

EXTENDED REPORT

A multicentre, randomised, double blind, placebo controlled phase II study of subcutaneous interferon beta-1a in the treatment of patients with active rheumatoid arthritis

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Objective: To assess the efficacy of interferon beta (IFN β) in combination with methotrexate in treatment of patients with rheumatoid arthritis.

Methods: 209 patients with active rheumatoid arthritis, who had been on methotrexate for at least six months and at a stable dose for four weeks before study entry, were randomised in double blind fashion to receive placebo (0.05 ml or 0.5 ml), IFN β 2.2 μ g (0.05 ml), or IFN β 44 μ g (0.5 ml), given subcutaneously three times weekly for 24 weeks. The primary efficacy measure was a change in radiological scores at week 24. The secondary endpoint was the proportion of patients who met the ACR 20% improvement criteria at the end of the study. Synovial biopsy specimens were obtained before and after treatment from a subset of patients. Immunohistochemistry was used to detect the presence of inflammatory cells and the results were measured by digital image analysis. Collagen crosslinks were measured in urine at different times throughout the study.

Results: Analysis of radiological scores and clinical variable showed no changes in any of the groups, and there were no differences between the groups. On microscopic analysis of synovial tissue there was no significant change in the scores for infiltration by inflammatory cells after IFN β treatment. Urinary levels of collagen crosslinks were unchanged between the treatment groups.

Conclusions: At the doses tested, treatment with IFN β three times weekly in combination with methotrexate did not have a clinical or radiological effect in patients with rheumatoid arthritis.

Rheumatoid arthritis is a chronic inflammatory disease affecting synovial tissue in multiple joints, leading in most patients to bone destruction and severe morbidity and disability. Studies have shown the need for early diagnosis and early aggressive treatment to help prevent irreversible joint damage.^{1–3} The prevention of cartilage and bone erosions is an important therapeutic challenge in the treatment of this disease. At present, various disease modifying antirheumatic drugs (DMARDs) are used to control arthritis activity.⁴ However, DMARDs are only moderately effective and not all patients tolerate them. Therapeutic possibilities have improved with the introduction of agents that block tumour necrosis factor α (TNF α), although not all patients are responsive to these drugs.^{5,6} This has motivated the search for additional effective treatments that can reduce inflammation as well as limit bone destruction.

Several studies have shown beneficial effects of interferon beta (IFN β) on clinical and MRI measures in relapsing-remitting multiple sclerosis.^{7–9} The effects of IFN β on the cytokine profile and cell trafficking in patients and models of multiple sclerosis have stimulated studies on its potential for treatment of patients with rheumatoid arthritis, which is also considered to be an immune mediated disease. Immunological functions of IFN β include inhibition of TNF α and interleukin (IL) 1 β secretion, and enhancement of IL10 and IL1RA production.^{10–13} Conceivably, concurrent targeting of TNF α , IL1 β , and other proinflammatory cytokines by the use of a counterregulatory cytokine such as IFN β might be effective in suppressing arthritis activity. Indeed,

several studies in animal models of collagen induced arthritis (CIA) have shown a markedly beneficial effect of IFN β treatment. A small study done in four rhesus monkeys with CIA demonstrated clinical improvement and decreased serum levels of C reactive protein after seven days of daily IFN β treatment.¹⁴ Furthermore, constitutive expression of IFN β by gene therapy resulted in reduced paw swelling and histological improvement in CIA mice,¹⁵ and CIA mice treated with daily injections of murine IFN β had a significant reduction in inflammation and an inhibition of the development of bone erosions.¹⁶

The favourable effects on CIA in animal models and the well known safety profile of IFN β treatment in humans motivated us to conduct an open label phase I study in 12 patients with active rheumatoid arthritis.¹⁴ Patients were treated with IFN β subcutaneously three times weekly for three months with one of three different doses of fibroblast derived natural IFN β (22 μ g, 44 μ g, and 66 μ g) (Frone[®], Sero International). The treatment was generally well tolerated and there was, on average, a significant improvement in clinical outcome measurements.

We undertook the present double blind, randomised, placebo controlled phase II study of treatment with IFN β -1a (Sero International) in rheumatoid patients with active

Abbreviations: ACR, American College of Rheumatology; CCP, cyclic citrullinated peptide; CIA, collagen induced arthritis; DMARD, disease modifying antirheumatic drug; IFN, interferon; IL, interleukin; ITT, intention to treat; mAb, monoclonal antibody; TNF, tumour necrosis factor

Table 1 Patient demographics, clinical characteristics, and previous treatment at baseline (ITT population)

	Combined placebo group (n = 73)	IFN β , 2.2 μ g (n = 67)	IFN β , 44 μ g (n = 68)	All patients (n = 208)
<i>Demographics</i>				
Age (years)	53.6 (11.7)	53.7 (12.8)	52.0 (11.1)	53.1 (11.9)
Age range (years)	25.1 to 81.4	28.0 to 79.0	29.3 to 77.9	25.1 to 81.4
Female	65.8%	83.6%	83.8%	77.4%
<i>Disease status</i>				
Mean duration (years)	4.1 (2.4)	4.2 (2.7)	4.2 (2.7)	4.2 (2.6)
Duration range (years)	0.6 to 9.5	0.6 to 12.5	0.5 to 10.4	0.5 to 12.5
Rheumatoid factor positive	71.2%	65.2%	65.2%	
Anti-CCP positive	54.8%	58.2%	55.9%	
Functional class I	15.1%	13.4%	17.6%	
Functional class II	57.5%	62.7%	72.1%	
Functional class III	24.7%	22.4%	10.3%	
Functional class IV	2.7%	1.5%	0	
<i>Concomitant MTX treatment</i>				
MTX dose (mg/week)	15.3 (4.6)	14.7 (5.5)	15.2 (5.1)	15.1 (5.0)
Length of MTX use (months)	19.0 (14.1)	16.4 (13.6)	17.1 (13.8)	17.6 (13.8)

Values are mean (SD) and range, or per cent.

CCP, cyclic citrullinated peptide; DMARD, disease modifying anti-rheumatic drug; ITT, intention to treat; MTX, methotrexate.

disease while on methotrexate to determine whether IFN β -1a is effective in reducing radiological damage and arthritis activity.

METHODS

Patients

Patients over 18 years of age with a diagnosis of rheumatoid arthritis according to the American College of Rheumatology (ACR) criteria¹⁷ were eligible for inclusion in the study if the duration of their active disease was more than six months and less than eight years. Patients were also required to have at least eight swollen joints, and to fulfil no less than three of the following criteria: at least eight tender joints; physician's global assessment of disease activity between 2 and 4 on a five point scale; patient's global assessment of disease activity between 2 and 4 on a five point scale; serum C reactive protein above 15 mg/dl.

Patients were required to have used methotrexate for six months or more, and to have followed a stable regimen (≥ 7.5 mg/week) for at least four weeks before study entry. Patients who were on oral corticosteroids (<10 mg/day) or non-steroidal anti-inflammatory drugs (NSAIDs) were required to have been on a stable dose for at least four weeks before enrolment. Patients were required to have adequate bone marrow reserve, liver function, and renal function (haemoglobin ≥ 5.5 mmol/l, white blood cell count $\geq 3.5 \times 10^9/l$, platelet count $\geq 100 \times 10^9/l$; serum bilirubin, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase ≤ 1.5 times the upper limit of normal values, and serum creatinine ≤ 150 μ mol/l).

Women who were pregnant or lactating were excluded from the study, as were patients who had received biological agents or ciclosporin within the previous six months, had used a DMARD other than methotrexate within 28 days, or had used leflunomide within eight weeks of enrolment. All study participants were hospital outpatients at time of enrolment but were excluded if they were wheelchair bound or bed ridden, had major surgery or joint replacement planned, had a history of cancer in the preceding five years, or had a positive test for anti-dsDNA antibodies. Patients were excluded if clinically significant serious abnormalities were apparent on electrocardiography or chest x ray; if they had had an active severe infection or an opportunistic infection in the three months preceding study entry; had a history of allergy to paracetamol or human serum albumin; or had a history of alcohol abuse.

Study protocol

Patients were randomised to one of four treatment groups: 2.2 μ g (0.05 ml) IFN β -1a, 44 μ g (0.5 ml) IFN β -1a, placebo (0.05 ml), or placebo (0.5 ml). All treatments were given by subcutaneous injection three times weekly for 24 weeks. Patients remained on a stable dose of methotrexate. Both the patients and the assessors were blinded to the treatment given. As IFN β side effects are easily recognised, physicians responsible for general patient management, including safety assessments, were different from those responsible for efficacy assessments. Patients gave written informed consent before entering the study and the study protocol was approved by the local medical ethics committee at each of the participating sites.

Safety and efficacy analyses

The primary efficacy measure was defined as the change from baseline radiological score at 24 weeks of treatment, based on the presumed mechanism of action of IFN β on osteoclasts^{15 16 18} and in line with previous observations showing that even modestly effective treatments may have a demonstrable protective effect on radiological joint damage after only 24 weeks of treatment.^{19 20} Anteroposterior radiographs of the hands and feet were scored using the Van der Heijde modified Sharp score²¹ by two independent blinded observers. Erosions in the feet were scored from 1 to 10, erosions in hands were scored from 1 to 5, and joint space narrowing was scored from 1 to 4. Scores were combined from hand and foot radiographs to provide a total score ranging from 0 to 448, with joint space narrowing scores ranging from 0 to 168 and erosion scores ranging from 0 to 280, with a maximum of 160 for the hands and 120 for the feet. The change in Van der Heijde x ray scores was calculated by the differences between the scores at the end of treatment and the scores on the baseline radiographs.

Secondary efficacy end points were defined as a 20% improvement in the ACR criteria (ACR20) and a decrease in C reactive protein concentrations. Clinical assessment for disease activity was repeated at baseline, day 15, day 29, and then every four weeks until week 28. This included a 68 joint count for joint swelling and tenderness; physician's and patient's assessment of disease activity on a scale from 0 (asymptomatic) to 5 (severe symptoms); assessment of pain by visual analogue scale from 0 (no pain) to 10 (severe pain); quality of life (health assessment questionnaire) from 1 (no disability) to 3 (severe disability); and erythrocyte

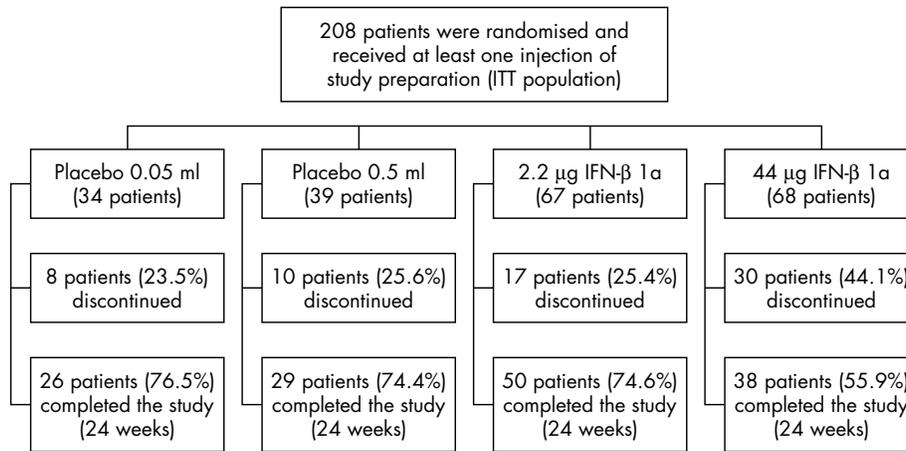


Figure 1 Patient disposition.

sedimentation rate (ESR) and C reactive protein measurement. In addition, rheumatoid factor, antibodies against cyclic citrullinated peptide (CCP),²² and anti-IFN β antibodies were measured by enzyme linked immunosorbent assay (ELISA) at screening, at week 12, and at week 24.

Safety assessments were completed at every visit by an independent observer, and included an interview, examination of vital signs, inspection of injection sites, and evaluation of current laboratory data. The use of concomitant drug treatment was recorded throughout the study.

Arthroscopy

Some patients underwent arthroscopy of an inflamed knee joint under local anaesthesia at baseline and at week 24. Patients gave separate written informed consent for this procedure. Arthroscopies, tissue sampling, and storage were carried out as described previously in detail.²³ All tissue samples were sent to the AMC, Amsterdam for immunohistochemical staining and digital image analysis.

Immunohistochemical analysis

Serial sections were stained with the following monoclonal antibodies (mAb): anti-CD68 (EBM11, Dako, Glostrup, Denmark), anti-CD55 (Clone-67, Serotec, Oxford, UK), and anti-CD3 (SK7, Becton-Dickinson, San Jose, California, USA). Sections with non-assessable tissue—defined by the

absence of an intimal lining layer—were omitted before analysis. For control sections, the primary antibodies were omitted or irrelevant isotype matched mouse antibodies were applied. Staining was done according to a three step immunoperoxidase method as previously described.²⁴

Digital image analysis

The slides were evaluated by digital image analysis. All sections were coded and analysed in random order by an independent observer, who was blinded to the clinical data as described previously.²⁵

Urinary analysis of hydroxy pyridinium collagen crosslinks

The presence of the collagen hydroxy pyridinium crosslinks pyridinoline and deoxypyridinoline in urine is an indication of the breakdown of mature collagen. It has recently been shown that the total amount of pyridinium crosslinks excreted correlates with disease activity in rheumatoid arthritis.²⁶ The urinary excretion of pyridinoline (released primarily from collagens type I and II of bone and cartilage) and deoxypyridinoline (released primarily from collagens type I and II of bone and dentin) was measured at baseline, at week 12, and at week 24. Urinary crosslink levels were investigated using gradient ion-paired reversed phase high performance liquid chromatography.²⁶

Table 2 Summary of adverse events occurring in more than 5% of the patients

Events	Placebo, 0.05 ml (n=34)	Placebo, 0.5 ml (n=39)	IFN β , 2.2 µg (n=67)	IFN β , 44 µg (n=68)
Injection site reaction	1 (3)	2 (5)	9 (13)	16 (24)
Flu-like symptoms	4 (12)	4 (10)	12 (18)	27 (40)
Headache	4 (12)	1 (3)	9 (13)	9 (13)
Fever	0	1 (3)	4 (6)	10 (15)
Fatigue	3 (9)	1 (3)	4 (6)	5 (7)
Rhinitis	5 (15)	4 (10)	11 (16)	4 (6)
Coughing	2 (6)	2 (5)	5 (7)	7 (10)
Upper respiratory tract infection	0	5 (13)	6 (9)	1 (1)
Nausea	4 (12)	0	5 (7)	6 (9)
Diarrhoea	2 (6)	2 (5)	3 (5)	4 (6)
SGPT increased	0	1 (3)	3 (5)	8 (12)
SGOT increased	0	1 (3)	2 (3)	8 (12)
Arthralgia	3 (9)	1 (3)	2 (3)	5 (7)
Rheumatoid arthritis aggravated	5 (15)	5 (13)	17 (25)	19 (28)
Increased ESR	2 (6)	2 (6)	6 (9)	11 (16)
Increased CRP	11 (32)	6 (15)	10 (15)	11 (16)

Values are n (%).

CRP, C reactive protein; ESR, erythrocyte sedimentation rate; IFN β , interferon beta; SGOT, aspartate aminotransferase, SGPT, alanine aminotransferase.

Table 3 Change from baseline in Van der Heijde x ray scores of the hands and feet before and after placebo or interferon beta treatment in patients with rheumatoid arthritis (evaluatable population)

	Change from baseline (%)		
	Placebo, 0.05 ml	IFNβ, 2.2 μg	IFNβ, 44 μg
Van der Heijde x ray score	1 (-12 to 17)	1 (-3 to 12)	0 (-5 to 47)
No of patients evaluatable	56	48	37

Values are median (range).
IFNβ, interferon beta.

Statistical analysis

The primary efficacy dataset was defined as all randomised patients for whom there were two sets of evaluatable hand and foot x rays (one set for baseline and one set for week 24; patients who withdrew from study between week 12 and week 24 had x rays as soon as possible after the last injection of study drug) and who were not major protocol violators. The intention to treat (ITT) population comprised 208 patients who received at least one dose of study drug. For statistical analysis, the two placebo groups were combined and compared with the groups having IFNβ-1a treatment. The results before and after treatment were compared by paired *t* test. Two non-parametric tests were used: the Kruskal–Wallis test for several group means (comparing clinical assessment and histological scores in more than two treatment groups), followed by the Mann–Whitney U test for comparison of two groups. For the ACR20 response, non-completers of the study were considered to be non-responders.

RESULTS

Patient characteristics and disposition

In all, 209 patients were recruited from 30 centres in 10 countries during an eight month period. Their baseline characteristics are summarised in table 1. The mean (SD) age of the 161 women (77%) and 47 men (23%) was 53 (11.9) years. The median duration of disease was 3.6 years (range 0.5 to 12.5 years); 140 patients (67%) were rheumatoid factor positive and 117 (56%) had antibodies against CCP. All patients had active disease at study entry; 165 patients (79%) were in functional class I or II, 40 (19%) were in class III, and three (1%) were in class IV. There were no statistically significant differences between the treatment groups with regard to dose or duration of methotrexate.

All 209 patients were randomised and 208 received treatment (ITT population). The disposition of the patients across the study groups is shown in fig 1.

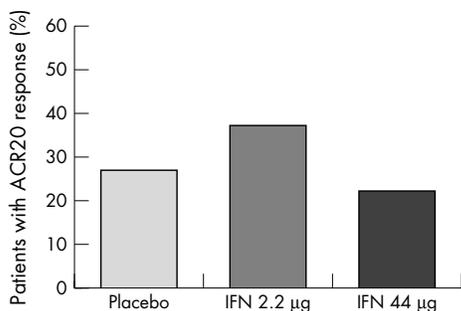


Figure 2 American College of Rheumatology 20% (ACR20) response in the treatment groups (placebo groups combined).

Safety and tolerability

Injection site reactions were the most commonly reported adverse events during the study and affected a higher proportion of patients on active treatment than on placebo. Similarly, general disorders including flu-like symptoms, headache, and increased ESR occurred at a higher frequency in the active treatment groups than in the placebo groups (table 2). Aggravated rheumatoid arthritis and raised C reactive protein concentrations were reported in a substantial number of patients in each treatment group, and there was a comparable incidence of respiratory system and gastrointestinal disorders in all treatment groups. A marginally increased incidence of raised liver enzymes appeared to be associated with the administration of IFNβ 44 μg. All events were of a mild or moderate nature, and severity was comparable between active and control groups: 10 patients in the IFNβ 44 μg group developed liver enzyme elevations of mild severity and six of moderate severity; five patients in the IFNβ 2.2 μg group developed elevations of mild severity and two of moderate severity; two patients in the placebo groups developed elevations of moderate severity.

More withdrawals were caused by adverse events in the 44 μg group (11 patients) than in the other treatment groups (two patients in the IFNβ 2.2 μg group and four in the placebo groups). In the 44 μg group, injection site reactions caused the most adverse event related withdrawals (seven patients); however, in the other groups no withdrawals were caused by injection site reactions. Flu-like symptoms, aggravated rheumatoid arthritis, and increased liver enzyme levels each caused the withdrawal of two patients from the total study population. Two patients tested positive for neutralising antibodies against IFNβ-1a at the end of treatment: both were receiving IFNβ 44 μg.

Clinical efficacy

There was no significant reduction in the progression of joint damage associated with treatment with IFNβ-1a at either of the doses tested compared with the control groups, as measured by the change from baseline in Van der Heijde x ray scores of the hands and feet (table 3). The 56 evaluatable patients in the control group showed a median change of 1 (range -12 to 17), 48 patients in the 2.2 μg IFNβ group showed a median change of 1 (range -3 to 12), and the 37 evaluatable patients in the 44 μg IFNβ group showed a median change of 0 (range -5 to 47). There were no statistically significant differences between the median ACR20 and ACR50 response rates between patients on active therapy and those in the control groups (fig 2).

Immunohistochemical analysis

Twenty five patients underwent synovial biopsy procedures, of whom 23 had results at baseline and 20 at the end of treatment (only 19 patients had both). The results of the immunohistochemical analysis are shown in table 4. After IFNβ 44 μg treatment there was a decrease in the number of

Table 4 Changes from baseline in synovial tissue indices in the 19 patients who underwent arthroscopy

Cell type	Combined placebo groups	IFN β , 2.2 μ g	IFN β , 44 μ g
CD68+ macrophages	-12 (-212 to 150), n=7	85 (-59 to 515), n=5	-46 (-320 to 111), n=7
Intimal lining CD68+ macrophages	-22 (-169 to 160), n=7	22 (-43 to 109), n=5	-7 (-228 to 23), n=7
CD55+ fibroblasts	1 (-347 to 290), n=7	106 (-94 to 293), n=5	1 (-370 to 532), n=7
CD3+ lymphocytes	-4 (-446 to 517), n=7	-2 (-356 to 32), n=5	-4 (-253 to 65), n=7

Values are median per cent change (range).

Data represent total cell count in 18 high power fields corrected for the percentage of actual tissue in the analysed area for cellularity, CD68+ macrophages, CD55+ fibroblasts, and CD3+ lymphocytes. There were no significant differences in change between the placebo group and either IFN β group.

IFN β , interferon beta.

CD68+ macrophages and a slight decrease in the number of intimal lining layer macrophages and CD3+ T cells, whereas CD55+ fibroblast-like synoviocytes increased slightly. None of these changes reached statistical significance. The IFN β 2.2 μ g treatment group showed a different trend, with an average increase in CD68+ intimal macrophages and CD55+ fibroblast-like synoviocytes and a slight decrease in CD3+ T cells; however, no statistically significant differences were detected when compared with placebo.

Collagen crosslinks analysis

There were no significant differences in urinary levels of the collagen crosslinks pyridinoline and deoxypyridinoline between patients treated with IFN β and those treated with placebo. Levels of crosslinks were similar before treatment and after treatment in all groups. Thus median (range) pyridinoline concentrations, in nmol/nmol creatinine, were 69 (27 to 233) at baseline and 70 (28 to 234) at week 24 with placebo; 75 (24 to 194) and 76 (30 to 212) with IFN 2.2 μ g; and 75 (35 to 254) and 67 (39 to 188) with IFN 44 μ g. Median (range) deoxypyridinoline concentrations, also in nmol/nmol creatinine, were 17 (7 to 170) at baseline and 16 (5 to 88) at week 24 with placebo; 19 (9 to 113) and 21 (9 to 38) with IFN 2.2 μ g; and 18 (6 to 47) and 16 (9 to 65) with IFN 44 μ g.

DISCUSSION

We report the results of a double blind, placebo controlled trial that evaluated the efficacy of subcutaneous IFN β -1a on radiological and clinical variables in patients with rheumatoid arthritis who were concomitantly receiving methotrexate. Treatment with IFN β -1a for 24 weeks was not associated with clinical or radiological improvement, neither was there a statistical change in biomarkers.

The absence of improvement in radiological scores after IFN β treatment reported here is in clear contrast to previous *in vitro* and animal studies. In these studies, IFN β has been shown to partly inhibit osteoclastogenesis and in consequence to reduce the development of erosive disease in CIA models.^{15–18} The discrepancy between the present study and previous animal work might relate to the mode of administration and the difference in IFN β -1a dosages used. In the present study IFN β was given three times weekly, following the regular treatment regimen in multiple sclerosis patients. In contrast, successful preclinical studies were done either with gene therapy, which leads to continuous IFN β release, or with daily IFN β injections at a dose of 2.5 μ g/mouse/day. Although IFN β is known to have a short half life, we chose not to use daily injections with higher IFN β concentrations because it was anticipated that this would be less tolerable to the patients. It is possible that more frequent injections, higher dosages, or the use of compounds with a longer half life is required to induce clinically meaningful effects in patients with rheumatoid arthritis. In addition, we cannot exclude the possibility that we were unable to detect a

modest protective effect on joint integrity in the light of the relatively short duration of the study.

There was a surprisingly high rate of discontinuation in our study. This was most pronounced in the IFN β 44 μ g treatment group. The most common reason for discontinuation was lack of efficacy—all treatment groups had similar percentages of drop out for this reason. However, withdrawals caused by adverse events such as injection site reactions and flu-like symptoms were more common in the 44 μ g IFN β group than in the other treatment groups. The high rate of withdrawal contrasts with results from placebo controlled trials with IFN β -1a in multiple sclerosis.⁸ This discrepancy may be explained by a difference in disease pathogenesis or differences in the study populations. The most likely explanation is, however, the use of drug titration at the start of treatment. In trials of IFN β in multiple sclerosis, the study drug is usually titrated over the first month from 20% to 50% and subsequently to full dose of treatment to reduce the incidence of adverse events.⁸ In the present study patients started treatment with their full dose of IFN β -1a.

Previous work has shown that analysis of serial synovial tissue samples in patients with rheumatoid arthritis is likely to reflect the biological effects of the treatment given; for example, patients who received either placebo or unsuccessful treatment with recombinant human IL10 did not show any significant synovial changes.²⁷ In contrast, beneficial clinical effects of anti-TNF α therapy are associated with decreased infiltration of synovial tissue by inflammatory cells.²⁸ Consistent with this, our data show that persistent disease activity is associated with unchanged synovial inflammation in serial biopsy specimens in IFN β treated rheumatoid patients. An earlier study suggested a modest reduction in CD3 positive T cells after one month of IFN β treatment in synovial tissue of 11 rheumatoid patients. However, it was noted in the same study that the number of CD3 positive T cells returned to baseline levels after three months of treatment.¹¹

In conclusion, the results of this study show that there was no apparent reduction in the progression or activity of rheumatoid arthritis compared with placebo when methotrexate treatment was supplemented with IFN β -1a in doses of either 44 μ g or 2.2 μ g over 24 weeks.

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REFERENCES

- 1 **Emery P**. Therapeutic approaches for early rheumatoid arthritis. How early? How aggressive? *Br J Rheumatol* 1995;**34**(suppl 2):87-90.
- 2 **van der Heide A**, Jacobs JW, Bijlsma JW, Heurkens AH, van Booma-Frankfort C, van der Veen MJ, *et al*. The effectiveness of early treatment with antirheumatic drugs. A randomized, controlled trial. *Ann Intern Med* 1996;**124**:699-707.
- 3 **Boers M**, Verhoeven AC, Markuse HM, van de Laar MA, Westhovens R, van Denderen JC, *et al*. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. *Lancet* 1997;**350**:309-18.
- 4 **Weinblatt ME**, Kaplan H, Germain BF, Block S, Solomon SD, Merriman RC, *et al*. Methotrexate in rheumatoid arthritis. A five-year prospective multicenter study. *Arthritis Rheum* 1994;**37**:1492-8.
- 5 **Maini R**, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, *et al*. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet* 1999;**354**:1932-9.
- 6 **Moreland LW**, Schiff MH, Baumgartner SW, Tindall EA, Fleischmann RM, Bulpitt KJ, *et al*. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. *Ann Intern Med* 1999;**130**:478-86.
- 7 PRISMS-4: Long-term efficacy of interferon-beta-1a in relapsing MS. *Neurology* 2001;**56**:1628-36.
- 8 Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) study group. *Lancet* 1998;**352**:1498-504.
- 9 **Li DK**, Paty DW. Magnetic resonance imaging results of the PRISMS trial: a randomized, double-blind, placebo-controlled study of interferon-beta 1a in relapsing-remitting multiple sclerosis. Prevention of relapses and disability by interferon-beta 1a subcutaneously in multiple sclerosis. *Ann Neurol* 1999;**46**:197-206.
- 10 **Coclet-Ninin J**, Dayer JM, Burger D. Interferon-beta not only inhibits interleukin-1beta and tumor necrosis factor-alpha but stimulates interleukin-1

- receptor antagonist production in human peripheral blood mononuclear cells. *Eur Cytokine Netw* 1997;**8**:345-9.
- 11 **Smeets TJ**, Dayer JM, Kraan MC, Versendaal J, Chicheportiche R, Breedveld FC, *et al*. The effects of interferon-beta treatment of synovial inflammation and expression of metalloproteinases in patients with rheumatoid arthritis. *Arthritis Rheum* 2000;**43**:270-4.
 - 12 **van Holten J**, Plater-Zyberk C, Tak PP. Interferon-beta for treatment of rheumatoid arthritis? *Arthritis Res* 2002;**4**:346-52.
 - 13 **Palmer G**, Mezin F, Juge-Aubry CE, Plater-Zyberk C, Gabay C, Guerne PA. Interferon beta stimulates interleukin 1 receptor antagonist production in human articular chondrocytes and synovial fibroblasts. *Ann Rheum Dis* Jan 2004;**63**:43-9.
 - 14 **Tak PP**, Hart BA, Kraan MC, Jonker M, Smeets TJ, Breedveld FC. The effects of interferon beta treatment on arthritis. *Rheumatology (Oxford)* 1999;**38**:362-9.
 - 15 **Triantaphyllopoulos KA**, Williams RO, Taylor H, Chernajovsky Y. Amelioration of collagen-induced arthritis and suppression of interferon-gamma, interleukin-12, and tumor necrosis factor alpha production by interferon-beta gene therapy. *Arthritis Rheum* 1999;**42**:90-9.
 - 16 **Van Holten J**, Reedquist K, Sattonet-Roche P, Smeets TJ, Plater-Zyberk C, Vervoordeldonk MJ, *et al*. Treatment with recombinant interferon-beta slows cartilage destruction and reduces inflammation in the collagen-induced arthritis model of rheumatoid arthritis. *Arthritis Res Ther* 2004;**6**:R239-49.
 - 17 **Arnett FC**, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315-24.
 - 18 **Takayanagi H**, Kim S, Matsuo K, Suzuki H, Suzuki T, Sato K, *et al*. RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-beta. *Nature* 2002;**416**:744-9.
 - 19 **Bresnihan B**, Alvaro-Gracia JM, Cobby M, Doherty M, Domljan Z, Emery P, *et al*. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. *Arthritis Rheum* 1998;**41**:2196-204.
 - 20 **Smolen JS**, Kalden JR, Scott DL, Rozman B, Kvien TK, Larsen A, *et al*. Efficacy and safety of leflunomide compared with placebo and sulphasalazine in active rheumatoid arthritis: a double-blind, randomised, multicentre trial. European Leflunomide Study Group. *Lancet* 1999;**353**:259-66.
 - 21 **van der Heijde D**. How to read radiographs according to the Sharp/van der Heijde method. *J Rheumatol* 2000;**27**:261-3.
 - 22 **Vencovsky J**, Machacek S, Sedova L, Kafkova J, Gatterova J, Pesakova V, *et al*. Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis. *Ann Rheum Dis* 2003;**62**:427-30.
 - 23 **Kraan MC**, Reece RJ, Barg EC, Smeets TJ, Farnell J, Rosenberg R, *et al*. Modulation of inflammation and metalloproteinase expression in synovial tissue by leflunomide and methotrexate in patients with active rheumatoid arthritis. Findings in a prospective, randomized, double-blind, parallel-design clinical trial in thirty-nine patients at two centers. *Arthritis Rheum* 2000;**43**:1820-30.
 - 24 **Tak PP**, van der Lubbe PA, Cauli A, Daha MR, Smeets TJ, Kluijn PM, *et al*. Reduction of synovial inflammation after anti-CD4 monoclonal antibody treatment in early rheumatoid arthritis. *Arthritis Rheum* 1995;**38**:1457-65.
 - 25 **Kraan MC**, Haringman JJ, Ahern MJ, Breedveld FC, Smith MD, Tak PP. Quantification of the cell infiltrate in synovial tissue by digital image analysis. *Rheumatology (Oxford)* 2000;**39**:43-9.
 - 26 **Kaufmann J**, Mueller A, Voigt A, Carl HD, Gursche A, Zacher J, *et al*. Hydroxypyridinium collagen crosslinks in serum, urine, synovial fluid and synovial tissue in patients with rheumatoid arthritis compared with osteoarthritis. *Rheumatology (Oxford)* 2003;**42**:314-20.
 - 27 **Smeets TJ**, Kraan MC, Versendaal J, Breedveld FC, Tak PP. Analysis of serial synovial biopsies in patients with rheumatoid arthritis: description of a control group without clinical improvement after treatment with interleukin 10 or placebo. *J Rheumatol* 1999;**26**:2089-93.
 - 28 **Tak PP**, Taylor PC, Breedveld FC, Smeets TJ, Daha MR, Kluijn PM, *et al*. Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor alpha monoclonal antibody treatment in patients with rheumatoid arthritis. *Arthritis Rheum* 1996;**39**:1077-81.