Phase I-II Trial of a Monoclonal Anti-Tumor Necrosis Factor α Antibody for the Treatment of Refractory Severe Acute Graft-Versus-Host Disease


In a multicenter pilot study, 19 patients with severe acute graft-versus-host disease (aGVHD) refractory to conventional therapy and serotherapy with a monoclonal anti-interleukin-2 receptor antibody were treated by in vivo infusion of a monoclonal anti-tumor necrosis factor α (TNFα) antibody (B-C7). Ten patients were grafted from a genotypically identical sibling, five from an HLA-mismatched family member, and four from an HLA-matched unrelated donor. Before B-C7 treatment, 15 patients had grade IV and four had grade III GVHD. In all cases, patients received cyclosporine/methotrexate as aGVHD prophylaxis. Patients were administered increasing doses of antibody (from 0.1 to 0.4 mg/kg). The antibody was infused in bolus daily for 4 days and then every other day twice (6 doses). No side effects were observed during treatment regardless of the dose level used. Changes in peripheral blood cell counts occurred in 8 of the 19 patients and appeared to be unrelated to B-C7. No truly complete response was observed; eight patients achieved a very good partial response (42.6%) and six a partial response (31.5%). The treatment was ineffective in five patients (26.4%). When present, the response occurred early (<3 days). In the 14 responding patients, gut lesions responded best (100%), followed by skin (85%) and liver (35.7%) lesions. In 9 of 11 evaluable patients (81%), GVHD recurred when treatment was discontinued in a median delay of 3 days (range, 2 to 120 days). All except one died from aGVHD. Two patients did not experience GVHD recurrence and are still alive 13 and 18 months post-bone marrow transplantation. This pilot study shows that a monoclonal anti-TNFα antibody may be of benefit to some patients with severe refractory aGVHD, but is ineffective to prevent GVHD recurrence in the majority of cases.

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of a phase I-II multicenter pilot study, including 19 patients
and assessing the potential clinical efficacy of this MoAb in
the treatment of severe forms of refractory aGVHD.

PATIENTS AND METHODS

Monoclonal Anti-TNFα Antibody (clone B-C7)

B-C7 MoAb (J. Wijdenes, Besançon Regional Blood Transfu-
sion Center) is a murine IgGl k MoAb with a Ka value of 1.8 × 10⁹
mol/L obtained by immunizing mice (Balb/c X 63/Ag 8653) with
human recombinant TNFα. After adaptation of the B-C7 cell line
to serum-free medium, B-C7 MoAb was produced in a bioreactor.
The MoAb is able to block the cytotoxic activity of natural and
recombinant human TNFα on the murine cell line L929 with a
specific biologic activity of 10 ng.

Patients

From November 1989 to January 1991, 19 patients aged 4
months to 43 years (median, 21 years) entered this pilot study.
Their clinical characteristics are summarized in Table 1. Ten
patients received a marrow transplant from HLA-matched related
donors (MRD), five from partially matched related donors (PMRD; 
one pheno-identical, one with one incompatible locus, and two
with two incompatible loci), and four from HLA-matched unre-
related donors (MUD). BMT conditioning regimens were variable
(fractionated total body irradiation [12 to 14 Gy], associated with
direct single drug chemotherapy [4 patients] or multiple drug chemother-
apy [4 patients]) or exclusive multiple drug chemotherapy (busul-fan
associated with another chemotherapeutic agent [eight patients]). Two patients (unique patient nos. [UPN] 1 and 19) were
transplanted for the second time after the first BMT. One patient
with SCID was not conditioned before transplantation. All patients
were nursed under strict protective isolation using laminar air-flow
or closed plastic bubble systems. They received a total nonspecific
gut decontamination using nonabsorbable antibiotics.

As aGVHD prophylaxis, three patients received a T-lymphocyte–
depleted donor marrow without postgraft immunosuppressive
therapy, and 16 patients received cyclosporine, either alone (n = 3)
or associated with methotrexate (n = 9) and with an anti-IL-2
receptor antibody (n = 4).

The criteria we adopted for diagnosis and grading of aGVHD
are those established at Seattle.7 aGVHD was histologically con-
firmed by skin biopsy. Due to the clinical and/or hematologic status
of the patients, gut and liver biopsies were seldom performed. The
intervals between BMT and the onset of GVHD and from
refractory GVHD to B-C7 treatment were 27 ± 21 days and 40.7 ±
36.6 days, respectively. Three patients initially presented grade I
GVHD, five grade II, five grade III, and six grade IV (4 of 6 had a
clinical picture of toxic epidermal necrolysis). Eighteen patients
were administered corticosteroids before B-C7, either at standard
dose (2 mg/kg), intermediate dose (5 mg/kg), or high dose (10
mg/kg) combined with monoclonal anti-IL-2 receptor antibodies
(12 patients) or antithymocyte globulin (6 patients). Eighteen
patients received B-C7 either as second-line (n = 5), third-line
(n = 5), or fourth-line therapy (n = 8). In one infant with SCID
(UPN 7) who presented acute transfusional GVHD before BMT,
B-C7 was initiated as first-line therapy.

Before B-C7 treatment was initiated, four patients had grade III
and 15 had grade IV refractory GVHD (Table 1).

During B-C7 administration, all patients were administered corticosteroids at standard or intermediate dose and all except one
received cyclosporine (Table 1).

Before B-C7 treatment, 13 patients did not exhibit any infection,
but six patients did (three had cytomegalovirus [CMV] infections,
one had aspergillosis, one had sepsis, and one had interstitial
pneumonitis).

At the initiation of B-C7 treatment, hematopoietic recovery was
complete in six patients, partial in 10, two had poor graft function,
and one had pancytopenia justifying a second marrow transplant 1
month later.

B-C7 Treatment Protocol

As part of this phase I-II protocol and with the goal of assessing
the feasibility, tolerance, and kinetics of this MoAb, the patients
included in this pilot study were administered increasing doses of
MoAb in vivo: 0.1 mg/kg (n = 4), 0.2 mg/kg (n = 9), 0.3 mg/kg
(n = 2), 0.4 mg/kg (n = 4) (Fig 1). The MoAb was diluted in 100 or
150 mL of saline and was infused for 15 to 30 minutes every day for
4 days and then every second day for a further 4 days (6 doses). To
avoid GVHD recurrence when B-C7 was discontinued, eight
patients received high-dose corticosteroids followed by rapid dose
reduction over 4 days. Three patients received anti-T MoAbs
(CD25 ± CD5; 10 mg/day for 10 days).

The sera of patients treated in Besançon were collected before
each B-C7 infusion for a study of B-C7 pharmacokinetics. Their
sera were also collected before and after treatment to determine
the levels of TNFα,22 soluble IL-2 receptor (sIL-2R), and soluble
CD8 (sCD8).

The protocol received the approval of the Committee of Ethics
of the University of Besançon (France). Patients or their legal
guardians gave their written informed consent.

GVHD response to B-C7 treatment was assessed upon comple-
tion of B-C7 treatment and fell into four groups. The complete
response (CR) patients were those in whom GVHD lesions totally
resolved in all organs involved. The very good partial response
(VGPR) patients were those in whom greater than 50% of GVHD
lesions disappeared in all of the organs initially involved. In
particular, improvement of gastrointestinal symptoms was assessed
by the number of stools per day (bloody or not), the volume of
diarrhea, and the disappearance of abdominal pain. Partial re-
sponse (PR) was considered to be a reduction of lesions in at least
one organ involved and no response (NR) to be an unchanged
status or progressive evolution of aGVHD.

Measurement of BC-7 Serum Levels

Circulating BC-7 was measured by serial dilutions in a double
sandwich enzyme-linked immunosorbent assay (ELISA) with
a sensitivity of 10 ng/mL. Rabbit antimouse Ig was coated overnight
in carbonate buffer. Control and patient sera were incubated
for 1 hour with streptavidin peroxidase. O-Phenylenedi-
amine was used as a substrate and optical density (OD) was
measured after 30 minutes at 405 nm.

Laboratory Assays

All assays were performed on frozen (−20°C) aliquoted samples.
Consecutive evaluable samples were assayed for sCD8, sIL-2R,
and TNFα. sCD8 and sIL-2R levels were measured with two-site
sandwich enzyme immunoassays (Cell Free test kits; T Cell
Science, Cambridge, MA). In our laboratory, control values were
346 ± 116 U/mL and 473 ± 180 U/mL for sCD8 and sIL-2R levels,
respectively. TNFα levels were determined with an immuno-
radiometric assay (IRA-Medgenix, Brussels, Belgium). Polypro-
pylene tubes coated with MoAbs directed against distinct epitopes
of TNFα (polyclonal system) were incubated with a mixture of

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### Table 1. Clinical and Treatment Characteristics of Patients Treated With an Anti-TNFα Antibody for Severe aGVHD

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age/ Sex</th>
<th>Diagnosis</th>
<th>HLA Matching</th>
<th>GVHD Prophylaxis</th>
<th>Organ Involvement (grading)</th>
<th>B-C7 Dose (mg/kg)</th>
<th>Medications Administered With B-C7</th>
<th>Sites of Response</th>
<th>Side Effects</th>
<th>aGVHD Recurrence</th>
<th>Time to Recurrence (d)</th>
<th>Treatment After B-C7</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/F</td>
<td>SAA</td>
<td>PMRD (3/6)</td>
<td>TCD + CSP</td>
<td>S4 + G4 (IV)</td>
<td>0.1</td>
<td>MP + CSP</td>
<td>S1 + G1</td>
<td>No</td>
<td>Yes</td>
<td>12</td>
<td>MP (SD) anti IL2R antibody</td>
<td>Dead day 74 post-BMT aGVHD multiorgan failure</td>
</tr>
<tr>
<td>2</td>
<td>30/M</td>
<td>CML</td>
<td>MRD</td>
<td>CSP + MTX</td>
<td>S2 + G4 + L4 (IV)</td>
<td>0.1</td>
<td>MP + CSP</td>
<td>S1 + G2 + L4</td>
<td>No</td>
<td>Yes</td>
<td>2</td>
<td>MP (HD)</td>
<td>Dead day 135 post-BMT aGVHD IP</td>
</tr>
<tr>
<td>3</td>
<td>35/F</td>
<td>CML</td>
<td>MUD</td>
<td>CSP + MTX</td>
<td>S3 + G4 + L1 (IV)</td>
<td>0.1</td>
<td>MP + CSP</td>
<td>S1 + G2 + L1</td>
<td>No</td>
<td>Yes</td>
<td>120</td>
<td>MP (HD)</td>
<td>Dead day 175 post-BMT liver failure (aGVHD)</td>
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<tr>
<td>4</td>
<td>25/M</td>
<td>CML</td>
<td>MRD</td>
<td>CSP + MTX</td>
<td>S1 + G2 + L3 (III)</td>
<td>0.1</td>
<td>MP + CSP</td>
<td>S0 + G2 + L2</td>
<td>No</td>
<td>Yes</td>
<td>3</td>
<td>MP (HD)</td>
<td>Dead day 202 post-BMT aGVHD IP</td>
</tr>
<tr>
<td>5</td>
<td>6/M</td>
<td>AML</td>
<td>PMRD (8/9)</td>
<td>CSP + MTX</td>
<td>S4 + L3 (IV)</td>
<td>0.2</td>
<td>MP + CSP</td>
<td>S3 + L1</td>
<td>No</td>
<td>No</td>
<td>—</td>
<td>MP (ID)</td>
<td>Alive and well 15 mo+</td>
</tr>
<tr>
<td>6</td>
<td>6/M</td>
<td>SAA</td>
<td>PMRD (5/6)</td>
<td>CSP + MTX</td>
<td>S4 + G3 + L3 (IV)</td>
<td>0.2</td>
<td>MP + CSP</td>
<td>—</td>
<td>No</td>
<td>—</td>
<td>—</td>
<td>ATG</td>
<td>Dead day 47 post-BMT aGVHD cerebral hemorrhage</td>
</tr>
<tr>
<td>7</td>
<td>4 mo/F</td>
<td>SCID</td>
<td>PMRD (3/6)</td>
<td>TCD</td>
<td>S4 + G2 + L4 (IV)</td>
<td>0.2</td>
<td>MP + CSP</td>
<td>S2 + G0 + L1</td>
<td>No</td>
<td>Yes</td>
<td>3</td>
<td>MP (HD)</td>
<td>Dead day 155 post-BMT ARDS no aGVHD</td>
</tr>
<tr>
<td>8</td>
<td>39/F</td>
<td>Hodgkin</td>
<td>MRD</td>
<td>TCD</td>
<td>S3 + G4 (IV)</td>
<td>0.2</td>
<td>MP + CSP</td>
<td>S1 + G1</td>
<td>No</td>
<td>NE</td>
<td>—</td>
<td>MP (HD)</td>
<td>Dead day 94 post-BMT suicide</td>
</tr>
<tr>
<td>9</td>
<td>4/F</td>
<td>AUL</td>
<td>MRD</td>
<td>CSP + MTX</td>
<td>S4 + G2 (IV)</td>
<td>0.2</td>
<td>MP + CSP</td>
<td>S1 + G1</td>
<td>Septic shock</td>
<td>NE</td>
<td>—</td>
<td>MP (SD)</td>
<td>Dead day 79 post-BMT septic choc (candida)</td>
</tr>
<tr>
<td>10</td>
<td>6 mo/F</td>
<td>ADA</td>
<td>deficiency</td>
<td>PMRD (4/6)</td>
<td>TCD</td>
<td>S3 + G4 (IV)</td>
<td>0.2</td>
<td>MP + CSP</td>
<td>—</td>
<td>No</td>
<td>—</td>
<td>—</td>
<td>MP (ID)</td>
</tr>
<tr>
<td>11</td>
<td>5/M</td>
<td>ALL</td>
<td>MUD</td>
<td>CSP + MTX</td>
<td>S4 + G2 (IV)</td>
<td>0.2</td>
<td>MP + CSP</td>
<td>—</td>
<td>No</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>ATG</td>
</tr>
<tr>
<td>12</td>
<td>2/M</td>
<td>AMML</td>
<td>MRD</td>
<td>CSP + MTX</td>
<td>S2 + G3 (III)</td>
<td>0.2</td>
<td>MP + CSP</td>
<td>S1 + G1</td>
<td>Pancreatic sepsis</td>
<td>Yes</td>
<td>3</td>
<td>MP (ID) CDS + CD25 ATG</td>
<td>Dead day 143 post-BMT IP + aGVHD</td>
</tr>
<tr>
<td>13</td>
<td>27/M</td>
<td>ALL</td>
<td>MRD</td>
<td>CSP + MTX</td>
<td>S2 + G3 + L2 (IV)</td>
<td>0.2</td>
<td>MP + CSP</td>
<td>S1 + G0 + L2</td>
<td>No</td>
<td>Yes</td>
<td>2</td>
<td>MP (ID)</td>
<td>Dead day 50 post-BMT aGVHD toxoplasmosis</td>
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<tr>
<td>14</td>
<td>32/M</td>
<td>CML</td>
<td>MUD</td>
<td>CSP + MTX</td>
<td>S2 + G3 + L3 (III)</td>
<td>0.3</td>
<td>MP + CSP</td>
<td>S1 + G1 + L3</td>
<td>No</td>
<td>Yes</td>
<td>3</td>
<td>Anti-IL2R antibody MP (ID)</td>
<td>Dead day 115 post-BMT aGVHD fungal infection</td>
</tr>
<tr>
<td>15</td>
<td>17/M</td>
<td>Blackfan Diamond</td>
<td>MUD</td>
<td>CSP + MTX</td>
<td>S2 + G4 + L3 (IV)</td>
<td>0.3</td>
<td>MP + CSP</td>
<td>S1 + G1 + L1</td>
<td>No</td>
<td>NE</td>
<td>—</td>
<td>MP (SD)</td>
<td>Dead day 100 post-BMT aGVHD</td>
</tr>
<tr>
<td>16</td>
<td>21/M</td>
<td>AML</td>
<td>MRD</td>
<td>CSP + MTX</td>
<td>S1 + G1 + L3 (III)</td>
<td>0.4</td>
<td>MP + CSP</td>
<td>Q0 + L1</td>
<td>No</td>
<td>No</td>
<td>—</td>
<td>MP (SD)</td>
<td>Alive and well 15 mo+</td>
</tr>
<tr>
<td>17</td>
<td>26/M</td>
<td>AML</td>
<td>MRD</td>
<td>CSP + MTX</td>
<td>S3 + G4 + L1 (IV)</td>
<td>0.4</td>
<td>MP + CSP</td>
<td>S1 + G0 + L0</td>
<td>No</td>
<td>Yes</td>
<td>27</td>
<td>MP (HD)</td>
<td>Alive and well mild chronic GVHD 6 mo+</td>
</tr>
<tr>
<td>18</td>
<td>43/F</td>
<td>AML</td>
<td>MRD</td>
<td>CSP + MTX</td>
<td>S2 + G4 + L2 (IV)</td>
<td>0.4</td>
<td>MP + CSP</td>
<td>NR</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>MP (HD) ATG</td>
<td>Dead day 121 aGVHD multiorgan failure</td>
</tr>
<tr>
<td>19</td>
<td>35/F</td>
<td>CML</td>
<td>MRD</td>
<td>CSP + MTX</td>
<td>S2 + G4 + L3 (IV)</td>
<td>0.4</td>
<td>MP + CSP</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>MP (HD)</td>
<td>Dead day 68 aGVHD ARDS</td>
</tr>
</tbody>
</table>

Abbreviations: SAA, severe aplastic anemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AUK, acute undifferentiated leukemia; AMML, acute myelomonocytic leukemia; ADA, adenosine deaminase; CML, chronic myeloid leukemia; SCID, severe combined immune deficiency; CSP, cyclosporin; MTX, methotrexate; TCD, T-cell depletion; S, skin; G, gut; L, liver; NE, not evaluable; HDM, high-dose methylprednisolone; ATG, antithymoglobulins; ARDS, acute respiratory distress syndrome; MP, methylprednisolone; HD, high dose; ID, intermediate dose; SD, standard dose.
125I-labeled anti-TNFα MoAb and the sample to be tested. After decantation, the bound fraction was counted in a gamma counter. Control values for serum TNFα levels were less than 15 pg/mL.

**RESULTS**

**B-C7 MoAb Tolerance**

The infusion of B-C7 antibody was very well tolerated over the 8 days of treatment. No clinical signs of intolerance (chills, hypotension) were observed regardless of the dose level used. In 11 patients, no significant change in white blood cell (WBC), absolute neutrophil count (ANC), monocyte, lymphocyte, or platelet counts was detected during B-C7 administration. In eight patients, changes in blood cell counts occurred during B-C7 treatment (Table 2). These variations of peripheral blood cell counts appeared to be random and are probably attributable to direct or indirect effects of severe GVHD. Of the eight patients presenting documented infection or interstitial pneumonitis (IP) before B-C7 treatment, no deleterious effects of B-C7 on the evolution of infection were observed during the treatment. When the treatment was completed, one patient (UPN 11) presented a septic shock due to candida. The clinical symptoms of this candida septicemia, which occurred during the course of B-C7 treatment, might have been masked by the anti-TNFα MoAb. Another patient (UPN 12) presented acute pancreatitis, as well as neurologic deterioration with a seizure, both of which appeared unrelated to B-C7 treatment and resolved completely with no sequelae.

**Response to B-C7 Treatment**

B-C7 treatment was initiated in a median delay of 25 days (range, 5 to 116 days) after the onset of aGVHD. The response was clinically assessed at the end of B-C7 treatment. Responses are given in Tables 1 and 3. Eight patients (42.1%) achieved a VGPR (6 grade IV, 2 grade III). Six patients (31.5%) achieved a PR (4 grade IV, 2 grade III) and five patients (26.3%) did not respond (5 grade IV). Skin and gut lesions responded best (11 of 18 [61%] and 13 of 18 [72%], respectively), followed by liver lesions (5 of 13 [38.5%]). Among the 14 responding patients (VGPR + PR), 14 of 14 (100%) gut lesions, 12 of 14 (85.7%) skin lesions, and 5 of 14 (35.7%) liver lesions, respectively, responded to B-C7 administration. The clinical response was particularly clear in gut lesions, because clinical manifestations disappeared in six patients (43%) and eight patients (57%) achieved a greater than 50% response.

In all the gut responders (10 adults, 4 children) the decreasing in stoolsing was clinically obvious: 1,726 ± 869 mL diarrhea before B-C7 treatment and 566 ± 324 mL diarrhea after treatment was completed. In five patients with bloody diarrhea, three patients changed to nonbloody diarrhea.

Skin improvement was also satisfactory, although no truly complete response was observed. Liver improvement was mediocre (8 failures), although two patients achieved a complete response. Response delays for skin and gut were very short (<3 days).

Clinical responses were seldom histologically documented.

Nevertheless, when skin (n = 6), rectum, or liver biopsies (n = 3) were performed, they consistently showed a staging decrease in histologic GVHD manifestations. There was no significant correlation between B-C7 dose levels and GVHD response (p = .8, Fischer’s exact test). The small sample size did not make it possible to establish a response curve. In terms of GVHD response, there was no difference whether patients received standard (2 mg/kg, n = 7) or higher doses (>2 mg/kg, n = 11) of prednisolone during B-C7 treatment.

**Table 2. Leukocyte and Platelet Modifications During B-C7 Administration in Eight Informative Patients**

<table>
<thead>
<tr>
<th>UPN</th>
<th>B-C7 Dose (mg/kg)</th>
<th>Pre WBC</th>
<th>Post WBC</th>
<th>Pre ANC</th>
<th>Post ANC</th>
<th>Pre Lymphocytes</th>
<th>Post Lymphocytes</th>
<th>Pre Monocytes</th>
<th>Post Monocytes</th>
<th>Pre Platelets</th>
<th>Post Platelets</th>
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<tr>
<td>2</td>
<td>0.1</td>
<td>2,500</td>
<td>1,500</td>
<td>2,000</td>
<td>1,275</td>
<td>75</td>
<td>85</td>
<td></td>
<td></td>
<td>3,100</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>5,400</td>
<td>3,600</td>
<td>4,644</td>
<td>3,132</td>
<td>54</td>
<td>180</td>
<td>640</td>
<td>252</td>
<td>80</td>
<td>50</td>
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<tr>
<td>5</td>
<td>0.2</td>
<td>2,600</td>
<td>4,200</td>
<td>1,946</td>
<td>1,780</td>
<td>178</td>
<td>1,210</td>
<td>182</td>
<td>812</td>
<td>130</td>
<td>83</td>
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<tr>
<td>10</td>
<td>0.2</td>
<td>11,000</td>
<td>15,500</td>
<td>10,160</td>
<td>13,125</td>
<td>330</td>
<td>875</td>
<td>220</td>
<td>875</td>
<td>26</td>
<td>45</td>
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<td>11</td>
<td>0.2</td>
<td>3,410</td>
<td>1,330</td>
<td>2,100</td>
<td>254</td>
<td>688</td>
<td>674</td>
<td>605</td>
<td>470</td>
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<td>13</td>
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<td>600</td>
<td>100</td>
<td>420</td>
<td>400</td>
<td>180</td>
<td>50</td>
<td>0</td>
<td>20</td>
<td>20</td>
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<tr>
<td>17</td>
<td>0.4</td>
<td>14,600</td>
<td>16,300</td>
<td>12,550</td>
<td>14,507</td>
<td>438</td>
<td>326</td>
<td>1,022</td>
<td>863</td>
<td>70</td>
<td>80</td>
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<tr>
<td>18</td>
<td>0.4</td>
<td>4,100</td>
<td>600</td>
<td>2,500</td>
<td>224</td>
<td>1,066</td>
<td>70</td>
<td>328</td>
<td>280</td>
<td>40</td>
<td>20</td>
</tr>
</tbody>
</table>

In the other 11 patients, no change in blood cell counts was detected the day of B-C7 MoAb completion.
### Table 3. Response to B-C7 Treatment Categorized by Individual Organs

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Skin</th>
<th>Gut</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluable</td>
<td>18</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>CR</td>
<td>—</td>
<td>6 (33)</td>
<td>2 (15.5)</td>
</tr>
<tr>
<td>VGPR</td>
<td>5 (28)</td>
<td>5 (28)</td>
<td>2 (15.5)</td>
</tr>
<tr>
<td>PR</td>
<td>6 (33)</td>
<td>2 (11)</td>
<td>1 (7.5)</td>
</tr>
<tr>
<td>NR</td>
<td>7 (39)</td>
<td>5 (28)</td>
<td>8 (61.5)</td>
</tr>
</tbody>
</table>

Percentages in parentheses.

Abbreviations: CR, complete response; VGPR, very good partial response (≥50%); PR, partial response (<50%); NR, no response (stable, progression).

**B-C7 Pharmacokinetics**

Pharmacokinetic analysis showed that peak serum B-C7 levels were dose-dependent and ranged from 1.2 to 13.4 μg/mL (Fig 2).

**Alloreactivity Markers**

**TNFα.** TNFα levels were measured in 10 patients immediately before B-C7 treatment (control values, <15 pg/mL). Five had high levels (118 ± 71 pg/mL) and five had levels less than 15 pg/mL. There was no correlation between the initial TNFα level and the response to B-C7 treatment. A follow-up of serum TNFα levels was performed on five patients during and after B-C7 treatment. Although the number of patients was too low for statistical analysis, the posttreatment TNFα level was higher in the PR/NR group (94 ± 53 pg/mL) as compared with the VGPR group (<20 pg/mL).

**siL-2R and sCD8.** Before B-C7 treatment in the eight evaluable patients, siL-2R and sCD8 levels were, respectively, 2,270 U/mL (range, 200 to 5,000 U/mL) and 294 U/mL (range, 160 to 410 U/mL). In patients evaluated posttreatment, siL-2R levels were 2,300 U/mL (range, 200 to 5,000 U/mL) and sCD8 levels were 423 U/mL (range, 110 to 1,200 U/mL).

**GVHD Recurrence**

When B-C7 treatment was completed, all responding patients received additional immunosuppressive therapy (Table 1). Among the 14 responders, only 11 were evaluable for GVHD recurrence. Three patients died immediately after treatment was discontinued (one suicide, one septic shock, one interstitial pneumonitis). In two patients (14%), GVHD did not relapse; both are alive 15 and 13 months post-BMT. In nine patients, GVHD recurred in a median delay of 3 days after B-C7 treatment was discontinued (range, 2 to 120 days). Except for a female patient whose GVHD recurrence involved only liver (UPN 3), all patients presented multiorgan involvement at the time of recurrence.

Although salvage treatments were administered at the time of relapse, only one of the relapsing patients survived and is currently alive on day +180, with moderate chronic GVHD.

**Survival**

Among the 19 patients included in this study, three are alive (15.8%) at 6, 13, and 15 months post-BMT. Two do not exhibit chronic GVHD and one presents moderate chronic GVHD. Sixteen patients died (84.2%). The causes of death (Table 4) are mainly due to GVHD recurrence.

**DISCUSSION**

In murine models, Piguet et al. showed the protective role of a polyclonal or monoclonal anti-TNFα antibody against aGVHD-induced endothelial lesions, but not against those induced by chronic GVHD. These antibodies also enhanced engraftment and prevented cachexy. These combined effects lead to a clearly reduced mortality (70% animals surviving in the group treated with anti-TNFα

**Table 4. Primary Causes of Death Among Patients Treated With Anti-TNFα (B-C7) for Severe aGVHD**

<table>
<thead>
<tr>
<th>Causes of Death</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>aGVHD</td>
<td>5</td>
<td>56%</td>
</tr>
<tr>
<td>aGVHD + IP</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Acute respiratory distress syndrome</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Interstitial pneumonitis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Suicide</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Septic shock</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fungal infection</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>
ANTTI-NF ANTIBODY FOR THE TREATMENT OF GVHD

Anti-TNF antibody versus 10% in the control group. High serum TNFα levels are reported to be associated with severe aGVHD.20 However, this association is not true in each individual patient undergoing allogeneic BMT. Indeed, severe aGVHD can be observed with low serum TNFα levels.21

B-C7 was well tolerated at all dose levels. Changes in blood cell counts were observed in eight patients, but appeared to be unrelated to the B-C7 administration. In one patient (UPN 9), a septic shock was observed when B-C7 was discontinued. The occurrence of infectious complications on discontinuation of a treatment with monoclonal anti-TNFα antibody administered in a context of infection is a potential risk, given the role played by TNFα in the mechanisms of antibacterial resistance.22 Nevertheless, the target-specificity of an anti-TNFα MoAb suggests that such a therapy, with respect to infectious complications, could be less dangerous than immunosuppressive agents with a broader action such as ATG. The maximal tolerated dose of this anti-TNFα MoAb is unknown. Doses greater than 1 mg/kg have been used in the treatment of septic shock with serum TNFα concentrations in excess of 1,400 pg/mL without any clinical intolerance.23 In our study, the dose escalation was interrupted at 0.4 mg/kg/d because patients did not appear to get any additional benefit, at this dose level, in terms of GVHD response.

With a 72.8% response rate, our pilot study shows that anti-TNFα may be of benefit to some patients with severe aGVHD after BMT from HLA-matched or mismatched donors. B-C7 has an influence on all the target organs of GVHD, with a decreasing efficacy on the following organ involvements: gut (72% response, among which 33% were complete responses and 39% partial responses), skin (61% response), and liver (38.5% response). Regression occurs particularly early in the case of gut disorders. The reasons why the most dramatic responses were observed in gut lesions are presently unknown. In our phase II trial involving an anti-IL-2R MoAb,6 the best responses were also observed in skin and gut lesions.

In our series, only 5 of 10 evaluable patients had high levels of TNFα just before B-C7 treatment. Serum TNFα levels less than 10 pg/mL do not exclude a possible GVH response to B-C7 treatment. Low serum TNFα levels may correspond to a tissue cell fixation of TNFα. In accordance, serum TNFα levels in a GVH murine model were never detectable, also suggesting that local versus systemic production of TNFα is predominant in most cases of GVH.16

The major problem encountered in MoAb serotherapy is GVHD recurrence when the treatment is discontinued.9,24 In our study, 9 of 11 (81%) evaluable patients relapsed. The rate of relapse is higher than that observed with MoAbs directed against the presumed effector cells of aGVHD.25 One can assume that a monoclonal anti-TNFα antibody has an influence only on a GVHD effector cytokine, and not on an effector cell.

Several alternatives exist to reduce the incidence of GVHD recurrence. An anti-T-cell MoAb (like anti-CD2 or anti-CD25) could be associated with an anti-TNFα MoAb and this type of serotherapy could be maintained for several weeks discontinuously. Preliminary data suggest that a 1- to 2-month maintenance treatment with anti-IL-2R MoAb (B-B10) is well tolerated and might be effective in preventing GVHD relapse (unpublished data). When anti-TNFα is discontinued, MoAbs conjugated with a toxin (ricin-conjugated CD25 or CD5) could be adopted to obtain a better target effect, because this type of conjugated MoAb is less dependent on the host’s natural effectors as compared with unconjugated MoAbs.8 High doses of corticosteroids, which also prove to be an effective anti-TNFα agent, could be associated with or follow B-C7 treatment.25

Progress in the treatment of aGVHD will clearly depend on our understanding of the cytokine network and its role in allospecific immune organ cytotoxicity.26

The rapidly occurring responses observed in severe potentially lethal aGVHD lesions and the high relapse rate observed after B-C7 treatment both suggest that anti-TNFα serotherapy could be used as an “emergency” medicine in severe GVH situations ideally associated with effective therapy directed against the allo-reactive cellular aGVHD component. B-C7 MoAb could also be used earlier in the therapeutic strategy of aGVHD, as second-line therapy, as soon as steroid resistance is established.

In summary, the possible efficacy of an anti-TNFα MoAb has been shown in severe refractory aGVHD. A major problem remains yet to be solved as to how to achieve long-term control of steroid-resistant GVHD. Associating an anti-TNFα MoAb with effective innovative therapy directed against the allo-reactive cellular GVHR component could be very promising.

ACKNOWLEDGMENT

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