INFLAMMATORY BOWEL DISEASE

A dose escalating, placebo controlled, double blind, single dose and multidose, safety and tolerability study of fontolizumab, a humanised anti-interferon γ antibody, in patients with moderate to severe Crohn's disease

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Introduction: This study was designed to evaluate the safety of fontolizumab, a humanised anti-interferon γ antibody, in patients with moderate to severe Crohn's disease (CD).
Patients and methods: Forty five patients with a CD activity index (CDAI) of 250–450 were randomised in a double blind, placebo controlled, dose escalating fashion to receive single doses of fontolizumab (0.1, 1.0, and 4.0 mg/kg) or placebo. By day 29, patients with clinical response were re-randomised to receive three additional doses of one half their initial fontolizumab dose or placebo at four weekly intervals. Primary objectives were safety and tolerability. Secondary outcomes included assessments of immunogenicity, clinical activity, and potential pharmacodynamic surrogates.
Results: Treatment was generally well tolerated. There were slightly more reports of chills, flu-like syndrome, asthenia, nausea, and vomiting in the 1.0 mg and 4.0 mg/kg fontolizumab cohorts. Two serious adverse events rated as worsening of CD occurred under fontolizumab. Antibodies to fontolizumab were confirmed in one patient. No differences in clinical activity parameters were noted between any of the active treatment groups and placebo, with the placebo group having a particularly favourable outcome (60% response and 40% remission). By day 29, a more enhanced decrease in median Crohn's disease endoscopic index of severity ($p=0.02$) and serum C reactive protein ($p<0.001$) was observed in the 4.0 mg/kg ($n=14$) fontolizumab cohort compared with placebo ($n=10$). Pharmacodynamic effects were observed by immunohistochemistry.
Conclusions: Fontolizumab was well tolerated with minimal immunogenicity at doses of up to 4.0 mg/kg in patients with CD. A biological activity of fontolizumab is suggested.

Crohn's disease (CD) is a chronic relapsing inflammatory disease which affects any part of the gastrointestinal tract, most frequently the terminal ileum and colon.[1] It is most prevalent in individuals 25–35 years old.[2] The clinical course is highly variable, including stricturing and penetrating complications requiring intestinal resection in 70–80% of patients.[1 3] Luminal bacteria, environmental factors, and genetic susceptibility contribute to its pathogenesis.[1] The intestinal immune response is skewed towards a type 1 helper T cell (T_H1) response characterised by upregulation of interferon γ (IFN-γ) and tumour necrosis factor α (TNF-α), thus providing the rationale for anticytokine therapy in CD.[4–6]

Among the biological agents, anti-TNF-α strategies are those that have been most extensively examined.[7] Repeat doses of infliximab, a chimeric anti-TNF-α monoclonal antibody, achieve durable responses in patients with CD refractory to standard therapy.[8 9] However, use of this agent in maintenance therapy may be limited due to increased susceptibility to opportunistic infections, including tuberculosis, and development of antibodies to infliximab associated with infusion reactions, delayed type hypersensitivity, and loss of response.[9–11] Humanised antibodies may be less immunogenic compared with chimeric antibodies and might prove to be better candidates for long term use. Furthermore, targeting the Th1 cytokine IFN-γ may result in the potential advantage of interrupting the cytokine cascade that leads to further TNF-α upregulation, resulting in decreased IFN-γ and TNF-α levels,[12 13] along with downstream mediators of inflammation.

Fontolizumab (HuZAF) is a humanised antibody directed against recombinant human IFN-γ (Protein Design Labs, Inc., Fremont, California, USA). The antibody binds to natural human IFN-γ and inhibits expression of IFN-γ regulated genes known to be upregulated in CD.[12 13] An initial study of fontolizumab in healthy volunteers showed fontolizumab to be well tolerated at single intravenous doses of up to 4.0 mg/kg. The current study is the first clinical assessment of fontolizumab in a patient population and the first in humans of multiple fontolizumab doses. The primary objective of the study was therefore to assess the safety, tolerability, and immunogenicity of fontolizumab as a single and then as multiple doses in patients with moderate to

Abbreviations: AE, adverse event; ALP, alkaline phosphatase; AUC, area under the concentration curve; CD, Crohn's disease; CDAI, Crohn's disease activity index; CDEIS, Crohn's disease endoscopic index of severity; CRP, C reactive protein; ELISA, enzyme linked immunosorbent assay; HRP, horseradish peroxidase; HuZAF, fontolizumab; IBDQ, inflammatory bowel disease questionnaire; IFN-γ, interferon γ; OD, optical density; Stat, signal transducer and activator of transcription; TNF-α, tumour necrosis factor α

severe CD, as well as exploring the potential pharmacodynamic surrogates.

MATERIALS AND METHODS

Patient eligibility

Patients were eligible for the study if they were between 18 (19 in Austria) and 65 years of age, with CD diagnosed by radiological, endoscopic, or histological criteria, with moderate to severe disease defined by a CD activity index (CDAI)[14] of 250–450, diagnosed at least six months prior to treatment, and who had received prior CD treatment. Patients were excluded from the study if they had at least one of the following: were receiving intravenous prednisone at a daily dose equal to or greater than 60 mg (or its equivalent); had received antibiotics for CD within two weeks before baseline; started or had a dose change of sulfasalazine or 5-aminosalicylic acid within four weeks before baseline; received investigational drugs or therapies within 60 days before baseline; started or had a dose change of ciclosporin, azathioprine, 6-mercaptopurine, or methotrexate; or received treatment with murine, chimeric, or humanised antibody therapy within 90 days before baseline. Ethics committee approval was obtained at all centres prior to activation, and all patients gave signed informed consent before any study related procedures.

Study design, treatment, and objectives

This double blind, randomised, placebo controlled, single and multiple dose, dose escalation, two stage study was conducted at seven European centres in four countries.

Study medication was fontolizumab 10 mg/ml in an isotonic buffer. Placebo contained the same formulated buffer with an appearance indistinguishable from fontolizumab. Study drug was infused intravenously over 60 minutes.

In stage A, patients received a single dose of fontolizumab (0.1, 1.0, or 4.0 mg/kg) or placebo in a 3:1 fontolizumab to placebo ratio in dose escalating cohorts of 6–12 fontolizumab treated patients and 2–4 placebo patients. In any cohort, if less than three of the first six fontolizumab patients in a cohort (or less than 6 of the full 12 patients in the full cohort) experienced a treatment related adverse event of clinical concern within seven days of dosing then escalation to the next dose level was permitted.

In stage B, patients who demonstrated a 70 point or greater reduction from baseline CDAI and experienced no adverse events of clinical concern on day 29 were re-randomised, again in a 3:1 ratio, to receive three doses of fontolizumab at 50% of the dose received in stage A (stage B doses were 0.05, 0.5, or 2.0 mg/kg) or placebo at four weekly intervals. The first dose in stage B was initiated within one week of stage A day 29. The decision to treat in stage B at 50% of the initial dose was made because this was the first experience with multiple intravenous doses in humans, and because fontolizumab has a relatively long half life of 2–3 weeks.

During screening, patient demographics, medical history, and concomitant medications were assessed. CDAI scores were calculated at baseline and 29 days post dosing. Fontolizumab treated patients with a CDAI score reduction of at least 70 points on day 29 were considered responders and advanced to stage B, provided they were otherwise eligible. Enhanced response was defined as a 100 point or greater decrease in CDAI score from baseline; clinical remission was defined as a CDAI score of 150 or less.

The study's primary objective was to determine the safety and tolerability of fontolizumab when administered as single or multiple doses to patients with CD. Secondary objectives included assessment of immunogenicity, clinical activity, and exploratory evaluations of potential pharmacodynamic surrogates.

Safety and disease activity evaluations

Safety

Adverse events (AEs), defined as any undesirable experience occurring during the trial regardless of relationship to study drug, were recorded and classified based on intensity and relationship to study drug. Events that were fatal, life threatening, required inpatient hospitalisation or prolonged hospitalisation, or surgical intervention were classified as serious AEs. Patients were followed for serious AEs and laboratory abnormalities for three months after the final dose of study medication. Physical examination, vital signs, haematology, serum chemistries, and urinalyses were performed for all patients at baseline and at scheduled time points post-dose in stage A and stage B. Chest radiographs and electrocardiograms were performed at baseline.

Disease activity

CDAI, inflammatory bowel disease questionnaire (IBDQ),[15] and serum concentrations of C reactive protein (CRP) were determined at baseline, on days 15 and 29 during stage A, on days 29, 57, and 85 during stage B, and at the three month follow up visit. CD endoscopic index of severity (CDEIS)[16] was evaluated within two weeks before baseline and on day 29 in stage A. Patients kept a daily diary of CD symptoms, temperature, and use of antidiarrhoeal medications, including opiates.

Immunogenicity

Serum samples for detection of circulating antibodies to fontolizumab were obtained predose and on days 15 and 29 during stage A; during stage B, samples were obtained predose on days 1, 29, and 57, and on day 85. For both stages, samples were obtained at the three month follow up visit. Positive samples in the screening assay were further analysed in a confirmatory bioassay; samples testing positive in the confirmatory assay were reported as positive and subsequently analysed for neutralising activities.

Bridging enzyme linked immunosorbent assays (ELISA) were used for screening and confirmatory assays. For the screening assay, fontolizumab coated plates were used to capture anti-fontolizumab antibodies from serum, and horseradish peroxidase (HRP) 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 cut off optical density (OD) value was set at mean + 2 SDs of the baseline OD value from 32 untreated individuals.

The confirmatory assay procedure was similar to the screening assay, except that excess fontolizumab was added. The assay end point was reduction in the signal of a sample preincubated with excess fontolizumab relative to the signal of the same sample preincubated with buffer. Positive samples were defined as having a signal reduction equal to or above the signal reduction for the assay's low level positive control.

The neutralisation bioassay tested 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.

Immunohistochemistry

Mucosal biopsies were obtained during ileocolonoscopy with standard forceps. Samples were fixed in formalin and routinely processed in paraffin or frozen in an optimal cutting temperature embedding medium. A single pathologist (KG) blinded to sample identity performed the evaluations. Immunohistochemistry evaluated the influence

Table 1 Patient demographics and baseline disease characteristics

Characteristic	Placebo (n = 10)	Fontolizumab (mg/kg) 0.1 (n = 6)	1.0 (n = 14)	4.0 (n = 15)
Sex (M/F)	4/6	0/6	7/7	3/12
Age (y) (median (min, max))	30.5 (22, 67)	39 (19, 63)	37 (23, 49)	33 (26, 42)
Smoking status (n (%))				
Non-smoker	1 (10)	2 (33)	3 (21)	1 (7)
Ex-smoker	2 (20)	0 (0)	5 (36)	1 (7)
Smoker	7 (70)	4 (67)	6 (43)	13 (87)
Duration of disease (y) (median (min, max))	7.3 (2, 20)	16.2 (1, 21)	8.0 (1, 19)	5.1 (2, 13)
Disease location (n (%))				
Ileal	3 (30)	0 (0)	3 (21)	5 (33)
Ileocolonic	6 (60)	5 (83)	8 (57)	9 (60)
Colonic	1 (10)	1 (17)	3 (21)	1 (7)
Disease behaviour (n (%))				
Non-stricturing-non-penetrating	5 (50)	2 (33)	5 (36)	8 (53)
Stricturing	2 (20)	2 (33)	3 (14)	4 (27)
Penetrating	3 (30)	2 (33)	6 (43)	3 (20)
Previous intestinal resections (n (%))	4 (40)	1 (17)	7 (50)	6 (40)
Concomitant CD medications				
5-ASA	3 (30)	4 (67)	5 (36)	3 (20)
Azathioprine	2 (20)	1 (17)	4 (29)	5 (33)
Antibiotic	0 (0)	0 (0)	1 (7)	2 (13)
Methotrexate	0 (0)	0 (0)	2 (14)	0 (0)
Steroids	4 (40)	1 (17)	7 (50)	7 (47)
Median CDAI	304	340	289	304
Median CDEIS	5	11	6	7
Median IBDQ	126	113	122	131
Median CRP (mg/l)	15.3	12.9	12.7	12
CRP ⩾10 mg/l (n (%))	6 (60)	4 (67)	10 (71)	9 (60)

5-ASA, 5-aminosalicylic acid; CDAI, Crohn's disease activity index; CDEIS, Crohn's disease endoscopic index of severity; CRP, C reactive protein; IBDQ, inflammatory bowel disease questionnaire.

of fontolizumab on the level of mucosal T cell infiltration using the marker CD3, the proliferative status of infiltrating cells using Ki67, and expression of three IFN-γ inducible biomarkers, signal transducer and activator of transcription (Stat) 1, CXCR3, and HLA-DR.

CD3, Stat1, CXCR3, and Ki67 staining was performed on serial cryostat sections (5 μm). Sections were incubated sequentially in dual endogenous enzyme block and protein block, and primary antibodies were diluted in antibody diluent (DakoCytomation, Carpinteria, California, USA). The primary antibodies used were rabbit monoclonal antihuman CD3 (Biocare Medical, Walnut Creek, California, USA; clone SP7, 1 μg/ml), mouse monoclonal antihuman Stat1 (Becton Dickinson, Franklin Lakes, New Jersey, USA; clone 1, 0.25 μg/ml), mouse monoclonal antihuman CXCR3 (BD; clone 1C6, 1 μg/ml), and rabbit monoclonal antihuman Ki67 (Biocare; 1/400). Rabbit IgG1 (Lab Vision, Fremont, California, USA) was used as the isotype control for CD3 and Ki67, and mouse IgG1 (DakoCytomation; 100 mg/ml) was used as the isotype control for Stat1 and CXCR3. Following three washes with the wash buffer (DakoCytomation), slides were incubated with MACH2 secondary antibodies (Biocare), which were polymerised to either HRP (poly-HRP) or alkaline phosphatase (poly-ALP). CXCR3 and Ki67 were detected with poly-HRP labelled goat antimouse IgG and goat antirabbit IgG, respectively; CD3 and Stat1 were stained with poly-ALP

Table 2 Adverse events considered possibly, probably, or related to treatment in ⩾10% of patients by cohort

Adverse event by preferred term†	No (%) of patients by stage A dose* Placebo (n = 10) (22)	0.1 mg/kg fontolizumab (n = 6) (13)	1.0 mg/kg fontolizumab (n = 14) (31)	4.0 mg/kg fontolizumab (n = 15) (34)	Total (n = 45)
Nausea	1 (10)	1 (17)	3 (21)	4 (27)	9 (20)
Asthenia	1 (10)	2 (33)	1 (7)	3‡ (20)	7 (16)
Headache	0	1 (17)	3 (21)	2 (13)	6 (13)
Vomiting	0	0	1 (7)	4§ (27)	5 (11)
Arthralgia	1 (10)	0	3 (21)	1 (7)	5 (11)
Abdominal pain	0	0	1 (7)	3§ (20)	4 (9)
Dizziness	1 (10)	0	2 (14)	1 (7)	4 (9)
Chills	0	0	0	3‡ (20)	3 (7)
Fever	0	0	0	3‡ (20)	3 (7)
Amblyopia	1 (10)	0	1 (7)	0	2 (4)
Injection site inflammation	1 (10)	0	0	0	1 (2)
Peripheral oedema	1 (10)	0	0	0	1 (2)

*Events counted by unique preferred term per patient.
†Events listed in descending order of frequency.
‡Patient 0703 reported asthenia, chills, and fever attributed to Crohn's disease flare; all were considered possibly related to study drug and each is counted here.
§Patient 0713 reported vomiting and abdominal pain due to functional stenosis; both events were considered possibly related to study drug and each is counted here.

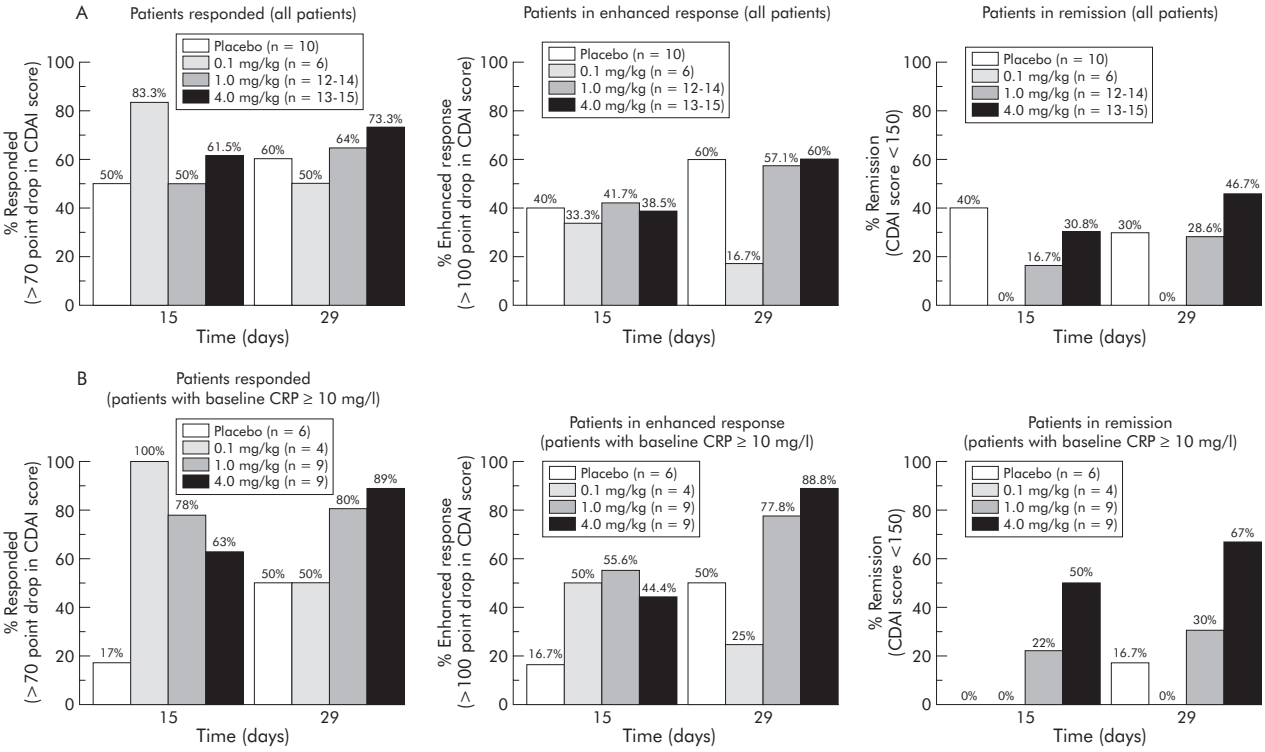

Figure 1 Rates of response, enhanced response, and remission for cohorts treated with placebo, or 0.1, 1.0, and 4.0 mg/kg fontolizumab, analysed for all enrolled patients (A) and for patients with baseline C reactive protein (CRP) levels ⩾10.0 mg/l (B) by days 15 and 29 (thus after a single dose of fontolizumab; stage A). CDAI, Crohn's disease activity index.

labelled goat antirabbit IgG and goat antimouse IgG, respectively. Colour development was achieved using DAB (DakoCytomation) for poly-HRP and Vulcan Fast Red (Biocare) for poly-ALP. Slides were analysed using an Olympus BX40 microscope. Staining was scored on a scale of 0 (negative) to 4 (most positive) based on the intensity and density of positively stained cells. Each slide was scored based on an average of five fields from the most severe lesions.

Immunohistochemical staining for HLA-DR was performed using the monoclonal anti-HLA-DR antibody, clone TAL, 1B5, which reacts with the alpha chain of monomorphic HLA class II DR antigen (Dako, Glostrup, Denmark; dilution 1/100), as previously published.[17] HLA-DR was considered to be expressed whenever epithelial cells showed positive staining.

Statistical methods

Statistics included mean, SD, sample size, median, and minimum and maximum changes in disease state, as assessed by calculating changes from baseline in CDAI, IBDQ, and CDEIS scores, and in serum levels of CRP at specified time points for stages A and B. Changes were assessed and compared between the four treatment groups. Fisher's exact test was used to compare treatment differences for categorical variables such as number of patients achieving response and remission. The Kruskal-Wallis test and the Wilcoxon rank sum test were used to analyse CDAI and CRP changes from baseline. All p values were based on two sided tests and an alpha level of 0.05 was used to determine statistical significance.

RESULTS

Patient demographics and disease baseline characteristics

Fifty four patients were screened and 45 patients were randomised to one of four treatment groups. Of the nine screen failures, eight did not meet entry criteria and one refused to enrol because the medication was not received in time. The first patient was enrolled in January 2001 and the last three month follow up visit was in July 2002. Demographic and baseline disease characteristics were generally balanced across treatment groups; however, patients in the 0.1 mg/kg cohort had higher CDAI and CDEIS scores and a higher proportion of ileocolonic involvement (table 1) than patients in the other cohorts. Also, the proportion of female patients was greater in the 0.1 and 4.0 mg/kg groups.

Safety

Among all AEs reported in this study, regardless of relationship to study treatment, chills (five patients, 11%), headache (13 patients, 29%), flu-like syndrome (five patients, 11%), nausea (10 patients, 22%), and vomiting (seven patients, 16%) were reported predominantly by the 1.0 and 4.0 mg/kg fontolizumab groups. Mild chills, asthenia, or fever transiently occurred within 24 hours of the first dose in four of the 15 patients (27%) in the 4 mg/kg fontolizumab cohort, requiring either no treatment or mild analgesics only, but were not reported with subsequent doses. Adverse events considered possibly, probably, or related to treatment with a frequency of 10% or more in any group are shown in table 2.

Five serious AEs were reported, one of which occurred in the placebo group. The four events reported in fontolizumab treated patients, two each in the 1.0 and 4.0 -mg/kg cohorts, were most likely related to underlying CD, and included abdominal cramping and vomiting after a fibre rich meal in a patient with ileal stenosis; anal pain in a patient with perianal fistulae; chills, fever, and fatigue associated with a flare of disease in the third patient; and, polyarthritis in a patient with a history of this extraintestinal CD manifestation. No deaths occurred in this study.

Table 3 Changes in C reactive protein levels from pretreatment during stage A

		Fontolizumab (mg/kg)			
Stage A time point	Placebo (n)	0.1 (n)	1.0 (n)	4.0 (n)	p Value
Day 8	−4.1 (10)	−1.9 (6)	−5.8 (13)	0 (14)	0.59
Day 15	−1.1 (10)	−2.0 (6)	0 (13)	0 (13)	0.95
Day 29	0 (9)	0.2 (6)	−2.4 (14)	−6.0 (15)	0.26

Values are median (mg/l).

Immunogenicity

One sample (collected on day 15, stage A) from a single patient treated with 4.0 mg/kg tested positive for anti-fontolizumab antibodies. All subsequent samples from this patient were negative, indicating that this was likely a transient response. Furthermore, the pharmacokinetic profile for this patient (not shown) did not appear to be different from other patients in the cohort, and the antibody was demonstrated to be non-neutralising. None of the other 34 fontolizumab treated patients developed anti-drug antibodies.

Disease activity

Using CDAI scores for analysis, a dose dependent increase in response, enhanced response, or remission at day 29 was observed with the fontolizumab groups (0.1 mg/kg fontolizumab: 50%, 17%, and 0%, respectively; 1.0 mg/kg fontolizumab: 64%, 57%, and 29%, respectively; 4.0 mg/kg fontolizumab: 73%, 60%, and 47%, respectively). However, no statistically significant difference was noted when any of the fontolizumab groups were compared with the favourable outcome in the placebo group (60% response, 60% enhanced response, and 40% remission) (fig 1, top row).

In light of the unexpected high placebo response and remission rates, a post hoc analysis of patients with baseline CRP values ⩾10 mg/l (n = 29; 64% of study population) was performed. In this subpopulation, a clear trend for differences between placebo and the two higher dose fontolizumab cohorts became apparent with respect to response, enhanced response, and remission at days 15 and 29. By day 29, remission rates in patients with elevated baseline CRP levels for the placebo (n = 6), 0.1 mg/kg (n = 4), 1.0 mg/kg (n = 10), and 4.0 mg/kg (n = 9) cohorts were 16.7%, 0% (p = 1.00), 30% (p = 1.00), and 67% (p = 0.12), respectively (fig 1B).

Median changes from baseline in CDEIS scores at day 29 were as follows: 0 in the placebo group (n = 10); −0.7 in the 0.1 mg/kg (n = 6; p = 0.41 versus placebo); −2.8 in the 1.0 mg/kg (n = 11; p = 0.62 versus placebo); and −3.9 in the 4.0 mg/kg (n = 14; p = 0.02 versus placebo) fontolizumab cohorts (p = 0.17 when compared across all four treatment groups).

Median changes in CRP values from baseline to days 8, 15, and 29 are shown in table 3. At day 29, CRP changes in the four treatment groups were not distinguishable from one another (p = 0.26). However, there was a tendency towards a greater reduction in CRP levels with greater fontolizumab dose. At day 29, the 1.0 and 4.0 mg/kg fontolizumab cohorts experienced statistically significant declines in CRP from baseline (p = 0.02 and <0.001, respectively), in contrast with the placebo and 0.1 mg/kg groups (p>0.31) where no such decline was observed.

Median changes from baseline in IBDQ scores at day 15 were 37, 25, 27, and 25 (p = 0.997 compared between all treatment groups) in the placebo, 0.1, 1.0, and 4.0 mg/kg fontolizumab cohorts, respectively. At day 29, IBDQ changes from baseline were 38, 6.5, 32.5, and 43 in the analogous groups (p = 0.31 compared between all treatment groups).

Eighteen patients advanced to stage B (table 4). Maintenance of a 70 point response up to day 85 was achieved in 11/13 patients who received fontolizumab in stage B and 3/5 patients who received placebo in stage B.

Immunohistochemistry

Expression of HLA-DR was evaluable in 19 patients (four each from the placebo and 0.1 mg/kg fontolizumab group; six from the 1.0 mg/kg fontolizumab group; and five from the 4.0 mg/kg fontolizumab). Expression of CD3, Stat1, CXCR3, and Ki67 was evaluable in eight patients (one in the placebo group; two each in the 0.1 mg/kg and 1.0 mg/kg fontolizumab groups, and three in the 4.0 mg/kg fontolizumab group). Due to the small sample size, no statistical analysis was performed on these results.

Aberrant HLA-DR expression was absent at baseline and at day 29 in five patients (one in the placebo group and four in the 1.0 mg/kg fontolizumab group, all presenting with ileal disease). Aberrant epithelial HLA-DR expression disappeared by day 29 in one of two patients in the 1.0 mg/kg fontolizumab group and in three of five patients in the 4.0 mg/kg group, but in no subject from the placebo (n = 3) or 0.1 mg/kg (n = 4) group.

With respect to CD3, Stat1, CXCR3, and Ki67, patients treated with placebo, or 0.1 mg/kg or 1.0 mg/kg fontolizumab did not show consistent reduction in any of these markers at day 29. In contrast, at 4.0 mg/kg fontolizumab, all three patients examined demonstrated reduction of greater than one grade in either three (one patient) or four (two patients) of the four markers evaluated. Results from a representative patient are shown in fig 2.

Table 4 Maintenance of response during stage B (fontolizumab treated patients)

Stage A (fontolizumab mg/kg)	0.1	1.0	1.0	4.0	4.0
Stage B (fontolizumab mg/kg)	0.05	0	0.5	0	2.0
Patients with stage A baseline value who entered stage B (n)	2	1	5	4	6
No of patients with at least a 70 point response					
Day 29 (n (%))	2 (100)	1 (100)	5 (100)	3 (75)	6 (100)
Day 57 (n (%))	2 (100)	1 (100)	5 (100)	1 (25)	6 (100)
Day 85 (n (%))	1 (50)	1 (100)	5 (100)	2 (50)	5 (83)
At 3 month follow up (n (%))	2 (100)	0 (0)	3 (60)	3 (75)	6 (100)

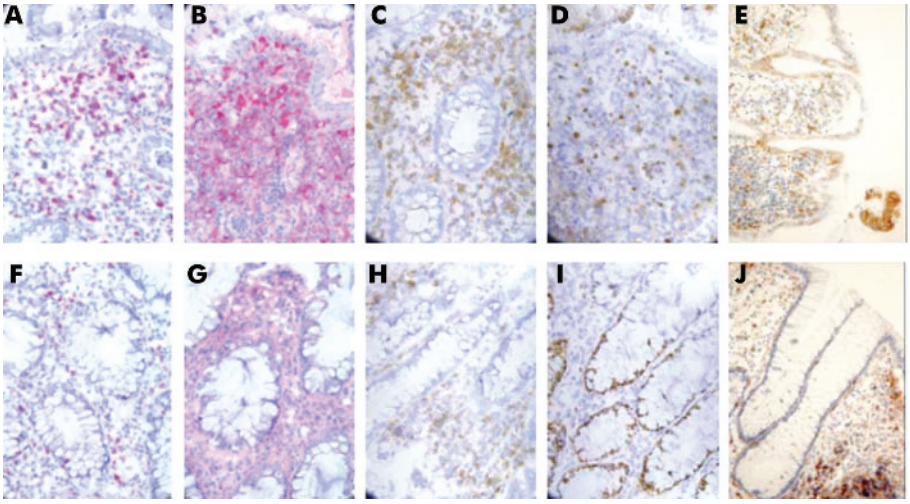

Figure 2 Immunohistochemistry analysis of CD3, signal transducer and activator of transcription (Stat) 1, CXCR3, Ki67, and HLA-DR from mucosal biopsy predosing and at day 29 after a single dose of 4 mg/kg fontolizumab in a single patient. CD3 (A, F), Stat1 (B, G), CXCR3 (C, H), Ki67 (D, I), and HLA-DR E, J). (A–E) Baseline; (F–J) day 29.

DISCUSSION

Because CD is widely regarded as a Th-1 type immune mediated inflammatory disorder, IFN-γ provides a rational therapeutic target.[4–6] Here we present results from the first study with a humanised anti-IFN-γ antibody, fontolizumab, in patients with moderate to severe CD. The results suggest safety and biological activity of single and multiple doses of fontolizumab in these patients.

Fontolizumab was well tolerated in the range 0.1–4.0 mg/kg as a single dose and in the range 0.05–2.0 mg/kg for three additional monthly doses. Most AEs were mild to moderate in intensity. Flu-like syndrome and chills were reported predominantly from the higher dose groups of fontolizumab. Notably, no increased susceptibility to infections was observed. Whether drug related or not, the events were not immunogenic in origin because they occurred with the initial dose, and in only one of 35 tested patients was an anti-fontolizumab antibody response detected. In this case, the anti-antibody response was transient, without neutralising capacity, and did not influence the pharmacokinetic profile of fontolizumab in this one patient. Therefore, the excellent safety profile and low immunogenicity observed favour fontolizumab as a reasonable candidate for long term use.

Rates of remission (60%), response (60%), and enhanced response (40%) at day 29 after a single dose of placebo in our study were among the highest reported in the literature in placebo controlled randomised trials in patients with moderate to severe CD.[18] This is worth mentioning, especially considering the short term evaluation and high baseline CDAI scores of the patients, since short study duration and high entry CDAI scores have been described as predictors of low placebo remission rates.[18] Against the background of the high placebo rate, an overall difference between the four treatment groups in any secondary end points based on CDAI could not be detected.

Although not designed to allow definite conclusions about clinical efficacy, the study provides evidence of the biological activity of fontolizumab. Recently, a high CRP serum level ⩾10 mg/l, indicative of inflammation, has been shown to correlate with a lower placebo response compared with TNF-α inhibitors.[19 20] Stratifying our patients according to CRP ⩾10 mg/l increased rates of enhanced response, and remission became apparent for the high dose fontolizumab groups compared with the placebo and 0.1 mg/kg fontolizumab groups. Furthermore, assessment of the exposure-response relationship revealed a positive correlation between CDAI score and serum fontolizumab area under the concentration curve (AUC), as well as between CDEIS score and serum fontolizumab AUC (data not shown), indicating dose dependent clinical activity. The decrease observed in CDEIS with 4.0 mg/kg fontolizumab is notable as this assessment tool is an objective score of mucosal involvement. As no dose limiting toxicity was observed in the present study, a higher dose level with longer duration of treatment may be further explored to optimise the dose regimen.

To evaluate the biological effects of fontolizumab at the lesion site, several biomarkers were analysed on biopsy specimens using immunohistochemistry. Stat1 is a major transcription factor activated by IFN-γ that regulates genes, including those coding for chemokines (IP10, MIG, I-TAC, and MCP-1), inducible nitric oxide synthetase, MHC class II, and intercellular adhesion molecule 1.[21] These molecules play important roles in recruitment of activated phagocytes and lymphocytes into intestinal lesions, and their expression has been shown to correspond with overall inflammatory activity.[22–25] Therefore, Stat1 activation may contribute to many of the pathophysiological features observed in inflammatory bowel disease, and indeed expression, phosphorylation, and nuclear translocation of Stat1 are increased in the lesional mucosa of patients with CD.[26] Expression of the chemokine receptor CXCR3 as well as its ligands, IP-10 and MIG, has also been observed in the inflamed mucosa from patients with CD.[22 27] Particularly expressed on Th1 cells, CXCR3 may be involved in the continuous migration of activated T lymphocytes into mucosal lesions in CD. In patients examined from the 4 mg/kg fontolizumab group, treatment was associated with decreased expression of HLA-DR, Stat1, and CXCR3. Additionally, there was a profound decrease in the total number of T cells (CD3) and proliferating activities (Ki67) in the inflamed mucosa following treatment. Results from this small group of patients suggest that fontolizumab inhibits lesional IFN-γ mediated gene transcription.

There is a potential advantage of targeting IFN-γ for the treatment of CD. Neutralisation of IFN-γ may interrupt the cytokine cascade that leads to TNF-α upregulation, resulting in decreased TNF-α and IFN-γ levels.[12 13] Inhibition of IFN-γ reduces induction of class II MHC molecule expression and blocks the maturation of naive T cells into T_H1 cells. In fact, some of the clinical effects of anti-TNF-α therapy have been

attributed to downregulation of IFN-γ. In targeting a distinct pathway, fontolizumab may be effective in patients who do not respond to anti-TNF-α therapies.

In conclusion, fontolizumab was generally well tolerated and minimally immunogenic in patients with moderate to severe CD. Biological and clinical responses were evident in patients treated with single dose fontolizumab and in multiple dose maintenance of remission.

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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

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