Exclusion criteria included treatment with DMARDs with unstable doses within 3 months of screening, prior treatment with anti-TNF $\alpha$  agents, current use of more than 10 mg per day prednisone or treatment with intra-articular corticosteroid injection within 3 weeks of screening. Patients with current infection or previous malignant tissue were also excluded. Individuals with abnormal blood tests or renal or liver function were not eligible for inclusion.

All patients gave written and informed consent at the time of enrolment. The protocol was approved by ethics committees at local centres and notified to the appropriate local regulatory authority. The study was done in accordance with the International Conference on Harmonisation of Good Clinical Practice and with the Helsinki Declaration.

### Procedures

The trial was a randomised, double-blind, multicentre study with three treatment arms of 5 mg, 7.5 mg, or 10 mg chaperonin 10 given intravenously twice a week. A blocked random allocation sequence was generated with Microsoft Excel. An unblinded person at each study site called a central coordination centre, who allocated the next available randomisation number for eligible patients. This unblinded person was responsible for preparation of chaperonin 10 injections. Once the injection was prepared in the syringe the dose of chaperonin 10 was indistinguishable. The unblinded person at each site did not do any trial assessments. During the treatment period, patients attended the study centre twice per week for 12 weeks, with safety and efficacy assessments done every 2 weeks. Final efficacy assessments were done at the end of 12 weeks treatment (day 84), with safety followup 4 weeks later (day 112).

The primary efficacy endpoint was the disease activity score (DAS28); the improvement of core disease measures (ACR) was also assessed. DAS28 is a composite score (scale 2–10) where a score of more than 5.1 indicates high disease activity, under 3.2 low disease activity, and below 2.6 disease remission, as defined and validated by criteria established by the European League against Rheumatism (EULAR).<sup>19</sup> The ACR response is a means to assess improvement in core disease measures; ACR20, 50, and 70 indicate a 20%, 50%, and 70% improvement, respectively. Secondary endpoints included tender and swollen joint counts, mean duration of morning joint stiffness, and the physicians' and patients' assessment of overall disease activity. A health assessment questionnaire and the short form 36 (SF36) were used to assess physical function and health-related quality of life for every patient. Safety assessments included analysis of treatment-emergent adverse events, changes in vital signs, and standard laboratory tests. An ELISA was used to detect the development of antibodies against chaperonin 10 in serum samples.<sup>20</sup>

We used an in-vitro assay to measure the in-vivo biological activity of chaperonin 10. PBMC were isolated from fresh whole blood in CPT heparin tubes (BD Biosciences, San José, CA, USA), and stimulated with lipopolysaccharide, as described previously;<sup>2</sup> endotoxin-free conditions were maintained throughout the isolation and stimulation procedures. Cytokine production was measured by cytometric bead array technology (Human Inflammation Kit, BD CBA software, BD Biosciences) according to manufacturer's instructions. To reduce interassay variability, patient PBMC samples were obtained and frozen in liquid nitrogen over the course of the trial

and were assayed together on the same day and within the same microplate. Identical lots of critical reagents were used throughout the analysis process. No significant variation was noted between replicates for any sample done on separate days (data not shown).

### Statistical analysis

With the exception of ACR, a parametric analysis of variance (ANOVA, repeated measures model) was used to compare any apparent differences between groups at the  $p < 0.05$  level. We also used ANOVA at all time points after screening (days 14, 28, 42, 56, 70, 84) to compare the change in value of indices from baseline across the three treatment groups. Furthermore, at every time point, values were compared with baseline for the individual groups and for all groups combined with a paired  $t$  test.

The ordinal data resulting from the use of DAS28 and other assessments of rheumatoid arthritis have much the same distributions, and no gross outliers, so that the use of a means-based analysis such as that done with ANOVA could be expected to be reasonably robust.

We compared the differences in proportions of patients achieving the various ACR response levels with  $\chi^2$  tests. All  $p$  values are post-hoc, had no adjustment made for multiple testing, and are regarded as measures of interest. Baseline cytokine assay samples were missing for four patients (two in the 5 mg group and one in both of the other groups) because of laboratory error during PBMC isolation. For these individuals, day 14 cytokine production values were carried backward to baseline—ie, no change assumed between baseline and day 14. Statistical analysis was done by external independent statisticians. All analyses were done by intention to treat.

This study is registered with the Australian Clinical Trials Registry, number ACTRN01260600041550.

### Role of the funding source

This study was sponsored and coordinated by CBio Ltd. The principal investigator and co-investigators obtained patient data for the study and the sponsor was responsible for data collection and analysis. All authors had access to all the data. All investigators and the sponsor designed the study, interpreted the data, wrote the manuscript, and decided to submit for publication. Cytokine and antichaperonin 10 antibody analyses were done at CBio Ltd.

## Results

Figure 1 shows the trial profile. The baseline characteristics of the three groups are shown in table 1. The ratio of women to men was higher in the 7.5 mg group than the other groups and three patients in the same group were not taking any concurrent DMARDs.

Three patients withdrew early from the study (figure 1). One patient in the 10 mg group reached the end of the study but failed to attend the safety follow-up visit. One patient in the 7.5 mg group withdrew because of an adverse event of cellulitis. The other early withdrawal was a 30-year-old woman who had a disease history of only 6 months at the time of study enrolment. At day 28, the patient showed no improvement and the investigator decided it was in the best interest of the patient to withdraw her from the study to initiate more intensive therapy with DMARDs.

DAS28 significantly improved from baseline to day 14 in all three groups (5 mg:  $p = 0.0449$  vs baseline; 7.5 mg:  $p = 0.0126$  vs baseline; 10 mg:  $p = 0.0182$  vs baseline) and continued to improve through to day 84 in all three groups (5 mg:  $p = 0.0045$  vs baseline; 10 mg:  $p = 0.0039$  vs baseline; 7.5 mg:  $p = 0.0294$  vs baseline; table 2 and figure 2). There was no evidence of a difference in DAS28 response between the three groups (table 2;  $p = 0.1538$ ).

On the basis of EULAR definitions of response according to DAS28 at day 84, six (75%) patients in the 5 mg group achieved a moderate response. One (13%) patient in the 7.5 mg group achieved a moderate response and one (13%) a good response. In the 10 mg group, two (29%) individuals achieved a moderate response and four (57%) achieved a good response.

Table 2 shows the number of patients who achieved ACR20, 50, or 70 by day 84; the rates of onset of such improvements are presented in figure 3. A 20% improvement in core disease measures was rapidly achieved in all three groups, with seven (30%) patients showing such an improvement by day 14 (figure 3). At day 84, a 70% improvement was seen in two patients in the 5 mg group and two in the 10 mg group, although onset was more rapid in the 10 mg group compared with the other groups (figure 3). No individuals achieved such an improvement in the 7.5 mg group, possibly because of sample size. There were generally

no significant between-group differences in ACR response at end of study, although there was some indication of a difference in the ACR50 score ( $p=0.0422$ ; table 2). The lower response rate seen in the 7.5 mg group did not seem related to the sex ratio of the group or the fact that three patients were not taking concurrent DMARDs.

All secondary endpoint measures, with the exception of ESR and CRP concentration, improved from baseline to day 14 and continued to show improvements across the study period to day 84 in the 10 mg group (table 3). We did not require raised ESR or CRP concentration for a patient to be included in this study, since these measures are often controlled with DMARDs, or prednisolone. Before treatment, only eight (35%) patients showed raised ESR and six (26%) had an increased CRP concentration. Apart from one patient in the 7.5 mg group, ESR and CRP concentrations improved in patients with raised baseline values (mean ESR fell by 41% and mean CRP concentration by 43% by day 84).

A significant change in health assessment questionnaire score from screening to day 14 ( $p=0.0175$ ) was sustained to day 84 in the 5 mg group ( $p=0.0119$ ), and from day 70 (mean change 0.376 [SD 0.32];  $p=0.0218$ ) onwards in the 10 mg group. The SF36 total score in the 10 mg group showed significant improvement at day 56 ( $p=0.0212$ ), which was sustained through to day 84 ( $p=0.0028$ ). Subgroup analysis of SF36 showed the improvements were within the role physical ( $p=0.0186$ ), body pain ( $p=0.0326$ ), and vitality ( $p=0.0185$ ) sections of the questionnaire.

When all treatment groups were combined, the production of a range of cytokines fell significantly by day 56 of treatment compared with baseline (table 4), including TNF $\alpha$  (two-tailed  $p=0.0118$ ), interleukin 1 $\beta$  ( $p=0.0055$ ), interleukin 6 ( $p=0.0086$ ) and interleukin 10 ( $p=0.0106$ ; day 28 only). This study is regarded as exploratory, and when treatment groups were examined individually, one-tailed  $p$  values were used in a post-hoc manner. Chaperonin 10 showed some effect on interleukin 6 and interleukin 1 $\beta$  by end of study, with the greatest effect with the higher doses (figure 4). Little effect was seen on the other cytokines, although 7.5 mg chaperonin 10 showed some effect on TNF $\alpha$  by end of study (figure 4).

No infusion reactions were reported after intravenous administration of chaperonin 10. The most commonly noted adverse events were recurrence or exacerbation of symptoms of rheumatoid arthritis; upper respiratory tract infections were the next most common adverse event. There did not seem to be any dose-dependent adverse events. Investigators rated adverse events as unrelated or unlikely to be related to study drug in four (17%) cases, possibly related to study drug in 14 (61%), and probably related in four (17%). Only one adverse event—myalgia—was rated as severe in intensity, with all other adverse events mild (12 cases; 52%) or moderate (ten cases; 43%) in severity. There were no consistent laboratory abnormalities or changes in vital signs noted in any patients during the study. Seven patients recorded an adverse event of rheumatoid arthritis flare 10–32 days after their last dose of chaperonin 10 (table 5). Furthermore, one patient had pain in the metacarpophalangeal and shoulder joints (arthralgia) 10 days post-dose.

One patient had cellulitis of the left forearm, and was admitted for intravenous antibiotic treatment. The patient was discharged from hospital the next day and continued a course of oral antibiotics; the cellulitis resolved completely. This patient had taken prednisone (10 mg per day) for 12 years after failure to respond to multiple DMARDs. Only two (9%) patients showed a four-fold increase in titre of antibodies against chaperonin 10 over the course of the study (data not shown). These patients were in the low-dose and medium-dose groups. There was no increase in antibodies in the high-dose group.

## Discussion

Our findings suggest that treatment of individuals with active rheumatoid arthritis with chaperonin 10 results in short-term improvement of disease activity indicators, with improvements in symptoms seen as early as day 14 and sustained to day 84 of treatment. Rapid improvements in physical functioning and quality of life, as well as inhibition of cytokine production *in vitro*, were also seen.

This protocol was designed without placebo as an exploratory proof-of-principle investigation of the first signals of safety and efficacy of chaperonin 10 and to examine elements of dose-ranging treatment effects. We recognise the limitations of the study design; however, trial outcomes were intended to inform the design of a definitive, randomised, placebo-controlled study. The US Food and Drug Administration have proposed the use of ACR70 as a measure of a major clinical response. In reported controlled trials of anti-TNF compounds,<sup>21–23</sup> although patients receiving placebo could achieve ACR20 (19–33%) or ACR50 (0–8%),

ACR70 responses (0–2%) were seen only rarely. The percentage of patients reaching ACR70 in this study (17%, all treatment arms combined) suggests a true treatment response. The patients enrolled in this study had moderate to long-standing treatment-resistant disease. The response rate seen (70% DAS responders, 57% ACR20 responders when all treatment arms were combined), together with the number of adverse events related to rheumatoid arthritis reported post-study, would suggest a positive response to chaperonin 10. Although the group sizes in this study were small, this limitation is partly balanced by the multicentre protocol, which was designed to minimise site bias.

There was some evidence of a dose response, with the high-dose group having consistently greater response than the other two groups for all primary and secondary endpoints, except ESR and CRP. However, the mid-dose group—7.5 mg chaperonin 10—did not in general lie between the other two groups in its response levels. This finding could be attributable, at least in part, to the small sample size of this study (eg, with sample sizes of this order, simulation experiments indicate that we might expect only about 25% of such studies to yield data consistent with a dose-response in ACR50; personal communication, P Mullins, University of Auckland, Auckland, New Zealand).

Chaperonin 10 was well tolerated, with no difference noted in the incidence or types of adverse events between treatment groups. Recombinant chaperonin 10, as used in this study, differs by only one amino acid residue from the naturally occurring protein, and therefore its immunogenic potential is thought to be negligible, as indicated by the low frequency of increased titre of antibodies against chaperonin 10. Although the neutralising activity of such antibodies was not assessed, they are unlikely to have inhibited the biological activity of chaperonin 10, since clinical response to treatment with chaperonin 10 seemed to be uncompromised. We need to fully assess the safety and long-term efficacy of chaperonin 10 in larger and longer term placebo-controlled studies.

Biological agents that modify production of TNF $\alpha$  or interleukin 1 $\beta$  are rapidly gaining acceptance as early treatment of rheumatoid arthritis. Inhibition of TNF $\alpha$  and interleukin 1 $\beta$  reduces the extent of inflammation in arthritis, together with the erosion of cartilage and other components of rheumatoid joints.<sup>9</sup> Interleukin 1 $\beta$  is the main cytokine implicated in joint destruction in rheumatoid arthritis and it might interact synergistically with TNF $\alpha$  to promote inflammation *in vivo*.<sup>24</sup> We used a simple *in-vitro* model of lipopolysaccharide-stimulated inflammatory cytokine response to assess the effect of chaperonin 10 on such an immune response. The *in-vitro* effects are thought to occur as a result of *in-vivo* exposure of cells to recombinant chaperonin 10, resulting in interaction with, and uptake of, chaperonin 10 by certain leucocyte subpopulations. The results of our *in-vitro* assay are unlikely to have been affected by carry-over effects of chaperonin 10 in the sample, since pharmacokinetic analyses of patient sera indicate there was little chaperonin 10 still present when PBMC samples were taken (8 h post-injection). Furthermore, the presence of residual recombinant chaperonin 10 in patient PBMC is also unlikely because of the processing involved in preparation of these samples for *ex-vivo* analysis: we have shown *in vitro* that cells pre-incubated with chaperonin 10 then washed to remove unbound chaperonin 10 retain an altered pro-inflammatory response to immune challenge (data not shown).

*In-vitro* and *ex-vivo* studies<sup>2,8</sup> have shown that chaperonin 10 exerts its anti-inflammatory activity by inhibiting events downstream in the TLR signalling pathway in both healthy individuals and patients with multiple sclerosis. One should note that activation of specific TLRs initiates signalling and generation of an intracellular cascade of events that culminates in the activation of several signalling pathways, including those with NF $\kappa$ B and MAP kinase,<sup>25</sup> and, ultimately, inflammatory cytokine production. A delicate balance is necessary when dampening the immune response associated with chronic inflammation. More than one member of the intracellular cascade might need to be targeted to achieve inhibition of the inflammatory response and disease remission.<sup>10,12</sup> This is the proposed mechanism of action of chaperonin 10. The data presented here confirm and extend our phase I findings<sup>8</sup> and show that chaperonin 10 inhibits the production of a range of cytokines, including TNF $\alpha$ , interleukin 1, and interleukin 6, between day 28 and day 56, with the production of pro-inflammatory cytokines reduced by about 30–40%, rather than ablating them. Similar results were seen in our therapeutic trial in multiple sclerosis (data not shown). By contrast, the p38 MAP kinase inhibitors inhibit lipopolysaccharide-induced TNF $\alpha$ , interleukin 1, and interleukin 6 production by more than 75% and also result in toxicity and safety concerns in the clinic.<sup>26</sup>

The results of this exploratory phase II study are encouraging in terms of the reduction of signs and symptoms of rheumatoid arthritis, as indicated by the inhibition of all clinical indices studied during 12 weeks of treatment. Furthermore, there seem to be no toxicity or tolerability issues after administration of chaperonin 10. The drug inhibits the production of a range of cytokines within the TLR signalling pathway in

the innate immune system. The design of this exploratory study means that comparison of the effect of chaperonin 10 with that of anti-TNF agents is difficult; nevertheless, the suggestions of efficacy and safety provide a basis on which to plan future studies of the clinical effect of chaperonin 10 in rheumatoid arthritis. The optimum dose range and route of administration of chaperonin 10 appropriate for achieving accelerated improvement in the signs and symptoms of rheumatoid arthritis needs to be identified.

#### **Contributors**

D Vanags, B Williams, and D Feeney had the main responsibility for writing the manuscript, with input from all other authors. B Williams was responsible for coordination of clinical trial sites. B Johnson and D Vanags contributed to the protocol design, and the design and optimisation of the in-vitro assays. D Vanags and J Weiss were responsible for coordination and assay of cell biomarkers. The study site investigators were S Hall (Melbourne, Victoria, Australia), P Nash (Brisbane, Queensland, Australia) and A Taylor (Perth, Western Australia, Australia).

#### **Conflict of interest statement**

D Vanags, B Williams, B Johnson, J Weiss, and D Feeney are employees of CBio Ltd. S Hall, P Nash, and A Taylor declare that they have no conflict of interest.

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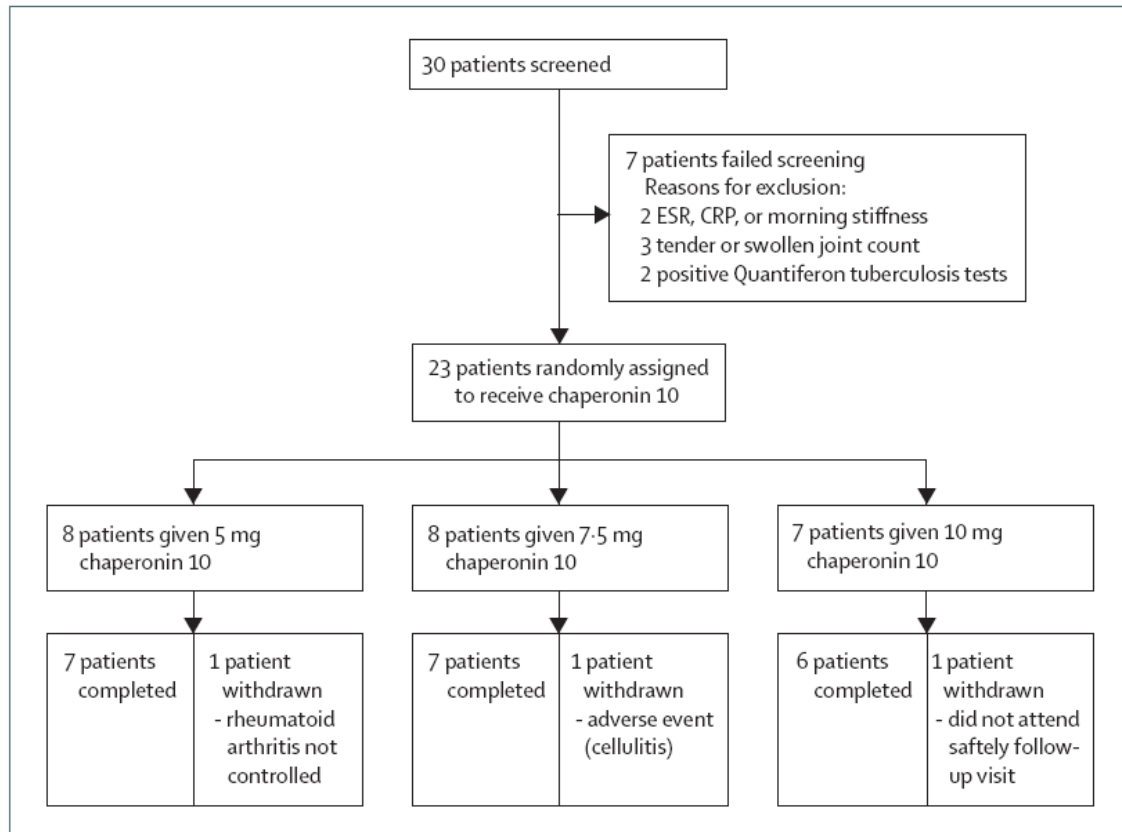


Figure 1: Trial profile

	5 mg group (n=8)	7.5 mg group (n=8)	10 mg group (n=7)
Age (years)	51 (15)	52 (15)	48 (8)
Women	3 (38%)	6 (75%)	4 (57%)
Duration of rheumatoid arthritis (years)	8 (9)	7 (6)	6 (5)
Rheumatoid factor positive	7 (88%)	6 (75%)	5 (71%)
Rheumatoid factor concentration (kIU/L)	182 (213)	180 (317)	133 (158)
Prior DMARDs	0.9 (0-3)	1.9 (0-4)	1.9 (0-5)
Receiving DMARDs	8 (100%)	5 (63%)	7 (100%)
Number of current DMARDs	1 (1-1)	0.75 (0-2)	1.43 (1-3)
Methotrexate	5 (63%)	4 (50%)	4 (57%)
Hydroxychloroquine	2 (25%)	1 (13%)	2 (29%)
Sulfasalazine	1 (13%)	1 (13%)	4 (57%)
Receiving glucocorticoids	3 (38%)	3 (38%)	3 (43%)
Glucocorticoid dose range (mg per day)	2.5 (5-10)	2.8 (2.5-10)	1.7 (3-5)
Receiving NSAIDs (%)	5 (63%)	5 (63%)	7 (100%)

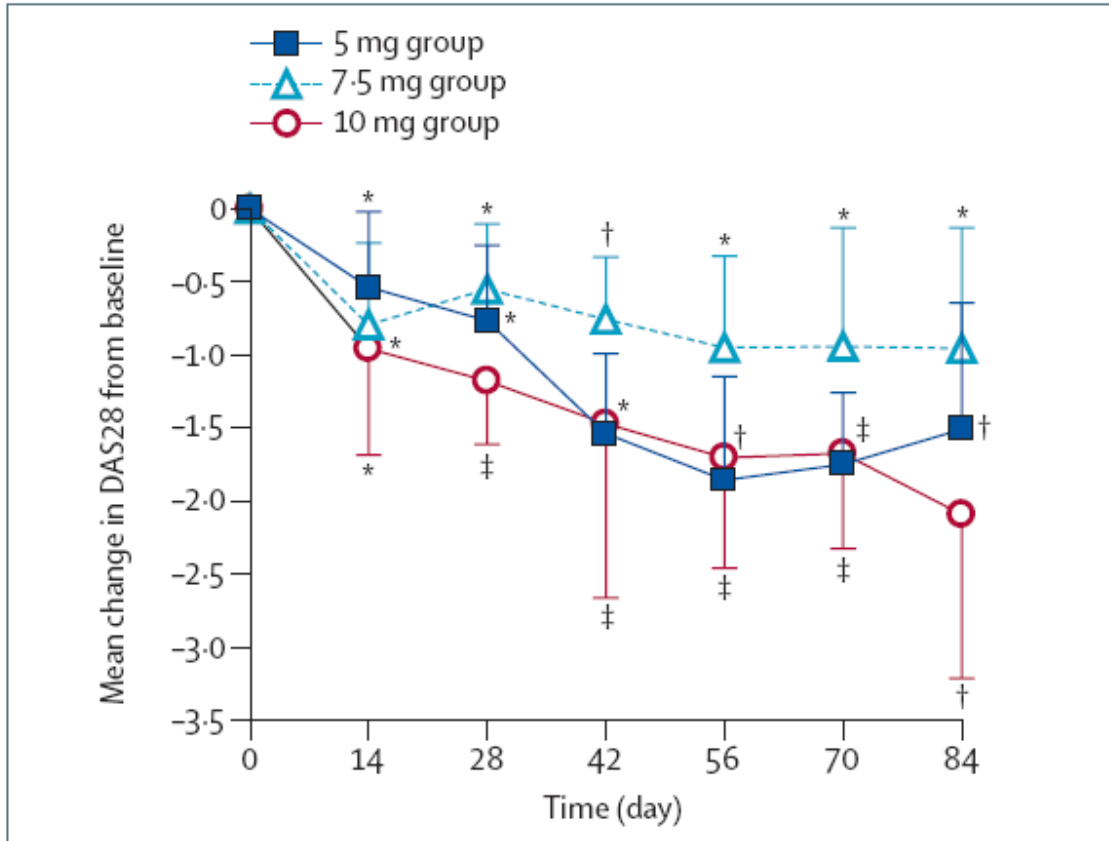
Data are mean (SD), mean (range), or n (%). DMARDs=disease-modifying anti-rheumatic drugs. NSAIDs=non-steroidal anti-inflammatory drugs.

**Table 1: Patient demographic and baseline clinical characteristics**

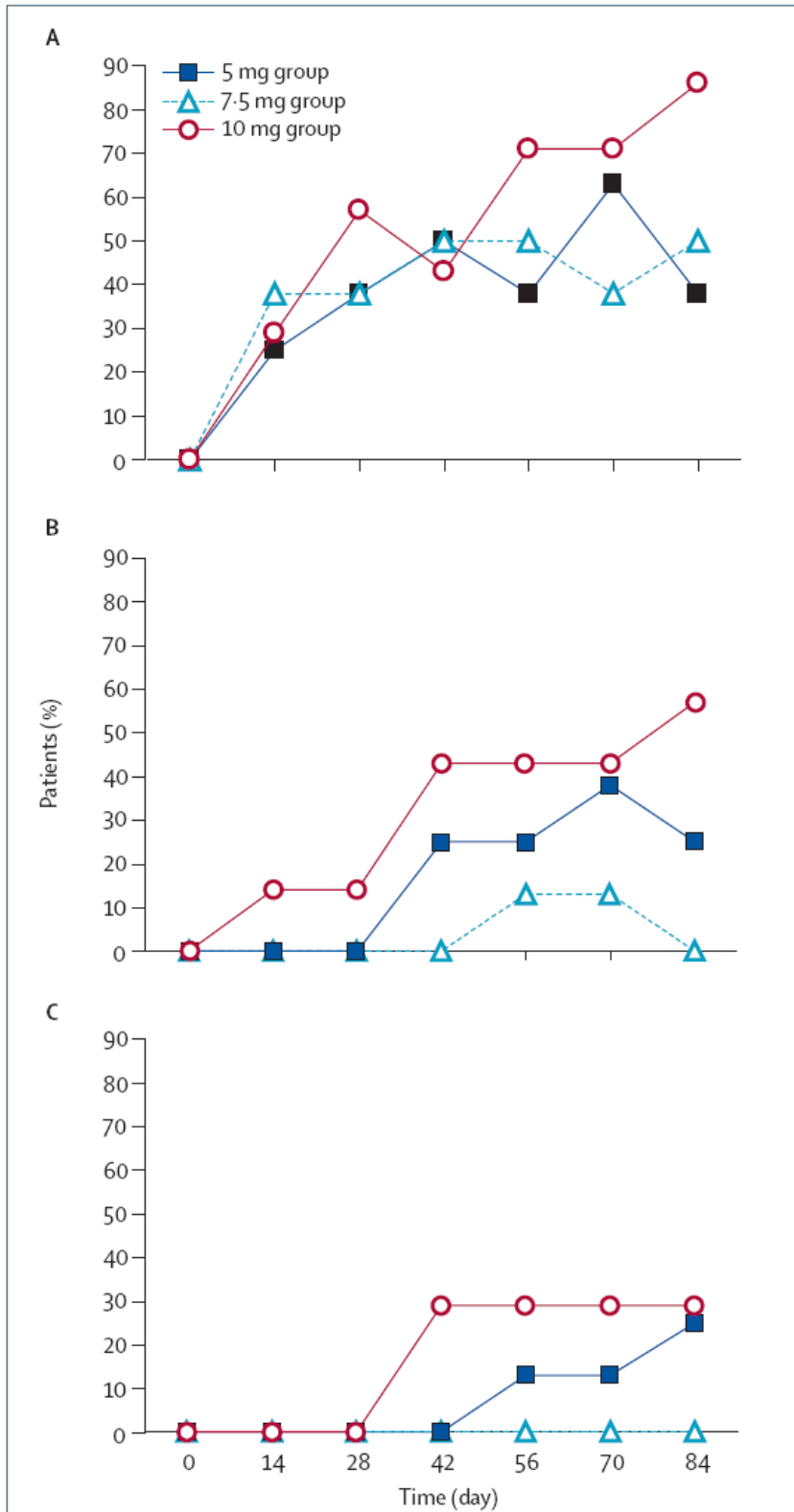
	5 mg group (n=8)	7.5 mg group (n=8)	10 mg group (n=7)	p value*
<b>DAS28</b>				
Baseline	6.17 (0.53)	5.99 (1.07)	5.93 (1.10)	..
14 days	5.64 (0.83)	5.19 (1.28)	4.97 (1.05)	0.4822
84 days	4.67 (1.13)	5.03 (1.17)	3.84 (1.34)	0.1819
Absolute change†	-1.51 (1.04)	-0.96 (0.99)	-2.09 (1.22)	0.1538
p value‡	0.0045	0.0294	0.0039	
<b>ACR responses by day 84</b>				
ACR20	3 (38%, 0-63)	4 (50%, 0-75)	6 (86%, 43-100)	0.1538
ACR50	2 (25%, 0-50)	0 (0, 0-38)	4 (57%, 14-86)	0.0422
ACR70	2 (25%, 0-50)	0 (0, 0-38)	2 (29%, 0-57)	0.2704

DAS28 are mean (SD), ACR are n (%; 95% CI). \* Comparison of differences between groups. †From baseline at day 84. ‡Comparison of day 84 value with baseline.

**Table 2: Primary measures of efficacy of chaperonin 10 by dose**



**Figure 2: Disease activity score during treatment with chaperonin 10**  
 Error bars are 95% CI. \*p<0.05; †p<0.01; ‡p<0.001.



**Figure 3: Proportion of patients with rheumatoid arthritis who had improvement relative to baseline according to ACR response criteria (A) ACR20; (B) ACR50; (C) ACR70.**

	5 mg group (n=8)	7.5 mg group (n=8)	10 mg group (n=7)	p value*
<b>Tender joint count (0-68 joints)</b>				
Baseline	32.0 (10.57)	29.4 (16.27)	29.1 (15.32)	..
Day 14	25.9 (8.48)	22.0 (15.98)	19.4 (19.97)	0.7161
Day 84	15.9 (12.29)	22.5 (11.63)	6.3 (5.53)	0.0242
Absolute change	-16.1 (13.40)	-6.9 (10.92)	-22.9 (14.67)	0.0803
Percentage change	-50%	-24%	-79%	
p value†	0.0114	0.1182	0.0062	
<b>Swollen joint count (0-66 joints)</b>				
Baseline	17.8 (6.36)	17.5 (9.84)	14.7 (4.54)	..
Day 14	16.8 (7.59)	9.0 (9.17)	9.3 (6.45)	0.1125
Day 84	14.5 (8.14)	9.3 (8.58)	5.7 (7.16)	0.1269
Absolute change	-3.3 (8.58)	-8.3 (4.03)	-9.0 (7.30)	0.2275
Percentage change	-19%	-47%	-61%	
p value†	0.3196	0.0007	0.0172	
<b>ESR (mm/h)</b>				
Baseline	24.12 (19.94)	21.50 (10.25)	21.571(19.11)	..
Day 14	18.63 (9.84)	20.75 (12.16)	14.43 (4.65)	0.6019
Day 84	16.50 (10.80)	23.88 (20.50)	19.86 (14.31)	0.6518
Absolute change	-7.62 (15.82)	2.37 (19.92)	-1.71 (13.31)	0.4978
Percentage change	-32%	11%	-8%	
p value†	0.215	0.7458	0.745	
<b>CRP (mg/L)</b>				
Baseline	10.75 (14.05)	15.23 (20.52)	6.01 (7.45)	..
Day 14	7.04 (9.49)	16.39 (11.43)	5.90 (6.03)	0.0774
Day 84	5.94 (6.67)	27.88 (39.08)	7.03 (5.67)	0.0853
Absolute change	-4.81 (11.59)	12.63 (39.19)	1.01 (4.25)	0.3629
Percentage change	-45%	82%	17%	
p value†	0.2787	0.3921	0.5513	
<b>Patient disease activity score (VAS, 0-100)</b>				
Baseline	69.1 (13.87)	75.5 (11.55)	69.0 (14.00)	..
Day 14	54.3 (34.78)	61.4 (23.32)	59.9 (14.14)	0.9917
Day 84	38.0 (35.45)	50.3 (25.29)	34.0 (28.72)	0.5566
Absolute change	-31.1 (26.14)	-25.3 (20.07)	-35.0 (25.75)	0.7338
Percentage change	-45%	-34%	-51%	
p value†	0.012	0.0092	0.0114	
<b>Patient assessment of pain (VAS, 0-100)</b>				
Baseline value	58.0 (29.47)	73.9 (21.13)	69.7 (14.74)	..
Day 14	51.3 (34.52)	61.5 (29.21)	54.9 (19.23)	0.7729
Day 84	36.9 (39.60)	54.9 (22.56)	32.6 (27.43)	0.3420
Absolute change	-21.1 (26.24)	-19.0 (21.86)	-37.1 (24.45)	0.3151
Percentage change	-36%	-26%	-53%	
p value†	0.0569	0.0436	0.007	

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<b>Physician's global score (VAS, 0-100)</b>				
Baseline value	57.0 (15.84)	55.4 (20.55)	48.3 (8.16)	..
Day 14	38.0 (23.77)	37.5 (22.06)	36.9 (24.11)	0.9955
Day 84	31.9 (26.37)	37.6 (29.64)	12.7 (6.45)	0.0863
Absolute change	-25.1 (27.29)	-17.8 (22.90)	-35.6 (9.54)	0.2766
Percentage change	-44%	-32%	-74%	
p value†	0.0352	0.0645	0.0001	
<b>Disability Index of HAQ (scale 0-3)</b>				
Baseline value	1.16 (0.69)	1.73 (0.41)	1.05 (0.41)	..
Day 14	0.85 (0.66)	1.52 (0.66)	0.86 (0.36)	0.0569
Day 84	0.72 (0.74)	1.07 (0.69)	0.59 (0.56)	0.3811
Absolute change	-0.44 (0.36)	-0.65 (0.52)	-0.466 (0.42)	0.5811
Percentage change	-38%	-38%	-44%	
p value†	0.0119	0.0097	0.0277	
<b>Morning joint stiffness (minutes)</b>				
Baseline value	152.5 (128.56)	174.4 (140.24)	180.0 (129.61)	..
Day 14	110.6 (124.45)	69.4 (74.95)	104.3 (118.44)	0.7183
Day 84	99.4 (134.39)	127.5 (167.03)	10.8 (13.57)	0.1667
Absolute change	-53.1 (127.50)	-46.9 (194.99)	-189.2 (126.27)	0.2007
Percentage change	-35%	-27%	-105%	
p value†	0.2771	0.5184	0.0144	
<b>SF36 (total score)</b>				
Baseline value	49.43 (19.74)	39.51 (14.75)	52.69 (18.64)	..
Day 14	57.57 (21.55)	49.63 (17.85)	58.32 (23.00)	0.6648
Day 84	56.99 (21.04)	53.53 (15.68)	74.61 (17.20)	0.0823
Absolute change	7.56 (11.54)	14.03 (18.21)	21.92 (11.94)	0.1792
Percentage change	15%	36%	42%	
p value†	0.1064	0.0658	0.0028	

Data are mean (SD). HAQ=health assessment questionnaire, scale ranges from 0=no difficulty to 3=unable to do activity. VAS=visual analogue scale, ranging from 0=no pain to 100=severe pain, or 0=no disease activity to 100=extreme disease activity. \* Comparison of differences between groups. †Comparison of day 84 value against baseline.

**Table 3: Secondary efficacy outcomes by dose of chaperonin 10**

	Baseline	Day 28	Day 56	p*
TNF $\alpha$	1301.6 (1241.5)	944.5 (977.94)	852.8 (803.6)	0.0118
Interleukin 1 $\beta$	2924.5 (2617.8)	2335.7 (1969.6)	1989.3 (1746.7)	0.0055
Interleukin 6	19063 (15483)	12673 (12123)	12970 (8461.9)	0.0086
Interleukin 10	1012.9 (807.6)	700.9 (662.52)	805.2 (673.5)	0.0106†

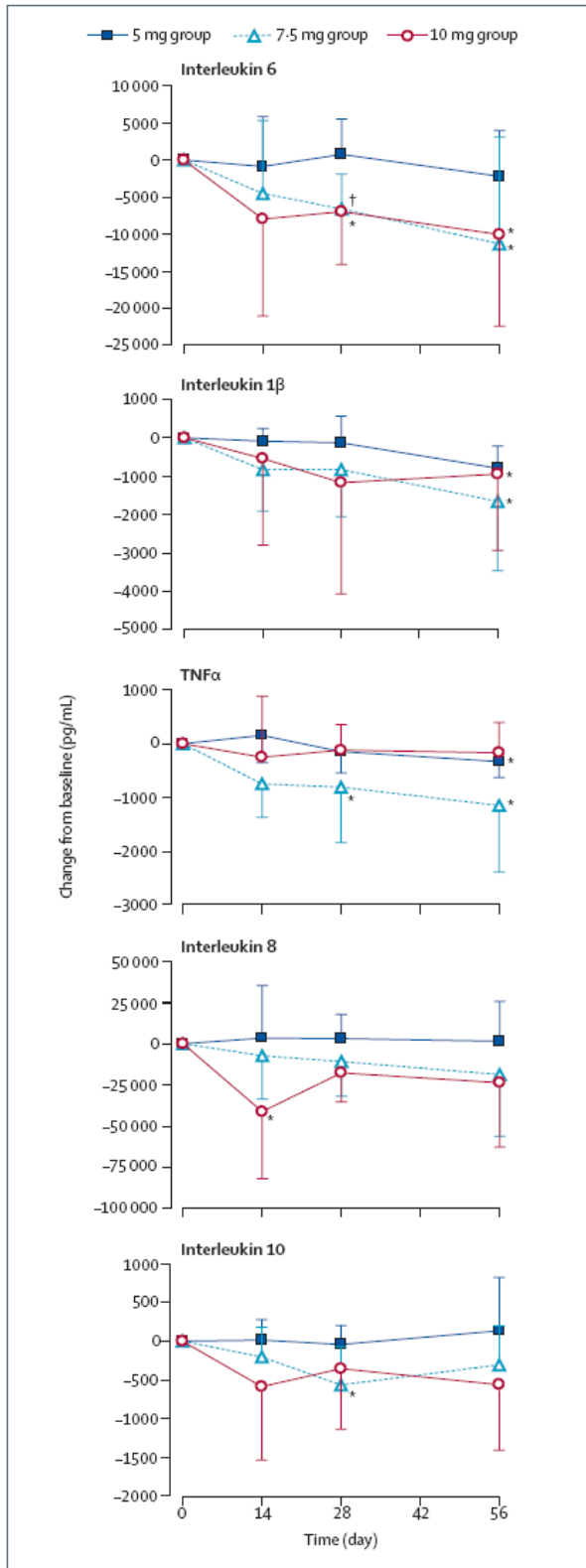
Data are mean (SD). \*Comparison of day 56 values with baseline. †Day 28 only.

**Table 4: Actual cytokine concentrations (pg/mL) during treatment with chaperonin 10 (all dose groups combined)**

	5 mg group (n=8)	7.5 mg group (n=8)	10 mg group (n=7)	Overall (n=23)
Exacerbation of rheumatoid arthritis during study	1 (13%)	2 (25%)	0 (0)	3 (13%)
Exacerbation of rheumatoid arthritis post-study	3 (38%)	2 (25%)	2 (29%)	7 (30%)
Upper respiratory tract infections	1 (13%)	2 (25%)	1 (14%)	4 (17%)
Nausea	2 (25%)	1 (13%)	0 (0)	3 (13%)
Arthralgia	2 (25%)	1 (13%)	1 (14%)	4 (17%)
Hot flush	0 (0)	1 (13%)	2 (29%)	3 (13%)
Myalgia	2 (25%)	0 (0)	0 (0)	2 (9%)
Lethargy	0 (0)	1 (13%)	1 (14%)	2 (9%)
Injection site extravasation	1 (13%)	1 (13%)	0 (0)	2 (9%)

Data are number (%). \*Occurred in  $\geq$ 5% of patients in any treatment group.

**Table 5: Most frequently reported adverse events\***



**Figure 4: Production of lipopolysaccharide-induced proinflammatory and anti-inflammatory cytokines during chaperonin 10 treatment**  
 Data are mean change from pre-dose baseline. Error bars are 95% CI. p values are one-sided and indicate comparison of mean change from baseline. \*p<0.05; †p<0.01.

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