TNF neutralization in MS

Results of a randomized, placebo-controlled multicenter study

The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group*

Article abstract—*Objective:* A double-blind, placebo-controlled phase II study was conducted in 168 patients, most with relapsing-remitting MS, to evaluate whether lenercept would reduce new lesions on MRI. *Background:* Tumor necrosis factor (TNF) has been implicated in MS pathogenesis, has been identified in active MS lesions, is toxic to oligodendrocytes in vitro, and worsens the severity of experimental allergic encephalomyelitis (EAE) in animals. Lenercept, a recombinant TNF receptor p55 immunoglobulin fusion protein (sTNFR-IgG p55), protects against EAE. *Methods:* Patients received 10, 50, or 100 mg of lenercept or placebo IV every 4 weeks for up to 48 weeks. MRI scans and clinical evaluations were performed at screening, at baseline, and then every 4 weeks (immediately before dosing) through study week 24. *Results:* There were no significant differences between groups on any MRI study measure, but the number of lenercept-treated patients experiencing exacerbations was significantly increased compared with patients receiving placebo ($p = 0.007$) and their exacerbations occurred earlier ($p = 0.006$). Neurologic deficits tended to be more severe in the lenercept treatment groups, although this did not affect Expanded Disability Status Scale scores. Anti-lenercept antibodies were present in a substantial number of treated patients; serum lenercept trough concentrations were detectable in only a third. Adverse events that increased in frequency in treated patients included headache, nausea, abdominal pain, and hot flushes. *Conclusions:* Lenercept failed to be beneficial, but insight into the role of TNF in MS exacerbations was gained.

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MS is believed to be an inflammatory autoimmune disorder of the CNS with unknown myelin components as target. A number of findings have suggested that tumor necrosis factor (TNF) contributes to propagating the inflammatory response and to tissue injury in MS. In autopsy specimens, TNF has been demonstrated within active MS foci.[1] TNF has been shown to have a direct toxic effect against oligodendrocytes and a proliferation-inducing effect on astrocytes in in vitro studies.[2,3] In patients with MS, elevated TNF levels in the serum and CSF have been correlated in some studies with disease progression.[4,5] Blood mononuclear cells from MS patients, studied just before an exacerbation, secrete greater amounts of TNF in response to mitogen stimulation than at other times.[6] Blood mononuclear cells from MS patients with active disease express higher levels of TNF mRNA than do cells from MS patients with stable disease or healthy controls.[7,8]

Studies of experimental autoimmune encephalomyelitis (EAE) have profoundly shaped views of MS pathogenesis. EAE is an autoimmune disease with pathologic features reminiscent of those seen in MS. TNF treatment worsens EAE,[9] and TNF neutralization by anti-TNF antibody treatment consistently protects animals from EAE.[10-12] Similarly, TNF capture by lenercept, a TNFα receptor–immunoglobulin G (IgG)1 fusion protein, protects in EAE.[13] The above indicates that TNF functions in EAE as a proinflammatory mediator and suggests that TNF depletion might be protective in MS. The hypothesis that neutralization of TNF may reduce or halt MS progression was evaluated in a phase II randomized, multicenter, placebo-controlled study of three doses of lenercept (sTNFR-IgG p55). Lenercept is a dimeric recombinant protein molecule built from two copies of the 55 kDa TNF receptor extracellular domain fused to a fragment of the human immunoglobulin

See also pages 444 and 466

*See the Appendix on page 464 for a listing of members of The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group.

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IgG1 heavy chain.[14,15] In accordance with recently published recommendations, efficacy was assessed by means of MRI.[16]

Methods. *Patients.* A total of 168 patients with clinically definite or laboratory supported definite MS were enrolled in a double-blind, placebo-controlled study. The study was approved by the institutional review boards of the participating centers, and all subjects gave informed consent. At enrollment, patients were between the ages of 18 and 55 years and had an Expanded Disability Status Scale (EDSS) score ≤5.5. For patients with an EDSS score ≤3, the history of MS was limited to a maximum duration of 10 years. All patients had at least two exacerbations within the previous 2 years, but were clinically stable for 4 weeks before the screening MRI and during the 4 weeks between screening and study entry. With the exception of glucocorticoids, any prior administration of agents with a putative effect on MS (including interferons, cyclophosphamide, or azathioprine) led to exclusion. Treatment with glucocorticoids was not permitted within a 4-week period before the screening visit or between screening and baseline. Other exclusion criteria included the diagnosis of primary progressive MS and inability to undergo MRI scanning. A randomization list with treatment blocks (four patients per block) was computer generated by Hoffmann–La Roche (Basel, Switzerland) for each investigation site. During the conduct of the study, the randomization list was available only to the Safety Review Board (SRB) members (see below). A limited number of Roche staff were unblinded at the time of the first analysis of efficacy as defined in the protocol. On study termination, each investigational site was provided with the site-specific randomization code.

Eligible patients were randomized to 10, 50, or 100 mg of lenercept or to placebo, administered IV every 4 weeks. Study duration was 48 weeks, consisting of a 24-week, double-blind treatment period and a 24-week follow-up period. Of the 168 patients randomized to treatment, one patient (randomized to placebo) was identified as ineligible prior to the baseline visit; this patient did not receive treatment, have a baseline MRI scan, or return for follow-up. For the 167 patients who received treatment, compliance to treatment and study procedures was excellent. During the first 24 weeks, 99% (991/1002) of all planned doses were administered and 98% (1303/1336) of all MRI scan sets were performed.

During the follow up period (study weeks 25–48), patients could continue double-blind treatment on a voluntary basis and 130 elected to do so. Those patients who opted not to continue treatment remained in the study and were followed on an intent-to-treat basis. For the full study duration, 10 doses (median) were administered to each treatment group.

For safety purposes, three cohorts of up to 16 patients were enrolled in an ascending-dose design at approximately 6-week intervals. The first cohort was randomized to placebo or 10 mg of lenercept whereas subsequent cohorts were randomized to placebo or 50 mg and finally to placebo or 100 mg of lenercept. An independent SRB evaluated the unblinded study data before each dose escalation during the ascending dose phase of the study. Following The SRB reviewed data at 3-month intervals throughout the study. This review included the MRI safety data but did not include a review of the MRI efficacy data.

Magnetic resonance imaging. MRI scans were performed according to a predefined MRI protocol at screening, baseline, and every 4 weeks (before each dose) throughout the first 24 weeks of the study for a total of eight scanning time points. At each time point, three scans with a slice thickness of 5 mm were obtained: 1) proton density/T2-weighted scan, 2) T1-weighted unenhanced scan, and 3) T1-weighted gadolinium (Gd)-enhanced scan 5 minutes after the administration of Gd-DTPA 0.1 mmol/kg. All scans were analyzed according to a prospectively defined MRI analysis plan by the UBC MS/MRI Analysis Group in Vancouver, Canada. After comparison of each MRI follow-up scan with the prior scan, the number of newly active lesions was ascertained by summing the new, recurrent, or enlarging T2 lesions, and the new or recurrent Gd-enhancing lesions. Newly active lesions identified on both the enhanced T1 scan and the T2 scan were counted as single lesions. The primary efficacy measure was the cumulative number of newly active lesions identified on the six treatment scans. Definitions of new, recurrent, and enlarging lesions have been reported previously.[17,18] Persistently enhancing or enlarging lesions were separately identified as persistently active lesions, a secondary measure of efficacy. In this way, new lesions could easily be separated from other types of activity. Other secondary efficacy measures included the percentage of active scans, defined as the proportion of scans with one or more newly active lesions, and the burden of disease, which was assessed as reported previously, at baseline, and at 24 weeks.[17,18] Burden of disease was determined by outlining each MS lesion identified on the T2-weighted MRI scan. These areas were summed slice by slice for a total lesion area recorded as mm^3. In addition, the total number of Gd-enhancing lesions (a measure of safety) was counted for each patient at each scanning time point on an ongoing basis to allow MRI data to be reviewed by the SRB.

Clinical assessments. At the baseline visit and every 4 weeks thereafter for the first 24 weeks, a history was taken, physical and neurologic examinations performed, and adverse events noted. Study drug was administered at the end of each visit. Patients were encouraged to come for additional visits should exacerbations occur between visits. During the second 24-week period, two formal visits were planned at weeks 36 and 48. Whenever possible, patients who withdrew from treatment were asked to continue all study procedures including all MRI scans.

Clinical endpoints. Exacerbations were defined as the appearance of a new sign or symptom or the worsening of an old sign or symptom attributable to MS, lasting at least 24 hours in the absence of fever, and preceded by a period of stability of 28 days. An exacerbation was deemed to have ended when signs and symptoms had begun to improve. For those patients with permanent deficits, the first day of a 28-day period of stability was taken as the ending of an attack. The Neurological Rating Scale (NRS)[19] was completed each time the neurologic examination was performed. As in other clinical trials in MS, a decline in NRS score of 15 points or more was considered to reflect a severe change in the patient's neurologic condition, whereas

moderate or mild changes, respectively.[17,20] A difference of 0 points was categorized as no change; an increase in the score as an improvement. The EDSS as recommended by Kurtzke[21] was scored at screening and at weeks 24 and 48 by the study neurologist. In 120/167 (72%) of patients, the EDSS was performed at all time points by the same neurologist.

In accordance with the protocol, a first analysis was undertaken after all patients had completed 24 weeks of double-blind treatment and after all MRI scans had been evaluated. An increase in the exacerbation rate was noted in lenercept-treated patients. This finding resulted in the sponsor's decision to terminate the study and to release the treatment code. All study drug administration was stopped promptly and, after a final visit, data collection was discontinued. For this reason, study data through week 48 are incomplete. The follow-up period through week 48, however, was similar in all treatment groups.

Pharmacokinetic/dynamic parameters. Serum samples were obtained at baseline and before dosing every 4 weeks for 24 weeks and at study weeks 36 and 48. The concentration of lenercept and titers of antibodies to lenercept were determined. All samples were analyzed centrally. Lenercept concentrations were measured using an enzyme-linked immunologic and biologic binding assay (ELIBA) developed by Roche (Hoffmann-LaRoche Ltd., Basel, Switzerland); antibodies to lenercept were identified by means of a double antigen antibody test. Samples with detectable anti-lenercept antibodies were further evaluated to determine the neutralizing potential of the antibodies (Medi-Lab, Medicinsk Laboratorium A/S, Copenhagen, Denmark).

Autoantibodies. Serum samples were obtained at baseline and at study weeks 24 and 48 and assayed for IgM–rheumatoid factor (RF), antinuclear antibodies (ANA) (Hep 2), and antibodies to dsDNA (DAKO; Carpinteria, CA) in a central laboratory (A. Wiik, Statens Seruminstitut Copenhagen, Denmark).

Statistical analyses. The cumulative number of newly active lesions was tested with a closed test procedure based on an analysis of variance (ANOVA) of the Ln(x + 1) transformed sum of the lesions. The protocol required that the analysis of the primary efficacy criterion be performed after imputation of the median number of lesions at a specific time point so as to compensate for missing values at that time point. Of the 1,008 expected values, 34 were missing, resulting in data imputation as noted above. The results of this analysis showed no differences among the treatment groups ($p = 0.417$) or between the pairs of treatment groups. Data imputation was not performed for the analyses presented herein.

A closed tests procedure, based on an ANOVA with the factor "treatment" of Ln(1 + x), where x denotes the cumulative number of newly active lesions, was used to compare the cumulative number of newly active lesions between the treatment groups. The closed tests procedure was first used to compare the means among all four treatment groups (global test)[22]; then, to compare the means among all combinations of three of the four treatment groups; and finally, to compare the means of each lenercept treatment group with that of the placebo group. For all comparisons, F tests were performed at the same significance level ($\alpha =$ of a treatment group is regarded as significantly different from that of the placebo group when all comparisons that include the two relevant treatment groups result in a p value ≤ 0.05; i.e., the adjusted p value is the maximum of the p values of these comparisons. The procedure stops early if the global test is nonsignificant. This procedure guarantees a multiple $\alpha = 0.05$. The closed tests procedure described above was also used to assess the cumulative number of persistently active lesions. Center effects were assessed using descriptive methods.

The Kruskal-Wallis test was used to compare the mean change in EDSS scores, change in the burden of disease, and percent of active scans. To assess the influence of baseline imbalances in MRI activity among the treatment groups, covariance analyses using the corresponding transformed baseline MRI values as covariate were performed.

Survival analysis methods (Kaplan-Meier estimates; i.e., product-limit estimates and logrank tests)[23] were applied to analyze the time to first exacerbation and the duration of exacerbations because of right censoring at the end of the observation period. Logrank tests, with a Bonferroni adjustment of the significance level ($\alpha = 0.017$), were used for the multiple comparisons among the three lenercept treatment and the placebo treatment Kaplan-Meier curves. As an exacerbation duration can only be observed in the presence of an exacerbation, we investigated the conditional distribution of their durations. After inspection of the exacerbation data, we assumed a counting process model according to Anderson and Gill[24] with independent increments because the process is slow. For the same reason, exacerbation durations were assumed to be independently and identically distributed between patients and within patients for those patients who had more than one exacerbation.

A chi-square was used to evaluate the number of patients with no, one, two, three, or four exacerbations in each treatment group after 24 weeks of treatment and at the end of the study (through week 48). Because of small frequencies in some cells, the table was collapsed to counting patients with and without exacerbations to allow a valid chi-square test. For the multiple comparisons the unadjusted p values should be compared with the Bonferroni adjusted α of 0.017 ($0.05 \div 3$). Chi-square tests were also used to evaluate the NRS and the rate of RF or ANA among the treatment groups. Cox regression analysis was performed to assess potential predictive factors for the occurrence of exacerbations. The data and analyses were performed on all data available, i.e., through week 48, unless otherwise stated in the text or tables. Two-tailed analyses were used throughout.

Results. Figure 1 depicts the trial profile. The treatment groups were comparable at entry on all baseline disease characteristics and demographics (table 1). The protocol permitted enrollment of both relapsing-remitting and secondary progressive patients, and from 4 to 10 patients with secondary progression were enrolled in each group (see table 1). Prestudy MRI characteristics were likewise comparable among the treatment groups (table 2) although there was a tendency (nonsignificant) for the higher lenercept dose groups to have more MRI activity (median).

MRI results. The results of the cumulative number of newly active MRI lesions, the percentage of persistently

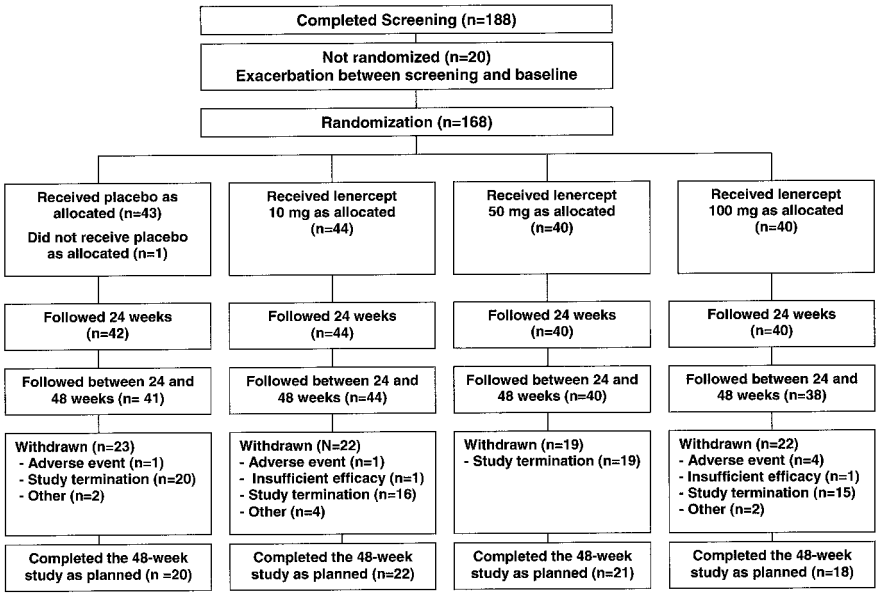

Figure 1. Profile of the lenercept MS clinical trial.

change in burden of disease over 24 weeks of treatment are shown in figure 2 and table 3. There were no significant differences between the treatment groups for any measure. The results of the analyses of the primary efficacy criterion according to the protocol specifications were similar to those presented here. Because of the tendency for higher activity at baseline in the high-dose groups (see table 2), covariance analyses using the corresponding transformed baseline MRI values as covariate were performed, but these, too, failed to show a significant difference between the groups.

Clinical endpoints. Exacerbations. The number of patients who developed exacerbations by week 24 and through study week 48 were both increased in the lenercept groups as compared with the placebo treatment group as shown in table 4. A center effect was not present. Over the entire study period, a total of 36 exacerbations was reported in patients taking placebo as compared with 38,

Table 1 *Demographic and baseline characteristics of patients entered into the lenercept MS trial*

Characteristics	Placebo, n = 44	Lenercept, mg		
		10, n = 44	50, n = 40	100, n = 40
% Female	66	80	78	73
Age, y, mean (range)	36.5 (21–50)	34.6 (23–51)	35.1 (19–47)	34.9 (21–51)
% White	98	100	100	93
No. with SPMS	10	5	10	4
Mean (range) no. exacerbations in prior 2 years	2.7 (2–5)	2.8 (2–8)	2.8 (2–8)	3.0 (2–6)
EDSS, mean (range)	2.45 (0–5.5)	2.52 (0–5.0)	2.83 (1.0–5.5)	2.55 (0–5.5)
NRS, mean (range)	83.2 (51–100)	83.7 (44–100)	81.8 (54–100)	83.0 (57–99)

SPMS = secondary progressive MS; EDSS = Expanded Disability Status Scale; NRS = Neurological Rating Scale.

Table 2 *MRI measurements at baseline of patients entered into the lenercept MS trial*

Variable	Placebo, n = 44	Lenercept, mg		
		10, n = 44	50, n = 40	100, n = 40
Newly active lesions, mean	1.8	1.5	2.1	1.9
Median (range)	0 (0–16)	0 (0–55)	1 (0–11)	1 (0–14)
Persistently active lesions, mean	1.2	0.5	1.6	0.6
Median (range)	0 (0–15)	0 (0–6)	0 (0–22)	0 (0–7)
Burden of disease, mean	2,459.9	2,236.2	3,757.2	2,707.2

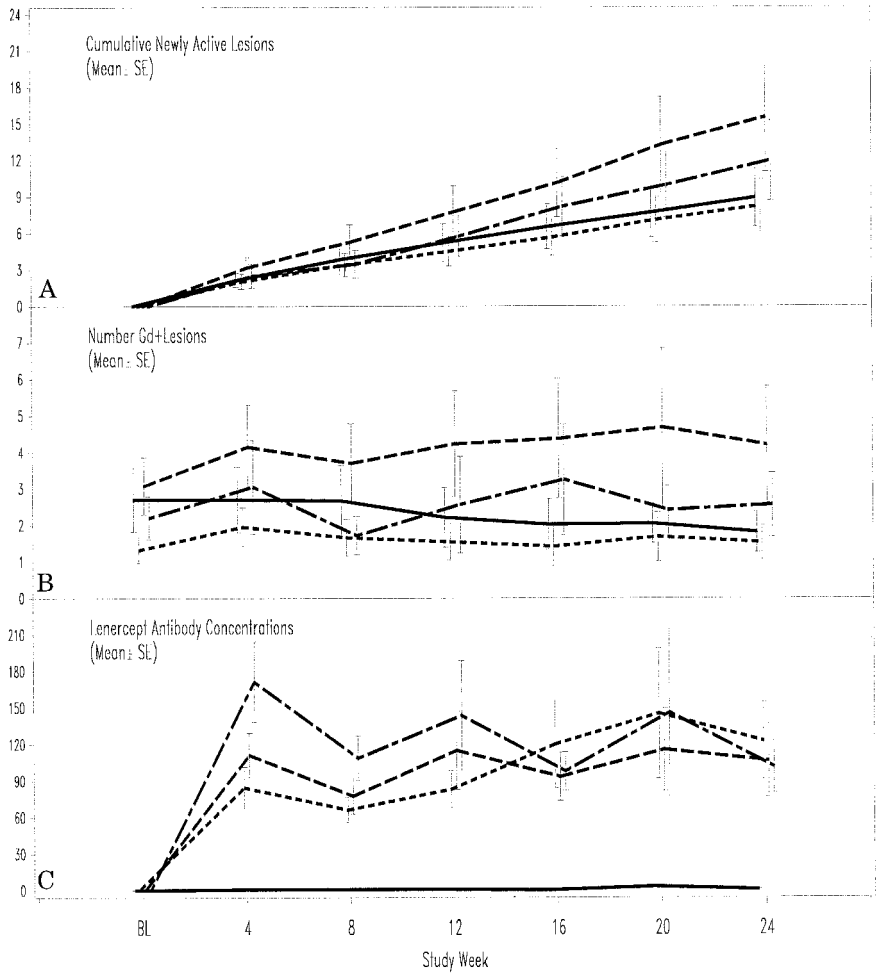

Figure 2. (A) Number of cumulative newly active lesions as determined by MRI (see Methods) over the first 24 weeks of the lenercept MS trial: placebo ——, lenercept 10 mg - - - -, lenercept 50 mg — · — ·, lenercept 100 mg – – – –. The vertical bars give the standard error at the four weekly intervals at which MRI scans were performed. (B) The mean number of gadolinium (Gd)–positive lesions every 4 weeks over the first 24 weeks of the trial. Vertical bars give the standard error. (C) Mean anti-lenercept antibody titers at four weekly intervals.

57, and 49 exacerbations in patients taking 10, 50, and 100 mg of lenercept, respectively. Exacerbation duration showed a tendency to increase with lenercept treatment, but this did not reach statistical significance (see table 4). This assessment was limited to exacerbations with an onset date within the first 24 weeks of the study as exacerbation resolution dates were available in all but four exacerbations (one per treatment group).

Exacerbation rate. The overall exacerbation rate in patients treated with placebo was approximately one exacerbation/patient/year (the expected placebo rate). The exacerbation rate was increased over the placebo rate by 2%, 68%, and 50% in patients treated with lenercept at doses of 10, 50, and 100 mg, respectively (see table 4). To control for a possible effect of unequal follow-up of patients between treatment groups, exacerbation rates were determined for each treatment group by 4-week intervals. The mean 4-week exacerbation rates were then converted to annual rates as presented in table 4.

There was a dose-dependent decrease in the time to first exacerbation as shown in figure 3 (logrank test: global, $p = 0.0006$; 10 mg versus placebo, $p = 0.498$; 50 mg

***Table 3** MRI measurements over the first 24 weeks of the lenercept MS trial*

Variable	Placebo, n = 43	Lenercept, mg			*p* Value
		10, n = 44	50, n = 40	100, n = 40	
Newly active lesions, mean	8.9	8.2	15.5	12.0	0.43*
Median (range)	4.0 (0–92)	3.0 (0–55)	5.5 (0–124)	4.5 (0–102)	
Persistently active lesions, mean	6.2	3.3	9.6	3.3	0.36*
Median (range)	1.0 (0–100)	1.0 (0–49)	1.0 (0–128)	1.0 (0–24)	
Percent of active scans,† mean	45.6	40.6	51.6	49.2	0.58†
Median (range)	50 (0–100)	33 (0–100)	50 (0–100)	55 (0–100)	
Percent change in burden of disease,† mean	6.0	9.9	4.3	4.9	0.74†
Median (range)	−2.2 (−28.8–280.9)	0.0 (−30.5–251.0)	1.4 (−35.2–62.8)	−0.2 (−36.0–81.4)	

* Analysis of variance of Ln(1 + x)

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