A clinical study assessing the tolerability and biological effects of infliximab, a TNF-α inhibitor, in patients with advanced cancer


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**Background:** Tumour necrosis factor-α (TNF-α) is an important regulator of the chronic inflammation contributing to tumour progression. Infliximab, an anti-TNF-α monoclonal antibody was investigated in this trial of patients with advanced cancer. The primary objectives were to determine the safety profile and biological response of infliximab in a cancer population. Clinical response was a secondary objective.

**Patients and methods:** Forty-one patients received infliximab at 5 mg/kg (n = 21) or 10 mg/kg (n = 20) i.v. at 0 and 2 weeks and then every 4 weeks. Post-treatment samples were measured for changes in plasma and serum TNF-α, CCL2, IL-6 and C-reactive protein (CRP).

**Results:** Infliximab was well tolerated with no dose-limiting toxic effects. At both doses of infliximab, neutralisation of serum TNF-α was observed after 1 h while plasma CCL2, IL-6 and serum CRP were decreased 24 and 48 h following infliximab administration. Seven patients experienced disease stabilisation (range 10–50+ weeks). There was no evidence of disease acceleration in any patient.

**Conclusions:** Infliximab treatment was safe and well tolerated in patients with advanced cancer. There was evidence of biological activity with baseline TNF-α and CCL2 being correlated with infliximab response.

**Key words:** cytokine, inflammation, infliximab, TNF-α, trial

**Introduction**

Chronic inflammation may play a critical role in the malignant process and tumour progression [1]. An increasing number of clinical and translational studies have provided evidence for the role of the pro-inflammatory cytokine tumour necrosis factor-α (TNF-α) in the promotion and progression of cancer [2–4].

TNF-α is a 17-kDa polypeptide that binds as a homotrimer to two receptors, TNF-α receptors TNFR1 (p55) and TNFR2 (p75); it signals via many pathways, including NF-κB and AP-1 [5]. Although TNF-α is usually undetectable in serum/plasma of healthy individuals, picogram concentrations of TNF-α protein have been detected in serum/plasma of patients with advanced cancer [6]. Both tumour and stromal cells in the tumour microenvironment of a variety of solid and haematological malignancies have increased TNF-α expression [7–9]. This inflammatory cytokine is a key regulator of the interactions between the tumour and stromal cells, which result in upregulation of TNF-α-regulated genes stimulating tumour cell growth, survival, invasion and metastasis, inflammatory cell trafficking to the tumour site and neoangiogenesis [4].

Infliximab (Remicade®) is an anti-TNF-α antagonist used successfully in a number of inflammatory conditions including Crohn’s disease and rheumatoid arthritis [10, 11]. Infliximab is a chimeric murine monoclonal antibody that binds with high affinity to soluble and membrane TNF-α and inhibits binding of TNF-α to its receptors [12]. Neutralisation of TNF-α results in reduction of TNF-α-regulated cytokines, proteases and other growth factors at the inflammatory site, minimising clinical symptoms and thus reversing the clinical disease [10, 11].

The rationale for this study is the link between chronic inflammation and cancer, a role for TNF-α in promotion of experimental cancers and TNF-α in blood and tumour
tissues of patients with advanced cancer. Furthermore, in chronic inflammatory diseases, anti-TNF-α therapy reduces cytokines and growth factors that are also involved in tumour growth, invasion and metastasis [13].

Despite all the beneficial effects of infliximab treatment in chronic inflammatory diseases, there are concerns over its safety profile. Infliximab may increase the risk of serious infection, including re-activation of latent tuberculosis, as well as the risk of cancer [14, 15].

The primary objectives of this study were to establish the safety profile and biological response to infliximab in patients with advanced cancer. The secondary objective was to determine clinical response so as to identify suitable tumour types or patient characteristics that may be selected for phase II trials.

patients and methods

patient eligibility

Patients were eligible if they had a histologically confirmed diagnosis of advanced and/or metastatic solid cancer for which no satisfactory treatment exists or against which established treatments had failed; World Health Organisation performance status 0 or 1, and life expectancy of at least 3 months. Adequate organ function was required including Hb > 10 g/dl, neutrophils > 1.5 × 10⁹/l, platelets > 100 × 10⁹/l, bilirubin < 1.5 × upper limit of normal, alanine aminotransferase < 2.5 × upper limit of normal (or less than five times if liver metastases are present) and creatinine < 1.25 the upper limit of normal. Patients were ineligible if they had a recent history of serious infections, a history of chronic or recurrent infectious disease in the previous 3 months or a prior history of tuberculosis. Patients with congestive heart failure were also ineligible. No investigational agent or chemotherapy was allowed within 4 weeks of study entry (6 weeks for nitrosoureas, mitomycin C or melphalan). Concurrent corticosteroids were not permitted.

ethical considerations

The study was approved by North East London and the City Health Authority Research Ethics Committee (LREC/P/02/150) and Lothian Research Ethics Committee (LREC/2002/8/31) and conducted according to the declaration of Helsinki. All patients gave voluntary, written informed consent.

trial design

This was a two-centre, open-label, study of infliximab at two dose levels (5 and 10 mg/kg) in patients with advanced cancer with intended cohorts of 20 patients at each dose level. Patients with clinical disease progression before the end of the 10-week assessment period were removed from the study.

dosing and administration

Patients received infliximab (Centocor R&D Inc., Malvern, PA) as an i.v. infusion on weeks 0, 2 and 6. Patients who showed evidence of clinical benefit or had stable disease at week 10 continued the treatment with dosing every 4 weeks. We selected the infliximab doses and schedule at the higher end of the well-characterised range that has been shown to be safe and known to maintain blood concentrations that have efficacy in other trials in patients with graft versus host disease [16], myelodysplastic syndromes [17, 18] and renal cell cancer [19]. Patients continued therapy until (i) disease progression, (ii) the development of unacceptable toxicity, (iii) patient withdrawal of consent or (iv) the responsible clinician’s decision that further treatment would not be in the patient’s best interest.

definition of dose-limiting toxicity

All toxic effects were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. Dose-limiting toxicity (DLT) was defined as grade 4 neutropenia, grade 4 thrombocytopenia and any grade 3 nonhaematological toxic effects (excluding alopecia, nausea and vomiting) or drug-related death. Allergic reactions, which were expected, were not considered as DLT.

pre-treatment and follow-up studies

A complete patient history and examination were performed at baseline and before each course of treatment. Complete blood counts, electrolytes and blood chemistries were performed weekly. Clinical tumour measurement and radiological assessments were carried out at study entry and at the off-study visit (4 weeks after last infusion). The Response Evaluation Criteria in Solid Tumors (RECIST) was employed for assessment of tumour response.

sample collection

Whole blood (10 ml) was collected for serum before and 1 h after the end of each infliximab infusion and 4 weeks after the final infusion (end of study). A further 10-ml sample of whole blood was collected in sterile preserve-free heparinised tubes (30 U/ml) before, then at 24 and 48 h after the first dose of infliximab; before second and third doses of infliximab and 4 weeks after the final infusion of infliximab. All aliquots were snap frozen in liquid nitrogen and stored at −80°C.

serum infliximab concentration analysis

Serum infliximab concentrations were measured at Centocor R&D Inc. using a monoclonal antibody-based enzyme immunoassay as previously described [20]. The assay sensitivity limit was 0.1 µg/ml infliximab.

cytokine measurement

Serum TNF-α and plasma TNF-α, IL-6 and CCL2 concentrations were measured using ELISA (R&D Systems, Abingdon, UK). The limits of sensitivity were 15.6, 3.12 and 31.2 pg/ml for TNF-α, IL-6 and CCL2 assays, respectively. Low concentrations of TNF-α were extrapolated using the TNF-α standard curves generated in the assay.

C-reactive protein measurement

Serum C-reactive protein (CRP) concentrations were analysed by a quantitative immunoturbidimetric methodology on an Olympus AU2700 analyzer [Olympus Diagnostica GmbH (Irish Branch), Lismeehan, Co., Clare, Ireland]. Assay sensitivity limit was 5 mg/l. For data analysis, samples <5 mg/l were assigned 3 mg/l. CRP concentrations in healthy volunteers are <10 mg/l [6].

statistical analysis

The Mann–Whitney U-test (a nonparametric t-test) was used to evaluate the difference between time points for the two doses, while the Wilcoxon signed rank test was used to analyse differences in pre- and post-treatment samples. Relationship between baseline CRP and IL-6 was performed using linear regression analysis. Analysis of contingency tables was performed using Fisher’s exact test. Prism version 4.0c was used (GraphPad Software Inc, San Diego, California, USA).

results

patient characteristics

Forty-one patients were recruited. As one patient with ovarian cancer was unable to continue beyond the first infliximab treatment due to clinical deterioration, an additional patient...
was recruited to the first dose level. Thus, the first cohort treated with 5 mg/kg infliximab consisted of 21 patients and 10 mg/kg cohort recruited 20 patients. Table 1 summarises the demographic data and tumour types of all patients treated.

toxicity
Toxic effects considered as possibly, probably, or definitely related to the infliximab are documented in Table 2. The few grade 3 toxic effects were clinically manageable. The most common toxicity (all grades) was allergic infusion reaction (15% of all treated patients had at least one infusion reaction, 4.5% of the total number of infusions resulted in an infusion reaction, consistent with http://www.remicade.com/pdf/HCP_PPI.pdf). Allergic reactions included skin rash, rigours, urticaria and chest pain and required the administration of corticosteroids and antihistamines in five cases. Three patients went on to receive the complete dose and the remaining three patients received 80%, 75% and 24% of the expected dose. No DLTs were experienced.

tumour responses
No objective responses (complete or partial) were achieved, but seven patients who were progressing before commencement of therapy experienced stable disease after the three initial infliximab treatments. Three of these patients were from the 5-mg/kg infliximab cohort (ovarian, renal and endometrial stromal cell sarcoma, duration 13–20 weeks). The four patients from the 10-mg/kg infliximab cohort had melanoma, cervical, adenocarcinoma of the appendix and colorectal cancer (duration 10–50 weeks).

Only five of the seven patients with stable disease went on to receive further treatment (patient 15, 20, 27, 37, 39). A median total number of five infliximab treatments (range 4–10) were administered to this group of patients. Time to progression in these patients ranged from 13 to 20 weeks in the 5-mg/kg cohort and 10 to 50 weeks in the 10-mg/kg cohort. Prolonged disease stabilisation after six treatments was noted in patient 39. This patient had metastatic colon cancer that had previously been treated with three lines of chemotherapy. This patient received a total of 12 infliximab treatments and had stable disease for 50 weeks. Patient 27, a patient with metastatic melanoma who had received no previous chemotherapy, experienced a significant improvement in systemic symptoms for the duration of therapy (nine doses of 10 mg/kg infliximab). However, despite continued stable disease, the drug was discontinued because of allergy (urticaria) to infliximab.

serum infliximab concentrations
There was a dose-dependent elevation in the serum infliximab concentrations in 1-h post-infusion samples in patients treated with 5 and 10 mg/kg infliximab on three occasions (supplemental figure s1A and B, available online). The median (range) infliximab concentrations 1 h after the first infusion of 5 and 10 mg/kg infliximab were 107 µg/ml (19–230 µg/ml, n = 20) and 252 µg/ml (128 – 326 µg/ml, n = 15), respectively.

changes in TNF-α protein concentrations
One of the primary objectives was to determine the biological response to infliximab. Pre-treatment plasma TNF-α protein was detected in nine patients in the 5-mg/kg infliximab

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**Table 1.** Patient characteristics (n = 41)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
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<tr>
<td>Median</td>
<td>58</td>
</tr>
<tr>
<td>Range</td>
<td>31–76</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
</tr>
<tr>
<td>Colorectal cancer</td>
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</tr>
<tr>
<td>Ovary</td>
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</tr>
<tr>
<td>Renal</td>
<td>4</td>
</tr>
<tr>
<td>Melanoma</td>
<td>3</td>
</tr>
<tr>
<td>All other tumours</td>
<td>14</td>
</tr>
<tr>
<td>Prior chemotherapy</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>One line</td>
<td>8</td>
</tr>
<tr>
<td>Two lines</td>
<td>13</td>
</tr>
<tr>
<td>Three lines or more</td>
<td>17</td>
</tr>
</tbody>
</table>

**Table 2.** Number of patients experiencing toxic effects related to infliximab

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>CTC grade</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1</td>
</tr>
<tr>
<td>Allergic reaction</td>
<td>2 2 2</td>
</tr>
<tr>
<td>Alopecia</td>
<td>1</td>
</tr>
<tr>
<td>Chest pain</td>
<td>1</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>1</td>
</tr>
<tr>
<td>Dry skin</td>
<td>2</td>
</tr>
<tr>
<td>Hot flushes</td>
<td>3 1</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
</tr>
<tr>
<td>Itch</td>
<td>2 1</td>
</tr>
<tr>
<td>Leg cramps</td>
<td>1</td>
</tr>
<tr>
<td>Leg weakness</td>
<td>1</td>
</tr>
<tr>
<td>Low mood</td>
<td>1</td>
</tr>
<tr>
<td>Malaise</td>
<td>2 1</td>
</tr>
<tr>
<td>Myalgia</td>
<td>1 1</td>
</tr>
<tr>
<td>Neurosensory</td>
<td>2</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>1</td>
</tr>
<tr>
<td>Night sweats</td>
<td>2</td>
</tr>
<tr>
<td>Oedema</td>
<td>1</td>
</tr>
<tr>
<td>Papitations</td>
<td>1</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>1</td>
</tr>
<tr>
<td>Rash</td>
<td>2 1</td>
</tr>
<tr>
<td>Rigours</td>
<td>1</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>4 1</td>
</tr>
<tr>
<td>Weight loss</td>
<td>1</td>
</tr>
</tbody>
</table>

Maximum toxic effects reached per patient until withdrawal are reported. CTC, Common Toxicity Criteria version 2.0, March 1998.
cohort (range 0.3–22 pg/ml) and seven patients in the 10-mg/kg infliximab cohort (range 1–8 pg/ml, supplemental table s1, available online).

Plasma TNF-α protein showed a nonsignificant decrease 24 h following the first infusion of 5 and 10 mg/kg infliximab and then rose steadily and significantly before second dose (Figure 1A and C). This is consistent with infliximab treatment in other diseases and is thought to be due to assay interference by the TNF-α-infliximab complexes. There were no significant differences in plasma TNF-α protein concentrations between the two cohorts at any of the pre- or post-treatment sample times.

To further elucidate the changes in TNF-α protein concentrations at earlier sampling times, we investigated the serum TNF-α protein concentrations in a subset of patients after 1 h following dose 1 and 2 (n = 5 for both cohorts). At both doses, significant reductions in TNF-α protein were observed 1 h after the end of infusion in dose 1 and 2 (P < 0.05, Figure 1B and D). We then investigated the effect of infliximab on two plasma proteins, IL-6 and CCL2, that may be regulated by TNF-α [21, 22].

changes in plasma IL-6 protein concentration
Plasma IL-6 protein was detectable in all patients treated in this study (data presented in supplemental table s1, available online). Plasma IL-6 concentration was significantly reduced 24 and 48 h after the first dose of infliximab in both cohorts (5 mg/kg: P = 0.002 and P = 0.04; 10 mg/kg: P = 0.006 and P = 0.04, respectively, Wilcoxon signed rank test, Figure 2A and B).

changes in plasma CCL2 protein concentration
Plasma CCL2 protein was detectable in all patients treated in this study (supplemental table s1, available online). CCL2 concentration showed a significant decline at 24 and 48 h following the first dose of infliximab in both cohorts (5 mg/kg: P < 0.001 and P < 0.0039; 10 mg/kg: P = 0.0083 and P = 0.015, respectively, Wilcoxon signed rank test, Figure 2C and D).

changes in serum CRP concentrations
Twenty-seven patients had baseline serum CRP concentrations greater than normal healthy levels (>10 mg/l). Five patients in the 10-mg/kg infliximab cohort had no detectable CRP at baseline. There were, however, no significant differences in baseline serum CRP concentrations in the patients treated with 5 or 10 mg/kg infliximab (supplemental table s1, available online). CRP decreased nonsignificantly 24 and 48 h after the first infliximab dose in the 5-mg/kg cohort (Figure 2E) and significantly in the 10-mg/kg cohort (24 h: P < 0.001; 48 h: P < 0.0078, respectively, Wilcoxon signed rank test, Figure 2F).
As described above, there were seven patients who achieved disease stabilisation after three doses of infliximab. None of these patients had detectable plasma TNF-α. In contrast, 17 of 34 patients whose disease progressed during infliximab therapy had detectable TNF-α. This difference was significant ($P = 0.029$, Fisher’s exact test, Figure 3A). At later time points, the TNF-α levels in the stable disease patients were the same range as the remaining patients.

There were no significant differences observed in the baseline and post-treatment plasma IL-6 concentrations in either cohort nor plasma CCL2 concentrations in 5-mg/kg cohort between patients that had stable disease or progressive disease. However, in patients treated with 10 mg/kg infliximab showing stable disease there were significantly lower CCL2 concentrations at baseline and 24 h post-treatment compared with the non-responding patients (pre-treatment: stable disease median = 277 pg/ml, non-responding median = 463 pg/ml, $P = 0.02$; 24 h: stable disease median = 258 pg/ml, non-responding median = 356 pg/ml, $P = 0.04$; Figure 3B).

## Discussion

One of the primary objectives of this study was to study the safety profile of an antibody against TNF-α in patients with a range of advanced solid cancers. The doses and schedule selected were on the basis of safe and clinically effective doses in other trials of graft versus host disease [16], myelodysplastic syndrome [17, 18] and renal cell cancer [19]. In our trial in a variety of solid epithelial tumour types, we also found that infliximab was safe and tolerable at 5 and 10 mg/kg. In cancer patients previously treated with potentially immunosuppressive chemotherapy, there was a possibility that TNF-α antagonist therapy may have increased the risk of serious infection, but, at least in this cohort of 41 patients,
there were no infections of note. There have been concerns also about increased susceptibility to cancers in patients treated with TNF-α antagonists. A meta-analysis of randomised clinical trials and retrospective study analysing the effect of TNF-α antagonists (±methotrexate), or methotrexate alone, in rheumatoid arthritis showed evidence of increased rates of cancer that appeared to be related to dose, in patients receiving anti-TNF-α monoclonal antibodies [14]. A more recent meta-analysis, however, found that the rates of cancer in patients treated with anti-TNF-α drugs were not significantly different from the patients treated with methotrexate alone [23]. In our study, there was no evidence of disease acceleration in any patient treated.

We also investigated the biological activity of infliximab in this heterogeneous population of advanced cancer patients. Infliximab reduced the serum concentrations of TNF-α at 1 h and plasma CCL2 and IL-6 concentrations. All cytokines, however, returned baseline concentrations before the second dose of infliximab.

Was there any evidence of clinical benefit in our phase I trial? We believe that there may have been. Five of the 41 patients went on to receive more than the three doses of infliximab defined by the trial protocol. All these were patients with progressive disease before entrance to the trial. Of particular note were two patients in the 10-mg/kg cohort with prolonged stable disease. Patient 27, with melanoma, had stable disease for 44 weeks and received nine doses of infliximab before discontinuing due to allergy. Patient 39, with metastatic colon cancer, had stable disease for 50 weeks before progressing on treatment. Clinical trials of the anti-TNF-α antagonist etanercept in patients with haematological, breast and ovarian cancer have also observed disease stabilization or partial responses [24–26].

This study has also given us some indication of those patients likely to benefit from TNF-α antagonist therapy. All seven patients who had clinical stabilisation from infliximab were those in which no baseline TNF-α was detected in the plasma. In contrast, 17 of 34 (50%) patients whose disease continued to progress during the three infliximab infusions had detectable TNF-α at baseline. In addition, although numbers were small, there was a significant correlation between lower baseline and 24-h CCL2 plasma levels and disease stabilisation in the patients receiving 10 mg/kg infliximab. These results may reflect the level of systemic cancer-related inflammation in these patients, or disease burden, but also may be due to higher circulating concentrations of TNF-α, as observed in the nonresponding patients may result in quicker clearance of the antibody resulting in less antibody reaching the cellular target and thus biological efficacy. Our observations support those made in a recent phase II trial of infliximab in renal cell cancer [19] where there was a significant association between baseline TNF-α levels and achievement of a partial response or disease stabilisation. Moreover, in the renal cell cancer patients receiving the higher 10 mg/kg infliximab dose, a decline in plasma CCL2 levels correlated with RECIST-defined stable disease [19].

In our study, there was no evidence of a dose–response relationship, and the levels of infliximab achieved in the plasma were well in excess of those needed to exert biological activity in vitro (1–10 µg/ml) [7, 27]. However, in some patients (5 mg/kg), infliximab levels measured before the second and third infusion were below biologically active concentrations. In view of the latter, together with the patients in this study achieving prolonged stable disease and the renal cell patients referred to above, we recommend dosing at 10 mg/kg in future studies.

The antitumour mechanism of action may be through modulation of the cytokine-dependent communication between cells in the tumour microenvironment rather than direct antibody-mediated cytotoxicity. Our data suggest that in the nonresponding patients, higher circulating concentrations of TNF-α may result in less antibody reaching cell membrane-bound TNF-α due to the quicker clearance from the microenvironment and thus limit the biological efficacy of infliximab. The antibody may modulate the tumour microenvironment by decreasing TNF-α-regulated inflammatory molecules, such as CCL2 and IL-6. This mechanism is supported by previous results from this laboratory in an experimental model of ovarian cancer [28] and also in decreases in plasma CCL2 and IL-6 observed in this trial.
We believe that further clinical trials of infliximab and similar agents that target TNF-α and other inflammatory cytokines may be warranted in patients with renal cell, colorectal and ovarian cancer as well as melanoma. Inflammatory biomarkers, especially TNF-α and CCL2, may allow us to select those patients who would benefit from this therapeutic approach.

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references