Web Supplement: Supplementary Appendix and Consort 2010 Checklist

Short-Term Treatment with a Gonadotropin-Releasing Hormone Antagonist, Cetrorelix, in Rheumatoid Arthritis (AGRA): A Randomised, Double-Blind, Placebo-Controlled Trial

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Detailed Inclusion and Exclusion criteria

Patients had a disease-activity score based on 28 joint count (DAS28) >3.2, with an active disease defined as at least 2 of the following criteria: ≥ 6 painful joints, ≥ 3 swollen joints, erythrocyte sedimentation rate (ESR) ≥ 20 mm/h or a C-reactive protein (CRP) ≥ 10mg/L.

Menstruating women could only enter the study in the early follicular phase of their menstrual cycle. The concomitant use of stable doses of disease-modifying anti-rheumatic drugs (DMARDs) for at least 8 weeks, stable prednisolone ≤7·5 mg daily for at least 4 weeks, and stable non-steroidal anti-inflammatory drugs (NSAIDs) for at least 2 weeks was allowed if these doses were continued throughout the study. Key exclusion criteria were as follows: pregnancy or breastfeeding females; corticosteroid injections within the trial or 4 weeks prior to screening; biological agents were not permitted during the trial or within 4 weeks prior to inclusion in the trial (with the exception of infliximab or adalimumab which were not permitted within 3 months prior to inclusion in the trial; and rituximab which was not permitted within 6 months prior to inclusion in the trial). A history of hormone-dependent cancers ever or non-hormone-dependent cancers within 5 years prior to screening; infections requiring intravenous antibiotic treatment within 30 days, or oral antibiotics within 14 days prior to enrolment; significant renal or hepatic impairment; and any treatment with hormone replacement therapy or oral contraception were also exclusion criteria.

During the first three months, patients with disease duration >36 months or patients taking concomitant NSAIDs and prednisiolone were also excluded. Due to slow recruitment, these stricter criteria were removed after the first 6 patients were enrolled. This change was not expected to bias our results, and allowed data generated by this trial to be generalised to a wider RA population.

TNF-α Assay

TNF-α was measured using a high sensitivity bead-based fluorescence immunoassay (Luminex Inc., Austin, Texas, USA) with multiplex technology according to the manufacturers' instructions. No significant variation was noted between duplicates for any sample. Identical lots of critical reagent, of negligible cross reactivity <2%, was supplied by Biorad, Hercules, California, USA. Samples were assayed together within the same microplate on the same day. The assay sensitivity for TNF-α was 0·5 pg/mL. Due to its skewed distribution, the statistical analysis gives both the log and relative change (%) of TNF-α from baseline.

Hormone Assays

Non-competetive immunofluorometric assays were used for the quantitative determination of serum LH and FSH (Dissociation Enhanced Lanthanide Fluoroimmunoassay [DELFIA] kit, Turku, Finland). A competitive immunofluorometric assay was used for the determination of serum oestradiol (DELFIA kit, Turku, Finland). A competitive radioimmunoassay was used for the quantitative determination of serum testosterone (Orion Diagnostica, Espoo, Finland). A competitive luminoimmunoassay was used for the quantitative determination of serum cortisol (Immulite 2000, California, USA).

Further Statistical Analyses

The sample size calculation was based on a two-sided significance level of 5% and a power of 80%. Assuming a 10% dropout rate, the trial needed to enrol 49 patients per treatment group to detect a between-group difference of 0·6 DAS28 units with a standard deviation (SD) of 1·0.

Dichotomous endpoints were compared with the Pearson chi-squared test or the Suissa-Shuster exact unconditional test, depending on the distribution of expected values (1). The Newcombe hybrid score interval was used to estimate 95% confidence intervals (CI) for the difference between proportions (2). No adjustments for multiple analyses were made, owing to the highly correlated variables. All clinical, biochemical, and safety data were analysed by an intention-to-treat analysis. Only predefined endpoints are presented. The intention-to-treat population was predefined in the protocol as all randomised patients who received any injections of study drug. Missing values were <1% and could, as predefined, be imputed with the last observation carried forward. The assumptions of normality needed for analyses were approximately valid. The Spearman rank correlation coefficient (Spearman's rho) was used to estimate the association between pairs of continuous variables.

Supplementary Results

Cetrorelix onset and offset effect

LH and FSH remained stable in the placebo group (Figure 1C in main article shows change from baseline in LH). Although LH and FSH were reduced as early as day 2 in patients allocated to cetrorelix, there were no significant changes in clinical endpoints compared with patients allocated to placebo until maximal suppression of LH and FSH by day 5. As expected by day 10, LH and FSH increased towards baseline levels after cessation of cetrorelix, with further increases towards baseline by day 15. The same trend was observed with DAS28CRP and with secondary endpoints. No variables in the cetrorelix group exceeded baseline levels after drug cessation. This rapid offset effect was expected owing to the short half-life of cetrorelix.

Core set measures

Each core set measure of disease activity showed non-significant greater improvements in the cetrorelix group compared with the placebo group except for the physician global assessment which was equally reduced in both groups.

Figure S1: Trial Flow Chart

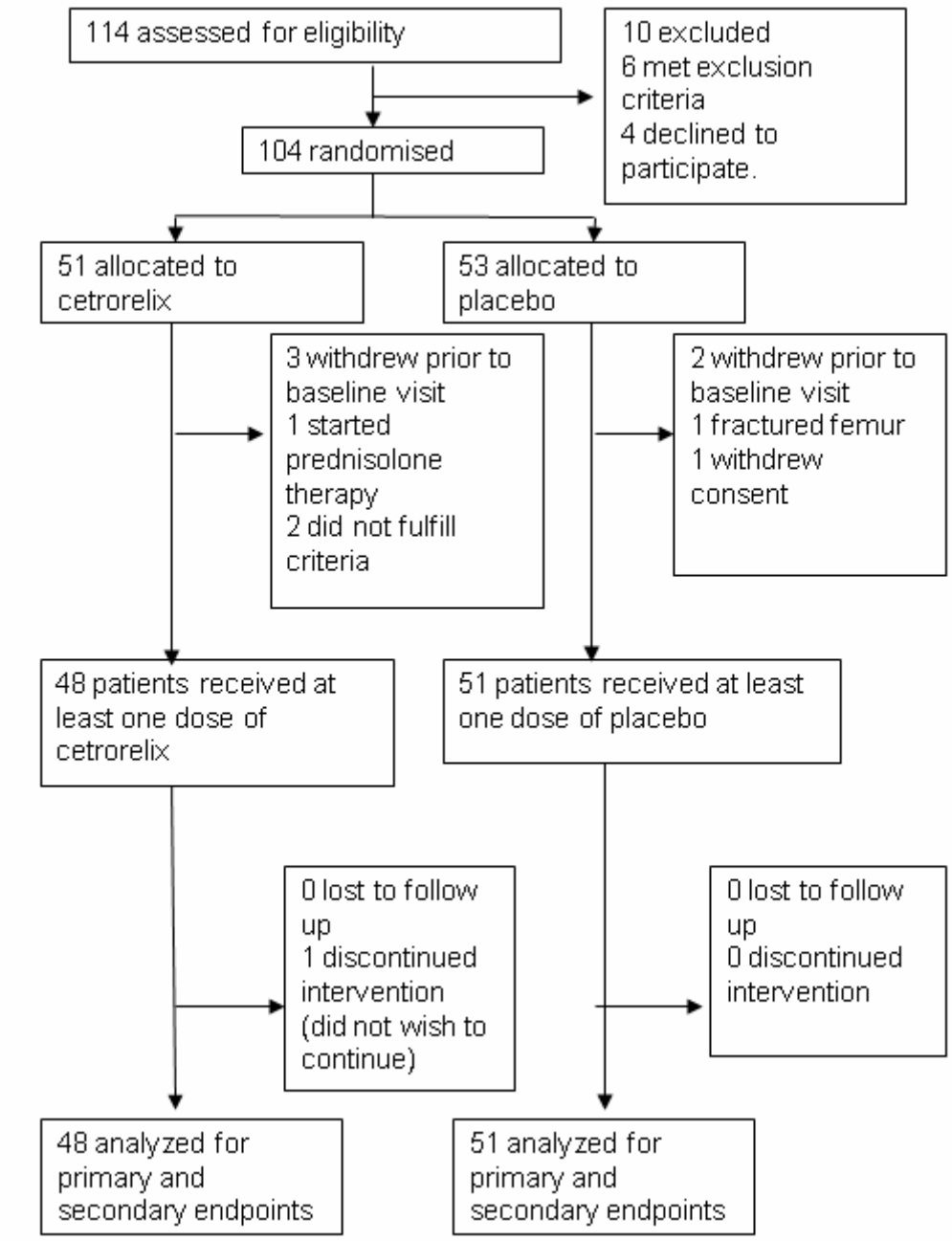

Table S1: All adverse events during the study period*

Event	**Cetrotide, N=48**	**Placebo, N=51**
	n (percent)	*n (percent)*
Headache	2 (4.2)	6 (11.8)
Injection site discomfort	3 (6.3)	0
Nausea	0	3 (5.9)
Menstrual spotting	2 (4.2)	0
Hot flushes	2 (4.2)	0
Toothache	0	1 (2.1)
Urinary tract infection	1 (2.1)	0
Nasopharyngitis	1 (2.1)	0

* There were no significant between-group differences.

References

1. Lydersen S, Fagerland MW, Laake P. Recommended tests for association in 2 × 2 tables. Stat Med 2009; 28: 1159-75.
2. Fagerland MW, Lydersen S, Laake P. Recommended confidence intervals for two independent binomial proportions. Stat Methods Med Res 2011; (In press, accessed 7th July, 2013, at http://smm.sagepub.com/content/early/2011/10/11/0962280211415469.long)

CONSORT 2010 checklist

Section/Topic	Item No.	Checklist Item	Page No.
Title and Abstract			
	1a	Identification as a randomised trial in the title	Title
	1b	Structured summary of trial design, methods, results and conclusion (see CONSORT abstract checklist)	Abstract
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	P3-4
	2b	Scientific obejectives or hypotheses	P4 (Further details in protocol)
Methods			
Trial design	3a	Description of trial design	P5
	3b	Important changes to methods after trial commencement (such as eligibility criteria). With reasons	Supplement
Participants	4a	Eligibility criteria for participants	P5+ Supplement
	4b	Settings and locations where the data were collected	P5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	P5+ Supplement
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	P5
	6b	Any changes to trial outcomes after the trial commenced with reasons	-
Sample size	7a	How sample size was determined	Supplement
	7b	When applicable, explanation of any interim analyses and stopping guidelines	- Protocol has criteria for

			termination of trial. No planned interim analyses
Randomization			
Sequence generation	8a	Method used to generate the random allocation sequence	P5
	8b	Type of randomization, details of any such restriction (such as blocking and block size)	P5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence	P5-6
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	P5-6
Blinding	11a	If done, who was blinded after assignment o interventions (for example, participants, care providers, those assessing outcomes) and how.	P6
	11b	If relevant, description of the similarity of interventions	P5
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	P5-6+ Supplement
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	-
Results			
Participant flow	13a	For each group, the numbers of participants who were randomly assigned received intended treatment, and were analysed for the primary outcome	Supplement: Figure S1
	13b	For each group, losses and exclusions after randomization, together with reasons	Supplement: Figure S1
Recruitment	14a	Dates defining the periods of recruitment and follow up	Removed due to word limit (was in original manuscript)

	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	All tables except where indicated in footnotes
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% CI)	Results in text/ tables.
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed including subgroup analyses and adjusted analyses, distinguishing prespecified from exploratory	Only predefined analyses presented with predefined statistics
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	P8 and Supplement: Table S1
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, impreceision, and if relevant, multilplicity of analyses	P10-11
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	P11
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	P9-11
Other information			
Registration	23	Registration and name of trial registry	Metadata
Protocol	24	Where the full trial protocol can be accessed, if available	Enclosed with submission,

			eventually can be accessed on institution website
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Title page