Absence of Efficacy of Subcutaneous Antisense ICAM-1 Treatment of Chronic Active Crohn’s Disease

STEFAN SCHREIBER,* SUSANNA NIKOLAUS,* HERBERT MALCHOW,† WOLFGANG KRUIS,§ HERBERT LOCHS, ‡ ANDREAS RAEDLER,¶ ECKHART G. HAHN,** THOMAS KRUNMMENERL,‡‡ and THE GERMAN ICAM–1 STUDY GROUP

*First Medical Department, University Hospital Kiel; ‡Krankenhaus Leverkusen; §Evangelisches Krankenhaus Köln-Kalk; †Fourth Medical Department, Charité University Hospital Berlin; ¶Tabea Center for Inflammatory Bowel Disease, Homburg; **Department of Medicine, University Erlangen/Nürnberg; ***Gastroenterology Offices, Münster; and ‡‡Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany

Background & Aims: ISIS-2302, an antisense oligonucleotide directed against intercellular adhesion molecule 1, was effective in steroid refractory Crohn’s disease in a pilot trial. The aim of this study was to investigate safety and efficacy of ISIS-2302 in chronic active Crohn’s disease (CACD).

Methods: A dose-interval, multicenter, placebo-controlled trial was conducted in 75 patients with steroid-refractory CACD (Crohn’s Disease Activity Index [CDAI], 200 – 400). The primary endpoint was steroid-free remission (CDAI <150) at week 14.

Results: Only 2 of 60 (3.3%) ISIS-2302–treated and no placebo patients reached the primary endpoint. Steroid-free remission at week 26 (secondary endpoint) was reached in 8 of 60 (13.3%) active treatment and 1 of 15 (6.7%) placebo patients. A greater proportion of ISIS-2302–treated than placebo patients achieved a steroid dose <10 mg/day at weeks 14 and 26 (48.3% vs. 33.3% and 55.0% vs. 40.0%, respectively, and a glucocorticoid dose of 0 mg [prednisone equivalent] at week 26 [23.3% vs. 6.7%, respectively]. Treatment with ISIS-2302 was safe. The most common side effects were injection site reactions in the active treatment group (23% in ISIS-2302–treated patients vs. none in placebo patients). No statistically significant differences in the frequency of side effects were detected between dose groups.

Conclusions: The trial did not prove clinical efficacy of ISIS-2302 based on the primary endpoint. Positive trends were observed in some of the secondary endpoints.

Crohn’s disease is a chronic inflammatory bowel disease of unknown origin with recurrent inflammatory episodes leading to progressive destruction and loss of function in intestinal tissue. Chronic immune activation of macrophages, B and T cells in the intestinal mucosa, is a hallmark of the disease.1 Various soluble mediators and cellular interactions control activation and suppression of the immune system. Adhesion processes play an important role. Several classes of cell surface molecules that mediate adhesion have recently been described and characterized.2 One of these mediators is the intercellular adhesion molecule 1 (ICAM-1). ICAM-1 is a member of the immunoglobulin superfamily, a transmembrane glycoprotein expressed on vascular endothelial cells, postcapillary endothelium, monocytes, tissue macrophages, keratinocytes, and a subset of T and B cells.3 In response to proinflammatory mediators (including tumor necrosis factor [TNF]-α, interleukin 1β, and interferon γ), many cells up-regulate expression of ICAM-1 on their surface.4 One of the most well-characterized functions of ICAM–1 in the inflammatory process is the facilitation of leukocyte emigration in response to inflammatory stimuli.3

Numerous studies have shown an increase in ICAM-1 expression in tissues obtained from patients with chronic inflammatory disorders including IBDs.6–10 The therapeutic use of monoclonal antibodies directed against ICAM-1 has produced beneficial effects in various animal models including inflammatory diseases of the lung, arthritis, asthma, glomerulonephritis, and colitis models.11–16

A new paradigm in drug discovery is the development of antisense oligonucleotides for therapeutic use.17,18 In contrast to the majority of drugs currently in use that modulate the activity of specific proteins involved in inflammatory immune regulation, antisense oligonucleotides target messenger RNA (mRNA) coding for a specific protein of interest. By hybridizing to the mRNA, they reduce the expression and therefore the amount of functional protein. In animal experiments, antisense ICAM-1 oligonucleotides have been successfully used as

Abbreviations used in this paper: AUC, area under the concentration-time curve; CCR, complete clinical remission; CDAI, Crohn’s Disease Activity Index; IBDQ, inflammatory bowel disease questionnaire; ICAM-1, intercellular adhesion molecule 1; mRNA, messenger RNA; TNF, tumor necrosis factor.

© 2001 by the American Gastroenterological Association
0016-5085/01/$35.00
doi:10.1053/gast.2001.24015
treatments in models of organ transplant rejection, chronic colitis, and collagen-induced arthritis.\textsuperscript{19—21}

ISIS–2302, a phosphothioate oligodeoxynucleotide, 20 bases in length, was designed for use in the human system. It selectively inhibits cytokine-induced ICAM-1 expression in a wide variety of human cells. Because of its specificity for human ICAM-1 mRNA, it is not suitable for use in animal pharmacology models. In a placebo-controlled pilot trial carried out with a small number of patients, intravenous application of ISIS-2302 in doses of 0.5–2 mg/kg showed possible therapeutic effects in steroid-refractory Crohn’s disease.\textsuperscript{22} This study was carried out in 20 patients who received 13 intravenous infusions of the study drug (0.5, 1, or 2 mg/kg or placebo) and were followed over 6 months. At the end of the treatment phase (day 26), 47% of the ISIS–2302–treated patients were in remission in contrast to 20% of the placebo patients.

Because of these promising results, a subcutaneous formulation of the drug was developed. In contrast to repeated daily intravenous infusion, subcutaneous administration would be a clinically applicable formulation of the drug. A phase I subcutaneous study conducted with 16 healthy volunteers indicated that doses up to 0.5 mg/kg were adequately tolerated. Subcutaneous injection of 0.5 mg ISIS–2302/kg body wt resulted in plasma levels that were about 5-fold higher than the levels needed to maximally inhibit ICAM-1 expression (100 ng/mL) in vitro in several model systems (TNF-induced adhesion of HL-60 cells to endothelial monolayer cultures, ICAM-1 expression on human umbilical vein endothelial cells). Intravenous injection of a similar dose resulted in about twice the peak plasma levels and plasma exposure (area under the concentration-time curve; AUC) in comparison with subcutaneous injection. In contrast to intravenous application, plasma levels reflect a secondary compartment after subcutaneous administration. Therefore, it was considered that systemic exposure through subcutaneous application was high enough to expect a suppression of ICAM-1 expression in vivo. However, because of the complexity of the system and the fact that endothelial and other tissue cells are the targets for an anti–ICAM-1 strategy, no pharmacodynamic endpoints were studied in the phase I program.

This study was designed to explore safety and efficacy of a chronic subcutaneous application of an antisense oligonucleotide against ICAM-1 (ISIS–2302) in steroid-refractory Crohn’s disease.

**Patients and Methods**

**Inclusion Criteria**

A double-blind, prospective, randomized, placebo-controlled, dose-interval, multicenter trial was conducted in patients with moderately active Crohn’s disease of at least 2 years’ duration (Crohn’s Disease Activity Index [CDAI], 200–400) with long-term steroid use (10–40 mg prednisone per day for more than 6 months, stable for the last 2 weeks) with at least 1 unsuccessful attempt to taper dosage within the last 6 months. Systemic corticosteroids other than prednisone had to be converted to prednisone equivalent at least 2 weeks before the trial. Budesonide had to be discontinued and converted to oral glucocorticoids at least 8 weeks before enrollment. Additional therapy with mesalamine, antibiotics, and parenteral or elemental diet was allowed but had to be kept stable 4 weeks before enrollment. All patients (men and women) were required to practice adequate birth control throughout the study and for at least 4 weeks after the last dose of study drug.

**Exclusion Criteria**

Pregnant or nursing women; patients with severe gastrointestinal or other diseases (including irritable bowel syndrome or gastrointestinal infections), human immunodeficiency virus or hepatitis B or C virus infection, or active malignancy (including pretreated hematologic malignancies); patients with evidence of current alcohol or drug abuse; patients with a body weight > 100 kg; and patients with laboratory abnormalities indicating severe anemia, leukopenia, or thrombopenia, renal or liver diseases, or abnormal coagulation laboratory parameters were excluded. Further exclusion criteria were the imminent need for surgery, known or suspected, fixed stenosis of the small or large bowel, and short bowel syndrome or ileostomy or colostomy.

Concomitant therapy with immunosuppressives (e.g., azathioprine) needed to be stopped at least 8 weeks before the trial. Budesonide in the previous 8 weeks and any prior treatment with TNF-binding proteins or ISIS–2302 were not allowed, as was any other investigational drug within 8 weeks before enrollment.

**Study Protocol**

The aim of the study was to assess the safety and efficacy of subcutaneous administration of 4 different dose-interval regimens of ISIS–2302 in patients with active, steroid-dependent Crohn’s disease.

The study was carried out as a double-blind, randomized, and placebo-controlled trial with a parallel group design. Plasma half-life of the drug is about 60 minutes. The maximum tolerable dose (0.5 mg/kg body wt) was administered and total exposure was varied through different treatment intervals at a constant dose level (dose-interval design) (Figure 1). Patients were randomized to 4 active treatment groups and a placebo group and received active treatment for 0 or 2 days, or 1, 2, or 4 weeks. All patients were followed up for 22 weeks after end of treatment for a total study duration of 26 weeks.

The primary endpoint was complete clinical remission (CCR; complete discontinuation of steroids, CDAI <150) at week 14. The primary endpoint was chosen at week 14 to ensure through subcutaneous administration was high enough to allow complete tapering of glucocorticoids (week 10) followed...
by a 4-week steroid-free remission period. For patients who entered the study on a daily dose of at least 20 mg prednisone (equivalent), the dose was held stable during week 1 and was reduced to 20 mg on day 8. The dose was then reduced in steps of 2.5 mg over the next 8 weeks. Patients entering the study on a daily dose of <20 mg prednisone (equivalent) were kept stable until the week at which their dosage was to be tapered according to the scheme used in the high-dose patients. Through this scheme, patients with different doses of glucocorticoids had the opportunity to be completely weaned at the same time point.

If the patient’s disease exacerbated before week 14, an increase in the prednisone (equivalent) dose was allowed once to a dose up to 40 mg/day. If the disease activity could not be controlled by the increase, the patient was withdrawn and counted as a treatment failure. After increasing the dose, an attempt to taper the daily dose was made with weekly decrements of 5 mg/day until 20 mg/day prednisolone (equivalent) was reached. From there, the dose was reduced weekly in steps of 2.5 mg/day.

Secondary endpoints were CCR at week 26, low-dose steroid-dependent remission (CDAI $<$150, $\leq$10 mg/day prednisone equivalent) at weeks 14 and 26, changes from baseline inflammatory bowel disease questionnaire (IBDQ) at weeks 14 and 26, changes from baseline in daily dose of corticosteroids consumed at weeks 14 and 26, partial clinical response (CDAI score decrease $>$70 points or $>100$ points, respectively) by weeks 14 and 26, and safety.

Statistics and Study Conduct

Data on baseline characteristics of the patient population were tabulated and summarized using descriptive statistics by treatment group. Comparability of treatment groups was evaluated using one-way analysis of variance for the continuous variables and a $\chi^2$ test for the categorical variables. In case this study had shown efficacy of ISIS-2302, a linear regression was planned to evaluate the influence of baseline characteristics. Safety data were listed, with the incidence of adverse experiences summarized by dose group, as were changes in World Health Organization grade from baseline in laboratory parameters.

Because this was an early phase 2 trial and little efficacy information was available regarding the effect of ISIS-2302 in Crohn’s disease, the study was sized to explore trends in efficacy and to aid in selection of the appropriate dose interval for a later phase III trial. Accordingly, the study plan did not call for the results of the primary efficacy analysis to be interpreted in the confirmatory (inferential) sense. Under these assumptions, a power of 80% was used to calculate the sample size. The frequency of reaching the primary endpoint (steroid-free complete remission at week 14) in the placebo population was estimated at less than 5%. It was felt that a remission rate of 40% in the active treatment group would be clinically meaningful. Using the formula of Casagrande, a necessary sample size of 30 evaluable patients per dose-group was calculated. Because a low dropout rate within the first 14 weeks was anticipated, a total of 175 subjects was planned to achieve the goal of 150 evaluable subjects. If all 175 patients would be evaluable, the power to detect a difference under these assumptions would be larger than 90%.

The primary efficacy analysis was a comparison of CCR, as defined above for each dose interval, with the rate for placebo using the unadjusted, 2-sided Fisher exact test. To minimize multiple testing, a step-down testing approach starting with a comparison between the group with the highest exposition (i.e., the longest dose-interval) and the placebo group was used. Data with 95% confidence intervals for each treatment group were calculated and used to further characterize the dose-interval/efficacy relationship. For the analysis of secondary endpoints, the $\chi^2$ test was used for pairwise comparisons of categorical variables. Fisher exact test was used if subgroups became too small for the $\chi^2$ test. All analyses are based on the intent-to-treat population. All patients who were randomized had received at least 1 dose of study drug and had at least 1 follow-up assessment, and were included in the efficacy analyses.

A planned interim analysis was carried out as specified in the protocol by an independent statistician after treatment of 40 patients, who reported the results to a 7-member management team from Isis Pharmaceuticals and Boehringer Ingelheim. The treatment blind was maintained for all persons involved in the conduct or evaluation of the trial. The interim analysis included all subjects who completed visit 24 by December 31, 1998. It was restricted to the primary endpoints measured at the end of week 14 (visit 24) and secondary endpoints derived from CDAI scores and daily glucocorticoid doses as well as toxicity. The purpose of the interim analysis was to analyze toxicity and to provide supportive data for administrative and ethical decisions regarding the development of ISIS-2302. Discussion about the early termination of the trial was not planned, therefore no discontinuation criteria were specified other than accumulation of toxic side effects. Upon reviewing the data, it was apparent that differences between ISIS-2302 and placebo patients were very small and
far from a clinically meaningful level. No patient in the entire interim analysis population had reached the primary endpoint and potential differences in the secondary endpoints investigated (clinical response and clinical remission) were too small to expect a meaningful benefit if continued. Therefore, the present trial was closed to further enrollment after enrollment of 78 patients, 75 of whom had received at least 1 injection. The study was carried out from March 1998 until August 1999 in 20 centers in Germany.

Results

Population

Eighty-three patients were screened, 78 randomized, and 75 treated with at least 1 dose of study drug. Overall, more women (63%) than men were enrolled. Baseline demographics and disease characteristics were not different between groups. All patients were white, mean CDAI was 262–294 points, and mean glucocorticoid dose at baseline (prednisone or equivalent) was 24–33 mg/day. Patients with open perianal fistula were included in all treatment groups with no differences in distribution, whereas patients with open abdominal wall fistula were only in the 2-day and 2-week treatment groups. A summary of other demographic and baseline characteristics is shown for each treatment group in Table 1.

Complete Clinical Remission

The primary endpoint, CCR at week 14, was defined as a CDAI <150 in conjunction with complete discontinuation of steroid treatment. Overall, 3.3% (2/60) of ISIS-2302–treated (all groups combined) and no placebo patients attained the primary endpoint (Figure 2). At week 26, 13.3% (8/60) of active-treatment and 6.7% (1/15) of placebo patients attained CCR (secondary endpoint) (Figure 2). No dose response was evident. CDAI scores decreased in all groups without significant differences between groups (Table 2).

Low-Dose Steroid-Dependent Clinical Remission

Low-dose steroid-dependent remission was defined as CDAI <150 with a maximum glucocorticoid dose of 10 mg prednisone/equivalent per day. Five active-treatment (8.3%) but no placebo patients attained low-dose steroid-dependent clinical remission at week 14 (Figure 3). Three of these patients were in the 1-week and 2 more in the 2-week treatment group. At week 26, 13 active-treatment (21.7%) and 2 placebo (13.3%) patients were in low-dose steroid-dependent remission, with the greatest response in the 2-week group (40%). No relationship was seen between exposure (dose-interval level) to active treatment and response (Figure 3). Ten of these active-treatment patients were from the low-dose baseline prednisone group (≤20 mg prednisone equivalent/day). Differences did not reach statistical significance.

Clinical Remission

Clinical remission was defined as a CDAI <150 and a glucocorticoid dose less than or equal to baseline.

Table 1. Characteristics of the 75 Patients Treated in the Study

<table>
<thead>
<tr>
<th>Group</th>
<th>2 Days (n = 14)</th>
<th>1 Week (n = 17)</th>
<th>2 Weeks (n = 15)</th>
<th>4 Weeks (n = 14)</th>
<th>Placebo (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>4/10</td>
<td>9/8</td>
<td>5/10</td>
<td>4/10</td>
<td>6/9</td>
</tr>
<tr>
<td>Age, median (minimum–maximum)</td>
<td>37 (29–56)</td>
<td>30 (20–61)</td>
<td>35 (21–53)</td>
<td>31 (20–61)</td>
<td>34 (23–55)</td>
</tr>
<tr>
<td>Smoker/never/exsmoker</td>
<td>7/4/3</td>
<td>12/5/0</td>
<td>9/3/3</td>
<td>9/5/0</td>
<td>8/4/3</td>
</tr>
<tr>
<td>Location: small + large bowel/small bowel only/ large bowel only/other</td>
<td>8/4/2/0</td>
<td>10/3/4/0</td>
<td>7/5/2/1</td>
<td>7/4/2/1</td>
<td>6/4/3/2</td>
</tr>
<tr>
<td>Baseline CDAI (mean ± SD)</td>
<td>266 ± 82</td>
<td>265 ± 74</td>
<td>262 ± 79</td>
<td>294 ± 73</td>
<td>283 ± 73</td>
</tr>
<tr>
<td>Baseline IBDQ (mean ± SD)</td>
<td>137 ± 31</td>
<td>141 ± 32</td>
<td>119 ± 29</td>
<td>123 ± 34</td>
<td>129 ± 28</td>
</tr>
<tr>
<td>Duration of steroid dependence (mo, mean ± SD)</td>
<td>39 ± 31</td>
<td>30 ± 19</td>
<td>33 ± 20</td>
<td>37 ± 37</td>
<td>31 ± 30</td>
</tr>
<tr>
<td>Glucocorticoid dose (mg prednisone, mean ± SD)</td>
<td>25 ± 20</td>
<td>33 ± 26</td>
<td>24 ± 17</td>
<td>30 ± 23</td>
<td>30 ± 14</td>
</tr>
</tbody>
</table>

Figure 2. Primary endpoint: CCR. The primary endpoint (CDAI <150 points, no steroid treatment at week 14) was only reached by 3.3% of active-treatment patients. No placebo patient attained CCR. At week 26 (secondary endpoint), 13.3% of active-treatment and 6.7% of placebo patients reached CCR.
Overall, clinical remission was attained by 11 (18.3%) active-treatment patients and 3 (20%) placebo patients at week 14. The 2-week treatment group had the highest number of patients in clinical remission at week 14 (5/15, 33.3%). Similar results were obtained at week 26 with 33.3% of active and 40% of placebo patients being in clinical remission (Figure 4). Differences did not reach statistical significance.

Clinical Response

Clinical response was defined as a CDAI decrease $>100$ or $>70$ points from baseline, respectively. The clinical response rate at the 100-point level in the active-treatment group was not different from the placebo group (week 14, 14/60 [23.3%] vs. 3/15 [20%]; week 26, 19/60 [31.7%] vs. 4/15 [26.7%]).

At week 14, 19 of 60 patients (31.7%) in the ISIS-2302 groups reached this secondary endpoint at the 70-point level in comparison with 4 of 15 (26.7%) in the placebo group (without statistical significance). At week 26, results were the same in active-treatment patients compared with the placebo group (33.3% vs. 33.3%). No dose-response relationship was observed.

Change From Baseline IBDQ

IBDQ scores improved in all treatment groups without showing a clear distinction between the active-treatment group and placebo. The mean change in IBDQ at week 14 was $+18$ in the active-treatment group vs. $+19$ for placebo patients. Results at week 26 were similar. Mean change in IBDQ in the active-treatment group was $+21$ vs. $+22$ in placebo patients.

Glucocorticoid Consumption

No statistically significant changes in the mean glucocorticoid consumption could be detected between the groups (Table 3). Overall, all active-treatment dose groups combined, 11.7% (7/60) of patients were able to discontinue glucocorticoid treatment (0 mg prednisone equivalent) at week 10 according to the steroid tapering schedule, in contrast to 6.7% (1/15) of placebo patients (not significant). At week 26, 23.3% of ISIS-2302–treated patients (14/60) had discontinued steroids in comparison with 6.7% of placebo patients. However, no ISIS-2302 dose effect was noted because the 1-week treatment group had the highest rate of steroid discontinuation (17.6%), followed by the 2-day and 4-week treatment groups (14.3% in both groups).

### Table 2. Mean Changes in CDAI Scores at Weeks 14 and 26 in Comparison With Baseline Scores, Respectively

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Baseline total</th>
<th>Week 14 Mean ± SD</th>
<th>Week 26 Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>282.5</td>
<td>-50.5 ± 113.2</td>
<td>-71.3 ± 125.2</td>
</tr>
<tr>
<td>2 Days</td>
<td>265.8</td>
<td>-37.2 ± 98.9</td>
<td>-36.6 ± 85.7</td>
</tr>
<tr>
<td>1 Week</td>
<td>265.0</td>
<td>-62.9 ± 139.9</td>
<td>-93.1 ± 107.5</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>261.9</td>
<td>-54.6 ± 109.3</td>
<td>-60.5 ± 103.2</td>
</tr>
<tr>
<td>4 Weeks</td>
<td>293.9</td>
<td>-39.1 ± 89.4</td>
<td>-65.9 ± 100.6</td>
</tr>
</tbody>
</table>

Figure 3. Secondary endpoints: low-dose steroid-dependent clinical remission. Some (8.3%) active-treatment patients but no placebo patients attained low-dose steroid-dependent clinical remission (CDAI $<150$ with $\leq 10$ mg prednisone [equivalent] per day) at week 14. At week 26, 21.7% of active-treatment and 13.3% of placebo patients were in low-dose steroid-dependent remission.

Figure 4. Secondary endpoints: clinical remission. For all active dose groups combined, 18.3% of active-treatment patients attained the secondary endpoint of clinical remission (CDAI $<150$ and glucocorticoid dose $\leq$ baseline) at week 14 (placebo, 20%). At week 26, 33.3% of active and 40% of placebo patients were in clinical remission. Differences did not reach statistical significance.
The proportion of patients receiving 10 mg prednisone equivalent at weeks 14 and 26, respectively, was higher in the ISIS-2302–treated patient groups (48.3% and 55%) than in the placebo group (33.3% and 40%). These differences did not reach statistical significance.

**Pharmacokinetics**

No drug accumulation was seen. No significant differences between the peak plasma concentrations of ISIS-2302 were seen between the treatment groups, although the 2-day treatment group showed a trend of less exposure to the drug (2-day treatment group, n = 18, 765 [362–1250]; 1-week treatment group, n = 23, 840 [396–1360]; 2-week treatment group, n = 15, 833 [296–1670]; and 4-week treatment group, n = 9, 651 [259–1580]; [Means (Min Cmax – Max Cmax), ng/mL]) (Figure 5). Mean 4-hour AUCs for ISIS-2302 and its main metabolite, 11279, were not different between groups (Figure 6). No accumulation was observed on the basis of the AUC analysis.

**Side Effects**

No deaths were reported in this study. Most of the adverse events seen in this trial were mild in severity (61%). Among events occurring at least 5% more commonly in ISIS-2302 patients than placebo were injection site reaction, headache, pain, fever, rash, arthritis, asthenia, and flu-like symptoms. Injection site reaction had the greatest differential between ISIS-2302–treated and placebo patients (23.3% vs. 0%), and 1 patient in the 4-week group was discontinued from the study because of severe injection site reaction. Side effects were not dependent on total exposure or any of the baseline demographic and clinical parameters.

There were 16 serious adverse events in the 60 patients treated with ISIS-2302 (0.27 serious adverse events/patient) and 2 serious adverse events in the placebo group (0.13 serious adverse events/patient). The majority of serious adverse events were related to progression of underlying Crohn's disease. The only serious adverse event that was considered potentially related to the study drug was a case of vomiting in the 1-week treatment group that required hospitalization during week 3 of the trial; at that time the patient had already received placebo for 2 weeks. Vomiting began in week 1 and the investigator considered the event as possibly caused by a conditioned response.

**Discussion**

ICAM-1, an adhesion molecule expressed on a host of different cells including endothelial cells, monocytes and macrophages, keratinocytes, and a subset of B and T cells is up-regulated in response to proinflammation.

---

**Table 3. Total Glucocorticoid Consumption (mg Prednisone Equivalent) at Baseline in Comparison With Weeks 14 and 26**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Baseline daily dose</th>
<th>Week 14</th>
<th>Change (mg/day)</th>
<th>Week 26 (mean ± SD)</th>
<th>Change (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>29.9</td>
<td>2513 ± 1179</td>
<td>−5.0 ± 9.4</td>
<td>4513 ± 1955</td>
<td>−4.6 ± 14.3</td>
</tr>
<tr>
<td>2 Days</td>
<td>25.2</td>
<td>2469 ± 2031</td>
<td>−4.3 ± 21.4</td>
<td>4133 ± 3445</td>
<td>−10.2 ± 16.2</td>
</tr>
<tr>
<td>1 Week</td>
<td>33.1</td>
<td>2741 ± 2520</td>
<td>−6.3 ± 10.8</td>
<td>4973 ± 4607</td>
<td>−5.0 ± 13.9</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>24.3</td>
<td>2049 ± 1384</td>
<td>−5.0 ± 8.0</td>
<td>3426 ± 2410</td>
<td>−8.1 ± 10.9</td>
</tr>
<tr>
<td>4 Weeks</td>
<td>29.7</td>
<td>2608 ± 2603</td>
<td>−4.3 ± 9.3</td>
<td>4563 ± 4784</td>
<td>−4.2 ± 12.1</td>
</tr>
</tbody>
</table>

---

*SCHREIBER ET AL. GASTROENTEROLOGY Vol. 120, No. 6*
influence cell adhesion molecule expression. Monoclonal antibodies directed against ICAM-1 significantly decreased immunologic changes and clinical scores in animal models of rheumatoid arthritis and colitis. Two phase I/II trials in human rheumatoid arthritis confirmed sustained clinical improvement up to 11 months in patients with moderate disease after a single course of therapy (5 days of intravenous treatment).

A more elegant method to reduce increased levels of ICAM-1 is to directly block the production of ICAM-1 on the mRNA level. In animal models of organ transplant rejection, ulcerative colitis, and collagen-induced arthritis, intravenous administration of antisense oligonucleotides directed against ICAM-1 resulted in an anti-inflammatory activity at doses ranging from 0.03 to 5 mg/kg. No clinically relevant side effects were seen in mice and monkeys. Clinical exploration of antisense oligonucleotides directed against ICAM-1 indicated beneficial effects in allograft kidney transplantation. Intravenous application of antisense oligonucleotides against ICAM-1 (ISIS-2302) in active, steroid-dependent Crohn's disease seemed well tolerated and promising in a pilot placebo-controlled clinical trial in 20 patients.

Based on these encouraging results, the subcutaneous trial presented here was conducted. Preclinical exploration of animal models and pharmacokinetic studies has shown that subcutaneous application of ISIS-2302 resulted in plasma levels that were in the immunologically effective range. We confirm that subcutaneous therapy with the antisense ICAM-1 oligonucleotide ISIS-2302 leads to measurable plasma levels of the compound, which are well above (about 5-fold) those needed in vitro to suppress translation of ICAM-1 mRNA. Therapy was safe and well tolerated, with the exception of injection site reactions and minor complaints, such as headaches, which were seen frequently and in a substantial proportion of patients.

However, the trial failed to show clinical efficacy of ISIS-2302 based on the primary endpoint, steroid-free remission at week 14. Only weak positive trends were observed in some of the secondary endpoints (e.g., complete glucocorticoid-free clinical remission at week 26, low glucocorticoid dose clinical remission at weeks 14 and 26). Because these trends show no relationship to cumulative exposure to ISIS-2302, it seems questionable how clinically meaningful these differences are.

The trial was stopped after interim analysis because response rates were felt to be not tolerable for further clinical exploration. The power of this study was chosen at 80%. The early end to recruitment has reduced the power substantially. Therefore, the possibility of a type-2 error exists. However, after 40 patients, the interim analysis showed that none of the patients had reached the primary endpoint; therefore, it seemed ethically problematic to continue recruitment.

The difference to the data reported earlier may be due to lower peak concentrations reached by subcutaneous application of ISIS-2302 in comparison to the intravenous route. Because there were no pharmacodynamic endpoints included in the protocol, it is unclear whether ICAM-1 protein expression was suppressed by ISIS-2302 in this trial as it had come to be expected from in vitro data. It should also be acknowledged that plasma concentrations reached through subcutaneous administration are about half of what is reached by intravenous infusion of the same dose. A large trial, which was conducted in 300 patients with refractory Crohn’s disease in parallel to this study, used a similar dose-interval concept for the intravenous administration of ISIS-2302 at a higher dose level (2 mg/kg). To our knowledge, this trial also failed to establish significance for the induction of CCR at week 14 through ISIS-2302. Therefore, a dose effect (i.e., the lowest maximum tolerable dose) may contribute to the failure of the subcutaneous formulation but is unlikely to be the only cause for ineffectivity of the drug.

Other options that could explain the apparent lack in therapeutic efficacy in refractory Crohn’s disease include the possibility that the antisense compound is not penetrating into the diseased tissue in vivo. However, it seems unlikely that suppression of ICAM-1 expression has worked in a variety of animal models (including colitis and rheumatoid arthritis models) but would fail in the human organism. In addition, the pilot trial of intravenous ISIS-2302 in Crohn’s disease indicated a down-regulation of ICAM-1 protein expression in the intestine.

Adhesion of inflammatory cells is a complex process that involves a host of molecules in addition to ICAM-1, which are redundant and overlapping in their biological specificities. Therefore, future studies are needed (i.e., using high-dose intravenous application of ISIS-2302 or monoclonal antibodies directed against ICAM-1) to evaluate the role of ICAM-1 as a therapeutic target in Crohn’s disease.

References
1. Schreiber S, MacDermott RP, Raedler A, Pinnau R, Bertovich M, Nash GS. Increased activation of isolated intestinal lamina pro-


5. Butcher EC. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell 1991;67:1033–1036.


Received August 29, 2000. Accepted December 27, 2001.

Address requests for reprints to: Stefan Schreiber, M.D., First Department of Medicine, Christian-Albrechts-University, Schittenhelmstrasse 20/21, 24105 Kiel, Germany. e-mail: s.schreiber@mucoса.de; fax: (49) 431-597-1842.

Supported by grants from ISIS Pharmaceuticals, Inc., Carlsbad, CA, Boehringer Ingelheim KG, Biberach/Riss, Germany, and the Competence Network “Inflammatory Bowel Disease” of the Bundesministerium fuer Bildung und Forschung.

Parts of the data have been presented at the 101st annual meeting of the American Gastroenterological Association, San Diego, CA, May 21–24, 2000.

Additional members of the German ICAM-1 study group were: Adler (Marburg), Bokemeyer (Minden), Buhr (Berlin), Caspary (Frankfurt), Emmerich (Rostock), Krakamp (Cologne), Kramm (Berlin), Malfertheiner (Magdeburg), Stange (Luebeck), Stoll (Muenster), Stremmel (Heidelberg), and Wellmann (Hannover).