MATERIALS AND METHODS

Study Design The study was designed as a two arm, randomized, double blind, placebo controlled clinical trial of UDCA plus methotrexate (MTX) versus UDCA plus placebo. The primary measure of treatment outcome was transplant free survival as measured by the distribution of time to transplant or death from all causes, whichever comes first. Secondary endpoints included comparison of treatment arms with respect to overall survival, time to clinical decompensation (development of ascites, hepatic encephalopathy, variceal bleeding, transplant, or death), development of varices, changes in biochemical tests, liver histology, and symptomatolgy and sense of well being. The study design was reviewed and approved by the Institutional Review Boards at each of the clinical centers.

Patient Selection Clinical investigators at 12 geographically diverse clinical centers in the U.S.A. (see appendix) screened 535 patients with PBC for possible entry into our treatment trial. During this screening period, patients' clinical records were reviewed and various clinical, laboratory, radiology, and pathology tests were performed to assure (1) that patients would satisfy our inclusion criteria for PBC; (2) that they had not already demonstrated exclusion criteria with would keep them from qualifying for the methotrexate/placebo phase, and (3) if eligible for the trial, that they would proceed to the next steps.

The intention was to study the effect of methotrexate on the progression of PBC in 20 to 69 year old patients of either sex and any race and with only moderately advanced disease at study entry. For documentation of sufficiently advanced PBC, patients were to have had a diagnosis of chronic cholestatic liver disease of at least 6 months duration, documented history of a positive antimitochondrial antibody test and alkaline phosphatase levels at least 1.5 times the upper limit of normal at their clinical center, and a liver biopsy within the 6 months prior to randomization (and on UDCA at

least 6 months) with histologic findings compatible with the diagnosis of PBC. To be judged adequate for staging of disease, the liver biopsy must have been at least 2 cm long if cirrhosis was not detected. Asymptomatic patients must have had a histologic stage greater than Stage I using the Ludwig classification. Patients could not have markedly advanced PBC, and thus patients ever having a history of serum bilirubin of 3.0 mg% or greater, a serum albumin less than 3.0 mg%, or a history of ascites, hepatic encephalopathy, or variceal bleeding were not eligible for randomization. As indicated in Table 1, at screening 393 of the 535 patients were judged to meet the defined inclusion criteria.

As also shown in Table 1, patients were excluded from the study if they had clinical, serologic, or histologic evidence of liver disease of other etiology, had a history of alcohol abuse within the two years prior to study enrollment, were treated with immunosuppressive agents, rifampin, or dilantin in the months preceding randomization, had a history of malignant disease, were HIV positive, had other major illnesses that could limit life span, or were pregnant or unwilling to use adequate forms of birth control to avoid pregnancy. Of the 385 patients meeting the screening inclusion and exclusion criteria, 300 patients progressed to a pre-entry evaluation phase during which they were treated with UDCA alone at a dose of approximately 15 mg/kg/day. At the end of this UDCA phase, the patients were again screened for meeting the inclusion and exclusion criteria given above, as well as for having an acceptable hematologic profile, adequate renal and pulmonary function, no radiologic or ultrasound evidence of biliary obstruction, and a liver biopsy within the last 6 months consistent with a diagnosis of primary biliary cirrhosis.

Table 1. Number of 535 screened patients satisfying inclusion and exclusion criteria.

All screened patients		535
Screening inclusion criteria		
Chronic cholestatic liver disease of at least 6 months duration	526	
Documented positive antimitochondrial antibody test	490	
Documented history of serum alkaline phosphatase levels at least 1.5 x ULN	494	
No history of serum bilirubin 3.0 mg % or greater	496	
Serum albumin 3.0 g % or greater	495	
No history of ascites, variceal bleeding, or hepatic encephalopathy	501	
Patients satisfying all screening inclusion criteria		393
Screening exclusion criteria (among those satisfying inclusion criteria):		
No clinical, serologic, or histologic evidence of liver disease of other etiology (e.g., chronic hepatitis B or C, autoimmune chronic active hepatitis, alcoholic liver disease, sclerosing cholangitis, drug-induced liver disease, symptomatic or obstructive gallstones)	391	
No history of alcohol abuse within past 2 years	393	
No treatment with immunosuppressive agents (e.g., azathioprine, chlorambucil, colchicines, corticosteroids, or d-penicillamine in the preceding 3 months; or with cyclosporine, FK-506, or methotrexate in the preceding 6 months)	381	
No treatment with rifampin in the preceding 6 months	391	
No treatment with dilantin	392	
No history of breast cancer or melanoma and no history of any other malignant disease (except basal cell skin cancer) within the past 5 years	391	
Documented HIV negative	392	
No other major illnesses that could limit life span	391	
If female, not pregnant and willing to use adequate birth control	392	
Patients satisfying all screening inclusion and exclusion criteria		385
Screen eligible patients signing consent and entering UDCA phase		300
Additional requirements for randomization		
Treated with UDCA (15 mg/kg) for at least 6 months	300	
Liver biopsy within last 6 months compatible with diagnosis of PBC	288	
If asymptomatic, liver biopsy stage > 1	293	
Ultrasound, CT, or cholangiography ruling out biliary obstruction	294	
Biliary: Bilirubin < 3 mg %	297	
Hematologic: WBC > 2,500; ANC > 1,500; Plt > 80,000	292	
Pulmonary: FVC > 50% predicted; DLCO > 50% predicted	293	
Renal: Creatinine clearance 60 cc per minute per 1.73 meters sq	289	
No history of ascites, variceal bleeding, or hepatic encephalopathy	297	
No clinical, serologic, or histologic evidence of liver disease of other etiology	298	
No treatment with immunosuppressive agents	299	
No treatment with rifampin in the preceding 6 months	299	
Patients randomized to UDCA-MTX or UDCA-Placebo Includes 10 patients granted exceptions to exclusion criteria despite: no liver biopsy in last 6 months (1 patient on MTX, 1 patient on placebo), percent predicted DLCO of 45% (1 patient on MTX arm), or creatine clearance between 50 and 60 cc per minute per 1.73 meters sq (5 patients on MTX arm, 2 patients on placebo arm)		265

Randomization Between January, 1994 and March, 1998, 265 subjects who signed

informed consent documents were randomized with equal probability in a double blind fashion to receive UDCA plus MTX (132 patients) or UDCA plus placebo (133 patients). Ten patients who failed to meet only one of the eligibility criteria were reviewed by the study principal investigator, and were judged suitable for randomization despite no liver biopsy within the last 6 months (1 patient on the MTX arm whose biopsy was 9.2 months prior to randomization and 1 patient on the placebo arm whose biopsy was 9.7 months prior to randomization), a percent predicted DLCO of 45% (1 patient on the MTX arm), or creatinine clearance between 50 and 60 ml / min / 1.73 meter sq (5 patients on the MTX arm and 2 patients on the placebo arm). In addition, in later, post-randomization review of medical records, two patients on the MTX arm were found to have had previous bilirubin measurements of 5.3 and 7.9 mg/dl.

Randomization was stratified according to histologic stage of liver disease according to the classification of Ludwig, et al. and as read by pathologists at the individual clinical centers. Of 126 patients initially reported to be stage 1 or 2, 62 were randomized to receive MTX and 64 to receive placebo, and of 139 patients initially reported to be stage 3 or 4, 70 were randomized to receive MTX and 69 to receive placebo. Two patients judged as stage 3 by the pathology reports at their respective clinical centers were erroneously randomized with the stage 1-2 group. In keeping with the principles of analysis by intention-to-treat, these patients were kept in the stage 1-2 group for all statistical analyses.

Drug Treatment All patients received UDCA in 300 mg capsules provided by Ciba-Geigy and, subsequently, Novartis, in a single dose of 13-15 mg/kg/day taken orally at bedtime. In addition, methotrexate or its placebo, provided as 2.5 mg tablets by Lederle Laboratories initially, then Wyeth-Ayerst Laboratories, was administered orally once a week in a single dose at bedtime. The initial dose was one-half of the maximum dose and was increased each month by 2.5 mg per week to the maximum dose of 15 mg per

1.73 m^2 body surface area, with a maximum dose of 20 mg per week, provided toxicity was absent or mild. Patients taking cholestyramine or colestipol were asked to take the medication at least 2 hours before or after intake of UDCA and methotrexate or its placebo. Patients were to be continued on UDCA along with methotrexate or its placebo until the closure of the study despite progression of disease unless liver transplantation or death without transplantation ensued, drug toxicity necessitated withdrawal, the patient developed a cancer, or voluntary withdrawal ensued.

Modification of Methotrexate Dose Because there is no current evidence that UDCA affects blood elements or induces side effects other than diarrhea in a small number of patients, the development of a cytopenia, of mucositis, significant nausea or anorexia were initially considered to be related to methotrexate, and methotrexate dose was altered in accord with the following rating for the common side effects and bone marrow toxicity of methotrexate (Table 2). Toxicity was rated as either mild (acceptable), moderate (requiring alteration of dose), or severe (requiring discontinuation of therapy).

For moderate toxicity, weekly dosage was reduced by a quarter or a third, and the toxicity was monitored weekly until resolved. The dosage of methotrexate was then increased by 2.5 mg per week until a dose of 2.5 mg less than the original toxic dose was reached, provided toxicity did not recur. Return to the original dose at which toxicity occurred was attempted carefully.

For severe toxicity, methotrexate was stopped completely while the toxic reaction was being managed. Gastrointestinal and hematologic findings usually improve fairly rapidly. Once better, methotrexate was to be restarted at half the toxic dose, and then increased 2.5 mg per week at monthly intervals provided toxicity did not recur, until a weekly dose 2.5 mg less than the original toxic dose was reached. If recurrent toxicity was not observed, cautious increase to full dose was attempted.

If severe toxicity did not improve within a week or two, or if it was judged to be life

threatening, leucovorin factor was to be administered at a dose of 5 mg po or IV every 12 hours for at least 48 hours in order to facilitate recovery. Controversy exists about duration and dosage of leucovorin factor in this type of toxicity.

Other reasons listed in the protocol for decreasing the dose or stopping methotrexate included the appearance of allergic reactions, severe skin rash, pulmonary symptoms or chest x-ray findings suggestive of pulmonary fibrosis, severe exacerbation of liver disease (as judged by liver biopsy histology or by prothrombin time, serum bilirubin and/or albumin levels), and worsening of renal function. Methotrexate was to be withdrawn if evidence of alcohol abuse arose or if the patient became pregnant or would no longer practice birth control. Study medication was stopped in patients developing a cancer.

Dose modifications could be carried out without the local investigator breaking the medication code, since in all instances dosage would be temporarily reduced or stopped. Nevertheless, when deemed necessary by our external safety monitors, the treatment code could be broken for their use in assisting with the management of our patients.

Schedule of Patient Visits and Investigations According to the study protocol, patients were to be seen and have blood drawn at weeks 2 and 4, then monthly for the first 6 months, bimonthly for the next 6 months, then at 3 month intervals for the duration of the study. Blood was to be drawn one week after the preceding dose of methotrexate and on the day of, but preceding the next dose of methotrexate. Symptoms of liver disease and of potential toxicity were to be assessed at each visit by history and with the aid of a diary. At each visit, blood was to be obtained for a CBC, differential and platelet count; at the monthly and each later visit for bilirubin, alkaline phosphatase, AST and ALT; at 3 monthly intervals for total protein and albumin, and at 6 monthly intervals for prothrombin time (INR). Complete histories, physical examinations, chest x-rays and pulmonary

function studies, including measurements of diffusing capacity (DLCO) were to be obtained at least annually. Patients were to have a liver biopsy and upper endoscopy after 24 months on methotrexate or its placebo, and subsequently at additional intervals of 2 years.

Evaluation of Compliance Patients were given known quantities of medicine at appropriate intervals and instructed in how to keep a log of medicine intake. The log was checked, and unused medicine counted at appropriate return visits, and before a new supply of medicine was given to the patient. The log and pill counts were kept in the permanent record for each patient.

Evaluation of Adverse Experiences The adverse experiences reported by patients during their study visits were grouped within broad categories defined by organ system. In addition, adverse experiences were categorized across all organ systems infections, bleeding events, neoplasms events, and cancers. Serious adverse experiences occurring at any clinical center were reported to a central committee monitoring such events.

Evaluation of Treatment Response The primary and several secondary measures of treatment outcome were based on the distribution of time to treatment failure as defined by a hierarchy of clinical and subclinical outcomes. Times to death, transplant, activation for transplant, and clinical deterioration as defined by development of variceal bleeding, hepatic encephalopathy, ascites, or disabling pruritus were obtained from the routine follow-up of patients. Subclinical deterioration was defined as a doubling of serum bilirubin from baseline to at least 2.5 mg/dl, a decrease in serum albumin to a level less than 2.5 g/dl, or an increase in PTINR to 1.3. In order to be judged a subclinical deterioration, the corresponding threshold must have been exceeded on two consecutive clinic visits.

Liver histology was evaluated as the average stage and fibrosis scores on liver

biopsies obtained every two years and scored independently by a panel of 5 pathologists in a central core. Development of varices was evaluated by endoscopies performed every two years according to the study protocol. Because patients with varices at screening were eligible for randomization, only a subset of the trial participants were evaluable for the endpoint of development of new varices.

Patients who terminated study treatment prior to liver transplant or death were encouraged to continue all regular clinic visits, and patients who agreed thus considered evaluable for all measures of treatment response. Some patients declined to have further biopsies, endoscopies, and/or serum chemistries measured, but were willing to be followed for clinical events, and these patients are considered fully evaluable for all endpoints that could be observed without invasive procedures. Patients who withdrew consent for all further follow-up contribute information only up to the time of their withdrawing their consent to be studied, although as described in the statistical methods, exploratory analyses imputed missing measurements for these patients.

Monitoring of the Clinical Trial The accruing data were monitored on a semiannual basis by an independent Data Safety and Monitoring Board (DSMB), who reviewed the data for safety, as well as making recommendations for early termination of the trial for reasons of demonstrated efficacy of methotrexate over placebo or for reasons of the inability to demonstrate a statistically credible, clinically important benefit. In making such a recommendation for early termination of the trial, the DSMB was guided by a formal stopping rule as described in Statistical Methods. Study procedures called for the DSMB to remain blinded to treatment assignment, unless specific safety issues arose that necessitated their becoming unblinded. Hence, at each of their meetings, the DSMB was provided with statistics broken down by study arm, but labeled only by treatment A or B. In the conduct of the study, the DSMB remained blinded until the formal interim analysis at which study termination was recommended.