Infliximab treatment for steroid-refractory acute graft-versus-host disease

Background and Objectives. Tumor necrosis factor α is one of the principal cytokines involved in the pathogenesis of acute graft-versus-host-disease (GVHD). Infliximab is an antibody to this cytokine.

Design and Methods. We performed a retrospective analysis to evaluate the activity of infliximab in 32 patients with severe steroid-refractory acute GVHD. The patients received a median of 3 weekly courses of infliximab. The main organs involved in the patients were skin (n=2) liver (n=1), bowel (n=19), liver and bowel at the same stage (n=10).

Results. Nineteen out 32 patients (59%) responded to infliximab with 6 (19%) complete and 13 (40%) partial responses. Age younger than 35 years, intestinal involvement and a longer time between hematopoietic stem cell transplantation and infliximab administration were factors predicting a favorable response. Infective episodes developed in 23/32 (72%) patients. All the 13 unresponsive patients died of GVHD shortly after infliximab. Thirteen of 19 responsive patients were alive at a median follow-up of 449 days (range 155–842) after infliximab, with no signs of chronic GVHD (n=5), limited (n=5) or extensive involvement (n=3). Six patients who responded subsequently died, one of chronic lung GVHD, the others of vascular complications or infections (2 fungal diseases).

Interpretation and Conclusions. We conclude that infliximab is active in the treatment of severe steroid-refractory acute GVHD, particularly when the intestine is involved. Infections commonly followed its administration. The clinical activity of infliximab and the possibility that it increases the risk of infections are worth investigating in prospective trials.

Key words: acute GVHD, TNF-α, infliximab.
GVHD. Infliximab, already approved for clinical use in rheumatoid arthritis and Crohn’s disease, is a chimeric mouse/human IgGκ antibody that binds with high affinity to soluble and transmembrane forms of human TNF-α. Binding to soluble TNF-α results in the neutralization of its activity, whereas binding to the transmembrane form of TNF-α causes lysis of the affected cells by activation of complement and induction of antibody-mediated cellular toxicity. In a mouse model, blocking LIGHT (a T-cell co-stimulatory molecule belonging to the TNF-α superfamily), with monoclonal antibodies led to a persistent anergy of CD4 T lymphocytes and prevented acute GVHD after infusion of immuno-competent allogeneic donor cells.

Based on these findings, we conducted a retrospective analysis to evaluate the activity of infliximab in steroid-refractory acute GVHD.

**Design and Methods**

**Patients**

Thirty-two patients receiving an allogeneic HSCT for a neoplastic disease between June 2000 and December 2004 were retrospectively analyzed. Clinical and laboratory data of the patients were collected through a questionnaire sent to 26 major Italian Transplant Units for children and adult patients. Eight Hematology Centers replied that they had some experience with infliximab treatment and sent the data on 32 patients. All subjects met the following eligibility criteria: (i) diagnosis of hematologic or solid tumor; (ii) non-T-cell-depleted transplant from an HLA-matched sibling or unrelated donor (fully matched for HLA-A,-B, -DR by serological and molecular testing); (iii) development of acute GVHD ≥ grade II refractory to prednisone therapy; (iv) salvage treatment with infliximab (Remicade).

The patients’ characteristics are shown in Table 1. The median age at transplant was 39 years (range 2-66). Seven patients were younger than 14 years. Twenty-two of the 32 patients were male (69%). The hematologic diseases necessitating transplantation were acute leukemia (n=14), chronic myeloid leukemia (n=6), lymphoma (n=4), multiple myeloma (n=5) Fanconi’s anemia (n=1) renal carcinoma (n=1), and prostatic carcinoma (n=1). The median time between diagnosis and transplant was 16 months (range 4-68). Nine out the 32 patients (28%) were transplanted in a late phase of disease after heavy pre-treatment (previous autologous stem cell transplantation and/or ≥ 3 chemotherapy regimens). All the patients provided written informed consent to the allogeneic HSCT and infliximab administration under studies approved by the local Ethical Committees.

### Table 1. Clinical characteristics of the patients treated with infliximab.

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<tr>
<th>Clinical characteristics</th>
<th>No. of patients</th>
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<td>Age at HSCT (years)</td>
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<tr>
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<td>Median (range)</td>
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<tr>
<td>BU±CY± melphalan</td>
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<td>Reduced intensity</td>
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<tr>
<td>Source of stem cells</td>
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<td>59</td>
</tr>
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<td>PB</td>
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</tr>
<tr>
<td>II</td>
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</tr>
<tr>
<td>III</td>
<td>8</td>
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</tr>
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<td>IV</td>
<td>20</td>
<td>63%</td>
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<td>Main organ(s) involved</td>
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<tr>
<td>Skin</td>
<td>2</td>
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<tr>
<td>Liver</td>
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<tr>
<td>Bowel</td>
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<td>60%</td>
</tr>
<tr>
<td>Liver + bowel</td>
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<td>31%</td>
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<tr>
<td>GVHD treatment prior to infliximab</td>
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<tr>
<td>Cyclosporine A</td>
<td>26</td>
<td>81%</td>
</tr>
<tr>
<td>Prednisone</td>
<td>32</td>
<td>100%</td>
</tr>
<tr>
<td>ATG</td>
<td>12</td>
<td>38%</td>
</tr>
<tr>
<td>Other drugs</td>
<td>11</td>
<td>34%</td>
</tr>
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</table>

(HSCT: hematopoietic stem cell transplantation; TBI: total body irradiation; BU: busulfan; CY: cyclophosphamide; BM: bone marrow; PB: peripheral blood; GVHD: graft-versus-host disease; ATG: anti-human T lymphocyte serum).

**Transplant-related data**

Stem cells were harvested from sibling donors for 18 patients (56%) and from unrelated donors for the other 14 patients (44%). Thirteen patients (41%) received
marrow cells and 19 (59%) peripheral blood stem cells. The conditioning regimen used conventional myeloab-
limative doses for 19 patients (59%), who received total body irradiation and cyclophosphamide (n=9) or busulf-
fan plus cyclophosphamide or melphalan (n=10) and was of reduced intensity for 13 patients (41%), who 
were treated with fludarabine, thiotepa, cyclophos-
phamide (n=10), fludarabine plus cyclophosphamide 
(n=1), fludarabine, thiotepa, melphalan (n=1), or 2 Gy total body irradiation (n=1).

Antibiotic prophylaxis with levofloxacin was admin-
istered after HSCT until complete neutrophil recovery. 
An antifungal drug, fluconazole or itraconazole, was 
used until all immunosuppressive drugs were with-
drawn. In addition, trimethoprim-sulphamethoxazole 
was used to prevent Pneumocystis carinii pneumonitis. 
Cytomegalovirus (CMV) infection was monitored weekly 
by CMV antigenemia. If CMV antigenemia tests 
became positive, patients were treated with ganciclovir 
or foscarnet.

GVHD prophylaxis included cyclosporine A (CyA) and 
methotrexate for 24 patients (75%), CyA alone for 5 
patients (16%), tacrolimus for 1 patient (3%) and CyA 
plus mycophenolate mofetil (MMF) for the other 2 
patients (6%). Anti-human T lymphocyte rabbit serum 
(ATG) was administered in association with CyA and 
methotrexate in 12 patients (37%) receiving unrelated 
transplants.

The assessment and grading of acute and chronic 
GVHD were primarily based on clinical findings and fol-
lowed the commonly accepted diagnostic criteria.15,16 
Diagnosis was supported by skin, liver or gut biopsies, 
whenever indicated and clinically possible. Patients with 
diarrhea who could not undergo colonoscopy had stool 
cultures negative for bacteria, fungi and protozoa.

Response was assessed for each organ involved 7 days 
after the initiation of infliximab. A complete response 
was defined as resolution of all manifestations of acute 
GVHD in all evaluable organs, a partial response was 
declared as a decrease in organ stage by 1 in at least one 
evaluable organ without deterioration of others. Unre-
sponsive patients had an increase of 1 in organ stage 
in at least one evaluable organ or absence of any change 
after infliximab.

All patients developing grade II–IV acute GVHD 
received 2–5 mg/kg/day methylprednisolone i.v. for 7 
days; if no partial or complete resolution of symptoms 
occurred, they were considered steroid-refractory and 
progressed to infliximab treatment. The dose of inflix-
imab was 10 mg/kg/day i.v. weekly and at least 2 cours-
es were given.

Statistical analysis
Patients responsive and unresponsive to infliximab 
treatment were compared on the basis of several of 
their clinical characteristics and features of the graft. 
Categorical variables were compared using χ² statistics 
or Fisher’s exact test and continuous values were com-
pared with the two-sided Student’s t-test. A p value 
<0.05 was considered to be statistically significant. 
Survival was estimated by the Kaplan–Meier product 
limit method and curves from different groups were 
compared by the log-rank test.

Results
The median time between the allogeneic HSCT and 
diagnosis of acute GVHD was 22 days (range 9–161). All 
but 2 cases were taking some form of prophylaxis (27 
CyA, 1 prednisone, 2 tacrolimus) at the moment of GVHD 
onset. The 2 patients who were not receiving any pro-
phylaxis were being given donor leukocyte infusions for 
disease relapse. All the patients showed clinical fea-
tures of acute GVHD: the main organs involved were 
the skin in 2 cases (6%), liver in 1 case (3%), bowel in 19 
cases (60%), liver and bowel at the same stage in 10 
patients (31%). The diagnosis of GVHD was confirmed 
by 7 intestinal, 2 cutaneous and 1 hepatic biopsies. 
GVHD was overall classified as grade 2 in 4 patients 
(12%), grade 3 in 8 patients (25%) and grade 4 in 20 
patients (63%).

First-line therapy for the acute GVHD was 2–5 
mg/kg/day methylprednisolone i.v. in all patients, in asso-
ciation with CyA (n=26) or tacrolimus (n=1) or MMF 
(n=5). All the patients were steroid-refractory, since 
they had no improvement after at least 7 days of treat-
ment. Twelve patients were subsequently treated with 
ATG, 2 with methotrexate, 3 with photophoresis and 1 
with cyclophosphamide without any improvement, 
before receiving infliximab. Overall, infliximab courses 
were administered to 14 patients (44%) as second-line 
therapy, after failure of prednisone and CyA (or 
tacrolimus or MMF) and as third-line therapy to anoth-
er 18 patients (56%) who did not respond to second-
line therapy.

The median time between HSCT and infliximab 
administration was 47 days (range 19–262); 27 of the 
32 patients (84%) were treated within 30 days after 
the onset of GVHD, the remaining 5 patients received 
infliximab after 70, 129, 134, 220 and 240 days. Inflix-
imab was administered at the dose of 10 mg/kg/day i.v.
weekly for a median of 3 courses (range 2–8) without 
infusion-related side-effects. Steroid doses were main-
tained between 1–2 mg/kg/day for all patients during 
the therapy with infliximab. Nineteen of the 32 patients 
(59%) responded to infliximab: responses were com-
plete in 6 patients (19%) and partial in 13 patients 
(40%). Fifteen out of the 19 responsive patients had 
premptual intestinal involvement, whereas 4 had liv-
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er involvement with significantly increased bilirubin levels, associated with skin or bowel disease. The median level of serum bilirubin of these cases was 5.5 mg/dL before infliximab and 1.7 mg/dL after treatment. After infliximab, Cy A and prednisone were gradually tapered down and then definitively stopped in 6 and 4 responsive patients, respectively. No patient who responded received ATG whereas 8 patients received other immunosuppressive therapies, such as tacrolimus (n=3), MMF (n=2), cyclophosphamide (n=1), or photophoresis (n=1) (Figure 1A). In the group of unresponsive patients, steroids were not suspended in any patients: 4 received ATG and 8 received other treatments, which were tacrolimus (n=2), MMF (n=2), methotrexate (n=2), cyclophosphamide (n=2) (Figure 1B).

Twenty-two out 32 patients (72%) developed one or more infectious episodes after infliximab: there were 7 cases of septicemias (5 Gram-positive and 2 Gram-negative), 2 cases of septic shock, 2 cases of pneumonia of bacterial origin and 5 caused by an undetermined etiologic agent, 4 cases of infectious enteritis, 1 case of encephalitis, 13 cases of CMV reactivation (documented by positive antigenemia) and 2 proven invasive mycotic infections (candidemia and pulmonary

Figure 1. Modification of immunosuppressive therapy in patients responsive (1A) and unresponsive (1B) to infliximab treatment. (CyA: cyclosporine A; PDN: prednisone; ATG: anti-human T lymphocyte serum; other: tacrolimus, mycophenolate mofetil, cyclophosphamide, methotrexate or photophoresis).
aspergillosis). All the 13 unresponsive patients died of GVHD at a median time of 43 days (range 14–450) after its onset. Thirteen of the 19 responsive patients (68%) were still alive at a median follow-up of 630 days (range 300–1170) after HSCT and 449 days (range 155–842) after the infliximab treatment. Five out of the 19 (26%) responsive patients had no signs of chronic GVHD, 5 (26%) had limited and 3 (16%) had extensive chronic GVHD. Six out the 19 (32%) responsive patients died: 3 because of infections (mycotic pneumonia, candidemia, encephalitis), 1 of thrombotic thrombocytopenic purpura, 1 of ischemic stroke with concomitant evidence of sepsis and 1 of respiratory failure due to lung GVHD. Four patients (12%) had evidence of disease relapse or progression after HSCT (3 lymphomas and one chronic myeloid leukemia transplanted in blastic phase). Survival curves after infliximab are shown in Figure 2. Patients who did or did not respond to infliximab were compared on the basis of several clinical features (sex, age, disease diagnosis, phase of disease, previous treatment), characteristics of their transplantation (myeloablative or reduced-intensity treatment, source of stem cells, type of donor) and GVHD features (time between HSCT and GVHD onset or first dose of infliximab, main organ involvement, infliximab as second or third-line treatment for GVHD). The analysis showed that age under 35 years, a predominant intestinal involvement (with no liver GVHD) and a longer time between HSCT and infliximab administration were associated with a favorable response to infliximab, as shown in Table 2. Patients with concomitant hepatic and intestinal GVHD had a significant adverse outcome after infliximab treatment.

Discussion

We conducted a multicenter retrospective study of 32 patients with steroid-refractory grade II–IV acute GVHD. More than half of them had grade IV GVHD and all but 3 had gastrointestinal involvement. Patients had a uniform conventional first-line treatment with high-dose prednisone and were evaluated as unresponsive after a 7-day course. Infliximab was added as a second-line drug in 14 patients whereas it was used as third-line salvage treatment in 18 patients. We report an encouraging response rate: 19% complete and 40% partial responses according to standard clinical criteria. It is worth noting that the amount of GVHD therapy could be reduced in patients responding to infliximab, whereas no drugs could be tapered down or withdrawn in the unresponsive patients and sometimes new therapies, such as ATG, had to be added.

That infliximab was clinically effective in acute GVHD had been suggested by a few case-reports and small series reported in the literature. Kobbe et al. described that 3 of 4 patients with acute stage III or IV intestinal GVHD refractory to high-dose steroids improved after infliximab administration. The MD Anderson group reported the largest experience in acute (37 patients) and chronic GVHD (26 patients), with an overall response rate of 70% and the greatest benefit in cases with lower gastrointestinal tract involvement. Recently, Couriel et al. confirmed these data in 21 patients in whom infliximab was added as a single agent to tacrolimus and corticosteroids for the initial treatment of steroid-resistant acute GVHD: complete responses were obtained in 62% with the best responses occurring in patients with gastrointestinal and cutaneous GVHD. Other groups observed some responses in series between 5 and 11 cases with acute or chronic GVHD unresponsive to steroids and several other drugs, such as ATG, daclizumab, MMF and tacrolimus. The majority of these studies did not allow definitive conclusions to be drawn because the groups of patients were small, quite heterogeneous, pre-treated with several conventional and experimental immunosuppressants and had a very short follow-up. The dose of infliximab was 10 mg/kg/day in the majority of the studies and was repeated weekly for a few courses: this schedule was drawn from clinical experience in rheumatology, but adequate pharmacokinetic studies are lacking in the field of HSCT. Other clinically promising but not conclusive data came from the experimentation of etanercept, a genetically engineered TNF-α receptor, which inhibits TNF-α activity by binding the soluble form of this cytokine. Clinical responses were reported in a pediatric case of chronic intestinal GVHD, in 15 patients with obstructive or restrictive patterns of lung injury associated with extensive chronic GVHD, and in
Infliximab for steroid-refractory GVHD

We observed that the strongest predictive factor for a favorable outcome after infliximab was when the intestine was the organ involved by GVHD; moreover, more frequent responses were reported in younger patients and those in whom the time between HSCT and infliximab administration was longer. The MD Anderson experience suggested that infliximab was most active on low intestinal tract GVHD.6,8,12 This observation was confirmed in our series; moreover, we found that patients who had predominant hepatic involvement or concomitant severe hepatic and intestinal involvement had a significantly lower response rate. It could be hypothesized that TNF-α is the principal mediator of intestinal GVHD, but that other cytokines, such as interleukin-6 and interleukin-10, are involved in skin and liver GVHD and that these are partially independent from infliximab inhibition of the TNF-α cascade.21-22 Patients who had greater benefit from infliximab had received it at a longer interval after HSCT; these patients included a few who developed GVHD late in the 3-month period after HSCT or after immune manipulation (donor leukocyte infusion or CyA withdrawal) and a few who had had GVHD conventionally treated in the first 3-month period and then received infliximab for a

<table>
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<th>Clinical characteristics</th>
<th>Responsive (%)</th>
<th>Unresponsive (%)</th>
<th>p value</th>
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<tbody>
<tr>
<td>Age at HSCT (years)</td>
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<tr>
<td>&lt;35</td>
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<td>6/13</td>
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<td>≤12</td>
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<td>Infliximab as second-line treatment for GVHD</td>
<td>6/19</td>
<td>8/13</td>
<td>0.56</td>
</tr>
</tbody>
</table>

**Table 2. Univariate analysis of possible differences in clinical characteristics between patients responsive and unresponsive to infliximab treatment.**

20 patients who received 2 mg/kg/day prednisone and etanercept as first-line treatment of acute GVHD.20

*HSCT: hematopoietic stem cell transplantation; BM: bone marrow GVHD: graft-versus-host disease; SD: standard deviation; ns: not significant.*
recurrence of GVHD with clinical features of the acute form (namely diarrhea and erythema) more than 100 days after the HSCT. The correlation between shorter time after transplantation and worse response might be explained by the conditioning regimen having damaged the intestinal mucosa, thus releasing large amounts of endotoxins, which primed GVHD. The shorter interval between the conditioning and the GVHD could produce very high levels of TNF-α that might not be effectively inhibited by anti-TNF-α therapy.

The outcome of responsive and unresponsive patients was dramatically different. In fact, all 13 unresponsive patients died of GVHD shortly after infliximab administration, while only one out the 19 patients who had some response died of chronic GVHD of the lung. Other causes of death in responsive patients were infections, vascular complications, disease recurrence or progression alone or in association in 5 out 19 patients. The 13 surviving patients were followed up for a median of 15 months after the infliximab treatment and the majority had no signs of chronic GVHD or only limited involvement. In our series we observed several infectious episodes: the etiological agents varied greatly (bacterial, viral, mycotic and unknown) and the infections were frequently localized to organs such as lungs, brain, and bowel, as was to be expected in a population of severely immunosuppressed patients. It should be emphasized that 2 patients who responded to infliximab died of proven invasive fungal infections. Moreover, there were 5 cases of pneumonia caused by an undetermined etiologic agent, which could have been a fungus. In other series, fungal infections were reported in 38 to 54% of patients treated with infliximab for acute GVHD.1,4,5,10,12 Recently, Jacobsohn et al.13 reported that all 6 out 11 patients who developed invasive mycosis after infliximab treatment had a fatal outcome. In these small retrospective series of patients treated with high-dose steroids and combinations of anti-GVHD drugs, it is difficult to assess how much the infliximab influenced the inability to control the fungal disease. However, it has also been reported that patients who received infliximab for treatment of Crohn’s disease or rheumatoid arthritis have an unusually high rate of extrapulmonary and disseminated tuberculosis or histoplasmosis, possible due to defective granuloma formation and absence of anti-TNF-α mediated macrophage apoptosis.24-25 The only controlled study in transplanted patients is that by Marty et al.,26 who demonstrated that patients who received infliximab to control severe GVHD had a significantly higher risk and a shorter time to diagnosis of non-Candida invasive fungal infections than did conventionally treated patients and that these differences were independent of the cumulative dose of steroids received.

Our study has several limitations due to its retrospective nature: the collection of data from 8 different centers, where infliximab was prescribed at the discretion of the attending physicians, and the heterogeneity of the additional immunosuppressive agents administered to the patients. However, infliximab produced clinical responses in 59% of the largest series of patients with steroid-refractory acute GVHD ever reported in the literature. The majority of these responses were obtained in patients with gastrointestinal involvement and many were durable: only 4 out of 19 responsive patients progressed to develop extensive (3 cases) or fatal (1 patient) chronic GVHD. Infective episodes occurred in 72% of the patients and 3 patients who responded to infliximab died of infections, 2 of them with proven fungal diseases. The clinical activity of infliximab and the possibility of an increased risk of infections are worthy of investigation in prospective trials. Given the suspicion of an increased risk of fungal infection, pre-emptive systemic antymycotic therapy should be considered in the study design for patients who develop severe GVHD and are treated with infliximab.

FP was the principal author responsible for the design of the questionnaires, the collection of the data and writing of paper. AS, DD, FB were responsible for the statistical analysis and interpretation of the data. GM, GB, AO, FC, GM, SC, GD were involved in the design of the study, the collection and the interpretation of the data. PC and RF gave the main contribution in the critical revision of the text. All the authors gave final approval of the version to be published. The authors reported no potential conflicts of interest.

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