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PHARMACOKINETICS, SAFETY, AND EFFICACY OF COMBINATION TREATMENT WITH METHOTREXATE AND LEFLUNOMIDE IN PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS

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Objective. **To examine the safety and pharmacokinetics of and clinical response to leflunomide, a de novo pyrimidine synthesis inhibitor, when administered to patients with active rheumatoid arthritis (RA) who have been receiving long-term methotrexate therapy.**

Methods. **This was an open-label, 52-week study in which 30 patients with RA that remained active despite therapy with methotrexate at 17 ± 4 mg/week (mean ± SD) for ≥6 months were given leflunomide, 10–20 mg/day. Patients were assessed for adverse effects, pharmacokinetic measurements of leflunomide and methotrexate, and clinical response by American College of Rheumatology (ACR) 20% response criteria.**

Results. **Twenty-three patients completed 1 year of treatment. No significant pharmacokinetic interactions between leflunomide and methotrexate were noted. This combination therapy was generally well tolerated clinically, with the exception of elevations of liver enzyme levels. Seven patients withdrew from the treatment regimen: 2 withdrawals were voluntary, 3 were due to persistent elevation of plasma transaminase levels, and 2 were due to lack of efficacy. Of the patients, 16 (53%) met ACR 20% response criteria. Two met ACR criteria for remission after 1 year.**

Conclusion. **The combination of methotrexate and leflunomide has therapeutic potential in RA.**

Leflunomide, a new disease-modifying antirheumatic drug (DMARD) of the isoxazole class, has shown efficacy in the treatment of rheumatoid arthritis (RA) (1). The active metabolite of leflunomide, A77 1726, inhibits dihydroorotate dehydrogenase, a critical enzyme for de novo production of pyrimidine (2–6). Since expansion of pyrimidine pools is required for mitogen-induced T cell proliferation to proceed, the net effect of inhibition of pyrimidine synthesis is to halt this process, which is thought to be a key step in the pathogenesis of RA.

Given the high failure rate of RA monotherapy and the multifactorial nature of the pathogenesis of RA, an increasing emphasis is being placed on combinations of therapeutic agents that act to inhibit different pathophysiologic processes in the disease (7). Considering its mechanism of action, leflunomide could be useful in combination therapy with methotrexate. Unlike leflunomide, methotrexate, at the dosages used for RA therapy, appears to have little effect on T cell proliferation, but strongly inhibits cellular synthesis of polyamines and promotes adenosine release, effects that limit inflammation and joint destruction (8). Additionally, a recent in vitro study suggests that methotrexate promotes apoptosis of activated T cells, an action that would be complementary to the effect of leflunomide to limit T cell proliferation (9). Testing of this combination in primate models was not possible because of significant differences in the metabolism, and thus in the pharmacokinetics, of leflunomide in humans and other primates. The present study was undertaken to examine the safety and pharmacokinetics of, and the clinical response to,

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the addition of leflunomide to methotrexate treatment in patients with active RA that showed inadequate response to methotrexate alone.

PATIENTS AND METHODS

Patients. All patients recruited for this study had RA diagnosed according to the American College of Rheumatology (ACR) criteria (10) and active disease despite treatment with methotrexate (≥15 mg/week) for a minimum of 6 months. Patients were required to have been receiving a stable dosage of methotrexate for at least 4 weeks prior to study enrollment. Patients with active RA whose highest tolerated dosage of methotrexate, due to documented toxicity, was 10–12.5 mg/week were also considered eligible. Active disease was defined by the following criteria: ≥8 swollen joints, ≥10 tender joints (both by 28-joint count) (11), and either Westergren erythrocyte sedimentation rate (ESR) ≥28 mm/hour or morning stiffness ≥45 minutes. Men and women ages 18–75 years were eligible for enrollment. Female subjects had to be neither pregnant nor nursing. Treatment with stable doses of nonsteroidal antiinflammatory drugs (NSAIDs) and oral prednisone (≤10 mg/day) was allowed. Intraarticular, intramuscular, and soft tissue injections of corticosteroids were not allowed. All patients received folate (1 mg once or twice per day) during the study.

Patients excluded from the study included those with a history of acute inflammatory joint disease other than RA, clinically significant drug or alcohol abuse, or persistently abnormal results on liver function tests. Additional reasons for exclusion included hematopoietic disorders, human immunodeficiency virus infection, active hepatitis B or C infection, persistent or severe infection within 3 months of enrollment, uncontrolled diabetes, unstable ischemic heart disease, active inflammatory bowel disease, active peptic ulcer, stroke within 3 months of enrollment, regular treatment with cholestyramine, or history of sensitivity to the study medication.

Study design. This 52-week preliminary study was a 2-center, open-label evaluation of the safety and pharmacokinetics of, and clinical response to, the addition of leflunomide to methotrexate treatment. The study was approved by each institution's human subjects research committee, and written informed consent was requested and received from all patients in the study.

Patients accepted for the study continued to receive their stable weekly dose of methotrexate, which was given orally on the same day of the week. Because of concern regarding potential toxicity and lack of relevant safety data in animal models, the dosing regimen of leflunomide was modified from that normally used for treatment of RA. A loading dose of leflunomide of 100 mg/day was given for the first 2 days of treatment, rather than the 3 days normally used. Thereafter, 10 mg of leflunomide was given orally each morning. After 3 months of treatment, physicians could increase the dosage of leflunomide to 20 mg/day in patients with continuing active disease. In the event of toxicity, the dosage of leflunomide could be reduced to 10 mg every other day or the drug could be discontinued. If medication had to be discontinued for reasons of poor tolerability, subjects received 3 doses of 8 gm cholestyramine in a 24-hour period and 5–15 mg of leucovorin in 1 dose if methotrexate had been administered within 36 hours of the incident. Primary outcome was assessed after 1 year, and patients exhibiting clinical benefit were allowed to continue the therapy.

Drug concentration measurements. The pharmacokinetics of leflunomide and methotrexate during combination therapy were studied in all of the patients at 1 center (n = 12). Blood samples were collected and analyzed for plasma concentrations of the metabolites of leflunomide and methotrexate at baseline and at weeks 6, 12, and 24. Baseline levels of methotrexate were determined prior to administration of leflunomide, utilizing 8 blood samples obtained over an 8-hour period immediately following the weekly methotrexate dose. Leflunomide is a prodrug, and its active metabolite is A77 1726. Plasma concentrations and pharmacokinetics of A77 1726 were determined from blood samples obtained on day 3 (following the 2-day loading dose) and at weeks 6, 12, and 24.

Venous blood samples obtained before and at intervals of 0.5, 1, 2, 3, 4, 6, 8, and 24 hours after morning drug dosing were used for the pharmacokinetic analysis of methotrexate and for A77 1726. All subjects fasted overnight and were allowed to eat between the 2-hour and 3-hour blood samplings. A 24-hour urine sample was also collected. All blood and urine samples were analyzed for methotrexate concentration by fluorescence polarization immunoassay and for A77 1726 by high-pressure liquid chromatography.

The plasma concentrations of methotrexate and A77 1726 in each individual were plotted as a function of time, and the area under the curve (AUC) over 8 hours was calculated for each. AUC from 8 hours to 24 hours was determined using the log trapezoidal rule or Simpson's approximation. AUC from the last measured concentration to infinity was determined by dividing the concentration of methotrexate or A77 1726 in the final blood sample by the terminal elimination rate constant. This rate constant was calculated with a nonlinear curve-fitting program (RSTRIP; Micromath, Salt Lake City, UT). AUC from time 0 to infinity was calculated as the sum of AUC from 0 to 24 hours and AUC from 24 hours to infinity.

Clinical and outcome measures. Safety assessments were performed every 2 weeks for the first 8 weeks and once every 4 weeks thereafter. Measurements of RA-related clinical parameters were performed on the same schedule, except that they were not obtained at week 6. To eliminate examiner variability, clinical measurements for each patient were performed by the same rheumatologist throughout the study.

Safety assessment included a complete medical history at the baseline visit and a complete physical examination before the study and at weeks 24 and 52 or as clinically indicated. Vital signs and weight were recorded at each visit. A 12-lead electrocardiogram and chest radiographs were obtained at baseline and at week 52 or as clinically indicated. Pill counts were performed to monitor compliance.

Laboratory tests consisting of hematologic measurements, serum chemistry studies, and urinalysis were performed at each visit. If a plasma liver enzyme level was ≥5 times the upper limit of normal and this was confirmed on retesting, leflunomide was discontinued immediately and cholestyramine administered. If plasma liver enzyme levels were 2–5 times the upper limit of normal and this was confirmed on retesting within 72 hours, the dosage of leflunomide was halved. Levels

Table 1. Pharmacokinetic parameters for methotrexate in 12 rheumatoid arthritis patients receiving methotrexate and leflunomide combination therapy*

Parameter	Baseline (n = 10)	Week 6 (n = 12)	Week 12 (n = 11)	Week 24 (n = 9)	*P*
C_{max} (μM/mg)	0.049 ± 0.01	0.052 ± 0.01	0.049 ± 0.01	0.050 ± 0.02	0.436
T_{max} (hours)	1.74 ± 0.85	1.83 ± 0.83	1.63 ± 0.83	1.56 ± 0.73	0.870
AUC (hours × μM/mg)	0.24 ± 0.07	0.28 ± 0.06	0.25 ± 0.09	0.25 ± 0.1	0.174

* Measurements were obtained before (baseline) and 6, 12, and 24 weeks after addition of leflunomide to the methotrexate regimen. C_{max} = maximum plasma concentration; T_{max} = time of maximum concentration; AUC = area under curve for drug concentration versus time.

were checked again after 7–14 days and, if they remained at >3 times the upper limit of normal, the patient was withdrawn from the study.

The rheumatologic assessment included determination of the tender and swollen joint count based on evaluation of 28 joints (11). Both patient and physician global assessments of disease activity were measured with a visual analog scale (VAS) of 0 (very well) to 10 cm (very poor). Patient assessment of pain intensity was also measured with a VAS of 0 (no pain) to 10 cm (severe pain). Functional disability was measured with a Modified Health Assessment Questionnaire (M-HAQ) (12) that rated functional disability, as reflected in the performance of 8 daily activities, from 0 (no difficulty) to 3 (unable to perform). The ESR was also determined during each visit.

The primary efficacy variable of this study was the number of positive clinical responses defined by ACR criteria for response (13). By ACR criteria, a responder is a patient who has a ≥20% improvement in both tender and swollen joint count and in at least 3 of the following 5 criteria: patient global assessment, physician global assessment, pain intensity, M-HAQ, and ESR. Patients were also assessed to determine if they met the criteria for clinical remission of RA (14). Safety was evaluated based on laboratory assay results and the occurrence of adverse events.

All subjects who were enrolled in the study and received at least 1 dose of leflunomide were included in the intent-to-treat analysis of efficacy through week 52. If a patient dropped out of the study prior to week 52, data from the last observation were carried forward in the analysis.

Statistical analysis. All analyses were performed in the intent-to-treat population of 30 patients. Descriptive statistics of all variables were calculated and are presented as means and standard deviations. RA assessment parameters before and after treatment were compared by paired *t*-test or, if the data were not normally distributed, by Wilcoxon's signed rank test. *P* values less than 0.05 were considered significant.

RESULTS

Demographics and baseline clinical characteristics. A total of 30 subjects, 23 women and 7 men, were enrolled in the study. The mean ± SD age of the subjects was 52.4 ± 10.8 years, and the mean duration of RA was 13.6 ± 8.7 years. Over the course of their disease, subjects had been treated unsuccessfully with an average of 2.9 DMARDs, not including methotrexate. NSAIDs were being used by 63% of the subjects and prednisone (≤10 mg/day) by 67%. At baseline, the patients had a mean of 16.9 ± 7.8 tender joints and 16.3 ± 6.1 swollen joints despite methotrexate therapy.

Dosing and compliance. Prior to addition of leflunomide, subjects were receiving a mean ± SD of 17.2 ± 3.9 mg of methotrexate per week. The mean methotrexate dosage remained stable throughout the study as required by the protocol.

The dosage of leflunomide at the beginning of the study was 10 mg/day following a 2-day 100-mg/day loading dose. Twelve subjects (40%) continued with this dosage throughout the study. The dosage of leflunomide was increased to 20 mg/day in 16 subjects (53%) who failed to respond adequately and was transiently reduced to 10 mg/every other day due to toxicity in 2 subjects (7%). Subject compliance with leflunomide treatment was calculated by comparing the number of tablets returned by subjects with the number dispensed. Compliance was at least 90% for 97% of the subjects.

Pharmacokinetic measurements. Pharmacokinetic parameters of methotrexate and leflunomide were measured at baseline and at weeks 6, 12, and 24 after initiation of leflunomide treatment, in all 12 subjects at 1 clinical site. For methotrexate, the results of these analyses are shown in Table 1. There were no significant differences in the pharmacokinetic parameters of methotrexate at any time during leflunomide administration, compared with baseline (prior to leflunomide administration).

Mean values of maximum plasma concentration (C_{max}) of A77 1726, the active metabolite of leflunomide, are shown in Figure 1. The C_{max} for A77 1726 was stable throughout the 24 weeks of the pharmacokinetic study (despite the background methotrexate) and was comparable with reported values in unpublished phase II clinical trials of patients receiving leflunomide alone.

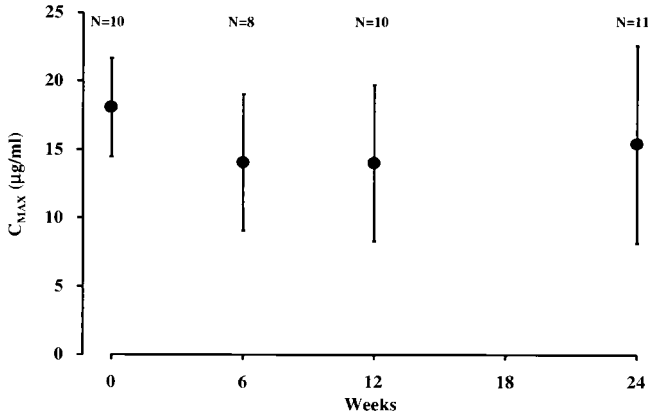

Figure 1. Mean values of maximum plasma concentration (C_{MAX}) for A77 1726, the active metabolite of leflunomide, in 12 patients with rheumatoid arthritis. Measurements were obtained before (time 0) and 6, 12, and 24 weeks after addition of leflunomide to the methotrexate regimen. The C_{MAX} for A77 1726 did not change significantly during the 24-week treatment period.

Safety. Twenty-seven subjects received combination therapy with methotrexate and leflunomide for at least 24 weeks, 25 subjects for at least 40 weeks, and 23 subjects for the full 52 weeks of the study. One patient was lost to followup and 1 withdrew from the study voluntarily. Three subjects withdrew because of adverse events and 2 because of lack of efficacy.

Combination therapy was generally well tolerated. Adverse events occurring in 2 or more patients are shown in Table 2. During the study, 155 adverse events were noted in 29 of the 30 patients. The most common adverse events were gastrointestinal (diarrhea, nausea, stomatitis) and respiratory (cough, dyspnea, infection). Alopecia and rash were also noted in 10% or more of the subjects and urinary tract infections in 13%. Most of the adverse events (152 of 155) were rated by the investigator as mild to moderate in severity.

There were 3 serious adverse events as defined by the protocol. One subject was inadvertently given an overdose of study medication and was treated with ipecac and cholestyramine. This subject continued in the study after 1 day. A second subject was hospitalized for esophagitis after 52 weeks of study. The subject recovered with treatment and the event was not considered to be related to the study medication. The third subject developed significant liver enzyme elevations during treatment with leflunomide and was eventually withdrawn from the study (see below).

No clinically noteworthy hematologic abnormalities were noted during treatment with methotrexate and leflunomide. There were also no clinically noteworthy changes in renal function.

The most common laboratory abnormality noted was asymptomatic elevation of plasma liver enzyme levels. Three subjects were withdrawn from treatment because of persistent elevation of plasma liver enzyme concentrations. The only serious adverse event reported during the trial that was considered to be drug related was noted in a 49-year-old woman. Plasma liver enzyme levels in this patient increased to 2 times the upper limit of normal within 3 weeks after initiation of leflunomide. When levels rose to >5 times the upper limit of normal, leflunomide was temporarily withdrawn, and at the next visit, transaminase levels were 2 times the upper limit of normal. When leflunomide was restarted, the patient's transaminase levels increased to >6 times the upper limit of normal. At this point, both methotrexate and leflunomide were discontinued and cholestyramine (3 8-gm doses over a 24-hour period) was administered as per protocol. Liver enzyme levels normalized within 25 days of treatment withdrawal, and the patient was withdrawn from the study. This patient subsequently began taking methotrexate again without recurrence of liver enzyme elevations.

Table 2. Adverse events among rheumatoid arthritis patients receiving methotrexate and leflunomide combination therapy (n = 30)*

Event	Mild, no. (%)	Moderate, no. (%)
Back pain	2 (7)	1 (3)
Hypertension	2 (7)	1 (3)
Stomatitis	3 (10)	1 (3)
Mouth ulcer	2 (7)	2 (7)
Diarrhea	6 (20)	4 (13)
Dyspepsia	2 (7)	1 (3)
Nausea	4 (13)	3 (10)
Abdominal pain	2 (7)	1 (3)
Myalgia	2 (7)	1 (3)
Depression	2 (7)	0 (0)
Dizziness	2 (7)	2 (7)
Increased cough	5 (17)	3 (10)
Dyspnea	3 (10)	1 (3)
Respiratory infection	7 (23)	3 (10)
Bronchitis	2 (7)	0 (0)
Rhinitis	5 (17)	1 (3)
Sinusitis	3 (10)	1 (3)
Rash	3 (10)	1 (3)
Alopecia	6 (20)	1 (3)
Subcutaneous nodules	2 (7)	0 (0)
Conjunctivitis	2 (7)	0 (0)
Urinary tract infection	0 (0)	4 (13)

* All adverse events experienced by 2 or more patients are shown.

Table 3. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) elevations >1.2 times the upper limit of normal (ULN) among rheumatoid arthritis patients receiving methotrexate and leflunomide combination therapy (n = 30)

	AST, no. (%)	ALT, no. (%)
No. with elevation	19 (63)	19 (63)
>1.2 × ULN to ≤2 × ULN	12 (40)	8 (27)
>2 × ULN to ≤3 × ULN	5 (17)	6 (20)
>3 × ULN to ≤8 × ULN	2 (7)	5 (17)
Discontinued treatment due to transaminase elevations*	3 (10)	3 (10)
Reversed to ≤1.2 × ULN at end point	12 (40)	13 (43)

* Total discontinued = 3.

Table 3 provides information on the subjects with normal baseline values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in whom levels of either of these enzymes became elevated to >1.2 times the upper limit of normal during treatment with methotrexate and leflunomide. Plasma levels of AST and ALT were elevated above the upper limit of normal in 19 subjects each. In 70% of the cases of liver enzyme elevations, levels reversed to <1.2 times the upper limit of normal without dosage reduction of leflunomide.

Three patients who had normal liver enzyme levels while receiving methotrexate alone had repeated elevations of serum transaminase levels over the 12-month treatment period in this study. These patients met the criteria for liver biopsy based on ACR guidelines for methotrexate monitoring (15). Liver biopsy showed no evidence of marked fibrosis or cirrhosis; 2 biopsies were scored as Roenigk grade IIIA (mild fibrosis) and 1 as grade I (normal). All of these patients continued to take methotrexate and leflunomide after biopsy, and their liver enzyme levels normalized in the second year of treatment.

Efficacy of combined treatment with methotrexate and leflunomide. Due to the open nature of this study, efficacy was not a primary outcome. Clinical response was measured in terms of the number of patients who met ACR 20% response criteria (Figure 2). After 1 month of leflunomide treatment, 20% of the subjects met ACR 20% response criteria. The percentage of ACR 20% responders increased with time and peaked at 57% after 9 months of treatment. This percentage remained relatively constant for the remainder of the 12-month study period. After 1 year of treatment, 2 patients met the ACR criteria for remission.

DISCUSSION

The observation that a large number of rheumatoid arthritis patients do not respond adequately to DMARD monotherapy has led many clinicians to resort to combinations of drugs, particularly methotrexate plus another DMARD, for the treatment of refractory disease. Early clinical trials of combination therapy with methotrexate gave disappointing results, perhaps due to lower-than-optimal dosing of methotrexate (16,17). Recently, however, 2 randomized, double-blind, controlled trials have yielded more positive results indicating that the combination of sulfasalazine, hydroxychloroquine, and methotrexate, or the combination of cyclosporin A and methotrexate, can result in significant benefit in terms of improvement of the symptoms of RA (18,19). The increased efficacy of combination therapy was achieved with little or no increase in the number or severity of adverse events.

We chose to examine pharmacokinetics, safety, and clinical response following addition of leflunomide to methotrexate treatment in patients with RA that had failed to respond adequately to methotrexate alone. This combination was chosen based on the complementary mechanisms of action of these 2 drugs. The primary action of leflunomide is to inhibit de novo pyrimidine biosynthesis and thus limit proliferation of activated T lymphocytes (1–4). Methotrexate, on the other hand, appears to act through multiple mechanisms, including inhibition of purine biosynthesis, inhibition of cellular

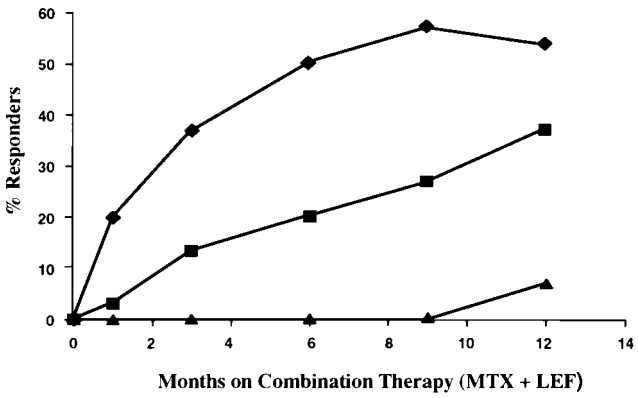

Figure 2. Percentage of patients with rheumatoid arthritis who met American College of Rheumatology criteria for 20% response (diamonds) and 50% response (squares) and for remission (triangles), as a function of the number of months receiving methotrexate and leflunomide (MTX + LEF) combination therapy. The percentage of 20% responders peaked after 9 months and remained constant thereafter, while the percentage of 50% responders continued to increase throughout the study period.

synthesis of polyamines, modulation of cytokine activity, promotion of adenosine release, and promotion of apoptosis of activated T cells (8,9).

One major objective of this study was to determine whether there were pharmacokinetic interactions between leflunomide and methotrexate. In a subset of patients in whom this was analyzed, there were no changes in the pharmacokinetic parameters of methotrexate or A77 1726, the active metabolite of leflunomide, over the course of the study. Measurement of the pharmacokinetic parameters of A77 1726 in the absence of methotrexate was not possible in this study because, based on the trial design, all patients had been continuously receiving methotrexate prior to beginning leflunomide treatment. However, the maximum concentrations of A77 1726 following dosing did not change significantly over the course of the study and were similar to values seen in patients receiving leflunomide alone in unpublished studies. These observations suggest that there is minimal interference of each drug with the metabolism of the other.

The combination of methotrexate and leflunomide also appeared to be reasonably well tolerated. Of the 30 patients who began the study, 23 (77%) completed the full year of combined treatment, and only 1 serious drug-related event, a significant elevation of serum transaminase levels, was reported. The most commonly reported clinical adverse events, mild-to-moderate gastrointestinal and respiratory symptoms and abnormalities on liver function tests, are similar to those reported with leflunomide or methotrexate monotherapy (1,20).

The most noteworthy adverse event observed during combination therapy with methotrexate and leflunomide was elevation of serum transaminase levels. Elevated transaminase levels were not seen with methotrexate alone, and became evident only following the addition of leflunomide to methotrexate. Three patients were withdrawn from the study by protocol due to persistent elevation of serum transaminase levels. A separate group of 3 patients met ACR guidelines for liver biopsy due to transaminase elevations (15) during the course of the study. Two of the biopsies revealed only mild fibrosis (Roenigk grade IIIA), and 1 biopsy result was normal. It should also be noted that a Roenigk grade IIIA biopsy result corresponds to a degree of fibrosis that is not considered to represent clinically significant disease and would not be an indication for discontinuation of methotrexate treatment (15). Both individuals continued to take leflunomide and methotrexate and have not had any further abnormalities in plasma transaminase levels 1 year post-biopsy.

The efficacy results of this open-label study suggest that the combination of methotrexate and leflunomide provides a potentially beneficial clinical response with acceptable tolerability. However, it should be stressed that interpretations of the efficacy data are limited by the open nature of this pilot study.

In summary, the findings of this small open-label study suggest that the combination of methotrexate and leflunomide may be useful for the treatment of RA patients who have an inadequate response to methotrexate alone. The occurrence of elevated liver enzyme levels with this drug combination is of concern, however. Because the overall risk of serious liver damage when methotrexate and leflunomide are used together is unknown, careful dose titration and patient monitoring will be necessary when this combination is used. A randomized trial to definitively establish the efficacy and safety of the methotrexate/leflunomide combination is currently in progress.

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