INFLAMMATORY BOWEL DISEASE

Fontolizumab, a humanised anti-interferon γ antibody, demonstrates safety and clinical activity in patients with moderate to severe Crohn's disease

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Introduction: Interferon γ is a potent proinflammatory cytokine implicated in the inflammation of Crohn's disease (CD). We evaluated the safety and efficacy of fontolizumab, a humanised anti-interferon γ antibody, in patients with moderate to severe CD.

Methods: A total of 133 patients with Crohn's disease activity index (CDAI) scores between 250 and 450, inclusive, were randomised to receive placebo or fontolizumab 4 or 10 mg/kg. Forty two patients received one dose and 91 patients received two doses on days 0 and 28. Investigators and patients were unaware of assignment. Study end points were safety, clinical response (decrease in CDAI of 100 points or more), and remission (CDAI $\leqslant$150).

Results: There was no statistically significant difference in the primary end point of the study (clinical response) between the fontolizumab and placebo groups after a single dose at day 28. However, patients receiving two doses of fontolizumab demonstrated doubling in response rate at day 56 compared with placebo: 32% (9/28) versus 69% (22/32, p = 0.02) and 67% (21/31, p = 0.03) for the placebo, and 4 and 10 mg/kg fontolizumab groups, respectively. Stratification according to elevated baseline C reactive protein levels resulted in a decreased placebo response and pronounced differences in clinical benefit. Two grade 3 adverse events were reported and were considered to be related to CD. One death (during sleep) and one serious adverse event (an elective hospitalisation) occurred, both considered unrelated.

Conclusion: Treating active CD with fontolizumab was well tolerated and resulted in increased rates of clinical response and remission compared with placebo.

I nterferon γ (IFN-γ), a type II interferon, is one of the most potent and pleiotrophic cytokines, exhibiting a multitude of immunoregulatory functions.[1] IFN-γ can activate a wide range of immunocompetent cell types, including macrophages, endothelial cells, and lymphocytes, and is a key factor for expression of class II MHC molecules on antigen presenting cells.[1] In addition, IFN-γ decreases epithelial barrier function and promotes neutrophil migration by increased expression of chemokines and their receptors.[2] In several Th1 mediated models for experimental colitis, including interleukin (IL)-10, $G\alpha_{i2}$, and IL-2 deficient mice, mucosal IFN-γ levels were increased.[3–5] Also, in human Crohn's disease (CD), which has a predominantly Th1 phenotype, elevated mucosal IFN-γ levels have been observed.[6–8] Therefore, strategies aimed at neutralising IFN-γ could prove efficacious in treating CD. Anti-IFN-γ strategies have shown benefit in another Th1 autoimmune disease, rheumatoid arthritis.[9]

Fontolizumab (HuZAF) is the humanised form of a murine antihuman IFN-γ antibody (Protein Design Labs, Inc., Fremont, California, USA) derived by using recombinant technology. It has shown excellent safety and tolerability in a phase I/II, double blind, placebo controlled, single and multiple dose, dose escalation study in patients with moderate to severe CD.[10] Therefore, we performed a phase II, randomised, double blind, placebo controlled study of intravenous fontolizumab in active CD.

MATERIALS AND METHODS

The study complied with the International Conference of Harmonisation E6 Good Clinical Practices, United States Food and Drug Administration regulations governing the protection of human subjects (21CFR parts 50 and 56), Declaration of Helsinki, and any local standards of ethics. This protocol was approved by the institutional review board at each centre prior to activation, and all patients provided signed informed consent before receiving any study related procedures.

Patient eligibility

The study was conducted at 25 hospitals in Europe (Belgium, Croatia, Hungary, Russia, Slovakia, the Netherlands, and the UK). Patients were eligible for study enrolment if they were 18–70 years old and had a Crohn's disease activity index (CDAI) score of $\geqslant$250 and $\leqslant$450. Patients were excluded from the study if they had changed corticosteroid dose two weeks before baseline; were receiving intravenous steroids or more than 30 mg/day of prednisone or its equivalent; had started or had a change in dose within three months of baseline of azathioprine, 6-mercaptopurine, methotrexate, or ciclosporin; had started or had a change in dose within four weeks of baseline of sulfasalazine, 5-aminosalicylic acid, or antibiotics for CD treatment; received murine, chimeric, or humanised antibody therapy within 60 days before randomisation; received investigational drugs or therapies within 30 days of randomisation; had a history of immune deficiency or autoimmune disorders other than CD; had prior or current malignancies; had serological evidence of infection with human immunodeficiency virus or hepatitis B

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Abbreviations: CD, Crohn's disease; CDAI, Crohn's disease activity index; CRP, C reactive protein; DSMB, Data Safety Monitoring Board; ELISA, enzyme linked immunosorbent assay; IFN-γ, interferon γ
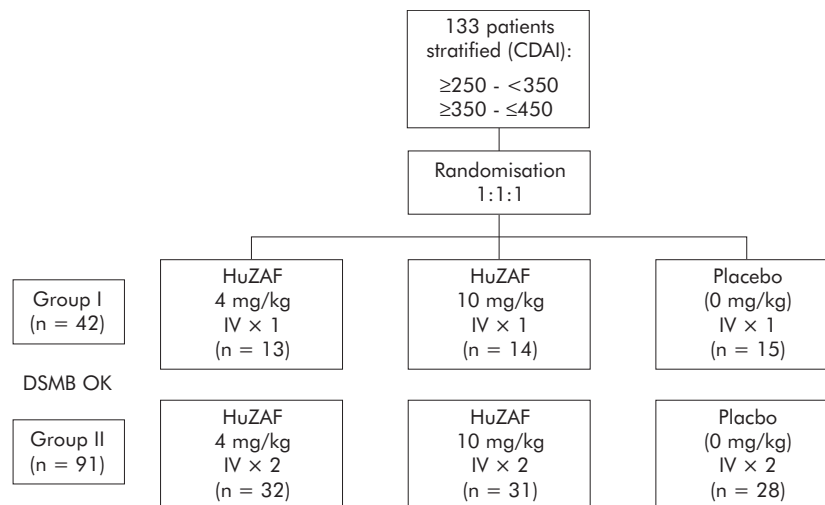
Figure 1 Study enrolment. $IV \times 1$ and $IV \times 2$ = one or two doses of study drug administered intravenously. CDAI, Crohn's disease activity index; HuZAF, fontolizumab.

or C; had evidence of *Clostridium difficile*, active infections requiring antibiotic therapy, or clinical history or radiographic evidence of tuberculosis; or had significant organ dysfunction or were likely to require surgery in the next six months. Patients were randomised within two CDAI strata (≥250 to <350 and ≥350 to ≤450).

Study design, treatment, and objectives

This phase II, randomised, double blind (except to the site pharmacist), placebo controlled study evaluated the safety and efficacy of intravenous fontolizumab (4 or 10 mg/kg) or placebo infused over 30 minutes in patients with moderate to severe CD. Patients who weighed ≥100 kg received 400 mg if in the 4 mg/kg group or 1000 mg if in the 10 mg/kg group.

Because multiple doses of 10 mg/kg had not previously been administered to patients, patients enrolled initially received a single intravenous dose of study treatment (group I). After reviewing safety data from the first 30 group I patients who completed day 28 assessments, a Data Safety Monitoring Board (DSMB), which was independent of the study sponsor and investigators, recommended enrolment of patients into group II. These patients received two doses of study drug, 28 days apart.

Clinical and laboratory assessments, including haematology and chemistry panels, immunogenicity, CDAI scores, adverse events, and use of concomitant medications, were determined at two week intervals up to day 56, and monthly thereafter, up to 3 months post dosing. The upper limit of normal for C reactive protein (CRP) for the assay used in this study was 4 mg/l. The National Cancer Institute Common Toxicity Criteria, version 2.0,[11] was used to rate the severity of adverse events. At six months post dosing, patients were queried about the occurrence of infectious diseases, including tuberculosis and malignancies.

Fontolizumab pharmacokinetics were assessed in a subset of patients. The sampling schedules differed slightly between groups I and II. In group I, samples were collected on day 0 at 15 minutes prior to dose and two hours post dose and on days 14, 28, 42, 56, and 84. In group II, samples were collected on day 0 at 15 minutes pre-dose and two hours after the first dose; on day 14; on day 28 at 15 minutes before and two and four hours after the second dose; and on days 42, 56, 84, and 112. Serum concentrations of fontolizumab were evaluated using validated enzyme linked immunosorbent assays (ELISAs). Pharmacokinetic parameters were estimated by two compartmental analysis using WinNonlin

Table 1 Baseline characteristics and values: groups I and II combined

Characteristic	Placebo (n = 43)	Fontolizumab 4 mg/kg (n = 45)	Fontolizumab 10 mg/kg (n = 45)
Female (%)	63	64	51
Age (y) (median (range))	39 (20–72)	43 (19–65)	39 (21–68)
CD mean duration (y)	6.7	6.4	7.4
Fistulae present (%)	16	13	9
Smokers (%)	30	27	18
CDAI (mean (range))	303 (228–401)	300 (241–457)	303 (241–456)
CDAI ≥350 (%)	16	7	13
Ileum only (%)	19	22	9
Colon only (%)	35	40	38
Ileum and colon (%)	46	36	49
Lower and upper GI (%)	0	2	4
C-reactive protein >4 mg/l (%)	47	56	53
Use of medications for treatment of CD (%)			
Prior infliximab use	19	22	22
5-aminosalicylic acid	54	49	40
Corticosteroids	33	36	38
Azathioprine	40	27	36
Methotrexate	7	2	4

CD, Crohn's disease; CDAI, Crohn's disease activity index; GI, gastrointestinal tract.

Table 2 Efficacy variables at day 28: groups I and II combined

Day 28 result	Placebo (n = 43)	Fontolizumab (n = 45 in each group)	
		4 mg/kg (p value*)	10 mg/kg (p value*)
Clinical response (CDAI drop of ≥100 points)	14 (33%)	17 (38%) (0.66)	19 (44%) (0.38)
Clinical remission (CDAI ≤150)	5 (12%)	14 (31%) (0.04)	8 (19%) (0.55)
CDAI change from baseline (mean)	−61.8	−99.8 (0.02)	−97.8 (0.04)
CRP change from baseline (median) (mg/l)	0.00	−0.05 (0.41)	−2.40 (<0.01)

*Compared with placebo group.
CRP, C reactive protein; CDAI, Crohn's disease activity index.

(enterprise version 3.2; Pharsight Corporation, Mountain View, California, USA).

Samples for testing for the presence of anti-fontolizumab antibodies were obtained on days 0, 14, 28, and 58, and three months after the last dose of study drug. On dosing days, samples were taken 15 minutes before dosing. Screening for circulating antibodies to fontolizumab was performed using a bridging ELISA. Samples that were positive in the screening assay were further analysed in a confirmatory test. Only samples giving positive or inconclusive results in the confirmatory assay were subsequently tested for the presence of neutralising antibodies.

For the screening assay, fontolizumab coated plates were used to capture anti-fontolizumab antibodies from serum, and horseradish peroxidase conjugated fontolizumab was used as the detecting reagent. An anti-idiotypic antibody against the fontolizumab murine parent antibody AF2 (anti-AF2id) was used for calibration. The assay positive cut off point was established using sera from 32 untreated individuals and was set as mean optical density + 2 SDs. The confirmatory assay was similarly conducted, except that excess fontolizumab was added. In order to confirm positivity, a sample had to show a reduction in signal in the presence of excess fontolizumab relative to the signal obtained without fontolizumab. The neutralisation bioassay tests the ability of anti-fontolizumab antibodies to neutralise the inhibitory function of fontolizumab on IFN-γ-induced IP-10 production in a susceptible cell line.

The primary objectives of the study were to determine the safety and response (≥100 point CDAI decline compared with baseline) and remission (CDAI ≤150 points) rates of each fontolizumab group versus placebo after a single dose at day 28. Secondary objectives included safety and response and remission rates of fontolizumab when administered as two doses, as well as the pharmacokinetics and immunogenicity results for all patients.

Sample size justification
Assuming a response rate of 70% in either of the active treatment groups and a 35% response in the placebo group, the study was designed to have 85% power to detect a difference of 35 percentage points (at alpha = 0.05 for the
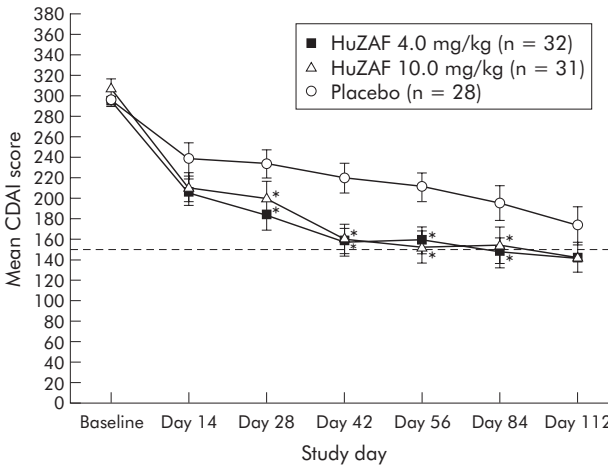
Figure 2 Mean (SEM) Crohn's disease activity index (CDAI) for patients receiving two doses of study drug. *Comparison is change from baseline value for each fontolizumab group versus placebo group. Significance is defined as p<0.05. HuZAF, fontolizumab.

end point), with 40 patients for each active treatment group and 40 patients for the placebo group. The total number of patients planned for the study was 120. The study was not powered to detect statistically significant differences between fontolizumab regimens.

Statistical methods
Demographics and baseline characteristics were summarised by treatment group. Continuous variables, including baseline CDAI scores and CRP serum levels, were compared across the three treatment groups using one way analysis of variance with treatment as factor. Categorical variables were compared across the three groups using the Fisher exact test.

The proportion of patients achieving response or remission at each visit was compared between each fontolizumab group and the placebo group using the Fisher exact test. The two sample t test was used for between treatment comparisons of change from baseline CDAI scores at each subsequent study visit. Change from baseline CRP value at each subsequent

Table 3 Clinical response and remission: group II

Parameter	Day	Placebo	Fontolizumab	
			4 mg/kg (p value*)	10 mg/kg (p value*)
Clinical response (CDAI drop of ≥100 points)	42	10/26 (39%)	19/32 (59%) (0.19)	20/31 (65%) (0.07)
	56	9/26 (35%)	22/32 (69%) (0.02)	20/30 (67%) (0.03)
Clinical remission (CDAI ≤150)	42	4/26 (15%)	15/32 (47%) (0.01)	14/31 (45%) (0.02)
	56	7/26 (27%)	13/32 (41%) (0.41)	16/30 (53%) (0.06)

*Compared with placebo group.
CDAI, Crohn's disease activity index.
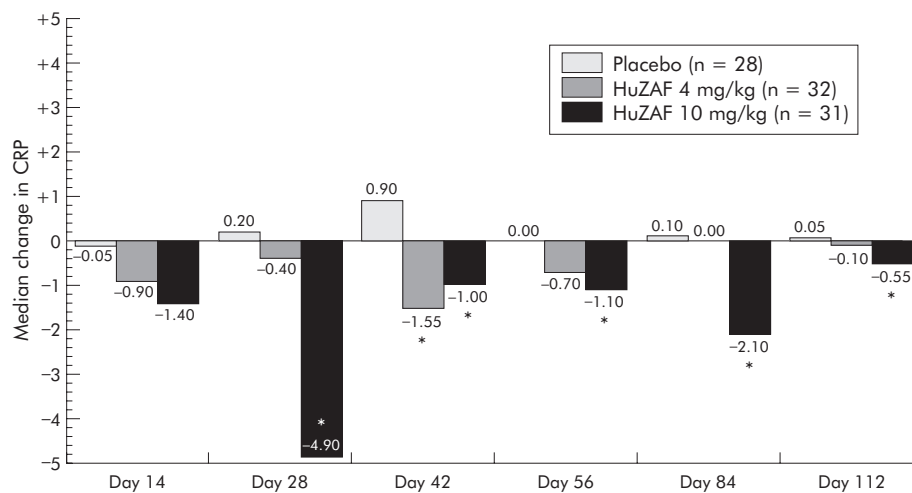
Figure 3 Median change in C reactive protein (CRP) in patients receiving two doses of study drug. *Each fontolizumab group compared with placebo. Significance is defined as p<0.05. HuZAF, fontolizumab.

visit was compared between each fontolizumab group and the placebo group using the Wilcoxon rank sum test. Survival time variables were analysed using the log rank test and presented in Kaplan-Meier plots. Patients who experienced regimen failure had their last observation carried forward. Regimen failure occurs when a patient is terminated from the study due to lack of efficacy or receives an increase in dose of a concomitant medication or new medications are added as therapy for CD.

Efficacy and safety analyses were performed on all patients who received at least one dose of study medication (intent to treat population). All statistical tests were performed using two sided tests with an alpha level of 0.05. The p values presented for the secondary exploratory analyses should be interpreted as descriptive measures of the strength of evidence, independent of the scale of measurement. They are not intended to be, nor should they be interpreted as, statistical proof of any actual or assumed differences.

RESULTS
Patient demographics and disease baseline characteristics
Of the 169 patients screened, 133 (79%) were enrolled and randomised into one of three dose groups, stratified according to CDAI score, as shown in fig 1. Approximately two thirds of patients who failed screening did not meet entry criteria (most commonly for CDAI <250); the remaining third elected not to participate. Of those enrolled, 119 completed the study, with approximately twice as many patients treated with placebo withdrawing from the study (n = 7), compared with either of the fontolizumab groups (n = 4, 4 mg/kg; n = 3, 10 mg/kg). The DSMB received clinical SAS databases after 30 patients in group I completed the day 28 assessments. No major safety concerns were noted, and enrolment into group II commenced. Forty two

patients received a single dose (the first 30 patients plus an additional 12 patients recruited prior to the DSMB decision) (group I), and 91 patients received two doses (group II) of study medication.

There were no statistically significant differences between the three treatment groups with regard to any demographic or baseline characteristic (table 1). Use of baseline concomitant CD therapies was similar across the three cohorts; 77%, 76%, and 85% of patients were receiving a glucocorticoid and/or an immunosuppressant in the placebo, 4, and 10 mg/kg cohorts, respectively.

Efficacy results
There was no statistically significant difference in the primary end point of the study (clinical response) between either of the fontolizumab groups versus the placebo group after a single dose at day 28. However, 14 of 45 (31%) patients receiving a single dose of 4 mg/kg demonstrated clinical remission at day 28 whereas only five of 43 placebo treated patients (12%; p = 0.04) achieved this (table 2).

For patients receiving two doses (group II), there was a doubling in the response rate at day 56 for each fontolizumab group compared with placebo: 35% (9/26) for the placebo group versus 69% (22/32, p = 0.02) and 67% (20/30, p = 0.03) for the 4 mg/kg and 10 mg/kg fontolizumab groups, respectively (table 3). There was a strong trend for increased remission, particularly at day 42, with the placebo remission rate being 15% versus 47% (p = 0.01) for the 4 mg/kg and 45% (p = 0.02) for the 10 mg/kg fontolizumab groups, respectively.

When change from baseline CDAI score was compared, the improvement in the fontolizumab groups was apparent as early as day 14; this was significant for the 10 mg/kg group compared with the placebo group (p = 0.02). By day 28 there was an approximate 50% greater reduction in mean CDAI

Table 4 Clinical response and remission in patients with baseline C reactive protein >4 mg/l: groups I and II combined

Day 28 results	Placebo (n = 20)	Fontolizumab	
		4 mg/kg (n = 25) (p value*)	10 mg/kg (n = 24) (p value*)
Clinical response (CDAI drop ≥100 points)	4 (20%)	11 (44%) (0.12)	11 (46%) (0.11)
Clinical remission (CDAI ≤150)	0	10 (40%) (<0.01)	4 (17%) (0.11)

*Compared with placebo group.
CDAI, Crohn's disease activity index.

Table 5 Adverse events with frequency of 5% or more in any treatment group

		Fontolizumab		
Preferred term	Placebo (%) (n = 43)	4 mg/kg (%) (n = 45)	10 mg/kg (%) (n = 45)	Total (%) (n = 133)
Abdominal pain	1 (2.3)	3 (6.7)	6 (13.3)	10 (7.5)
Crohn's disease	4 (9.3)	4 (8.9)	2 (4.4)	10 (7.5)
CRP increased	4 (9.3)	1 (2.2)	2 (4.4)	7 (5.3)
Vomiting	0	4 (8.9)	2 (4.4)	6 (4.5)
Headache	1 (2.3)	2 (4.4)	3 (6.7)	6 (4.5)
Nausea	2 (4.7)	1 (2.2)	3 (6.7)	6 (4.5)
Arthralgia	0	0	4 (8.9)	4 (3.0)
Asthenia	1 (2.3)	0	3 (6.7)	4 (3.0)
Cough	0	0	3 (6.7)	3 (2.3)

CRP, C reactive protein.

from baseline in the two fontolizumab cohorts compared with the placebo group: −61.8 for placebo versus −99.8 (p = 0.02) and −97.8 (p = 0.04) for the 4 and 10 mg/kg fontolizumab groups, respectively. Change from baseline CDAI score was statistically significant for patients receiving two doses of study treatment in the fontolizumab groups versus placebo patients at days 42 and 56 (fig 2, table 3). For patients in group II, time to remission demonstrated a dose-response trend. Median time to remission was 106 days, 50 days (p = 0.04), and 43 days (p = 0.08) for the placebo and 4 and 10 mg/kg fontolizumab groups, respectively, in patients who received two doses. This was significant for the 4 mg/kg group only.

When median change from baseline CRP values was compared, values were significantly improved (decreased) between the 10 mg/kg and placebo groups at day 28 for all patients (fig 3, table 2). At visits after day 28, compared with placebo patients, improved CRP values were significant for group II patients in the 4 mg/kg group at day 42 (p<0.01) and in the 10 mg/kg group at days 42 (p<0.01) and 56 (p = 0.05).

Approximately half of the patients in this study were found to have an elevated baseline CRP (>4 mg/l). The response rate to placebo at day 28 in this subset of patients was decreased by 40% compared with that of the total study population (from 33% to 20%). On the other hand, the response rate in the two fontolizumab groups for patients with elevated CRP was numerically increased compared with the total study population randomised to these doses: 44% versus 38% for the 4 mg/kg group and 46% versus 42% for the 10 mg/kg group (tables 2, 4). A similar pattern was observed for remission at day 28 (table 4) and for group II patients at days 42 and 56 (table 3).

Although there was a non-significant imbalance in the proportion of smokers and patients with fistulas at baseline between the placebo and active arms, there were insufficient numbers of patients to analyse these potential covariates.

Safety results

The percentage of patients reporting adverse events was similar across the dose groups: 42%, 44%, and 47% of patients in the placebo, 4 mg/kg, and 10 mg/kg groups, respectively, reported at least one adverse event. There were no evident patterns of adverse events specifically attributable to fontolizumab, and there were few events reported with an overall frequency greater than 5% (table 5). There were two grade 2 infectious events in the placebo (*Campylobacter* and influenza) and 4 mg/kg (jaw abscess and Herpes zoster) arms and five in the 10 mg/kg groups (influenza, pharyngitis, upper respiratory tract infection, viral infection, and condyloma accuminata). Only 4.5% of patients had serious adverse events, and all involved hospitalisation for exacerbation of CD except for one patient who was hospitalised for an elective excision of condyloma accuminata mass. At six months post dosing, there were no reports of opportunistic infections or malignancies. One death of unknown cause occurred in a 65 year old male 35 days after the second dose of 4 mg/kg fontolizumab. The death, which occurred while the man slept, was considered unrelated to the study drug by the investigator (no autopsy was performed).

Pharmacokinetics

Selected centres were identified to collect samples for pharmacokinetic assessment. Eighteen patients for whom samples were collected were dosed with placebo and thus excluded from the analysis. Forty one patients had sufficient data for pharmacokinetic analysis (four from group I and 37 from group II).

Fontolizumab pharmacokinetics were characterised by biexponential decline with an initial fast distribution phase followed by a slow elimination phase. Due to the limited number of patients in group I, this discussion focuses primarily on group II. Pharmacokinetic values are presented in table 6.

The observed serum concentrations and area under the drug concentration-time curve within one dosing interval ($AUC_{0-\tau}$) at steady state appeared to be proportional to the dose. Serum drug concentrations for most of the patients were maintained above 10 and 20 μg/ml for the 4 and 10 mg/kg dose groups, respectively, within the dosing period.

Consistent with previous findings, fontolizumab has low clearance and a long elimination half life (table 6), low initial

Table 6 Mean group II pharmacokinetic parameters

	First dose		Second dose				
Dose (mg/kg)	C_{max} (μg/ml)	C_{min} (μg/ml)	C_{max} (μg/ml)	C_{min} (μg/ml)	Clearance (ml/h/kg)	Steady state $AUC_{0-\tau}$ (h×μg/ml)	$t_{1/2,\beta}$ (days)
4	80	12	120	17	0.16	27271	18
10	184	27	224	40	0.18	61176	17.7

$AUC_{0-\tau}$, area under the drug concentration-time curve within one dosing interval; C_{max}, maximum drug concentration; C_{min}, minimum drug concentration; $t_{1/2,\beta}$, elimination half-life.

volume of distribution (V_1) (45 and 55 ml/kg for the 4 and 10 mg/kg groups, respectively), and low volume of distribution at steady state (V_{ss}) (83 and 94 ml/kg for 4 and 10 mg/kg groups, respectively). At steady state, moderate (1.5-fold) drug accumulation is expected with monthly intravenous infusions. These main pharmacokinetic parameters were constant across the two dose groups, indicating dose independent linear kinetics for fontolizumab. Variation in pharmacokinetic values between patients was less than 33%.

Immunogenicity

Immunogenic responses were confirmed in seven of 90 patients after fontolizumab treatment. These responses were detected as early as two weeks following the first dose and did not persist over time. Of these, four (two from each group) were positive in the neutralisation assay; however, levels were of low magnitude ($< \sim 200$ ng/ml). Two of these subjects, one from each dose group, had received two doses of fontolizumab. None of the positive responses was associated with either infusion reactions or obvious declines in fontolizumab exposure compared with other patients in the same treatment cohort.

DISCUSSION

This study was designed to evaluate one or two doses of fontolizumab at 4 or 10 mg/kg, doses expected to provide pharmacologically active concentrations based on in vitro assays. There was no significant difference in response between the three arms at day 28, with placebo resulting in a 33% response rate. On the other hand, the fontolizumab arms produced a 50% greater reduction in mean CDAI score compared with placebo, an advantage that was statistically significant as early as day 14 in the higher dose cohort. A subgroup analysis of approximately 50% of the study patients with an elevated CRP found that 20%, 44%, and 46% of placebo, 4, and 10 mg/kg fontolizumab patients, respectively, responded favourably to the study treatment at day 28. Although the difference was not statistically significant, both doses of fontolizumab were associated with a more favourable response than placebo.

Unlike infliximab, the target for fontolizumab, interferon γ does not exist as a membrane bound protein, and there is no in vitro evidence that target binding by fontolizumab would result in acute apoptosis of mucosal lymphocytes. Its onset of action might be more gradual, with maximum benefit observed after multiple dosing. Analysis of the patients in group II who received two doses of fontolizumab lends preliminary evidence that this might indeed be the case. On day 56, four weeks after the second dose of study drug, the response rates in the fontolizumab arms were doubled compared with placebo: 35% in the placebo versus 69% and 67% in the 4 and 10 mg/kg fontolizumab groups, respectively. This effect was enhanced for patients with an elevated baseline CRP who received two doses, where the day 56 response rate was 17% for placebo compared with 82% for the 4 mg/kg cohort (p<0.01) and 77% for the 10 mg/kg fontolizumab cohort (p<0.01).

This clear reduction in placebo response rate and a neutral to increased impact on the fontolizumab subset is consistent with a true anti-inflammatory effect of this molecule. Further corroboration of a direct anti-inflammatory effect of fontolizumab comes from analysis of median change from baseline in CRP value. There were statistically significant reductions in the 10 mg/kg cohort of patients in group II compared with the placebo group on days 28, 42, 56, and 84. It is interesting to note that the effect on CRP was greatest in the 10 mg/kg cohort whereas 4 mg/kg appeared at least as active in terms of CDAI related parameters. This discrepancy may be due to the fact that CDAI does not always correlate well with CRP.

One of the primary end points of this study was safety, especially as 10 mg/kg fontolizumab had never been administered to patients with CD. Based on animal studies and cases of humans with non-functional interferon γ receptors, the predicted toxicities are increased susceptibility to intracellular infections with microbacteria. Patients in this study were required to have no clinical or radiographic evidence of prior tuberculosis. All three arms were similarly well tolerated. There was no evidence of infusion reactions after one or two doses, and no clinical evidence of infectious complications of interferon γ inhibition. One patient died in his sleep 35 days after the second dose of 4 mg/kg fontolizumab. The cause of death was presumed to be unrelated to fontolizumab, but results of larger randomised studies will be required to rule out an unexpected drug related aetiology.

Long term use of other biological agents has been limited by immunogenicity. In this study, one or two doses of fontolizumab resulted in detection of neutralising anti-fontolizumab antibodies in only 4.4% of patients. These antibodies were of low titre, transient (appearing typically at two weeks and becoming undetectable in subsequent sampling), and with no clinical or detectable pharmacokinetic impact. If confirmed in larger studies, with longer term dosing, the low level immunogenicity of this molecule could be of significant clinical benefit for chronic or even intermittent dosing.

In vitro bioassays of fontolizumab indicate maximal activity in the concentration range 10–100 µg/ml.[12] Pharmacokinetic analysis from this study reveals an elimination half life of 18 days, a peak concentration of 120 µg/ml after two doses of 4 mg/kg fontolizumab, and a trough concentration of 17 µg/ml at four weeks after the second dose. The corresponding peak and tough concentrations for the 10 mg/kg dose were 224 µg/ml and 40 µg/ml, respectively. As a consequence of the long elimination half life, a clinically convenient dosing interval of every four weeks was sufficient to maintain the desired drug level in both the 4 and 10 mg/kg dose groups. Both doses thus provide sustained pharmacologically active concentrations over the dosing period, consistent with the similar levels of clinical activity between doses. Given the equivalent tolerability of both doses, the 10 mg/kg dose may allow for the development of regimens with longer, more convenient, dosing intervals.

This study has described the clinical and biological activity of fontolizumab at either one or two doses of 4 or 10 mg/kg. The favourable side effect profile, long half life, and low immunogenicity suggest that more sustained dosing, and more adequately powered studies, should be considered. The 10 mg/kg dose could offer the option of dosing every 6–8 weeks.

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Conflict of interest: declared (the declaration can be viewed on the *Gut* website at http://www.gutjnl.com/supplemental).

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APPENDIX

The following investigators participated in this study: Dr Slava Djoneva, MBAL ''St Ivan Rilski,'' Department of Internal Diseases and Clinical Pharmacology, Sofia, Bulgaria; Associate Professor Nadejda Kostova, MBAL ''St Ivan Rilski,'' Department of Internal Diseases and Clinical Pharmacology, Sofia, Bulgaria; Associate Professor Iskren Kotsev, MBAL ''St Marina,'' Department of Gastroenterology, Varna, Bulgaria; Dr Mario Markov, MBAL ''St Anna,'' Department of Internal Diseases, Sofia, Bulgaria; Dr Plamen Penchev, MBAL ''Tsaritsa Ioanna'', Department of Gastroenterology, Sofia, Bulgaria; Dr Plamen Petrov, MBAL ''St Anna,'' Department of Internal Diseases, Sofia, Bulgaria; Dr Megena Stamboliiska, MBAL ''St Marina,'' Department of Gastroenterology, Varna, Bulgaria; Associate Professor Simeon Stoinov, MBAL ''Tsaritsa Ioanna'', Department of Gastroenterology, Sofia, Bulgaria.

EDITOR'S QUIZ: GI SNAPSHOT ....

Answer

From question on page 1123

The *x* ray (fig 1) shows an abnormal collection of gas in the right side of the abdomen. Linear opacities suggest intramural gas.

Computerised tomography scan (fig 2) showed caecal intramural gas and localised perforation. At laparotomy, the small bowel looked normal but there was thickening of the pericaecal tissues. A right hemicolectomy was performed. Microscopic examination of the resected bowel showed irregular spaces within the submucosa and serosa consistent with pneumatosis coli, but no evidence of inflammatory bowel disease.

Pneumatosis coli is a rare condition characterised by gas within the wall of the large bowel. It can occur as a primary disorder but is more commonly the result of other pathologies (table 1). Primary pneumatosis coli usually affects the left colon. The pathophysiology is not understood but it has been suggested that either gas is forced through a mucosal breach under pressure or is produced in situ by gas forming organisms.
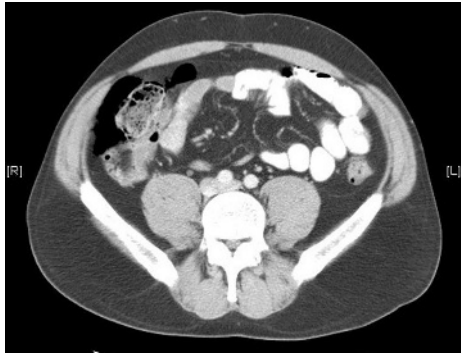
doi: 10.1136/gut.2005.085993

Figure 2 Computerised tomography section showing intramural gas.

Table 1 Causes of secondary pneumatosis coli

Gastrointestinal causes	Systemic causes
Bowel inflammation/necrosis	Pulmonary disease
UC/Crohn's	COPD/asthma
Pseudomembranous colitis	Barotrauma
Diverticulitis	Drugs
Appendicitis	Steroids
Bowel obstruction	Immunosuppressants
Mucosal trauma	Chemotherapy
Endoscopy	Immunosuppression
Infection with gas forming organisms	Hepatic/renal transplantation
	Leukaemia