

ORIGINAL ARTICLE

## Treatment of Ulcerative Colitis with a Humanized Antibody to the $\alpha_4\beta_7$ Integrin

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### ABSTRACT

#### BACKGROUND

Selective blockade of interactions between leukocytes and vascular endothelium in the gut is a promising strategy for the treatment of inflammatory bowel diseases.

#### METHODS

We conducted a multicenter, double-blind, placebo-controlled trial of MLN02, a humanized antibody to the  $\alpha_4\beta_7$  integrin, in patients with active ulcerative colitis. We randomly assigned 181 patients to receive 0.5 mg of MLN02 per kilogram of body weight, 2.0 mg per kilogram, or an identical-appearing placebo intravenously on day 1 and day 29. Eligible patients also received concomitant mesalamine or no other treatment for colitis. Ulcerative colitis clinical scores and sigmoidoscopic assessments were evaluated six weeks after randomization.

#### RESULTS

Clinical remission rates at week 6 were 33 percent, 32 percent, and 14 percent for the group receiving 0.5 mg of MLN02 per kilogram, the group receiving 2.0 mg per kilogram, and the placebo group, respectively ( $P=0.03$ ). The corresponding proportions of patients who improved by at least 3 points on the ulcerative colitis clinical score were 66 percent, 53 percent, and 33 percent ( $P=0.002$ ). Twenty-eight percent of patients receiving 0.5 mg per kilogram and 12 percent of those receiving 2.0 mg per kilogram had endoscopically evident remission, as compared with 8 percent of those receiving placebo ( $P=0.007$ ). For the minority of patients in whom an MLN02 antibody titer greater than 1:125 developed, incomplete saturation of the  $\alpha_4\beta_7$  receptor on circulating lymphocytes was observed and no benefit of treatment was identifiable.

#### CONCLUSIONS

In this short-term study, MLN02 was more effective than placebo for the induction of clinical and endoscopic remission in patients with active ulcerative colitis.

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**U**LKERATIVE COLITIS IS AN INFLAMMATORY disease characterized by bloody diarrhea, abdominal cramps, and fatigue.<sup>1</sup> Initial therapy for most patients consists of mesalamine compounds.<sup>2,3</sup> Although these drugs can be effective and have acceptable side effects,<sup>4</sup> many patients do not have a response and thus require treatment with corticosteroids.<sup>5</sup> Corticosteroid therapy, despite its efficacy, is frequently associated with adverse effects.<sup>6</sup> Accordingly, identifying alternative treatments is a priority.

One approach is to inhibit the migration of leukocytes into inflamed intestinal tissue by blocking cellular adhesion molecules.<sup>7</sup> Integrins are heterodimeric proteins that regulate cellular movement. The  $\alpha_4\beta_7$  integrin, which is primarily involved in the recruitment of leukocytes to the gut,<sup>8</sup> is present on the cell surface of a small population of circulating T lymphocytes. The major ligand for  $\alpha_4\beta_7$ , mucosal addressin-cell adhesion molecule 1,<sup>9</sup> is selectively expressed on the endothelium of the intestinal vasculature<sup>10</sup> and is present in increased concentrations in inflamed tissue.<sup>11</sup> Blockade of this interaction might be effective therapy for inflammatory bowel diseases.<sup>12-15</sup>

MLN02 (Millennium Pharmaceuticals), a humanized<sup>16</sup> monoclonal antibody, specifically recognizes the  $\alpha_4\beta_7$  heterodimer but does not cross-react with the individual component monomers. Theoretically, this characteristic should convey specificity for the vasculature of the gut. Preliminary studies in tamarins<sup>17</sup> and humans<sup>18</sup> suggested that a dose of MLN02 of 2.0 mg per kilogram of body weight was safe and possibly effective. Therefore, we assessed the efficacy of MLN02 therapy in patients with active ulcerative colitis.

## METHODS

### PATIENTS

We conducted this randomized, double-blind, placebo-controlled study at 20 university medical centers between December 2000 and February 2003. The investigational review board at each center approved the protocol. All patients gave written informed consent.

Eligible patients were adults with active disease. Active disease was defined as an ulcerative colitis clinical score<sup>18</sup> of 5 to 9 points, with a score of at least 1 on either stool frequency or rectal bleeding, and a modified Baron score<sup>19</sup> of at least 2 on sig-

moidoscopic examination, with the disease a minimum of 25 cm from the anal verge. Participants had received either no therapy for ulcerative colitis or mesalamine, provided it had been administered for at least four weeks, with a stable dose for two weeks before screening. Criteria for exclusion were therapy with oral corticosteroids within four weeks before screening or parenteral corticosteroids within six weeks, topical therapy with mesalamine or corticosteroids within one week before screening, immunosuppressive therapy within the preceding three months, severe disease (as evidenced by a hemoglobin concentration below 10 g per deciliter, toxic megacolon, or an ulcerative colitis clinical score above 10), abnormal laboratory results (white-cell count below 3000 per cubic millimeter; platelet count below 100,000 per cubic millimeter; serum aspartate aminotransferase, alanine aminotransferase, or alkaline phosphatase concentration greater than 2.5 times the upper limit of normal; serum creatinine concentration greater than 1.5 times the upper limit of normal; positive stool test for pathogens; or proteinuria), or the inability to comply with the protocol.

### BASELINE STUDIES

Patients were screened 14 and 7 days before randomization. They were given a physical examination and blood tests, and a stool sample for pathogens and demographic information were obtained. Eligible patients were scheduled for a screening visit immediately before randomization, which included sigmoidoscopy and the determination of baseline ulcerative colitis clinical scores, modified Baron scores, Riley scores,<sup>20</sup> and scores on the inflammatory bowel disease questionnaire.<sup>21</sup>

The ulcerative colitis clinical score, a modification of the scoring system of the Mayo Clinic,<sup>2</sup> consists of four items — rectal bleeding, stool frequency, functional assessment by the patient, and global assessment by the physician. Items are scored on a scale from 0 (normal) to 3 (severe disease). The composite score ranges from 0 (inactive disease) to 12 (severe disease activity). The modified Baron score, which represents an endoscopic classification, ranges from 0 to 4, with 0 denoting normal mucosa, 1 granular mucosa with an abnormal vascular pattern, 2 friable mucosa, 3 microulceration with spontaneous bleeding, and 4 gross ulceration. Inflammation in rectal-biopsy specimens was graded with the acute-inflammation subscale of the Ri-

**Table 1. Baseline Characteristics of the Patients.\***

| Characteristic                                    | MLN02,<br>0.5 mg/kg<br>(N=58) | MLN02,<br>2.0 mg/kg<br>(N=60) | Placebo<br>(N=63) |
|---|-------------------------------|-------------------------------|-------------------|
| Age — yr  | 41.6±14.7                     | 43.8±14.6                     | 38.9±13.4         |
| Male sex — no. (%)                                | 33 (57)                       | 30 (50)                       | 35 (56)           |
| Months since diagnosis                            | 78.0±84.9                     | 74.8±77.8                     | 82.9±83.5         |
| Current smoker — no. (%)                          | 0                             | 4 (7)                         | 4 (6)             |
| Mesalamine  |                               |                               |                   |
| Use — no. (%)                                     | 48 (83)                       | 50 (83)                       | 53 (84)           |
| Dose — molar equivalents/day†                     | 22.4±9.0                      | 21.9±9.7                      | 21.9±9.8          |
| Ulcerative colitis clinical score                 | 7.0±1.4                       | 7.3±1.5                       | 6.7±1.6           |
| Stool frequency                                   | 2.3±0.9                       | 2.3±0.8                       | 2.2±0.9           |
| Rectal bleeding                                   | 1.5±0.8                       | 1.6±0.8                       | 1.4±0.8           |
| Assessment by the patient                         | 1.3±0.7                       | 1.5±0.7                       | 1.2±0.8           |
| Assessment by the physician                       | 1.9±0.3                       | 1.9±0.3                       | 1.8±0.4           |
| Modified Baron score                              | 3.0                           | 3.0                           | 3.0               |
| Riley histopathological score                     | 5.9±1.3                       | 6.2±1.0                       | 5.7±1.5           |
| Score on inflammatory bowel disease questionnaire | 139.6±34.2                    | 131.4±30.1                    | 142.6±31.6        |
| Hemoglobin concentration — g/dl                   | 13.5±1.6                      | 13.2±1.8                      | 13.2±1.7          |
| White-cell count — $\times 10^{-3}/\text{mm}^3$   | 8.7±2.8                       | 8.2±2.6                       | 8.5±2.2           |

\* Plus-minus values are means  $\pm$ SD. The modified Baron scores are median values.

† Molar equivalents were derived by dividing the average daily dose of the 5-aminosalicylic acid compound by 1/100 of the molecular weight of the compound (i.e., 153.14, 346.21, and 398.4 for mesalamine formulations, osalazine, and sulfasalazine, respectively). For example, a total daily dose of 3.6 g of mesalamine equals 23.5 molar equivalents.<sup>23</sup>

ley score,<sup>20</sup> which ranges from 0 (no inflammation) to 7 (severe acute inflammation). Health-related quality of life was evaluated with the inflammatory bowel disease questionnaire.<sup>21</sup> Scores range from 32 to 224, with higher scores indicating a better quality of life.

#### RANDOMIZATION

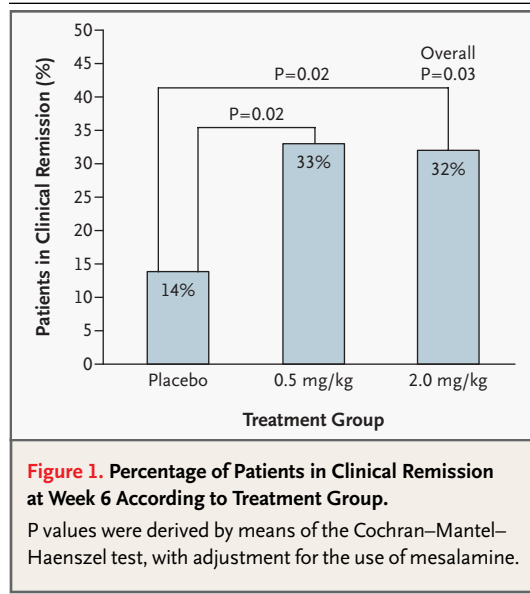
Patients were randomly assigned to receive 0.5 mg of MLN02 per kilogram, 2.0 mg of MLN02 per kilogram, or placebo in an equal ratio (according to a computer-generated schedule) with the use of permuted blocks of three. The randomization was stratified according to the use of mesalamine. Each patient received two intravenous infusions, one at the baseline visit (day 1) and a second on day 29. Neither the investigators nor the patients were aware of the treatment assignment. The placebo was identical in appearance to MLN02.

The use of corticosteroids, immunosuppressive

agents, antibiotic drugs, nicotine supplements, or antidiarrheal agents was not permitted. Use of mesalamine was continued at a stable dose.

#### FOLLOW-UP

Patients were seen one, two, four, and six weeks after randomization. At each visit, the ulcerative colitis clinical score was calculated and blood for serum chemistry was obtained. Sigmoidoscopy was repeated at weeks 4 and 6. The endoscopist was instructed to take biopsy specimens from the most severely affected area 15 cm from the anal verge. The inflammatory bowel disease questionnaire was administered at weeks 4 and 6. Blood samples for pharmacokinetic and pharmacodynamic studies were drawn in a subgroup of 30 patients. Serum MLN02 concentrations were measured with use of a competitive-binding enzyme-linked immunosorbent assay (ELISA). ELISA was also used to measure human antihuman antibodies in serum at weeks



4 and 8. An antibody titer of 1:5 or greater was considered positive.

#### OUTCOME MEASURES

The primary outcome measure was clinical remission at week 6, defined as an ulcerative colitis clinical score of 0 or 1 and a modified Baron score of 0 or 1 with no evidence of rectal bleeding. Secondary outcomes were the changes in the ulcerative colitis clinical scores, the modified Baron scores, the Riley scores, and the scores on the inflammatory bowel disease questionnaire. We also evaluated the proportion of patients with clinical response (an improvement of 3 points or more on the ulcerative colitis clinical score), endoscopically evident remission (a modified Baron score of 0), and endoscopic response (an improvement of the modified Baron score of at least 2 grades) at week 4 and week 6. Adverse events were classified with the use of the *Medical Dictionary for Regulatory Activities (MedDRA)*.<sup>22</sup>

#### STATISTICAL ANALYSIS

Descriptive statistics were used to evaluate differences in demographic characteristics. The effects of cigarette smoking, disease activity, and mesalamine use were evaluated in univariate analyses. For the primary analysis, the Cochran–Mantel–Haenszel chi-square test, with adjustment for mesalamine use, tested the null hypothesis that the rate of clinical remission was not different among the three treatment groups. Patients who withdrew prematurely were classified as not achieving remission.

A similar approach was used to compare the rates of clinical response.

The changes from baseline to weeks 4 and 6 in the ulcerative colitis clinical scores, the Riley scores, and the scores on the inflammatory bowel disease questionnaire were assessed by analysis of covariance with adjustment for mesalamine use and the baseline score. For patients who withdrew prematurely, the last observation available before withdrawal was carried forward for the ulcerative colitis clinical score and the score on the inflammatory bowel disease questionnaire. The modified Baron scores were compared with the use of analysis of covariance based on ranks.

The rates of endoscopic remission and response at weeks 4 and 6 were compared by means of the Cochran–Mantel–Haenszel chi-square test. The modified Baron score was compared by means of the Wilcoxon rank-sum test.

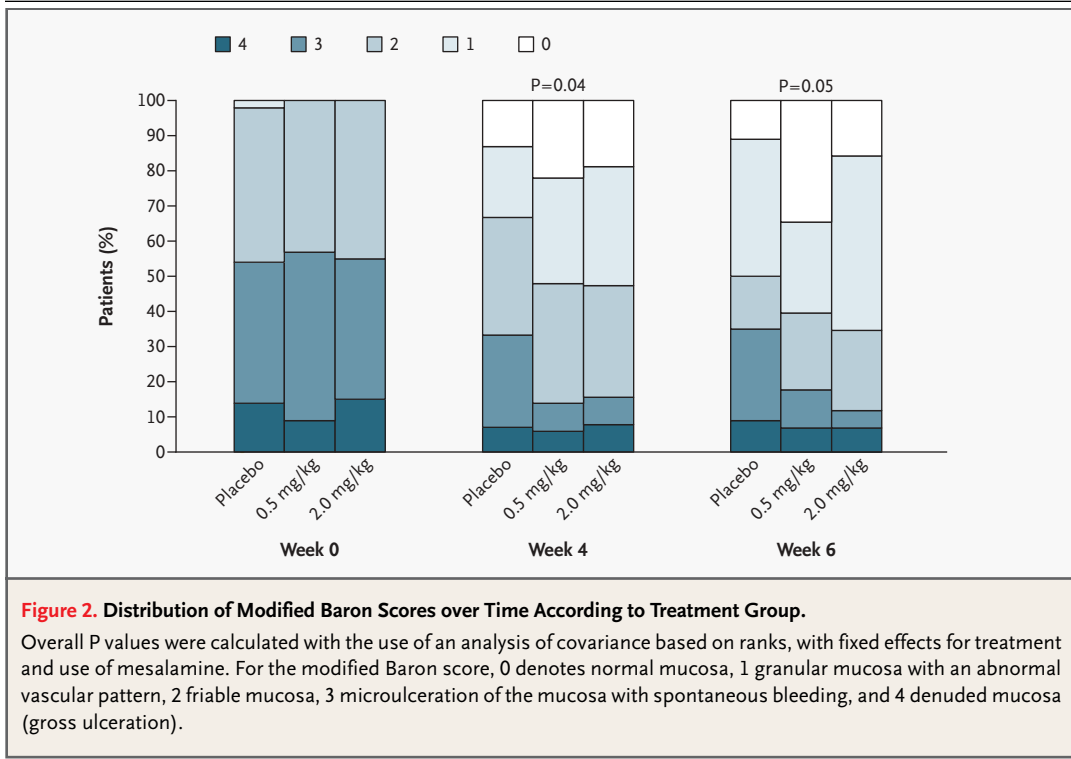
For all analyses of endoscopic and histopathological scores, missing values were not imputed. Fisher's exact test was used to compare the incidence of adverse events between the patients who received MLN02 and those who received placebo. Statistical tests were two-sided, with a P value of 0.05 as the criterion for statistical significance. Analyses were performed according to the intention-to-treat principle.

We anticipated that 10 percent of patients receiving placebo would enter clinical remission. We determined that randomization of 180 patients allowed for 80 percent power to detect a difference of 20 percent between the patients given MLN02 and those given placebo, with the use of a two-sided test with an alpha error of 0.05.

The study was designed and implemented by the steering committee in collaboration with Millennium Pharmaceuticals, which analyzed the data. The investigators wrote the manuscript. The academic authors had access to the data and vouch for the validity and completeness of the data and the data analysis. A data safety and monitoring board reviewed the safety data.

## RESULTS

Between December 2000 and November 2002, 249 patients were evaluated. Of these, 68 (27 percent) did not undergo randomization. The common reasons for exclusion were low disease activity (19 patients), laboratory abnormalities or serious diseases (14), a positive assay for *Clostridium difficile* toxin



(11), inadequate documentation of disease (9), and a requirement for contraindicated medication (7). Eight eligible patients withdrew consent.

Of 181 patients who underwent randomization, 58 were assigned to receive 0.5 mg of MLN02 per kilogram, 60 to receive 2.0 mg of MLN02 per kilogram, and 63 to receive placebo. The baseline characteristics of the groups were similar (Table 1).

The number of patients who withdrew from the study was 1 of 58 (2 percent) in the group receiving 0.5 mg of MLN02 per kilogram, 5 of 60 (8 percent) in the group receiving 2.0 mg per kilogram, and 3 of 63 (5 percent) in the placebo group. No important differences were observed among the three groups in the reasons for withdrawal.

**PRIMARY OUTCOME**

After six weeks, the proportion of patients in clinical remission differed significantly among the three groups. In the group receiving 0.5 mg of MLN02 per kilogram, 19 of 58 patients (33 percent) achieved remission, as compared with 19 of 60 (32 percent) in the group receiving 2.0 mg per kilogram and 9 of 63 (14 percent) in the placebo group (overall P=0.03) (Fig. 1). Each comparison between the MLN02 groups and the placebo group was significant

(P=0.02 for both the group receiving 0.5 mg per kilogram and the group receiving 2.0 mg per kilogram). Neither the use of mesalamine nor any other prognostic factor that was examined influenced these results.

**CLINICAL RESPONSE**

One of the secondary outcomes was the change in the ulcerative colitis clinical score. At week 6, the proportions of patients in the group receiving 0.5 mg per kilogram, the group receiving 2.0 mg per kilogram, and the placebo group whose score improved at least 3 points were 66 percent, 53 percent, and 33 percent, respectively (P=0.002).

**ENDOSCOPIC REMISSION AND RESPONSE**

Figure 2 shows the distribution of the modified Baron scores. Patients assigned to receive MLN02 had a lower median score than those assigned to placebo at week 4 (modified Baron score, 1.0 vs. 2.0; P=0.01) and week 6 (1.0 vs. 1.5, P=0.02). At week 6, 16 of 58 patients (28 percent) who received 0.5 mg of MLN02 per kilogram were in endoscopic remission, as compared with 7 of 60 patients (12 percent) who received 2.0 mg per kilogram and 5 of 63 patients (8 percent) who received placebo (P=0.007

**Table 2. Ulcerative Colitis Clinical Scores, Modified Riley Scores, and Scores on the Inflammatory Bowel Disease Questionnaire over Time According to Treatment Group.\***

| Variable  | MLN02,<br>0.5 mg/kg<br>(N=58) |               | MLN02,<br>2.0 mg/kg<br>(N=60) |               | Placebo<br>(N=63)  |               | P Value† |                                   |                                   |  |
|---|-------------------------------|---------------|-------------------------------|---------------|--------------------|---------------|----------|-----------------------------------|-----------------------------------|--|
|   | No. of<br>Patients            | Mean<br>Score | No. of<br>Patients            | Mean<br>Score | No. of<br>Patients | Mean<br>Score | Overall  | 0.5 mg of<br>MLN02 vs.<br>Placebo | 2.0 mg of<br>MLN02 vs.<br>Placebo |  |
| Composite ulcerative colitis clinical score           |                               |               |                               |               |                    |               |          |                                   |                                   |  |
| Baseline  | 58                            | 7.0±1.4       | 60                            | 7.3±1.5       | 63                 | 6.7±1.6       |          |                                   |                                   |  |
| Week 4  | 58                            | 3.5±2.8       | 60                            | 3.9±3.1       | 63                 | 5.0±2.7       | 0.001    | 0.006                             | 0.02                              |  |
| Week 6  | 58                            | 3.3±2.9       | 60                            | 3.9±3.2       | 63                 | 4.8±2.8       | 0.007    | 0.008                             | 0.06                              |  |
| Modified Riley score‡                                 |                               |               |                               |               |                    |               |          |                                   |                                   |  |
| Baseline  | 58                            | 5.9±1.3       | 60                            | 6.2±1.0       | 61                 | 5.7±1.5       |          |                                   |                                   |  |
| Week 4  | 49                            | 3.9±2.5       | 53                            | 4.5±2.5       | 53                 | 5.4±1.7       | <0.001   | 0.002                             | 0.08                              |  |
| Week 6  | 46                            | 3.6±2.7       | 44                            | 3.7±2.9       | 43                 | 4.8±2.4       | 0.03     | 0.03                              | 0.06                              |  |
| Score on the inflammatory bowel disease questionnaire |                               |               |                               |               |                    |               |          |                                   |                                   |  |
| Baseline  | 58                            | 139.6±34      | 60                            | 131.4±30      | 63                 | 142.6±32      |          |                                   |                                   |  |
| Week 4  | 58                            | 171.7±39      | 60                            | 164.6±37      | 63                 | 159.3±32      | 0.02     | 0.05                              | 0.28                              |  |
| Week 6  | 58                            | 175.5±42      | 60                            | 167.6±40      | 63                 | 162.5±34      | 0.03     | 0.03                              | 0.37                              |  |

\* Plus-minus values are means ±SD.

† P values were derived by analysis of covariance, with adjustment for mesalamine use and the baseline value.

‡ For all analyses of endoscopic and histopathological scores, missing values were not imputed.

for the comparison between the MLN02 groups and the placebo group). Furthermore, patients who received 0.5 or 2.0 mg per kilogram were more likely to improve two or more endoscopic grades than those who received placebo (48 percent and 35 percent, respectively, vs. 16 percent;  $P=0.001$ ).

#### OTHER MEASURES OF DISEASE ACTIVITY

Patients who received MLN02 had greater improvement in ulcerative colitis clinical scores and Riley scores than those who received placebo (Table 2). Their scores on the inflammatory bowel disease questionnaire also improved.

#### PHARMACOLOGY

The mean (±SD) maximum MLN02 concentration was  $12.5\pm 2.5$  µg per milliliter in the low-dose (0.5 mg per kilogram) group (11 patients) and  $52.0\pm 10.4$  µg per milliliter in the high-dose (2.0 mg per kilogram) group (11 patients). The serum half-lives were 9 and 12 days, respectively. In both groups, saturation of  $\alpha_4\beta_7$  on more than 90 percent of the CD4+CD45RO+ T cells in the peripheral circulation were observed at both week 4 and week 6.

#### ADVERSE EVENTS AND ANTIBODIES TO MLN02

There were no substantial differences among the three groups in the prevalence of adverse events (Table 3). Three noteworthy events were observed in patients treated with MLN02. A 50-year-old woman, in whom hives and mild angioedema developed during her second infusion, tested positive for MLN02 antibodies with a titer of 1:3125. Of 103 participants who received two infusions of MLN02, she was the only one in whom a clinically relevant infusion reaction developed. A primary cytomegalovirus infection developed in a second patient, whose condition improved without antiviral therapy. Lobar pneumonia developed in a patient three days after spinal surgery and was successfully treated.

No differences in laboratory results were identified among the treatment groups. Notably, there was no difference in the total blood lymphocyte, T-cell, and B-cell counts between patients treated with MLN02 and those who received placebo.

Human antihuman antibodies developed by week 8 in 44 percent of the patients who received MLN02. Overall, 24 percent of patients were positive for antibody at a titer of greater than 1:125.

Corresponding values for the groups receiving 2.0 mg per kilogram and 0.5 mg per kilogram were 11 percent and 38 percent, respectively. In patients with this concentration of antibody, the rate of clinical remission was 12 percent, which was similar to the rate in the placebo group (14 percent). Furthermore, in patients with this concentration of antibody,  $\alpha_4\beta_7$  binding sites on circulating CD4+CD45RO+ T lymphocytes became unsaturated. Conversely, in the 76 percent of patients who either tested negative or had lower titers of antibody, the clinical-remission rate was 42 percent and these binding sites remained saturated.

DISCUSSION

These results indicate that selective blockade of the movement of CD4+CD45RO+ T cells by MLN02 is an effective therapy for moderately severe ulcerative colitis. Patients who received MLN02 were more than twice as likely to enter remission as those who received placebo, a difference that was both statistically significant and clinically meaningful. Furthermore, endoscopic improvement, endoscopic remission, and histopathological improvement occurred more frequently in patients who were assigned to active treatment. Parallel improvement in health-related quality of life was also observed. Eighty-two percent of the patients had not responded to mesalamine and probably would have received corticosteroids. Given that many patients ultimately become dependent on corticosteroids,<sup>24</sup> MLN02 may be an attractive alternative for those in whom mesalamine treatment fails. However, despite a high rate of adverse events with prolonged use, corticosteroid therapy can be both effective and relatively inexpensive. Thus, future trials of MLN02 should include patients in whom therapy with corticosteroids is ineffective.

No important differences in the occurrence of adverse events were identified among the treatment groups. No deaths, cancers, or opportunistic infections were observed. A primary cytomegalovirus infection developed in one patient, but this case was not consistent with such reactivation of viral infection as might be seen in the setting of compromised immunity. The patient recovered without treatment. The only other complication related to infection, lobar pneumonia, occurred postoperatively; thus, it is difficult to ascribe a causal relation to treatment with MLN02.

Despite the use of techniques of molecular en-

**Table 3. Adverse Events.**

| Variable                                    | MLN02,<br>0.5 mg/kg<br>(N=58) | MLN02,<br>2.0 mg/kg<br>(N=60) | Placebo<br>(N=63) | P<br>Value* |
|---|-------------------------------|-------------------------------|-------------------|-------------|
| <i>no. of patients (%)</i>                  |                               |                               |                   |             |
| <b>Adverse event†</b>                       |                               |                               |                   |             |
| Ulcerative colitis aggravated               | 29 (50)                       | 22 (37)                       | 24 (38)           | 0.50        |
| Nausea                                      | 15 (26)                       | 11 (18)                       | 10 (16)           | 0.32        |
| Headache                                    | 12 (21)                       | 11 (18)                       | 13 (21)           | 0.85        |
| Frequent bowel movements                    | 10 (17)                       | 5 (8)                         | 10 (16)           | 0.56        |
| Fatigue                                     | 8 (14)                        | 5 (8)                         | 7 (11)            | 0.98        |
| Nasopharyngitis                             | 8 (14)                        | 8 (13)                        | 5 (8)             | 0.26        |
| Abdominal pain                              | 6 (10)                        | 5 (8)                         | 8 (13)            | 0.48        |
| Abdominal tenderness                        | 4 (7)                         | 1 (2)                         | 8 (13)            | 0.07        |
| Arthralgia                                  | 4 (7)                         | 7 (12)                        | 5 (8)             | 0.75        |
| Dizziness                                   | 6 (10)                        | 4 (7)                         | 1 (2)             | 0.10        |
| Rash  | 6 (10)                        | 4 (7)                         | 4 (6)             | 0.77        |
| Blood in stool                              | 6 (10)                        | 3 (5)                         | 8 (13)            | 0.27        |
| Vomiting                                    | 6 (10)                        | 2 (3)                         | 5 (8)             | 0.77        |
| <i>no. of events</i>                        |                               |                               |                   |             |
| <b>Serious adverse events</b>               |                               |                               |                   |             |
| Exacerbation of colitis                     | 4                             | 9                             | 5                 | 0.51        |
| Infusion reaction with angioedema           | 1                             | 0                             | 0                 | 1.00        |
| Infection                                   | 1                             | 2                             | 0                 | 0.55        |
| Nausea and vomiting                         | 0                             | 1                             | 1                 | 1.00        |
| Degenerative disk disease                   | 0                             | 2                             | 0                 | 0.54        |
| <i>no. of patients</i>                      |                               |                               |                   |             |
| <b>Patients with serious adverse events</b> | 6                             | 12                            | 6                 | 0.28        |

\* P values were calculated with the use of Fisher's exact test and are for the comparison of the combination of the groups receiving MLN02 with the placebo group.

† All events that occurred in at least 10 percent of patients in any of the three treatment groups are shown.

gineering to minimize sensitization, human anti-human antibodies can develop after treatment with monoclonal antibodies.<sup>25,26</sup> The clinical outcomes of antibody formation are more relevant than the frequency of their occurrence. Hypersensitivity reactions related to human antihuman antibodies are a clinically significant adverse event associated with the use of therapeutic antibodies.<sup>27,28</sup> Although antibodies to MLN02 developed in 44 percent of the patients treated with MLN02, only one patient had an infusion reaction. However, 24 percent of patients with a human antihuman antibody titer of greater than 1:125 showed a loss of saturation of  $\alpha_4\beta_7$  binding sites, and the clinical remission rate in this group was similar to that in the placebo

group. The development of neutralizing antibodies has been reported with other humanized antibodies.<sup>26,29,30</sup> Given these observations, interventions such as the administration of intravenous corticosteroid<sup>31</sup> at the time of MLN02 infusion, concomitant use of antimetabolites,<sup>32</sup> or as suggested by our data, use of a higher initial dose of MLN02<sup>33</sup> may be necessary to reduce the risk of sensitization. Whether these interventions are relevant for MLN02 therapy requires evaluation in future studies.

Our study had several limitations. First, only one third of patients who received MLN02 entered remission. Therefore, future studies should evaluate a higher dose of the drug. However, this approach may not yield greater efficacy, since no dose-response relationship was observed in the current trial and saturation of  $\alpha_4\beta_7$  on CD4+CD45RO+ peripheral T cells at both doses of MLN02 had occurred when the primary end point was assessed. Second, since we did not study patients treated with corticosteroids or antimetabolites, it is possible that coadministration of these drugs with MLN02 might either enhance or antagonize the effects of MLN02. Finally, we were not able to evaluate whether selective blockade of  $\alpha_4\beta_7$  is a preferable strategy to the more broadly inhibitory approach of blocking the  $\alpha_4$  integrin with natalizumab, which has been successful in the treatment of both Crohn's disease<sup>14</sup> and multiple sclerosis.<sup>15</sup>

The lymphocytosis that occurs after treatment with natalizumab, an antibody directed toward the  $\alpha$  integrin monomer, was not observed in our study.<sup>14,15</sup> This important finding supports the hypothesis that MLN02 selectively blocks a small population of T cells involved in intestinal immunity.

We speculate that this property will ultimately be shown to result in less impairment of systemic immunity than occurs with universal blockade of all  $\alpha$  integrin-mediated interactions between leukocytes and endothelium.

In conclusion, we have shown in this short-term study that MLN02 is an effective treatment for patients with active ulcerative colitis. However, the role of MLN02 in clinical practice is not yet known, and additional long-term studies are needed.

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#### APPENDIX

The following persons and institutions (all in Canada) participated in the study: London Health Sciences Centre, London, Ont.: B. Feagan, J.W.D. McDonald, W. McCaw, P. Walton-Mennill; Montreal General Hospital, Montreal: G. Wild, N. Pellerin, J. Cousineau; Hôtel-Dieu de Québec, Québec, Que.: R. Dubé, S. Lepire, P. Paré; Mount Sinai Hospital, Toronto: G.R. Greenberg, S. Irwin; Jewish General Hospital, Montreal: A. Cohen, N. Desjardins, J. Rivard; Mount Sinai Hospital, Toronto: A.H. Steinhart, S. Mikolainis; Ottawa Civic Hospital, Ottawa: A. Rostom, P. Waddell; Health Sciences Centre, Winnipeg, Man.: C. Bernstein, P. Rawsthorne, S. Chubey; St. Paul's Hospital, Vancouver, B.C.: S. Whittaker, S. Patterson, B. McDougall, H. Kooner; St. Michael's Hospital, Toronto: J. Baker, B. Winter, E. Dubcenco, G. Stewart; Queen Elizabeth II Health Sciences Centre, Halifax, N.S.: D. MacIntosh, C.N. Williams, J. Stewart; Saint-François d'Assise, Québec, Que.: C. Dal-laire, F. Bernard; University of Alberta Hospital, Edmonton: R.N. Fedorak, M. Harriott, S. Appelman-Eszczuk; Vancouver General Hospital, Vancouver, B.C.: F. Anderson, I. Wong; Royal Victoria Hospital, Montreal: A. Bitton, M. Bernier; Centre Hospitalier Affilié Universitaire de Québec-Hôpital St. Sacrement, Québec, Que.: P. Paré, S. Rousseau, J. Emond; Sunnybrook and Women's Health Sciences Centre, Toronto: F. Saibil, M. Morgan; Health Sciences Centre, Calgary, Alta.: R. Panaccione, N. Racicot; Hôpital Maisonneuve-Rosemont, Montreal: A. Archambault, S. Bélanger. *Steering Committee:* B. Feagan (chair), R.N. Fedorak, G.R. Greenberg, J.W.D. McDonald, P. Paré, G. Wild. *Robarts Clinical Trials Coordinating Center Personnel:* B. Bergman, M. Brine, T. Clayton, M. Irlam, B. Jasevicius, L. Jensen, W. Johnson, D. LeBer, E. Liddiard, S. MacDonald, J. Rémillard, I. Ruocco, B. Sarazin, B. Sharpe, L. Smith, H. Sun, M. Vandervoort, C. Wong. *Millennium Personnel:* J. Kuesters, M. Webster, J. Madruga, J. Ott, N. Herring, and K. Lloyd were responsible for the clinical study; M. Briskin and P. Ponath developed MLN02 through modeling and humanization design; M. Green assayed the antibodies; C. Horvath performed analyses of pharmacokinetics and pharmacodynamics; and K. Kishimoto and J. Guiterrez-Ramos provided advice. *Data Safety and Monitoring Board:* D. Jewell (chair), J. Mahon, R. Rothstein.

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