CLINICAL–ALIMENTARY TRACT

A Phase I Study of Visilizumab, a Humanized Anti-CD3 Monoclonal Antibody, in Severe Steroid-Refractory Ulcerative Colitis

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See CME quiz on page 1690.

Background & Aims: **To evaluate the safety and biological activity of visilizumab (a humanized anti-CD3 monoclonal antibody) and to determine a maximum tolerated dose in patients with severe ulcerative colitis that had not responded to 5 days of treatment with intravenous corticosteroids.** ***Methods:*** **In this open-label phase 1 study, 32 subjects received visilizumab at a dose of 10 or 15 μg/kg, administered intravenously on 2 consecutive days. Clinical response was defined as a Modified Truelove and Witts Severity Index <10 with a minimum decrease of 3 points; remission was <4 points. Endoscopic remission was a Mayo endoscopic subscore of 0 or 1.** ***Results:*** **Eight patients received 15 μg/kg visilizumab. Because of dose-limiting toxicities (T-cell recovery >30 days in 2 of 8 patients), the dose was reduced to 10 μg/kg in 24 patients. On day 30, 84% of patients demonstrated a clinical response, 41% achieved clinical remission, and 44% achieved endoscopic remission. Forty-five percent of patients did not require salvage therapies or colectomy during the first year postdose. Mild to moderate symptoms of cytokine release occurred in 100% and 83% of patients in the 15- and 10-μg/kg dose groups, respectively. All patients exhibited a rapid decrease in circulating $CD4^+$ T-cell counts, which returned to baseline values by day 30 in 26 of 30 evaluable patients (86%). There were no serious infections.** ***Conclusions:*** **Visilizumab had an acceptable safety profile at the 10-μg/kg dose level and may be clinically beneficial in patients with severe intravenous corticosteroid–refractory ulcerative colitis.**

Ulcerative colitis (UC) is a chronic inflammatory disorder of the colon of unknown etiology with clinical features of rectal bleeding and diarrhea. Approximately 15% of patients with UC will have a severe attack requiring hospitalization for treatment with intravenous (IV) corticosteroids at some stage in their illness.[1] Sixty percent of patients with severe UC who are treated with IV corticosteroids will be free of symptoms by the end of 5 days, 15% will have significant improvement, and 25% will not improve.[2] Those who fail to improve may be treated with cyclosporine, tacrolimus, or infliximab.[3–5] However, recent data suggest that the long-term failure rates after cyclosporine salvage therapy are relatively high; no long-term data are available for tacrolimus and infliximab.[6]

Patients who fail or decline salvage therapy may undergo colectomy. In patients with severe UC who initially respond to parenteral corticosteroids, colectomy rates vary from 38% to 60% during the following year.[2,4,7] Although advances in surgical therapy have provided more satisfactory outcomes for patients with UC, colectomy is associated with a variety of surgical complications (including abscess, anastomotic leaks, obstruction, chronic pouchitis, sexual dysfunction, female infertility, and poor functional results) that can result in repeated surgery, diminished quality of life, and mortality. New treatment options for patients with severe IV corticosteroid–refractory UC are needed.

CD3 antigen-positive T cells are believed to play an important role in the pathogenesis of the disease.[8] Managing T-lymphocyte activation and proliferation by tar-

Abbreviations used in this paper: DLT, dose-limiting toxicity; EBV, Epstein–Barr virus; MTWSI, Modified Truelove and Witts Severity Index.

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geting interleukin-2 synthesis through inhibition of the calcineurin pathway with cyclosporine and tacrolimus are current treatments for severe UC.[4,5] Therefore, newer biologic therapies that functionally inhibit activated T cells or their trafficking are an attractive therapeutic approach for the treatment of patients with severe UC. In humans, a murine anti-CD3 monoclonal antibody, OKT3, has activity in treating renal allograft transplant rejection, but side effects associated with systemic cytokine release after administration have limited its use outside of the transplant setting.[9] In addition, because of its high immunogenicity, use of this murine protein has been limited to a single course of therapy. To abrogate these issues with OKT3, newer generations of antibodies have been humanized and modified to attenuate binding in vivo to Fc receptors expressed on a variety of immune functional cells.[10]

Visilizumab is a humanized immunoglobulin G2 monoclonal antibody that specifically binds to human CD3 expressed on T cells. It was engineered to reduce FcR binding by incorporating 2 amino acid substitutions at positions 234 and 237 in the CH2 domain of the antibody's Fc region.[11] As a result of these changes in the Fc region, visilizumab elicits a substantially diminished cytokine release, complement fixation, and T-cell activation compared with the FcR-binding of OKT3 of resting human peripheral blood mononuclear cells in vitro.[11,12] We performed a phase 1 safety and dose-finding study of visilizumab in hospitalized patients with severe IV corticosteroid–refractory UC.

Materials and Methods

Patients

Male and nonpregnant female patients, 18–70 years of age, who were diagnosed with UC verified by colonoscopy or barium enema within 36 months before study entry and who had active disease were eligible for the study. Active disease was documented by a Modified Truelove and Witts Severity Index (MTWSI) score of 11–21 and ongoing treatment with IV corticosteroids for at least 5 days before study entry.[4,13] Each patient provided written informed consent to participate in the trial before commencement of screening procedures. Other pertinent inclusion and exclusion criteria are provided in Supplement 1 (see supplemental material online at www.gastrojournal.org).

Study Design

The institutional review boards at 9 investigative sites in the United States and Europe approved the protocol for this trial conducted between June 2002 and January 2004. The study was originally designed as a dose-escalation study to determine the maximum tolerated dose of visilizumab in this patient population. The starting dose was 15 μg/kg administered as an IV bolus on 2 consecutive days (day 1 and day 2 of the study); additional dose levels up to 120 μg/kg were planned. See Supplement 2 (see supplemental material online at www.gastrojournal.org) for description of dosing considerations.

The maximum tolerated dose was defined as the next lower dose level than the dose at which 2 or more of the first 10 patients experienced dose-limiting toxicity (DLT). A DLT was defined as either (1) a severe acute toxicity lasting longer than 24 hours and related to visilizumab administration or (2) failure to achieve adequate recovery of blood T-cell counts by day 30. Adequate T-cell recovery was defined as a $CD4^+$ T-cell count of $\geq$200 cells/μL or the patient's lowest predose value. The protocol was later amended to a dose de-escalation design due to the occurrence of DLTs at 15 μg/kg after the first 8 patients, and an additional 24 patients were treated at 10 μg/kg.

During the course of this first study in patients with IV corticosteroid–refractory UC, a standardized pretreatment and posttreatment regimen of medication and IV hydration was developed to prevent or reduce the discomfort of symptoms associated with the transient increase of serum cytokines. The premedication regimen included ondansetron, diphenhydramine, and the patient's entire daily dose of IV corticosteroids administered approximately 1 hour before administration of visilizumab; acetaminophen was administered 1 hour after visilizumab dosing. In addition, patients were hydrated with IV fluids for several hours before and after dosing with visilizumab. Morphine sulfate or meperidine and diphenhydramine were used as needed to treat chills, myalgias, arthralgias, and pruritus.

Data on all adverse events were collected through day 60; the occurrence of opportunistic infections, malignancies, and surgeries was followed for up to 1 year postdose.

Study Treatment

Study drug was supplied in single-use vials containing 1.0 mg/mL visilizumab in a solution of 20 mmol/L sodium citrate, 120 mmol/L sodium chloride, and 0.01% polysorbate 80 at a pH of 6.0.

Concomitant Medical Therapies

Patients were permitted to receive concomitant mesalamine derivatives, 6-mercaptopurine or azathioprine, and corticosteroids. Patients received IV methylprednisolone, hydrocortisone, or dexamethasone at a dose equivalent to 40–60 mg methylprednisolone per day for at least 5 days before and each day of visilizumab infusion. After day 2, patients were transitioned from IV corticosteroids to the equivalent of 40–80 mg oral prednisone per day. Corticosteroids were tapered after day 8 at the investigator's discretion.

Clinical End Points

The MTWSI and the Mayo Clinical Activity Index (Mayo) score were used to assess the overall severity of the

disease.[4,13,14] The MTWSI was assessed at baseline, day 1 of dosing, and on days 15, 30, 60, 90, and 180. Response was defined as an MTWSI score of <10 points with an improvement from baseline of at least 3 points. Remission was defined as an MTWSI score of <4 points. Although not a component of the original protocol, Mayo scoring at baseline and day 30 was added after an efficacy signal was observed in the first 8 patients enrolled. Mayo response was defined retrospectively as a decrease from baseline of 3 or more points in the total Mayo score, with a rectal bleeding subscore of 0 or 1, or a reduction in the rectal bleeding score of 1. Mayo remission was defined retrospectively as a total score <3, with no individual subscore >1. Endoscopic remission was defined as a Mayo mucosal subscore of 0 or 1, which implied no active bleeding, ulcers, or friability.[15]

Other evaluations included assessment of corticosteroid-sparing effects of visilizumab and duration of clinical response. Duration of clinical response was assessed retrospectively at 1 year by evaluating patients' requirement for colectomy or for the initiation of other salvage therapies such as IV corticosteroids, cyclosporine, infliximab, or re-treatment with visilizumab. Salvage therapies did not include initiation of or adjustments in outpatient medications, including mesalamine, azathioprine, 6-mercaptopurine, and oral corticosteroids.

Peripheral Blood T-Lymphocyte Counts

Absolute counts for major lymphocyte subsets were obtained using multiparameter flow cytometric analysis. Blood samples for flow cytometry were drawn at baseline; 15 minutes before dosing on days 1 and 2, and 1 hour after dosing on day 1; on days 8, 15, and 30; and every 7 days as necessary until T-cell levels had recovered. Major immune subsets were defined as total T cells ($CD3^+$), T-helper cells ($CD3^+CD4^+$), T-effector cells ($CD3^+CD8^+$), natural killer cells ($CD3^-$ $CD56^+\pm$ $CD16^+$), and B cells ($CD3^-$ $CD19^+$). See Supplement 3 for methodological details (see supplemental material online at www.gastrojournal.org).

Epstein–Barr Virus Whole Blood DNA Copies

Levels of Epstein–Barr virus (EBV)-specific nucleic acid sequences in whole blood were assessed using quantitative DNA amplification by a fluorogenic probe-based reverse-transcriptase polymerase chain reaction assay. Blood samples for EBV viral load were drawn at baseline and on days 8, 15, and 30; if the EBV titer was higher on day 30 than the patient's baseline value, EBV assays were repeated every 2 weeks until they returned to pretreatment levels. See Supplement 3 for methodological details (see supplemental material online at www.gastrojournal.org).

Immunogenicity

Antibodies to visilizumab were detected using a bridging enzyme-linked immunosorbent assay with a limit of detection of 50 ng/mL, and cell-based flow cytometry assay with a limit of detection of 100 ng/mL was used to detect the presence of neutralizing antibodies. Blood samples for immunogenicity assessments were drawn 15 minutes before dosing on day 1 and on days 15, 30, and 90.

Pharmacokinetics

Serum visilizumab levels were measured with an enzyme-linked immunosorbent assay with a lower limit of quantification of 25 ng/mL. Blood samples for pharmacokinetics were drawn at baseline; 15 minutes before and 1 and 4 hours after dosing on day 1; 15 minutes before and 1 and 6 hours after dosing on day 2; on days 8, 15, 30, and 90.

Analytical Methods

Results were summarized by dose level without formal statistical testing of differences between groups. Descriptive statistics and 95% confidence intervals were used where appropriate. Subjects with missing day 30 data were imputed as failures. The verbatim adverse events were mapped to the system organ class and preferred term using the Medical Dictionary for Regulatory Activities.[16] The severity of adverse events was graded based on the Common Toxicity Criteria of the National Cancer Institute.[17]

Results

Patient Screening and Demographics

Of 58 patients who were screened for enrollment, 32 were enrolled and received study drug. Hospitalized patients received IV corticosteroids for a median of 7 days (range, 5–17) before receiving visilizumab. Twenty-five potential patients who were hospitalized with severe UC and treated with IV corticosteroids had measurable EBV DNA levels in whole blood (median, 747 copies/mL; range, 97–10,500 copies/mL) at screening. Six patients with measurable whole blood EBV DNA levels were enrolled in the study (5 patients as protocol exceptions and 1 patient who experienced an increase in EBV DNA levels between screening and visilizumab administration). The presence of detectable EBV genome did not correlate with initial absolute peripheral blood $CD3^+$, $CD4^+$, or $CD8^+$ T-cell counts (data not shown). Two patients were excluded from the study because of clinical response to IV corticosteroids during the screening period. Five subjects were excluded for other reasons.

Baseline characteristics of this patient population (Table 1) were consistent with severe/fulminant IV corticosteroid–refractory UC. Patients were significantly immunocompromised. All were receiving high doses of IV glucocorticoids, and more than one third of the patients were receiving an immunomodulator drug. The patients' compromised immune status at baseline was indicated by

Table 1. Baseline Demographics

Dose	10 μg/kg (n = 24)	15 μg/kg (n = 8)	Total (N = 32)
Gender			
Male	18	4	22
Female	6	4	10
Median age, y (range)	42 (19–70)	45 (33–70)	43 (19–70)
Extent of disease			
Left-sided	6	3	9
>60 cm	18	5	23
Median days of IV corticosteroids	7	9.5	7
Mean baseline MTWSI score (±SD)	13.0 (2.1)	13.4 (1.5)	13.1 (2.0)
Concomitant medications, n (%)[a]			
Mesalamine	19 (79)	4 (50)	23 (72)
6-mercaptopurine or azathioprine	11 (46)	2 (25)	13 (41)
Enema	2 (8)	0	2 (6)
Mean laboratory values (±SD)			
Hematocrit (%)	36.9 (5.6)	39.2 (4.9)	37.3 (5.5)
White blood count (*k/μL*)	11.6 (5.9)	10.2 (3.0)	11.3 (5.4)
T-cell subsets, mean cells/μL (±SD)			
$CD3^+$	1195 (869)	1167 (818)	1189 (842)
$CD4^+$	694 (505)	569 (342)	666 (471)
$CD8^+$	479 (451)	577 (503)	501 (454)
Albumin (*g/dL*)	3.5 (0.6)	3.4 (0.5)	3.5 (0.6)
Erythrocyte sedimentation rate (*mm/h*)	23.4 (21.2)	25.4 (15)	24 (19)

[a]One patient received prior treatment with infliximab, and one patient received prior treatment with tacrolimus.

their generally low predose T-cell levels; the median peripheral blood $CD4^+$ T-cell count was 512 cells/μL (range, 58–1871 cells/μL). Notably, $CD4^+$ T-cell counts were <200 cells/μL in 16% of screened patients. In addition, 1 patient received prior treatment with infliximab (last dose 43 days before visilizumab) and 1 patient received tacrolimus (last dose 13 days before visilizumab).

Safety

All patients received both doses of study drug without interruption. No patients discontinued the study because of adverse events. However, DLTs occurred in 2 of the first 8 patients who received visilizumab at the 15-μg/kg dose level. One patient had delayed T-cell recovery (at day 61) and severe bilateral knee pain of 1-week duration, and a second patient had delayed T-cell recovery (at day 161). Thus, the maximum tolerated dose had been exceeded, and the dose of visilizumab was decreased to 10 μg/kg for subsequent patients.

Overall, 31 patients (97%) experienced at least 1 adverse event; the incidence of adverse events was similar in both dose groups (96% vs 100%; Supplementary Table 1; see supplemental material online at www.gastrojournal.org). Ninety-four percent of the events were mild or moderate. Overall, 25% of patients experienced severe adverse events, defined as events that caused disability and interfered with the activities of daily life. The incidence of severe events was higher in the 15-μg/kg dose group (38%) compared with the lower-dose group (21%). There were no life-threatening events in either dose group.

Twenty-seven patients (84%) experienced at least 1 adverse event related to study drug. Overall, the most commonly reported related adverse event was chills (15 subjects; 47%), followed by nausea (14 subjects; 44%), headache (12 subjects; 38%), pyrexia (11 subjects; 34%), and fatigue (10 subjects; 31%). Five patients (16%) reported a severe drug-related adverse event (2 of 8 in the 15-μg/kg dose group and 3 of 24 in the 10-μg/kg dose group). Drug-related severe adverse events were abdominal pain and distention (in 1 patient) and knee pain, chills, cytokine release syndrome, diarrhea, and nausea/vomiting (in 1 patient each). Many of the unrelated adverse events were ascribed to underlying UC activity.

The majority (77%) of drug-related adverse events occurred within 3 days after the first dose of visilizumab. All adverse events that occurred on study days 1–3 were potentially related to the transient increase in serum cytokines (Supplementary Table 2; see supplemental material online at www.gastrojournal.org). Overall, 88% of the patients experienced at least 1 event possibly associated with cytokine release; the incidence of cytokine release symptoms was lower in the patients who received 10 μg/kg visilizumab compared with patients who received 15 μg/kg (83% vs 100%; Supplementary Table 2). Most cytokine release symptoms were mild or moderate; 5 patients reported one or more severe symptoms. The incidence of severe cytokine release symptoms was lower in the 10-μg/kg dose group than in the 15-μg/kg dose group (12.5% vs 25%).

Symptoms of cytokine release affected more subjects and were more intense following the first dose of visilizumab compared with the second dose; cytokine release symptoms occurred in 27 patients (84%) on day 1, and symptoms were reported in 17 patients (53%) on day 2. The majority (84%) of the cytokine release symptoms resolved within 24 hours. Ten patients received an opioid drug on days 1, 2, or 3; 8 of the 10 patients received an opioid drug to treat one or more likely symptoms of cytokine release. Two patients received an opioid drug to treat adverse events deemed not related to study drug.

Eleven patients (34% total; 29% in the 10-μg/kg group and 50% in the 15-μg/kg group) reported 8 types of infections. Two patients had urinary tract infections, 2 developed cellulitis, 2 had oral candidiasis, and 1 each was diagnosed with bronchitis, nasopharyngitis, rhinitis, tonsillitis, and upper respiratory tract infection. All infections were mild or moderate and resolved with outpatient management. Four of the infections (rhinitis, oral

candidiasis, upper respiratory infection, and urinary tract infection) were deemed related to visilizumab by the investigator.

The clinical course of 2 patients merits further description. One patient experienced chest pain and had a modest elevation of troponin I level 30 minutes after the second visilizumab infusion. Symptoms resolved within 20 minutes. No enzyme evolution, electrocardiogram, or stress echocardiogram evidence of a myocardial infarction or ischemia was evident during subsequent evaluation. One patient experienced mild bilateral blurring of vision on the first day of visilizumab infusion. An ophthalmologic examination 1 month later revealed several small peripheral retinal hemorrhages of uncertain duration in 1 eye. This patient did not experience transient thrombocytopenia. All events resolved with no residual effects.

Administration of visilizumab resulted in the rapid reduction of blood $CD3^+$ T-cell counts in all 32 patients. $CD3^+$ T-cell counts declined from a mean (±SD) of 1041 (±770) cells/μL immediately before the first dose of visilizumab to 63 (±77) cells/μL 1 hour after the first dose in the 10-μg/kg group and from 1027 (±546) to 33 (±11) cells/μL in the 15-μg/kg group. Figure 1 depicts the time course of the decline and recovery of peripheral blood $CD3^+$ T cells and $CD4^+$ and $CD8^+$ T-cell subsets in the 10-μg/kg dose group.

In 6 of the 8 patients who received 15 μg/kg visilizumab, the $CD4^+$ T-cell counts recovered by day 30. The median T-cell recovery for this dose group was 15 days (range, day 7–161). In 2 patients, T-cell count recovery was documented on days 60 and 161. The T-cell counts may have recovered sooner, but in both instances the patients were not assessed between days 30 and either day 60 or 161. Twenty of 24 patients who received 10 μg/kg visilizumab had $CD4^+$ T-cell count recovery within 30 days from dosing. The median time to T-cell recovery for this dose group was 16 days (range, 8–72 days). In 2 patients, the T-cell counts did not recover until 42 and 74 days after dosing. Additionally, 2 patients withdrew from the study 2 and 15 days following dosing, without further assessments of T-cell levels. No serious or opportunistic infections occurred during the time of peripheral T-cell reduction.

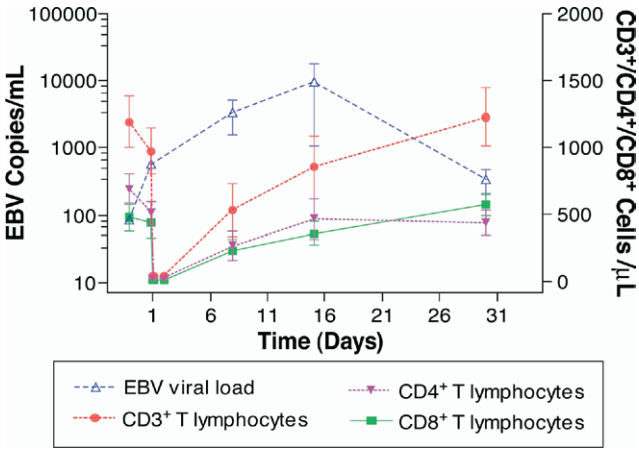


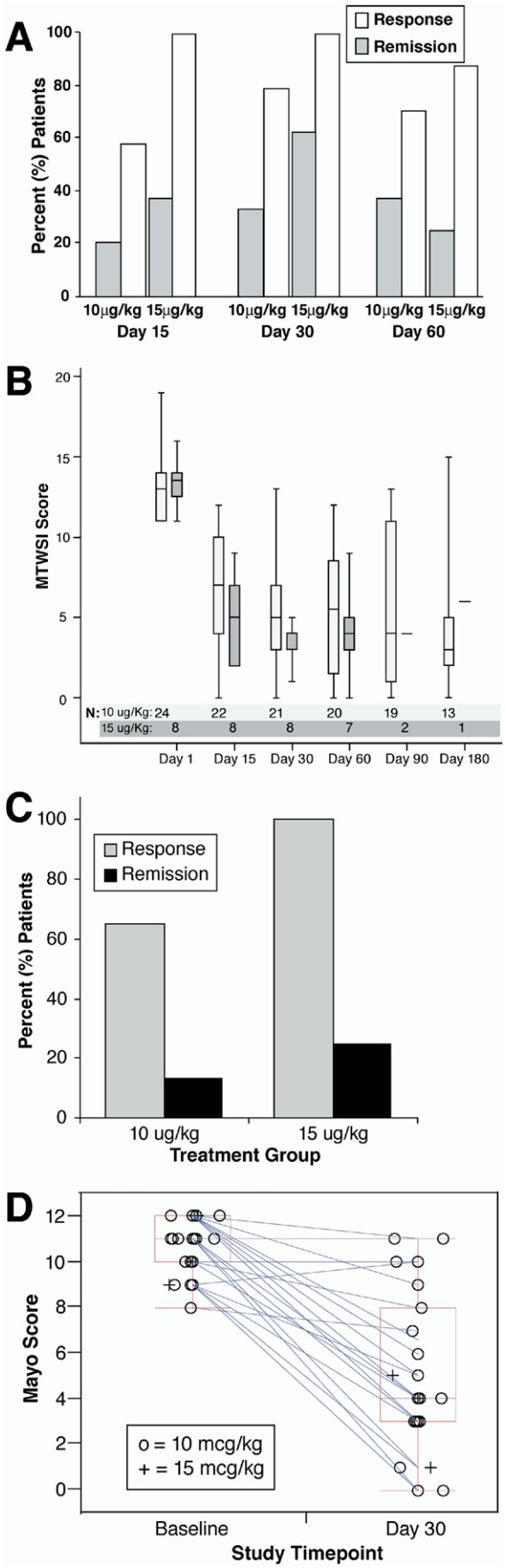
Figure 1. Pharmacodynamic effects on levels of EBV genomic DNA and $CD3^+$, $CD4^+$, and $CD8^+$ T-cell subsets in peripheral blood following treatment with visilizumab. Data were pooled from patients who received 10 μg/kg visilizumab on days 1 and 2 (data points shown are means; n = 18–24 per time point; *error bars* indicate ±1 SEM). Rapid reduction of all peripheral blood T cells was observed 1 hour after visilizumab administration on day 1. Recovery of total $CD3^+$ T-cell counts, as well as $CD4^+$ and $CD8^+$ T-cell subsets, was evident by day 7, and by day 15 all 3 sets had returned to baseline levels. The level of EBV genomic DNA copies in whole blood increased upon the reduction of T-cell levels and then returned to below the limit of assay quantification in 86% of patients by day 30.

Peripheral blood subsets that are not bound by the antibody also demonstrated a rapid decrease 1 hour after administration of visilizumab, for example, neutrophils (mean percent decrease, 44%), $CD19^+$ B cells (mean percent decrease, 52%), $CD16^+CD56^+$ natural killer cells (mean percent decrease, 73%), and monocytes (mean percent decrease, 73%). Neutrophil counts returned to baseline levels on day 2; other subsets of cells recovered by day 8 (Supplementary Figure 1; see supplemental material online at www.gastrojournal.org).

Two patients had transient platelet reductions during the study. Single platelet counts of 16,000/μL and 19,000/μL were observed 1 hour after the dose on day 1 in 1 subject and on the predose assessment on day 2 in the other. All other platelet counts were within the normal range, and there was no clinical evidence of thrombocytopenia (ie, petechiae or bruising).

Twenty-four of 32 patients were unequivocally negative for detectable EBV DNA in whole blood at screening; in addition, 3 patients had detectable EBV DNA in whole blood samples, but the levels were below the lower limit of quantification (80 copies/mL). At screening, 5 of 32 patients had quantifiable levels of EBV genome (110, 111, 97, 156, and 2321 copies/mL). Because of variability in the lower limit of quantification between the 2 laboratories used in this study, the medical monitor determined that all subjects with an EBV titer <200 copies/mL (the lower limit of quantification in the European laboratory) would be given exceptions and allowed to participate in the study. One patient (2321 copies/mL at screening) tested negative for EBV DNA in the study site's clinical transplantation immunology laboratory and was given a protocol exception.

After visilizumab administration, peripheral T-cell decline was associated with elevations of whole blood EBV DNA titers in the majority of patients (Figure 1). The EBV genome concentration increased to a median of 228 copies/mL (range, undetectable to 190,000 copies/mL) on day 15 in 24 of 32 patients. EBV DNA was undetectable after a median of 23 days in the 15-μg/kg dose group and after a median of 9 days in the 10-μg/kg dose



group. There were no clinical symptoms of EBV infection associated with elevations in EBV DNA.

Clinical Assessment of Disease Activity

All patients had an MTWSI assessment and a sigmoidoscopy at baseline. On day 30, the MTWSI was assessed in 21 of 24 patients in the 10-μg/kg group and in all 8 patients in the 15-μg/kg group. Three patients in the 10-μg/kg group experienced early disease progression and withdrew consent; they were unavailable for MTWSI assessment on day 30 and were imputed failures in the analysis. Clinical response was achieved at day 30 in 79% (19/24) and 100% (8/8) of patients in the 10- and 15-μg/kg dose groups, respectively (Figure 2*A*). At day 30, 33% (8/24) of the patients who received 10 μg/kg visilizumab and 62.5% (5/8) of those who received 15 μg/kg were in remission. The median time to clinical response was 16 days (range, 14–49 days) for the 10-μg/kg visilizumab group and 16.5 days (range, 15–19 days) for the 15-μg/kg group. Figure 2*B* shows the median MTWSI scores by dose group over time (day 1–180).

Mayo scores were available at baseline and day 30 for 25 patients; for an additional 2 patients, their last observation was carried forward to day 30 for the analysis. (For 4 of the patients in the 15-μg/kg dose group, Mayo scores were calculated retrospectively from baseline data, because Mayo scoring was not part of the protocol when they were enrolled.) Among the 27 patients for whom both baseline and day 30 Mayo scores were available, there was a median 7-point decrease in the 12-point Mayo score (range, 11-point decrease to a 1-point increase; Figure 2*D*). The day 30 Mayo response rate was 74.1%, and the remission rate was 14.9% (Figure 2*C*).

Endoscopic Assessment of Disease Activity

Baseline and day 30 Mayo endoscopic subscores were available for 27 patients; for an additional 2 patients, their last observation was carried forward to day 30 for the analysis. At screening, 25 of 29 patients (86%) had the most severe Mayo endoscopic subscore of 3, and 4 of 29 (14%) had a score of 2 (Figure 3*A*). At day 30, 3 patients had a Mayo endoscopic subscore of 0, and 13

Figure 2. (*A*) MTWSI response and remission following visilizumab administration at days 15, 30, and 60. (*B*) Mean and median MTWSI values following visilizumab administration. *Boxes* represent 25th to 75th percentile range. *Lines bisecting each box* represent the median; *whiskers* represent minimum and maximum values. (*C*) Response and remission rates by Mayo score. The overall Mayo response rate was 74.1%, and the overall Mayo remission rate was 14.9%. (*D*) Change in Mayo score from baseline to day 30. The *boxes* represent the central 50% of the data at baseline and day 30, respectively. The Mayo score determination is retrospective for patients in the 15-μg/kg dose group and prospective for patients in the 10-μg/kg dose group. Mayo scores were available for 27 patients at baseline (median, 11) and 26 patients at day 30 (median, 4). See Materials and Methods for the definitions of response and remission used in this analysis.

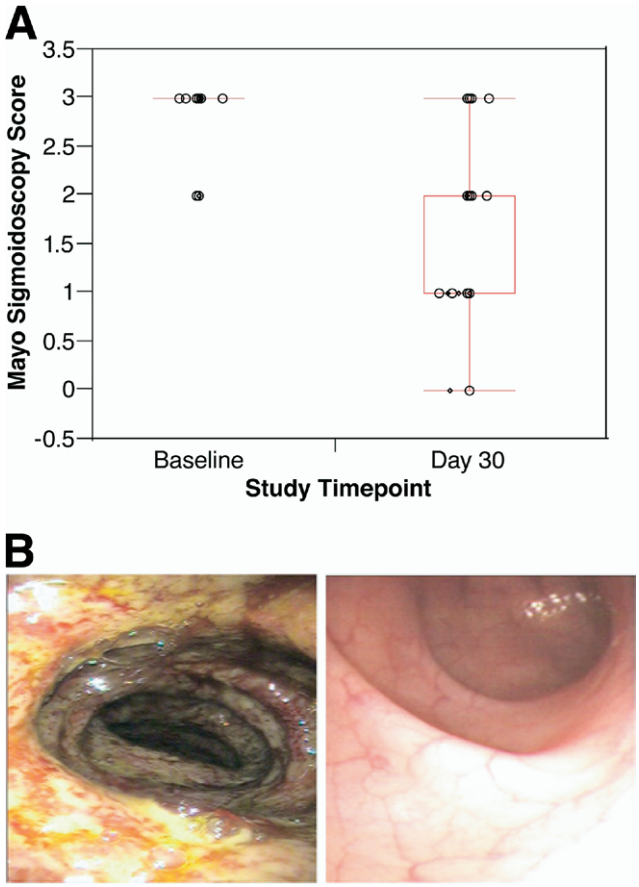


Figure 3. (*A*) Mayo sigmoidoscopy subscores were determined at baseline and at day 30 (±4 days), for the 10-μg/kg and the 15-μg/kg dose group. At day 30, 3 of 29 subjects had a score of 0 and 13 of 29 had a score of 1; thus, the overall endoscopic remission rate was 55.2% (16/29 subjects). Endoscopic remission was 47.8% in the 10-μg/kg dose group and 83.3% in the 15-μg/kg dose group. Mayo endoscopic scores were available for 29 patients at baseline (median, 3) and 27 patients at day 30 (median, 1). (Although day 30 endoscopic scores were not available for 2 patients in the 10-μg/kg dose group, their last observation was carried forward for the day 30 analysis.) (*B*) Photographs were taken of the sigmoid colon (at 20 cm) in a patient who received 15 μg/kg visilizumab on day 1 and day 2. The Mayo endoscopic score went from 3 at baseline (*left panel*) to 0 at day 30 (*right panel*) in this patient.

patients had a subscore of 1. Thus, the overall endoscopic remission rate was 55% (16/29) (Figure 3*A*; see Figure 3*B* and Supplementary Figure 2*A* for representative endoscopic photographs; see supplemental material online at www.gastrojournal.org). Sigmoidoscopic biopsy specimens from a patient at baseline and 30 days after visilizumab treatment also showed marked resolution of inflammatory cell infiltrates (Supplementary Figure 2*B*; see supplemental material online at www.gastrojournal.org).

Corticosteroid-Sparing Effects of Visilizumab

After day 2, patients were transitioned from IV corticosteroids to the equivalent of 40–80 mg oral prednisone per day. After day 8, the dose of prednisone was tapered further at the discretion of the patient's clinician. Corticosteroid-sparing effects were determined retrospectively in patients who responded to treatment and who did not require salvage therapy. Forty-four percent (12/27) of clinical responders were tapered completely off corticosteroids by day 60. An additional 4 responders were taking 5 mg/day of prednisone.

Duration of Response

Forty-five percent (10/22) of the patients who were evaluable for duration of response at 1 year had not required surgical or medical salvage therapy. Nine patients were lost to follow-up before 1 year, and 1 patient was determined to have Crohn's disease at the day 30 endoscopy. At 1 year, 2 patients in the 15-μg/kg group and 5 in the 10-μg/kg group had had a colectomy (Kaplan–Meyer plot; Figure 4). The median time to salvage was 446 days (range, 13–967 days). Seventy-eight percent of the patients did not undergo colectomy by the end of the study period. Among the 7 patients who did undergo colectomy during the study, the median time to colectomy was 160 days (range, 13–410 days). Administration of concomitant azathioprine or 6-mercaptopurine within 30 days following visilizumab dosing did not significantly affect the time to colectomy or administration of salvage immunosuppressive agents.

Pharmacokinetics

Preliminary pharmacokinetic analysis showed a mean maximum concentration of visilizumab after the first dose of 112 and 215 ng/mL for the 10- and 15-μg/kg dose groups, respectively. The mean maximum concen-

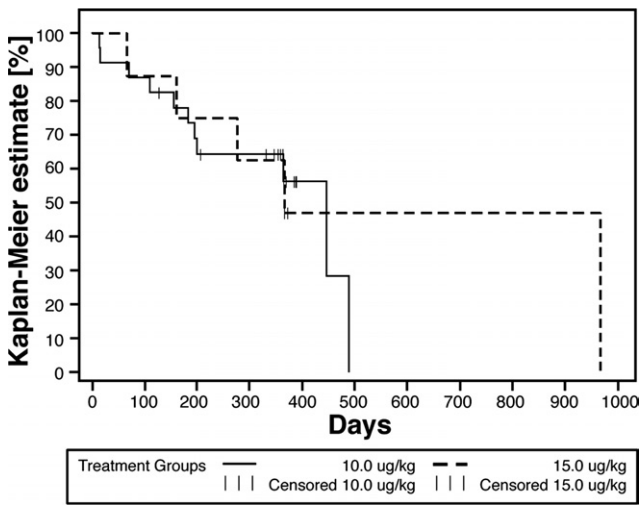


Figure 4. Kaplan–Meier estimate of the percent of patients who did not require salvage therapy after visilizumab treatment. Salvage therapy was defined as rehospitalization and treatment with IV corticosteroids, infliximab, cyclosporin A, or surgical proctocolectomy. Changes in oral corticosteroids, immunomodulators (azathioprine, 6-mercaptopurine), and mesalamine were not defined as salvage therapy in this study. The majority of patients did not require salvage therapy 1 year after treatment with visilizumab. (Patients who had not undergone salvage therapy at the end of their follow-up period were censored.)

tration for the second dose of visilizumab was 174 and 353 ng/mL for the 10- and 15-μg/kg dose groups, respectively. Visilizumab was undetectable in the serum 5 days after the last dose, with an elimination half-life of approximately 17 hours.

Immunogenicity

In 7 of 31 patients (23%) who had day 15 or day 30 blood samples available for testing, antibodies to visilizumab were detected. The antibodies in 6 of the 7 patients were neutralizing and therefore were likely anti-idiotypic. There was no evidence that the development of these antibodies affected the safety, pharmacokinetics, or efficacy of initial visilizumab treatment. This is consistent with pharmacokinetic data that showed visilizumab was undetectable in the serum within 1 week after the last dose. Twenty-four percent of patients (4/17) who received immunomodulators (6-mercaptopurine, azathioprine) and 20% of patients (3/15) who did not receive immunomodulators developed anti-visilizumab antibodies.

Discussion

In a phase 1 dose-finding study, visilizumab had an acceptable safety profile and demonstrated clinical activity in hospitalized patients with IV corticosteroid–refractory UC. There were no serious infectious complications during the study. In this preliminary open-label analysis, visilizumab appeared to produce durable clinical responses that exceeded its pharmacokinetic and pharmacodynamic properties (ie, elimination half-life of 17 hours and transient peripheral blood T-cell lymphopenia generally lasting less than 30 days). Forty-five percent of patients did not require medical or surgical salvage therapies during the first year following visilizumab treatment.

Cytokine release symptoms occurred commonly (88% of patients overall) and included fever, headache, chills, arthralgias, nausea, and vomiting. These infusion-related events were of relatively short duration, were successfully treated with standard measures, and did not result in serious morbidity or mortality. The incidence and severity of cytokine release symptoms were somewhat diminished in the 10-μg/kg cohort compared with the 15-μg/kg cohort. Although it is difficult to assess the relative contributions of the dose decrease and the addition of premedications and IV hydration to the amelioration of the cytokine release symptoms, standardized pretreatment in the lower-dose group appeared effective in reducing symptoms.

The pharmacokinetics of visilizumab differ from that of other biologic agents used to treat UC and Crohn's disease, which depend on sustained drug levels to exert their effects. Visilizumab was administered at microgram per kilogram doses as an IV bolus, and peak serum concentrations between 100 and 200 ng/mL were sustained briefly, with an elimination half-life of 17 hours. In vitro, visilizumab concentrations >50 ng/mL induce apoptosis and cytokine release in activated T lymphocytes.[11] However, blood concentrations of visilizumab were below this level within 4 hours after dosing and below the limit of assay detection (35 ng/mL) in less than 1 week. As early as 30 minutes after visilizumab administration on day 1, peripheral T cells were essentially undetectable. Although apoptosis of activated $CD3^+$ T cells likely occurs after intervention with visilizumab in vivo, the rapid kinetics of peripheral T-cell disappearance and the resting state of most peripheral T cells suggest other mechanisms for the transient changes in blood T-cell counts.

Patients who had evidence of EBV replication were excluded from the study because of previous experience with anti–T-cell antibodies in patients who had undergone solid organ and hematopoietic transplants, where T-cell depletion was associated with EBV reactivation and lymphoproliferative disorder.[18] In allograft recipients who received anti–T-cell antibodies along with multidrug immunosuppression regimens that included cyclosporine, corticosteroids, and mycophenolate mofetil, the incidence of EBV-associated lymphoproliferative disorder ranged from 1% to 20%.[19] In our study, EBV DNA concentrations were elevated in the peripheral blood in the majority of patients (24/32) at day 15 after visilizumab treatment. There were no clinical symptoms of EBV infection or lymphoproliferative disorder in these patients, and EBV DNA levels rapidly returned to undetectable levels after recovery of peripheral T cells. The relatively short-term effect of visilizumab on T cells suggests that it may be safely administered to patients who have baseline replication of EBV. This hypothesis is currently being explored in an ongoing phase 1/2 study. Caution should be exercised in extrapolating EBV DNA results between studies and diseases, because there is great variability between methods and laboratories.[18]

Although the current study was not placebo controlled, placebo rates in hospitalized patients with IV corticosteroid–refractory UC were reported in 3 studies.[3,4,20] Two studies reported 0% placebo response rates (vs cyclosporin A[4] and infliximab[20]), and one study reported a 31% placebo response (vs infliximab[3]). Therefore, the response rate with visilizumab of 74% appears to be an efficacy signal that merits further exploration. Caution should be exercised in comparing efficacy across these clinical trials because of differences in design, study populations, and definitions of response. Whether visilizumab provides a better safety and benefit profile in severe UC relative to infliximab or cyclosporin A remains to be determined.

In conclusion, visilizumab had an acceptable safety profile at the dose level of 10 μg/kg in patients with severe IV corticosteroid–refractory UC. The most prevalent toxicities were transient infusion-related cytokine

release symptoms and a transient decrease in T-cell levels. In this preliminary open-label analysis, rapid and sustained clinical and endoscopic improvements suggest that visilizumab merits further exploration in clinical trials in severe UC.

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at doi:10.1053/j.gastro.2007.08.035.

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