Biost 578 B: Introductory Applied Data Analysis

Emerson, Spring 2005

Homework #6

April 27, 2005

Written answers to the homework are to be handed in at the beginning of class Wednesday, May 4, 2005. (There will be no class on Monday, May 2, 2005.)

You are asked to prepare a statistical analysis detailing the analyses you would use to describe

1. Duration of the clinical study (i.e., length of time(s) patients were observed during follow-up).
2. Patient disposition over the course of the clinical trial (e.g., number of patients who died, who received a liver transplant, who discontinued follow-up, etc.)
3. Patient compliance with study drug over the course of the clinical trial.
4. Patient adherence to clinic visits and study procedures.

As a part of this analysis, prepare graphs of timelines for individual patients in which you indicate

1. Times that each patient was taking study drug. Differentiate between patients who stopped study drug per protocol and patients who stopped study drug due to their own personal preference.
2. Times that each patient permanently discontinued study drug but continued to be followed with clinic visits.
3. Times that each patient discontinued regular visits but consented to be followed for vital status (deaths, transplants).
4. Times of death or transplant for each patient, if observed.
5. Time between randomization for each patient and the date that the DSMB terminated the study on November 1, 2002.
6. Times that each patient has valid (nonmissing) laboratory data, endoscopy data, and biopsy data. You may choose to identify with which scheduled visit each measurement is labeled. Alternatively, you might consider providing descriptive statistics

Answer:

This key includes a general discussion of possible approaches, followed by relevant excerpts from the report I sent to the Principal Investigator. Note that I included parts that do not relate to this homework assignment (Materials and Methods, including analyses related to screening for accrual). I did not include all the Materials and Methods related to the analyses of endpoints.

A separate S-Plus script file containing some of the code that might be used is also available.

I addressed these question in several ways. First, I described the distribution of potential censoring times if no patient dropped out early. This was defined as the time from randomization until the study was stopped on November 1, 2002. Note that we know this data exactly, so there is no need to do anything beyond the usual descriptive statistics for complete data.

I also considered patients who stopped clinic visits early. Hence, I considered time to stopping the regular visits, as well as time to stopping follow-up for vital status. I am trying to assess when I lost track of patients. This is primarily a question of study quality. If very many patients are not followed according to protocol, I have to wonder how representative the data that I do have is. I note that deaths and liver transplantation are censored observations: I do not know when a subject who died might have stopped coming to clinic. Hence I estimate probabilities using Kaplan-Meier estimates, and I compare treatment groups either by those estimates or by a logrank test.

Similar analyses were used to assess the time until stopping study drug. It was also important to try to assess reasons for stopping study drug. I categorized the text reasons that were given. I also provided some more exploratory analyses addressing the question of whether stopping study drug was indicative of impending failure.

EXCERPTS FROM REPORT TO PRINCIPAL INVESTIGATOR

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 (B. Combes), 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 (PTID 7519) and 1 patient on the placebo arm whose biopsy was 9.7 months prior to randomization (PTID 4021)), a percent predicted DLCO of 45% (1 patient on the MTX arm (PTID 2005)), or creatinine clearance between 50 and 60 ml / min / 1.73 meter sq (5 patients on the MTX arm (PTID 5013, 7006, 7008, 7516, 8001) and 2 patients on the placebo arm (PTID 4090, 5017)). 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.

In their review of the data on October 31, 2002, the DSMB recommended termination of the study for reasons of futility: The estimate of treatment effect at that interim analysis was such that the hypothesized treatment effect for which the study was powered had been ruled out with high confidence. On November 1, 2002, clinical centers began advising patients to discontinue study drug. At the time of such notification, patients were asked to continue clinical visits for monitoring of clinical progression and laboratory measurement of blood chemistries. The more invasive study procedures of liver biopsy and endoscopy were no longer performed. It was hoped that such monitoring would continue for at least one year post discontinuation of study drug to observe the effect of withdrawal of MTX therapy.

Stopping Rule and Sample Size The clinical trial was conducted using a group sequential stopping rule. In November, 1997, prior to any formal interim analyses and when only two transplants and one death had been observed, the DSMB and investigators adopted a stopping rule based on a level .025 one-sided group sequential test with O'Brien-Fleming boundary relationships for the efficacy endpoint and a futility stopping boundary corresponding to a choice of boundary shape

parameters P=0.8, R=0, A=0 within the unified family of group sequential designs (Kittelson JM and Emerson SS, A unifying family of group sequential test designs. *Biometrics* 55:874-882, 1999). Such a stopping rule preserves the nominal one-sided type I error at .025 (corresponding to a two-sided level 0.05 test) while allowing early stopping either for strong evidence of efficacy of methotrexate or for the futility of further continuation of the trial in the absence of evidence for sufficient benefit due to methotrexate. It was planned to continue the trial until 50 primary events had been observed, unless stopping was recommended by our Data Safety Monitoring Board (DSMB) as guided by the specified stopping rule. Using the sampling plan suggested by this stopping rule, the planned maximal sample size of 50 transplant or death events would provide 80%, 90%, 95%, and 97.5% statistical power to detect a benefit of methotrexate therapy corresponding to hazard ratios of 0.45, 0.39, 0.35, and 0.32, respectively. Hence, a failure to reject the null hypothesis can be interpreted as having 95% confidence that the true treatment effect was less than would correspond to a 68% decrease in risk of transplant or death. At the time of study design, a hazard ratio of 0.32 was estimated to correspond roughly to five year transplantation-free survival rates of 83% on placebo and 94% on methotrexate.

Formal interim analyses were to be performed according to the funding cycle of this NIH sponsored study. At the first such competing renewal, the number of events was so small as to make early termination virtually impossible. In October, 2002, the second renewal period was drawing to a close with a total of 30 transplant or death events observed. Implementation of the stopping rule was effected using the constrained boundary approach (Burington BE and Emerson SS, Flexible implementation of group sequential stopping rules using constrained boundaries. *Biometrics* 59: 770-777, 2003). Computation of estimates of treatment effect, confidence intervals, and P values adjusted for the bias introduced by a stopping rule using the bias adjusted mean and the sample mean ordering (Emerson SS and Fleming TR, Parameter estimation following group sequential hypothesis testing. *Biometrika* 77:875-892, 1990). All reported P values are two-sided.

RESULTS

Baseline Characteristics This information is summarized in Table 3. The patients in each treatment arm were well matched. Over 90 percent of the randomized patients were women. Serum bilirubin was 1.0 mg per dl or less in 85 percent, 1.1 to 2.0 mg per dl in 13 percent, and 2.1 to 2.9 mg per dl in only 4 patients (approximately 1.5 percent).

Subject Disposition The interventional aspect of the study was stopped in November, 2002, when the time from randomization was a median of 7.2 years (range 4.6 to 8.8 years). Prior to that time, liver transplants were received by 7 patients on the MTX arm and 7 patients on the placebo arm. Deaths were observed in 7 patients on the MTX arm and 11 patients on the placebo arm. Two of the deaths observed on the placebo arm occurred post-transplantation. Hence a total of 14 and 16 liver transplants or deaths were observed on the MTX and placebo arms, respectively.

Forty-one (41) patients on the MTX arm and 47 patients on the placebo arm discontinued taking study drug prior to the end of the interventional phase and prior to experiencing the primary endpoint of liver transplant or death. Table 4 presents the actuarial estimate of the proportion of patients discontinuing study drug prematurely for any reason.

Table 4. Actuarial (Kaplan-Meier) estimates of the cumulative probability of a patient discontinuing study drug prior to the end of the interventional phase of the study and prior to experiencing the primary endpoint of liver transplant or death.

	Probability of Early Discontinuation of Study Drug (95% CI)		
Years post randomization	**MTX arm**	**Placebo arm**	**P value**
1	6.8% (2.4% - 11.0%)	3.8% (0.5% - 6.9%)	0.27
2	14.4% (8.2% - 20.2%)	12.0% (6.3% - 17.4%)	0.56
3	17.4% (10.7% - 23.6%)	16.5% (10.0% - 22.6%)	0.85
4	19.7% (12.6% - 26.2%)	18.8% (11.9% - 25.2%)	0.85
5	22.0% (14.6% - 28.7%)	22.6% (15.2% - 29.4%)	0.91
6	25.4% (17.5% - 32.6%)	29.2% (20.7% - 36.8%)	0.50
7	31.6% (22.7% - 39.5%)	35.7% (26.2% - 43.9%)	0.51
8	34.5% (24.9% - 42.9%)	42.7% (31.5% - 52.1%)	0.24

By the seventh year post-randomization, approximately one-third of patients on both arms discontinued study treatment, with no statistically significant differences between the treatment arms (P = 0.41 in proportional hazards model of time to premature discontinuation of study drug by treatment arm). Many of the patients discontinuing study treatment did so in accordance with

protocol specified modifications of therapy. Table 5 presents the numbers of patients discontinuing treatment early for each of several categories of reasons for early termination.

Table 5. Reasons provided for discontinuing study treatment (MTX or placebo) prior to the end of the inteventional phase and prior to experiencing the primary endpoint of liver transplantation or death.

	MTX (n=132)	Placebo (n=133)
Signs of bone marrow suppression	4	5
Signs/symptoms of gastrointestinal toxicity	5	1
Signs/symptoms of respiratory adverse effects	2	4
Cancer	9	5
Other indications for MTX	0	2
Pregnancy	0	1
PBC progression	5	5
Other medical conditions	3	8
Other signs/symptoms (AEs)	4	2
Study burden	9	14
ANY REASON	**41**	**47**

The overwhelming majority of patients who discontinued their study drug were still followed for occurrence of the study endpoints. Only 11 patients prematurely withdrew consent for follow-up of transplant free survival status: 3 in the MTX arm (PTID 1043, 5001, 8027) and 8 in the placebo arm (PTID 2058, 3051, 5027, 7011, 7045, 7501, 7512, 8025). The cumulative proportion withdrawing from the study in this manner was 1%, 3%, and 4.5% at 1, 2, and 6 years post randomization, respectively, with no statistically significant difference between treatment arms (P = 0.14 from proportional hazards model). Such continued follow-up of patients was crucial, because, as suggested by the data in Table 5, patients who discontinued study treatment early were most often symptomatic, and this was associated with an increased risk of liver transplantation and/or death in the time period immediately following that discontinuation. In an analysis of transplant free survival in which discontinuation of study drug is modeled as a time-varying covariate, patients who discontinued their study drug had a 42-fold higher risk of the primary endpoint (95% confidence interval 14-fold to 124-hold higher, P < 0.001). The actuarial estimate of the cumulative probability of liver transplantation or death during the first year post-discontinuation was 19.2% in the MTX arm

and 18.6% in the placebo arm; the estimated two year failure rates were 25.7% and 28.3%, respectively.

An additional 18 subjects (5 on the MTX arm (PTID 2007, 4014, 6016, 7023, 8035) and 13 on the placebo arm (PTID 2023, 4005, 4054, 4072, 4080, 5002, 6001, 6005, 6010, 6014, 7504, 8002, 8022)) declined to participate in regular clinic visits at some point after discontinuing study drug, but prior to experiencing the primary endpoint of liver transplantation or death and prior to the end of the interventional phase of the study. Observation of clinical events such as death, liver transplantation, bleeding varices, and hepatic encephalopathy was still possible for these patients, but measurements of subclinical progression according to serum bilirubin, serum albumin, or prothrombin time, as well as development of varices and progression of pathologic stage, were not available on these patients following their withdrawal of consent for these investigational procedures. As shown in Table 6, the loss of information in this way was greater for the placebo arm than for the MTX arm (P = 0.015 from proportional hazards model).

Table 6. Actuarial (Kaplan-Meier) estimates of the cumulative probability of a patient discontinuing clinic visits prior to the end of the interventional phase of the study and prior to experiencing the primary endpoint of liver transplant or death.

	Probability of Early Discontinuation of Clinic Visits (95% CI)		
Years post randomization	**MTX arm**	**Placebo arm**	**P value**
1	2.3% (0.0% - 4.8%)	3.0% (0.1% - 5.9%)	0.72
2	2.3% (0.0% - 4.8%)	6.0% (1.9% - 10.0%)	0.13
3	3.1% (0.1% - 6.0%)	7.6% (2.9% - 12.0%)	0.10
4	3.1% (0.1% - 6.0%)	7.6% (2.9% - 12.0%)	0.10
5	3.9% (0.5% - 7.2%)	9.1% (4.1% - 13.9%)	0.09
6	4.8% (1.0% - 8.6%)	14.0% (7.5% - 20.0%)	0.01
7	6.0% (1.5% - 10.3%)	17.5% (10.0% - 24.4%)	0.01
8	8.3% (1.9% - 14.4%)	19.4% (11.1% - 26.9%)	0.03

Following the DSMB's recommendation for early termination of the study on October 31, 2002, the clinical centers advised the 173 patients (89 on the MTX arm, 84 on the placebo arm) still taking their study drug to discontinue that the experimental therapy. The median time between the decision to terminate the study and patients' discontinuation of study drug was 13 days, with only 3

patients continuing to take study medication for more than 6 weeks after the end of the interventional phase of the trial (2 patients on the MTX arm (PTID 1020, 1004) continued for 50 and 87 days after the DSMB recommendation, and one patient on the placebo arm (PTID 9014) continued study drug for 118 days after the DSMB recommendation).

During the post-intervention phase of the trial (i.e., following the October 31, 2002 DSMB recommendation), 110 patients on the MTX arm and 98 patients on the placebo arm consented to continue to be followed in regular clinic visits for an average of 381 days (median 398 days, range 110 to 460 days). An additional 12 MTX patients and 19 placebo patients consented to be followed for liver transplantation and vital status during this period. These numbers include those patients who discontinued study drug and regular clinic visits during the interventional phase of the trial.

Subject Compliance with Prescribed Study Drug The majority of patients in both arms had their study drug stopped at least once (127 in MTX, 123 in placebo). Drug was stopped an average of 1.7 times per patient. More patients in the MTX arm had their drug dose reduced (51 vs 37). Adherence to prescribed drug dose was described both by the average percentage of times when the patient took the full prescribed amount and by the average ratio of amount taken versus amount prescribed. Both criteria suggested a high adherence rate – both arms were fully compliant 93% of the time. MTX patients averaged 97.7% of their prescribed doses, and placebo patients averaged 98.1%. The average number of tablets per week per person taken by the placebo arm was 0.38 more than that by the MTX arm (95% C.I. 0.015, 0.76, p=0.0413).