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A Randomised, Blinded, Placebo-Controlled, Dose Escalation Study Of The Tolerability And Efficacy Of Filgrastim For Haemopoietic Stem Cell Mobilisation In Patients With Severe Active Rheumatoid Arthritis

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Abstract

Autologous haemopoietic stem cell transplantation (HSCT) represents a potential therapy for severe rheumatoid arthritis (RA). As a prelude to clinical trails, the safety and efficacy of haemopoietic stem cell (HSC) mobilisation required investigation as colony-stimulating factors (CSFs) have been reported to flare RA. A double-blind, randomised placebo-controlled dose escalation study was performed. Two cohorts of eight patients fulfilling strict eligibility criteria for severe active RA (age median 40 years, range 24-60 years; median disease duration 10.5 years, range 2-18 years) received filgrastim (r-Hu-methionyl granulocyte(G)-(SF) at 5 and 10 μg/kg/day, randomised in a 5:3 ratio with placebo. Patients were unblinded on the fifth day of treatment and those randomised to filgrastim underwent cell harvesting (leukapheresis) daily until 2×10^6/kg CD34+ cells (haemopoietic stem and progenitor cells) were obtained. Patients were assessed by clinical and laboratory parameters before, during and after filgrastim administration. RA flare was defined as an increase of 30% or more in two of the following parameters: tender joint count, swollen joint count or pain score. Efficacy was assessed by quantitation of CD34+ cells and CFU-GM. One patient in the 5μg/kg/day group and two patients in the 10 μg/kg/day group fulfilled criteria for RA flare, although this did not preclude successful stem cell collection. Median changes in swollen and tender joint counts were not supportive of filgrastim consistently causing exacerbation of disease, but administration of filgrastim at 10 μg/kg/day was associated with rises in median C-reactive protein and median rheumatoid factor compared with placebo. Other adverse events were well recognised for filgrastim and included bone pain (80%) and increases in alkaline phosphatase (four-fold) and lactate dehydrogenase (two-fold). With respect to efficacy, filgrastim at 10 μg/kg/day was more efficient with all patients (n = 5) achieving target CD34+ cell counts with a single leukapheresis (median = 2.8, range = 2.3-4.8 $\times 10^6$/kg, median CFU-GM = 22.1, range = 4.2-102.9 $\times 10^4$/kg), whereas 1-3 leukaphereses were necessary to achieve the target yield using 5 μg/kg/day. We conclude that filgrastim may be administered to patients with severe active RA for effective stem cell mobilisation. Flare of RA occurs in a minority of patients and is more likely with 10 than 5 μg/kg/day. However, on balance, 10 μg/kg/day remains the dose of choice in view of more efficient CD34+ cell mobilisation.

Keywords: rheumatoid arthritis; stem cell transplantation; granulocyte colony-stimulating factor

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Based on animal data and anecdotal case reports, haemopoietic stem cell transplantation (HSCT) has been proposed for the treatment of autoimmune diseases.[1-9] Recent advances, including the use of cytokine mobilised peripheral blood stem cells, have resulted in major reductions in the mortality and morbidity of autologous transplantation for malignant diseases[10-11] and it now seems reasonable to consider HSCT as an experimental treatment for severe autoimmune diseases.

Rheumatoid arthritis (RA) is the most common systemic autoimmune disorder, affecting 1% of the population. RA incurs significant morbidity, with over 50% of individuals unable to work after 10 years of the disease, and has been estimated to shorten life by 5-10 years. Although various treatments may suppress disease activity, they require chronic administration, and do not cure the disease or prevent irreversible end organ damage. The associated economic costs are considerable, both to the individual and to the community.[12-13] Patients with severe refractory RA may be ideal candidates for experimental HSCT as, unlike other systemic autoimmune diseases, vital organ

function is usually uncompromised. In addition, RA is easily assessed by clinical and laboratory parameters.[7]

As a preliminary to studies of high-dose therapy, the safety and efficacy of peripheral blood stem cell mobilisation in RA requires confirmation. Both granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been shown to flare arthritis in mice.[14-15] Anecdotal clinical reports have reported exacerbation of autoimmune disease following treatment with G-CSF and GM-CSF. These have largely been in the context of Felty's syndrome[16-21] or drug-induced neutropenia.[22-23] Other reports have suggested that colony-stimulating factors may exacerbate vasculitis[19,24,25] and psoriasis[26] and precipitate bullous pyoderma gangrenosum[27] and neutrophilic dermatosis.[28] Potential causes for this effect may include leucocyte activation,[29] secondary release of proinflammatory cytokines[20] or possibly a direct effect on target tissues. However, there are many reports where CSFs have been used safely, for pro-longed periods, to treat neutropenia in rheumatoid arthritis.[26,30-42] In some cases,[43] it is unclear whether increased pain represents flare of disease or bone pain, which commonly accompanies CSF administration.

In addition, the quality of the stem cell product must be established. Abnormalities in the haemopoietic and immune systems in patients with RA[9,44] have the potential to influence numbers and repopulating ability of haemopoietic progenitor cells and the composition and function of other cellular effectors in the harvested product. Patients with severe RA may have been exposed to myelosuppressive anti-rheumatic drugs, in some cases for many years, potentially influencing the mobilisation product and function of the graft.[44,45]

The primary objective of this study was to investigate the tolerability of granulocyte colony-stimulating factor (r-Hu-methionyl G-CSF, filgrastim; Amgen, Thousand Oaks, CA, USA) in patients with severe active, but stable, rheumatoid arthritis using doses suitable for progenitor cell mobilisation. The secondary objectives were to determine whether laboratory parameters of inflammation, principally C-reactive protein, were affected by filgrastim and to investigate the yield of PBSC as measured by $CD34^+$ and CFUGM assays.

Patients and methods

Study design

This single centre study was approved by the Research Ethics Committee of St Vincent's Hospital and all patients gave informed consent. The design was a randomised, double blinded placebo-controlled phase I dose escalation study with two cohorts of eight patients, with five randomised to filgrastim and three receiving placebo in each cohort. The first cohort received filgrastim at 5 μg/kg/day. After acceptable tolerability (defined as less than two patients in the filgrastim group compared with the placebo group experiencing an increase of 30% or greater from treatment baseline in two of swollen joint count, tender joint count, and pain score, or more than two patients achieving a white cell count of $>75 \times 10^9/1$, or other intolerable events related to filgrastim) was confirmed for the first cohort, the dose was escalated to 10 µg/kg/day for the second cohort. Patients received four daily doses of study drug or placebo in a double blind manner. On day 5, following assessment of clinical parameters, patients and assessors were unblinded and those receiving filgrastim underwent leukapheresis on a Cobe Spectra cell separator (Cobe, Lakewood, CO, USA) aiming to process 2.5 × blood volume. Leukapheresis was continued for a maximum of 3 consecutive days until the target $CD34^+$ cell yield of 2×10^6/kg was achieved. Filgrastim was discontinued once the target yield was achieved or to a maximum of 6 days in total. Patients were reassessed at study completion on day 11, and also reviewed at a follow-up appointment 4-6 weeks following filgrastim administration.

Patient selection

Patients were enrolled 7-10 days before commencing filgrastim after satisfying the following eligibility criteria: age 18-65 years, a diagnosis of RA according to the criteria of the American College of Rheumatology (ACR)[46] for 2-20 years, failure of at least two second-line anti-rheumatic agents (i.e. anti-rheumatic drugs other than corticosteroids and non-steroidal anti-inflammatory drugs, NSAIDs). In addition, they fulfilled criteria for active disease, i.e. had six or more swollen joints, six or more tender joints, plus at least two of the following three criteria: (1) nine or more joints, capable of response, tender on pressure or motion; (2) 1 h or more of

morning stiffness; (3) ESR of >28 mm/h. Patients had no other serious prior or intercurrent illness, including any major haematological disorder. Full blood count including differential and coagulation screen were within normal ranges or compatible with RA and/or its treatment (e.g. anaemia of chronic disorder, reactive neutrophilia and thrombocytosis, and lymphopenia due to corticosteroids were permitted). Biochemical screens showed plasma creatinine <0.15 mmol/l, liver enzymes <3 × the upper limit of normal and plasma bilirubin <30 mmol/l. Premenopausal women had to have a negative pregnancy test, not be breast feeding and use contraceptive precautions. Informed consent was signed by all patients. Exclusion criteria included concurrent enrolment on any other protocol using an investigational drug or haemopoietic growth factor within 4 weeks of study entry, any disorder that compromised ability to give informed consent, known sensitivity to E. coli-derived drug preparations and previous entry to the study. Each patient was given an identification number in order of enrolment, with 1001 to 1008 for cohort 1 and 2001 to 2008 for cohort 2.

Concomitant anti-rheumatic medications

Patients were stabilised on corticosteroids and NSAIDs for at least 4 weeks prior to enrolment on the study and were continued at the same dose throughout the study. Minor fluctuations within the dose range of NSAIDs and paracetamol were permitted. Joint injections were not permitted within the 4-week 'run in' period or during the study. Additional paracetamol was permitted to manage side-effects associated with filgrastim (such as bone pain). Patients on methotrexate, azathioprine, gold, hydroxychloroquine, sulphasalazine, D-penicillamine or cyclophosphamide had received the drug at the same dose for 3 months prior to beginning the study. Myelosuppressive drugs were stopped 1 week prior to the first dose of the study drug and recommenced 8 days later. Hence, patients were stable on anti-rheumatic medication prior to administration of filgrastim. A 2-week cessation of myelosuppressive anti-rheumatic drugs, necessary to permit administration of filgrastim, was considered to be insufficient for their anti-rheumatic effect to be lost.

Assessment of safety

Prior to commencing treatment with the study drug, patients were assessed with baseline physical examination and measurement of swollen and tender joint counts (EULAR 28 joint count score), joint pain score on a 100 mm visual analogue scale (VAS), duration of morning stiffness (measured semi-quantitatively, 0 min, 0-30 min, 30-60 min, 1-2 h, 2-4 h, >4 h), adjectival patient and clinician global scales for disease (nil, mild, moderate, severe, very severe), vital signs, full blood count, plasma biochemistry, serum rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), and C-reactive protein. All patients had a normal chest X-ray and ECG, with the exception of one patient in whom the ECG suggested ischaemic heart disease which had been excluded by cardiac angiography. Pre-treatment bone marrow aspirates in 14 out of 16 patients agreeing to the procedure confirmed normal appearances or appearances consistent with RA and/or its treatment in all patients.

During the study, patients were assessed with daily full blood count on days 1-5 and on days 6 and 7 where leukapheresis was performed. Plasma biochemistry, C-reactive protein, ESR and RF were repeated on the day after the final administration of the study drug. Patients completed a daily diary detailing pain score, morning stiffness, adjectival patient global scale and specific symptoms. Vital signs were recorded when patients attended clinic. On day 5, double-blind measurement of swollen joint count, tender joint count and adjectival clinician global scale were performed, after which patient and assessor were unblinded.

The study was terminated at day 11 when all baseline assessments were repeated. Four to 6 weeks following study drug administration, a follow-up interview was con-ducted with repeat joint counts and blood tests. More than 95% of joint counts were performed by one assessor (AF), the remainder by a second assessor. Assessment before and after the blinded period was performed by the same assessor on all occasions.

Assessment of efficacy

$CD34^+$ cell counts were assessed by flow cytometry as previously described.[47] CFU-GM count was performed on thawed cells which had been frozen in 10% dimethyl sulphoxide using 14-day culture in methylcellulose media (Methocult GF H4434; Stem Cell Technologies, Vancouver, Canada) as previously described.[48]

Analysis of data
Descriptive statistical methods (medians, ranges) were used to compare both filgrastim cohorts with the combined placebo group and to look for a dose escalation effect between the 5 and 10 μg/kg/day cohorts. To detect a flare in the RA of individual patients, we defined a flare as an increase from baseline of 30% or more of two of the following parameters; tender joint count, swollen joint count or pain score on VAS.

Results

Patient demographics
Patient data are summarised in Table 1. All patients were Caucasian. One patient out of 10 randomised to filgrastim required central venous access for leukapheresis in view of poor peripheral venous access.

Safety data
Descriptive data for changes in rheumatological variables are summarised in Tables 2-5. Three patients (patients 1003, 2003 and 2006) fulfilled criteria for flare, although this did not prevent successful stem cell collection. In one of these patients (2003) symptoms were sufficient to warrant an increase in dose of corticosteroids for 2 weeks (from 5 mg to 20 mg prednisolone daily). These clinical flares were associated with rises in C-reactive protein, ESR and rheumatoid factor (Table 4). The increased disease activity was also reflected by worsening in the semi-quantitative parameters shown in Table 3.

Median values of swollen and tender joint counts and semi-quantitative assessment of morning stiffness and patient and clinician global assessments remained largely stable and were not supportive of filgrastim consistently causing exacerbation of disease (Tables 2, 3 and 4). Administration of filgrastim at 10 μg/kg/day was associated with a rise in median C-reactive protein level which corrected by day 11 and a progressive rise in rheumatoid factor at days 5 to 11 (Table 5). It is difficult to comment on changes in ESR as there was a progressive rise in the placebo group.

The median joint pain scores (by VAS) support a dose-dependent increase in pain during the blinded phase of the study, which improved after cessation of the filgrastim (Table 2). Here there may be some overlap with filgrastim induced bone pain, the most common adverse event occurring in 8/10 filgrastim vs 0/6 placebo patients. Other adverse events were routine for filgrastim and included headache (5/10 filgrastim vs 2/6 placebo patients) and fatigue/tiredness (5/10 filgrastim vs 2/6 placebo patients). There was an increase in median alkaline phosphatase from baseline on day 5 (3.5-3.9 × placebo) returning to 1.3-1.4 × placebo by day 11. Similarly, median LDH increased on day 5 (1.8-1.9 × placebo) and settled to 1.1-1.2 × placebo by day 11. The absence of significant change in disease activity of the placebo patients provided support that withdrawal of anti-rheumatic drugs for the short study period did not in itself exacerbate their RA.

Efficacy data
Filgrastim administration produced a significant rise in WCC (and neutrophil count) in both treatment groups, with a median peak of $42.7 \times 10^9/1$ (neutrophils $36.8 \times 10^9/1$) at day 6 in the 5 μg/kg/day cohort and of $43.7 \times 10^9/1$ (neutrophils $38.8 \times 10^9/1$) at day 3 in the 10 μg/kg/day cohort. Yields of haemopoietic progenitors, measured principally by $CD34^+$ cells/kg, are summarised in Table 6. Filgrastim at a dose of 10 μg/kg/day was more efficient in terms of mobilisation in that target $CD34^+$ yields were achieved with one leukapheresis and total CFU-GM counts from this group were higher.

Table 1 Patient data

	Filgrastim 5 μg/kg/day n = 5	*Filgrastim 10 μg/kg/day n = 5*	*Placebo n = 6*
Age median (range)/years	51 (28–60)	45 (37–58)	30 (24–51)
Sex	1M:4F	1M:4F	2M:4F
Duration of RA median (range)/years	9 (8–15)	12 (4–17)	6 (2–18)
No. previous myelosuppressives: median (range)	4 (1–5)	3 (2–7)	4 (2–5)
Previous myelosuppressive drugs:			
Methotrexate	5	5	6
Gold	4	4	4
Hydroxychloroquine	2	3	3
Sulphasalazine	2	3	6
Azathioprine	2	2	0
D-penicillamine	2	1	2
Cyclophosphamide	0	1	0

Table 2 Median rheumatological parameters pre-filgrastim, pre-unblinding, and day 11

	Pre-filgrastim day 1	*Pre-unblinding*	*Study completion day 11*
Tender joint count/28			
5 μg/kg/day	14	15	18
10 μg/kg/day	24	26	19
Placebo	20	18	19
Swollen joint count/28			
5 μg/kg/day	14	16	16
10 μg/kg/day	14	14	11
Placebo	14	14	13
Pain score (mm)			
5 μg/kg/day	62	55	58
10 μg/kg/day	48	60	55
Placebo	62	49	53

Table 3 Summary of changes in morning stiffness, clinician global and patient global (assessed semi-quantitatively)

	Filgrastim 5 μg/kg/day n = 5	*Filgrastim 10 μg/kg/day n = 5*	*Placebo n = 6*
Morning stiffness			
Number worse days 1–4	1 (patient 1003)	1 (patient 2003)	0
Number worse days 1–11	1 (patient 1003)	1 (patient 2003)	1 (patient 2008)
Clinician global			
Number worse days 1–5	0	0	0
Number worse days 1–11	0	1 (patient 2006)	0
Patient global			
Number worse days 1–4	0	2 (patient 2005, 2006)	0
Number worse days 1–11	0	2 (patient 2003, 2006)	0

Discussion

This placebo controlled study is the first to investigate the safety and efficacy of filgrastim in patients with severe active RA for stem cell collection. In a minority of patients, filgrastim administration was associated with an early or late transient flare of RA, but this did not prevent successful harvesting. In only one patient was the flare significant enough to warrant an increase in the steroid dosage. Pro-genitor cell yields were satisfactory in all patients based on both CD34+ counts and CFU-GM assays, and fulfilled recently published criteria in the EBMT/EULAR consensus guidelines for autoimmune disease (i.e. CD34+ count >2 × 106/kg and CFU-GM >2 × I04/kg).[6] In all patients receiving filgrastim at 10 *u*g/kg/day, the target threshold of 2 × 106/kg CD34+ cells was achieved with one leukapheresis.

Table 4 Summary of patients experiencing 30% or more increase in tender joint count, swollen joint count and pain score from baseline

Patient number	*1001*	*1003*	*2003*	*2005*	*2006*	*2007*
Dose filgrastim (μg/kg/day)	5	5	10	10	10	Placebo
Tender joint count						
% change days 1–5					44	
% change days 1–11			36			57
Swollen joint count						
% change days 1–5		40				
% change days 1–11						
Pain score						
% change days 1–4	36	50	69	38	30	
% change days 1–11	33		94			

Clinical flares were associated with rises in C-reactive protein (1003; 21 to 50 mg/l, 2003; 10 to 53 mg/l, 2006; 11 to 30 mg/l), ESR (1003; 17 to 26 mm/h, 2003; 4 to 12 mm/h, 2006; 16 to 26 mm/h) and rheumatoid factor (2003; 256 to 412 IU/ml).

Table 5 Median laboratory parameters pre-filgrastim, 1 day after the last dose of filgrastim, and at study completion on day 11

	Pre-filgrastim day 1	*Post-filgrastim*	*Study completion day 11*
CRP (mg/l)			
5 μg/kg/day	13	9	8
10 μg/kg/day	11	30	10
Placebo	49	36	42
ESR (mm/h)			
5 μg/kg/day	17	14	24
10 μg/kg/day	16	16	26
Placebo	38	54	63
Rheumatoid factor (IU/ml)			
5 μg/kg/day	269	201	240
10 μg/kg/day	256	271	337
Placebo	82	87	89

Table 6 Stem cell mobilisation

	Day 5 $CD34^+$ $\times 10^6$/kg	*Day 6 $CD34^+$ $\times 10^6$/kg*	*Day 7 $CD34^+$ $\times 10^6$/kg*	*Total $CD34^+$ $\times 10^6$/kg*	*Total CFU-GM $\times 10^6$/kg*
Dose filgrastim					
5 μg/kg/day					
n	5	4	1	5	5
median	0.9	1.4	0.3	2.3	12
range	0.9–2.2	0.8–2.4		2.0–3.4	5.3–19.6
10 μg/kg/day					
n	5			5	5
median	2.8			2.8	22.1
range	2.3–4.8			2.3–4.8	4.2–102.9

One other pilot study has recently addressed the safety of administering filgrastim to patients with RA 49 Five patients were given intramuscular or intra-articular methylprednisolone (median 80 mg, range 40-120 mg) prior to administration of filgrastim at 5 μg/kg/day to protect against flare. Disease activity remained stable although the pre-administration of corticosteroids may have inhibited any pro-inflammatory effect of filgrastim. The patients did not undergo leukapheresis, but efficacy, quantitated indirectly using peripheral blood $CD34^+$ count, was considered adequate.

Our study differs in that it has shown that RA flare may be a complication of filgrastim administration for stem cell mobilisation. However, routine pre-administration of corticosteroids is unnecessary in the majority of patients. Use of anti-inflammatory agents (which in our study were kept stable for 1 month prior to and during the study period) could be individualised to minimise flare. Alternatively, the risks of flare could be discussed with the patient, who could then decide whether or not to receive intra-articular or intramuscular steroid injections.

Our results also show that the target thresholds can be achieved in one leukapheresis without the use of cytotoxic agents, such as cyclophosphamide, for priming. Although cyclophosphamide priming may increase the yield of PBSC, it has the potential to add morbidity and even mortality.[11,50] In addition, a recent analysis suggests that the use of cyclophosphamide priming may contribute to an excess incidence of secondary myelodysplasia observed with peripheral blood stem cells compared with bone marrow in autologous transplantation.[51] The above study is reassuring in that sufficient HSC may be

mobilised from most patients with RA with filgrastim alone. Avoidance of additional cytotoxic agents in mobilisation may be desirable in RA patients who even without the risks of autologous transplantation are known to have a higher incidence of leukaemia from the disease and conventional treatments.[52]

Further studies are necessary on stem cell mobilisation in patients with RA before large scale clinical trials of autologous HSCT can commence. Analysis of greater numbers of patients will define the risk of flare more accurately. Mobilised stem cells should be evaluated in short- and long-term *in vitro* culture to reassure that such harvests are able to reconstitute long-term haemopoiesis. The composition of the harvest should be determined and the functional properties of contaminating lymphocytes and other cellular effectors should be investigated. Such information may be valuable in planning graft manipulation strategies although at present it is unknown whether such processing will be clinically advantageous. Further investigation will naturally depend on the success of autografting in RA patients, although early results of unmanipulated autografting in sporadic cases[53,54] and in our own clinical trial[55] appear promising.

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