MOR

Medical Officer's Review

DRAFT

NDA # 50,708
M.O. Review #1

Submission: 7/23/93
Review completed: 6/01/94

Drug name: Tacrolimus

Generic name: Tacrolimus

Research name: FK-506

Proposed trade name: Prograf™

Chemical name: [3*S*-[3*R**[*E*(1*S**,3*S**,4*S**)],4*S**,5*R**,8*S**,9*E*,12*R**, 14*R**,15*S**, 16*R**,18*S**,19*S**,26a*R**]]-5,6,8,11,12,13,14,15,16, 17,18,19, 24,25,26, 26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10.12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3H-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone, monohydrate.

Sponsor: Fujisawa USA, Inc.
Parkway North Center
3 Parkway North
Deerfield, IL 60015

Pharmacologic Category: Immunosuppressive drug

Proposed Indication: Prophylaxis of organ rejection in patients receiving allogeneic liver transplants.

Dosage Forms and Routes of Administration: 1 mg and 5 mg hard gelatin capsules for oral administration [NDA 50-708, see also NDA 50-709 for 5 mg ampules for intravenous infusion].

NDA Drug Classification: 1-P

Related Reviews: Statistical Review dated: Dec. 30, 1993
Biopharm Review dated: Pending

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3 Material Reviewed

Volumes dated 7/23/93: 1.1, 1.49 (Including Integrated Summary of Efficacy and Integrated Summary of Safety), 1.50-1.66 (Including Appendices to ISE and ISS, and Safety Report Submitted to FDA), 1.67-1.87 (Report of Controlled Study FPC-FK 506-7), 1.88-1.105 (Report of Controlled Study GHBA-157), 1.118-1.126 (Cited Literature and Clinical Literature).

Volumes dated 10/6/93: 5.1-5.5 (90-day Safety Update submitted by agreement with the FDA in lieu of a 120-day Safety Update).

4 Chemistry/Manufacturing Controls

No manufacturing and control problems of any clinical significance have been identified in consultation with the reviewing chemist.

5 Animal Pharmacology/Toxicology

5.1 Mechanism of Action

Tacrolimus has been shown to inhibit *in vitro* the mixed lymphocyte reaction (MLR) assay (a model for T-lymphocyte activation), and interleukin-2 (IL-2) formation by T lymphocytes. Tacrolimus has also been shown to prolong graft survival in several animal models of allograft rejection and xenograft rejection using a variety of dosing regimens.

The primary mechanism of rejection following allograft transplantation involves activation of T-lymphocytes and the subsequent production of cytokines including IL-2. Tacrolimus inhibits the activation of T-lymphocytes in both animals and humans, especially the activation that is calcium dependent. Tacrolimus interferes with the formation of active transcription factor NK-AT (nuclear factor of activated T-cells) and inhibits the production of cytokines including IL-2, IL-3, IL-4 and interferon-gamma. The net result is immunosuppression.

Although the precise molecular mechanism of action is not known, tacrolimus binds to cytoplasmic immunophilins that have been designated FK506-binding proteins (FKBPs). FKBPs are ubiquitous in many tissues. The predominant species of these immunophilins in the lymphoid system is FKBP-12. Tacrolimus bound to FKBP-12 forms a pentameric complex with Ca^{2+}, calmodulin and calcineurin (a calcium dependent serine/threonine phosphatase), and inhibits the enzymatic activity of calcineurin. The action of tacrolimus on calcineurin may inhibit the generation of active NF-TA by inhibiting the "trafficking" of the cytosolic component of NF-AT ($NF\text{-}TA_c$). This prevents the production of cytokines at the level of promotion of transcription and suppresses the immune response to antigens.

5.2 Animal Toxicology

No particular animal findings were the basis for focused searches in the review of human safety,

although some of the toxicities observed in animals mimicked some observed in humans. Based on the assessment of several studies, the order of sensitivity of target organs in rats is immune system (lymphoid atrophy) > kidney (increased BUN or serum creatinine) > pancreas (increase urine or serum glucose) > liver (decreased serum proteins) > blood (change in erythrocyte parameters) > reproductive organs (change in organ weights) > nerves (Sciatic nerve damage and tremors).

These study results may misrepresent the sensitivity of the endocrine pancreas to injury by tacrolimus. In a special toxicity study in rats, there was evidence of glucose intolerance which was unaccompanied by increasing fasting blood glucose levels, following 2 weeks of dosing at 1 mg/kg. These data were reported in a non-GLP study. If born out by further research, it would indicate that the pancreatic islets may be the most sensitive target for tacrolimus toxicity. This data must be considered in the light of observation that hyperglycemia and diabetes mellitus were associated with the administration of tacrolimus in humans.

A similar order of target organ sensitivity was observed in the baboon: immune system (lymphoid atrophy) = kidney (decreased serum calcium, increased urea) = pancreas (glucose intolerance) > liver (decreased serum proteins and increased LDH) > nervous system (tremors) = blood (decreased platelets, neutrophils and lymphocytes).

6 Clinical Background

6.1 Relevant human experience

Orthotopic liver transplantation (OLTX) is offered in the United States for patients with chronic end-stage disease, fulminant hepatic failure, inborn errors of metabolism and unressectable primary hepatic malignancies isolated to the liver. Since October 1987 data concerning the practice of OLTX in the United States has been collected by the Pittsburgh-UNOS Liver Transplant Registry (LTR) established as part of the United Network for Organ Sharing (UNOS) Scientific Registry. The following is a summary of information available in Belle SH, et al., The Pitt-UNOS Liver Transplant Registry, in Terasaki PI, Cecka JM, Eds. *Clinical Transplants 1992*, Los Angeles, UCLA Typing Laboratory, 1993; pp17-32.

Since the LTR began collecting data in October 1987, 9,868 OLTXs have been performed on 8,539 recipients including 1,510 pediatric (age < 16) and 7,029 adult (age ≥ 16) recipients. The total number of OLTX procedures increased from 1,711 in calendar year 1988 to 2,951 in 1991.

In 1991 there were 394 pediatric recipients and 2,198 adult recipients. Biliary atresia remains the most common indication for OLTX among pediatric recipients, accounting for 55% of the recipients in 1991. In adults alcoholic liver disease, the primary liver disease for 21.6% of the recipients in 1991, remains the most common indication for OLTX.

Data from LTR (October 1987 through 1991) indicate that the cumulative probability of

surviving (without retransplantation) one and 4 years after initial transplantation was 0.78 (0.68) and 0.74 (0.61) respectively for pediatric patients. Among adults, the cumulative probability of surviving (without retransplantation) one and 4 years following OLTX was 0.76 (0.69) and 0.65 (0.57) respectively.

In pediatric recipients, factors associated with survival in univariate (unadjusted) analyses included age (the youngest recipients had the worst survival), UNOS description (poorer functional status just prior to transplantation led to poorer survival), and primary liver disease (survival was worst for recipients transplanted due to fulminant hepatic failure, and best with patients with α-1 antitrypsin deficiency). "Retransplant-free" survival was also associated with ABO matching.

Among adults, factors associated with survival in univariate (unadjusted) analyses included race (Blacks and Asians had the poorest survival), age (the oldest recipients had the poorest survival), UNOS description (poorer functional status just prior to transplantation led to poorer survival), multiorgan transplantation (recipients of organs in addition to the liver had poorer patient survival than recipients of liver only), and primary liver disease (best survival for cirrhosis due to autoimmune disease or cholestatic cirrhosis, poorest survival for malignancies, hepatitis B, and fulminant liver failure).

These figures reflect current practice using a variety of immunosuppressive regimens based on cyclosporine for the prophylaxis of liver rejection. Cyclosporine is always used with adrenal corticosteroids (dual therapy) which may be tapered over time. Initial therapy azathioprine is often added to this regimen (triple therapy). Some centers prefer to add a brief course of antilymphocyte antibody (induction therapy) to the triple regimen.

Following the introduction of cyclosporine in the early 1980's, one year graft survival increased from approximately 30% to 70%. The principal adverse reactions of cyclosporine therapy are renal dysfunction, tremor, hirsutism, hypertension and gum hyperplasia.

Rejection is a common phenomenon. Sixty percent of liver transplant recipients will experience at least one rejection episode, commonly occurring between the fourth and fourteenth day postoperatively. Steroids are also always the first line treatment for rejection. There are several acute and chronic side effects that are associated with the use of steroids in transplantation. These include, but are not limited to, insulin-dependent diabetes, severe infection and bone disease.

Azathioprine is a purine analog which acts as an antimetabolite. The principal and potentially serious toxic effects of azathioprine are hematological and gastrointestinal. These include, but are not limited to, leukopenia and thrombocytopenia which are dose-related. The risks of secondary infections and neoplasia are also significant.

Since June 8, 1993, OKT3, a murine monoclonal antibody against a human pan-T-lymphocyte antigen has been approved by the FDA for the treatment of steroid resistant rejection.

6.2 Important information from related INDs and NDAs

Tacrolimus has not yet been approved for any indication in the United states. Its initial development in the United States began in 1989 under IND

Journal articles reporting results from clinical studies conducted under this IND have been include in this NDA submission. Initial clinical investigations of tacrolimus in liver transplantation sought to combine it with cyclosporine. Pharmacokinetics evaluations in the first 11 patients demonstrated increases in cyclosporine whole blood concentrations and prolongation of cyclosporine elimination with resultant renal dysfunction, as evidenced by elevations of serum creatinine. Therefore, the co-administration of cyclosporine with tacrolimus was discontinued.

6.3 Foreign experience

Tacrolimus is marketed only in Japan, following a launch in June, 1993. A submission for marketing approval was made for liver transplantation in December 1991 in Japan, where approval was received in March 1993.

A submission was made in Japan in April 1993 for use in the treatment of refractory graft-versus-host disease following bone marrow transplantation. A marketing application was also submitted for tacrolimus in liver, kidney and heart transplantation in Germany in June, 1993.

6.4 Human Pharmacology, Pharmacokinetics, Pharmacodynamics

The results of human pharmacokinetics studies indicate that the absorption of tacrolimus from the gastrointestinal tract is incomplete and variable. The absorption half-life of tacrolimus in 16 stable liver transplant patients averaged 5.7 hours. Peak concentrations (C_{max}) in whole blood and plasma were achieved at approximately 1.0-3.2 hours after oral administration. Compared to an intravenous infusion, the absolute bioavailability of tacrolimus capsules (based on whole blood) was 21.8% in liver transplant patients, 20.1% in kidney transplant patients, and 14.4%-17.4% in healthy volunteers. The relative bioavailability of tacrolimus was reduced by 27% when it was administered after a meal of moderate fat content. It should be noted that the latter finding was not incorporated into any dosing recommendations during the conduct of the two phase 3 clinical trials described in below in section 8.

The disposition of tacrolimus from whole blood is biphasic with a terminal half-life of 11.7 $\pm$ 3.9 hours in liver transplant patients and 21.2 $\pm$ 8.5 hours in healthy volunteers. The volume of distribution and total body clearance for tacrolimus following intravenous administration in liver transplant patients are 0.85$\pm$0.31 (mean$\pm$SD) L/kg and 0.053$\pm$0.017 L/h/kg, respectively.

Dose proportionality of tacrolimus was not documented in liver transplant patients; however, the sponsor did present a dose proportionality study conducted in kidney transplant patients. The results of this study do not support a dose proportional increase in AUC and C_{max} for tacrolimus.

Whole blood and plasma trough concentrations of tacrolimus from kidney and liver transplant recipients taken 10-12 hours after oral administration of tacrolimus (C_{min}) appear to correlate well with the AUC_{0-12h}, suggesting the utility of 12-hour trough levels for monitoring overall tacrolimus exposure. However, this relationship was linear only for the range of ng/mL.

The sponsor conducted a single dose bioequivalence study of the 1 mg and 5 mg capsules in healthy volunteers. An important finding is that the 1 mg and 5 mg tacrolimus capsules when administered as a 5 mg dose (5x1 mg and 1x5 mg) cannot be considered bioequivalent. The 1 mg capsule is estimated to be 17% more than bioequivalent.

The results from two separate protein binding studies, showed that 99% and 75% of tacrolimus was bound to plasma proteins, respectively, and that binding was concentration independent. The extent of protein binding, the identity of the plasma protein and binding site merit further investigation.

Tacrolimus is also highly bound to erythrocytes. The distribution of tacrolimus between whole blood and plasma depends on several factors such as: hematocrit, temperature of separation of plasma, drug concentration, and plasma protein concentration. The observed ratios of whole blood to plasma concentrations across individuals ranges from

Tacrolimus is presumed to be metabolized in the liver. *In vitro* studies suggest that tacrolimus is metabolized by the cytochrome P450 system of enzymes, specifically cytochrome P450IIIA and to a lesser extent, P450IA. Drug interaction studies with tacrolimus have not been conducted; however, what is known about its metabolism suggests a potential for interactions with other substrates, inhibitors, and inducers of this enzyme system. Data submitted to the NDA as a publication, suggest that dosages adjustments may be necessary in patients with severe hepatic impairment but not for those with mild impairment. Additional studies are under way in humans with mild hepatic impairment.

The clearance of tacrolimus is independent of renal function; less than 1% is recovered unchanged in the urine. However, it was recognized in early clinical development of tacrolimus, that dose adjustments may necessary in patients with impaired renal function to reduce the potential nephrotoxic effects of the drug.

6.5 Other relevant background information

In January 1990 Fujisawa Pharmaceutical Company (FPC) met with the Division of Oncology and Pulmonary Drug Products (HFD-150) for pre-IND consultation which led to the submission of IND This submission contained a protocol, FPC-FK506-03: "A phase III study of FK506 in liver transplantation with drug-resistant immune rejection". This study was placed on clinical hold, and remained so despite several exchanges of letters and a meeting with HFD-150, until the sponsor withdrew this protocol. Outstanding deficiencies included, but were not limited to, major disagreements about study design, the adequacy of historical controls, sample size estimates and the definition of treatment failure. This study was

withdrawn from the IND and resubmitted as a "compassionate use protocol" (Study FPC-FK506-9), permitting the use of tacrolimus in liver transplant recipients suffering from refractory allograft rejection despite conventional immunosuppressive treatment.

In June 1990, the sponsor requested Subpart E designation, which was granted July 25, 1990 "for prevention and treatment of liver allograft immune rejection following liver transplant".

In July 1990 a meeting was held between FPC and HFD-150, with the assistance of several outside expert consultants invited by both parties. One of the topics of discussion was a proposed study, FPC-FK506-07: "A randomized trial of FK506 *vs.* cyclosporine A as primary immunosuppressive treatment in liver transplantation", which had been placed on clinical hold, because of disagreement over the study design, definition of endpoints and because the proposed study as written was unlikely to be able to meet its stated objectives. A revised protocol -7 was resubmitted on August 2, 1990 and the study was allowed to proceed on August 9, 1990. In a removal-of-clinical-hold letter dated September 28, 1990, specific advice was given on the timing and nature of statistical analyses which would be the basis of the FDA's evaluation:

> "The FDA will base its evaluation and assessment of effectiveness and safety on one year patient and graft survival. The final analysis should be performed 12 months after patient accrual is complete. No other time point (prior to one year) will be acceptable as a final statistical analysis."

March 16, 1992 IND was transferred to the Division of Antiviral Drug Products (HFD-530). FPC met with HFD-530 on March 23, 1992 to present an overview of their clinical development.

In October 1993, FPC [now known as Fujisawa USA (FUSA)] met with the Division of Antiviral Drug Products for a pre-NDA meeting. During that meeting, FUSA was reminded that the basis of our evaluation of the efficacy of tacrolimus in the prevention of graft rejection after liver transplant would be based on 12 month patient and graft survival from at least two adequate well controlled studies. The sponsor agreed to collect this information and include it in the NDA submission.

The original NDA submission dated July 23, 1993, included a Clinical Study Report which the sponsor intended to use to support the claim that tacrolimus was safe and effective in the treatment of An internal meeting was held on 9/23/93 it was felt that the NDA did not contain, on its face, sufficient information to permit a substantive review of this particular indication. This was communicated to the sponsor (Telephone conversations dated 9/23/93 and 9/24/93) who agreed to withdraw this indication and Study Report from the NDA (Letter from the Sponsor dated 9/24/93). A revised proposed package insert omitting reference to this indication was submitted with the letter.

7 Description of Clinical Data Sources (both IND and non-IND)

The efficacy and safety of tacrolimus in preventing rejection after liver transplantation was evaluated in two large, controlled, FUSA sponsored studies. The study designs and number of patients enrolled are shown in table 1. Study FPC-FK506-7 was conducted under the sponsor's IND

TABLE 1: FUJISAWA CONTROLLED LIVER TRANSPLANT STUDIES

Investigator and Publication or Protocol	Completion Status (Start Date)	Study Design	Dose (mg/kg) IV/PO	Treatment Duration (Mean)	Subjects M/F
FPC-FK506-7	Completed (Started Aug 1990)	R,O,C,M	FK506: 0.075, then 0.05IV bid; 0.15 PO bid CyA: varied among centers (1-2 IV bid, 5 PO bid or adjust by trough	Up to 360 days	136/127 140/126
GHBA-157	Ongoing (Started Sep 90)	R,O,C,M	FK506: 0.075, then 0.03-0.05 IV bid; 0.015 PO bid CyA: 1-15/day	Up to 360 days	136/134 158/117

R = randomized
O = open-label
C = active control
M = multicenter

Additional controlled studies of FK506 in liver transplantation, some conducted by the sponsor (but not submitted to their US IND) and others extracted from published literature, are listed in Table 2. Studies FG-1-01 and FG-1-03 2 are ongoing and will not be discussed any further in this review. One study [Takaya et al.] was an open-label, historically controlled in 409 subjects, where tacrolimus was used according to each participating institution's standard dosing procedures. This study was not conducted under the sponsor's US IND and was only submitted as a publication. The remaining completed studies in Table 2 were either small, and/or without concurrent controls, and were submitted only as publications. They too will not be discussed any further in this review. Although data from these studies may be relevant to the efficacy and safety of tacrolimus in liver transplantation, the evaluation of the efficacy and safety will focus on the two large studies in Table 1.

TABLE 2 ADDITIONAL CONTROLLED STUDIES OF TACROLIMUS IN LIVER TRANSPLANTATION

Investigator and Publication or Protocol	Completion Status (Start Date)	Study Design	Dose (mg/kg) IV/PO	Treatment Duration (Mean)	Subjects
Fung et al.	Completed (started Feb 90)	R,O,C	FK506:0.01 daily/0.15 PO bid CyA: 4.0 daily/8.0 PO bid	343 days 346 days	41 50
Todo et al.; Jain et al.	Completed (Started Aug 89)	O,H	FK506: 0.15 daily IV/0.075 IV bid/ 0.15 PO bid	6-12 months	110
Tzakis et al.	Completed (Started Apr 91)	O,H	FK506: 0.15 daily/0.3 daily	18 months	59
Takaya et al.	Completed (Started Aug 89)	O,H	FK506: ISDP	13 months	409
FG-1-01	Ongoing (Started May 92)	R,O,C,M	FK506: 0.05 bid plus steroid vs. 0.03 bid plus steroids and azathioprine	(3 months)	(125)
FG-103 2 (Italy)	Ongoing (Started Feb 93)	R,O,C,S	FK506: 0.012/day IV; 0.03 bid PO + azathioprine + steroids + OKT3 CyA: 2/day IV or 5-22/day PO + azathioprine +steroids + OKT3	(12 months)	(20-30)

R = randomized
O = open-label
C = active control
M = multicenter
S = single center
H = historical control
ISDP = institutional standard dosing procedure

8 Clinical Studies

8.1 Reviewer's Trial # 1; Sponsor's protocol # FPC-FK506-7

8.1.1 Objective/Rationale

The primary objectives of this study were to compare the safety and efficacy of tacrolimus and a moderate dose of corticosteroids versus cyclosporine-based immunosuppressive regimens (CBIR) in controlling immune rejection of liver allografts.

8.1.2 Design

The study was designed as a multicenter, open-label, randomized, comparative, parallel-group, equivalence study, with CBIR as the active control. The trial was conducted in 12 centers:

Baylor Univ.; Mayo Clinic; Univ. Nebraska; Univ California, San Francisco and Los Angeles; California Pacific (formerly Pacific Presbyterian); Barnes Hospital; Univ. Wisconsin; Mt. Sinai Medical Center; New England Deaconess Hospital; Univ. Chicago; and Johns Hopkins Hospital.

The primary endpoints were 12 month patient survival and 12 month graft survival. The sample size estimates were based on ruling out a difference in 12 month survival of more than 10% between tacrolimus and CBIR. Secondary endpoints included acute rejection and steroid resistant rejection, chronic rejection with duct loss, and retransplantation for chronic rejection.

Because of the differences between study arms in use of corticosteroids and azathioprine defined by the protocol and because of the requirements to monitor tacrolimus or cyclosporine levels, the sponsor and investigators felt it would not be feasible to conduct a double blind study. The study design has several limitations.

The open-label design may have allowed for biased estimates of the treatment effects. Precautions taken included, randomization before surgery and keeping the patient and investigator blinded until the administration of the first dose of immunosuppressive drug. The primary analysis of 12 month patient and graft survival was conducted according to intent to treat. The open-label design also made difficult the ascertainment of secondary endpoints.

The active control arm included a combination of immunosuppressive drugs that in practice are used in a variety of ways across liver transplant centers in the US. Ten out of the 12 study sites agreed to use a common CBIR regimen. The assumption was made that the CBIR reflected "best available therapy" for this indication.

The study design did not allow one to assess the relative contribution of tacrolimus to the efficacy and safety outcomes. The assumption was made that if equivalence between a combination of tacrolimus plus moderate dose of corticosteroids and cyclosporine-based combinations could be supported by this study, it could not be accounted for by the use of corticosteroids alone.

8.1.3 Protocol

8.1.3.1 Population

Male or female patients, including pediatric patients, with end-stage liver disease who were approved for liver transplantation by their institution's patient selection committee were eligible for entry.

Significant exclusion criteria were multi-organ transplantation, a previous liver transplant failure, and ABO-incompatible transplants. High risk patients (including those with cancer, including hepatic tumor with high risk recurrence; renal failure; stage IVB hepatic encephalopathy; HIV seropositivity; and medically unstable patients were excluded.

8.1.3.2 Procedures

8.1.3.2.1 Tacrolimus Intravenous Dosing

Initially, patients randomized to tacrolimus were to receive the first dose of 0.075 mg/kg given as a 4-hour IV infusion in the operating or recovery room and repeated every 12 hours for 3 days or until the patient could tolerate oral medication.

Because of reported nephrotoxicity, three protocol amendments were made on December 13, 1990; January 7, 1991; and April 17, 1991. The effects on the IV doses are shown in Table 3.

TABLE 3
Tacrolimus Dosing Changes (IV & PO) and Time to First IV Dose

Parameter	Original	Amendment 1 Dec 13, 1990	Amendment 2 Jan 7, 1991	Amendment 3 Apr 17, 1991
IV Dose (mg/kg/12hr)	0.075	0.05	0.05	0.05
IV Infusion Duration	4 hr	4 hr	12 hr	12 hr
Timing of First IV Dose	OR and RR	RR: if urine ≤20 mL/hr hold up to 24 hr	≤6 hour after reperfusion; if urine ≤20 mL/hr hold up to 24 hr	6-24 hr after reperfusion; hold up to 48 hr for unstable patient or liver/renal dysfunction
PO Dose (mg/kg/12hr)	0.15 12 hr after start of last IV dose	No Change	0.15 Upon D/C of IV dosing	0.15 Upon D/C of IV dosing, PO or nasogastric tube; ideal weight dosing in obese patients

OR = Operating room
RR = Recovery room
D/C = Discontinuation

8.1.3.2.2 Tacrolimus Oral Dosing

Patients were to be converted to oral tacrolimus after 3 days of IV dosing, or when oral dosing was tolerated. Initially tacrolimus was to be dosed at 0.15 mg/kg every 12 hours, based on actual body weight. Dosing in overweight patients was changed to be based on ideal body weight by Amendment 3 to the protocol (See Table 3).

8.1.3.2.3 Corticosteroid Dosing in Tacrolimus Patients

Patients were to receive hydrocortisone 1000 mg IV intraoperatively or immediately postoperatively followed by a steroid cycle with methyl prednisolone at 100 mg, decreased by

20 mg daily over 5 days. Oral prednisolone or prednisone, at 20-mg prednisone equivalent in adult patients was to be initiated when tolerated and reduced over time. Lower prednisone-equivalent doses were to be used in pediatric patients and titrated down.

8.1.3.2.4 Changes to Tacrolimus Dosing

Tacrolimus dosing was to be adjusted according to plasma trough levels (target 0.2-5 ng/mL) and according to the presence of toxicity or rejection. Protocol Amendments 1, 2, and 3 were made to give the investigators greater latitude in determining necessary dose changes based on toxicity.

8.1.3.2.5 CBIR Dosing

Cyclosporine-based immunosuppressive regimens (CBIR) varied between centers in cyclosporine dosing and the nature and dosing of other immunosuppressants in the regimen (Table 4). Preoperative treatment with cyclosporine, azathioprine, and corticosteroids was allowed in the common regimen (10 centers), as were pretreatment with cyclosporine and steroids at the University of Nebraska (Univ. Neb) and azathioprine at the University of California, San Francisco (UCSF). Anti-lymphocyte globulin was also added at UCSF.

TABLE 4: COMPOSITION OF CBIR DOSING IN STUDY -7

Center	Cyclosporine	Azathioprine	Steroids	ALG
Common[a] (10 centers) N = 208	1 mg/kg IV q12 adjust by trough	2 mg/kg IV x 7 days; then 1 mg/kg	Yes, tapered	No
Univ. Neb.[b] N = 31	2 mg/kg IV q12 (days 1 to 2); then 5 mg/kg PO q12; then adjust by trough	No	Yes, tapered	No
UCSF[c] N = 27	Start about day 4; then adjust by trough level	2 mg/kg IV/PO x 7 days	Yes, tapered	IV x 5 days

a: Preoperative treatment allowed with cyclosporine, azathioprine, or steroids.
b: Preoperative treatment allowed with cyclosporine and steroids initially; steroids later allowed intraoperatively.
c: Preoperative treatment allowed with azathioprine.

8.1.3.2.6 Treatment of Rejection Episodes

Rejection episodes, as determined by biochemical parameters and/or biopsy, were to be treated similarly in the tacrolimus and CBIR treatment arms. The first episode of documented rejection was to be treated with methylprednisolone (MP) followed by a 6-day recycle of oral prednisone starting at 200 mg given q6h on day 1 of the episode. Pediatric patients were to receive lower methylprednisolone and oral prednisone recycle doses. OKT3, at 5 mg/day in adult and 2.5 mg/day in pediatric patients (<30 kg in weight)), was to be used for steroid-resistant rejection (See Table 5).

Table 5: Initial Treatment of Rejection Episodes (9/90 to 4/17/91)

Patient	First Episode	Steroid Resistant	Definition of Treatment Failure	Post Failure Treatment
Adult	MP* 1 gm IV x 1 & 6-day oral prednisone recycle	OKT3, 5 mg IV qd x 14 days	Lack of response to OKT3 plus 2nd steroid course; or recurrent rejection <30 days after OKT3; or recurrent rejection >30 days that is unresponsive to 2nd steroid course	Discontinue study drug; switch to alternative study drug allowed
Pediatric	MP 20 mg/kg IV x 1 and oral prednisone recycle	OKT3, 2.5 mg IV qd x 14 days	Same as adult	Same as adult

Amendment 3 to the protocol, dated April 17, 1991, changed the treatment of rejection episodes. These changes are in bold italic type in Table 6. Because of changes in the intensity of steroid usage for the first episode of rejection, the protocol's definition of "steroid resistant rejection" leading to use of OKT3 had a different meaning for the period of 9/ (enrollment of first patient on study) to 4/17/91 (Amendment 3) than it had for the period of 4/17/91 to 10/92 (when the last patient enrolled had completed 12 months on study). Since more investigator discretion was now allowed in the use of OKT3, the definition and therefore significance of "Treatment Failure" was different for these two periods.

Table 6: Treatment of Rejection Episodes After Amendment 3 (4/17/91)

Patient	First Episode	Steroid Resistant	Definition of Treatment Failure	Post Failure Treatment
Adult	MP* 1 gm IV x *two* & 6-day oral prednisone recycle	OKT3, 5 mg IV qd x *ten to fourteen* days	No change, *but prior steroid and OKT3 use may have changed.*	*Continue* study drug *or* switch to alternative study drug *or other experimental agent*
Pediatric	*Table by weight*	OKT3, *"reduced dose"* IV qd x *ten to fourteen days*	No change, *but prior steroid and OKT3 use may have changed.*	No change, *but now different from adults.*

8.1.3.2.7 Plasma/Blood Trough Levels

Plasma and whole blood trough concentrations of tacrolimus (10-12 hours after oral dosing) were to be determined using an ELISA method at regular intervals during the study and when toxicity or rejection occurred. In general tacrolimus dosing was to be decreased when plasma trough concentrations exceeded 5 ng/mL, except in the presence of rejection. Tacrolimus doses were to be increased in cases where plasma trough concentrations were below 0.2 ng/mL on 2 of 3

consecutive measurements, except in patients experiencing toxicity thought to be due to tacrolimus. However, in all cases clinical events (i.e., toxicity or rejection) took precedence over absolute concentrations in directing dosage adjustments.

Trough (whole blood or plasma) concentrations of cyclosporine were to be determined at each investigative site using a local assay. Dose adjustments were made according to local practice.

8.1.3.3 Endpoints

The primary endpoints measured were patient and graft survival. These were measured from the time of transplantation, although patients may have been randomized a day or two earlier and may also have received immunosuppressive drug prior to surgery. Graft loss was defined as patient death or retransplantation. These are considered appropriate endpoints to evaluate efficacy and are reasonably resistant to bias from the open-label design if the 12 month follow-up is complete and the primary analysis is conducted according to intent-to-treat.

Secondary endpoints such as acute rejection and/or steroid resistant rejection were not clearly defined in the written protocol. This is problematic in an open-label study. The definition of "response to rejection therapy" (including: complete response; partial response; improvement; and progressive disease) in the original written protocol was a complicated one which called in good part for the unblinded investigator's judgement. The diagnosis of rejection required confirmation by liver biopsy. The protocol required liver biopsies on Days 7 and 28. Additional biopsies could be performed at the discretion of the investigator. The written protocol did not require that the pathologist reading the biopsy be blinded as to the patient's assignment, chronology, rejection treatment or clinical status.

Because the treatment of rejection was modified by Amendment 3 to the protocol, the significance of steroid resistant rejection (leading to use of OKT3) and of treatment failure did not have the same meaning after April 17, 1991. The use of OKT3 per se was not a clinical endpoint defined in the written protocol nor was its evaluation included in the protocol's stated objectives. However, it was included as a secondary endpoint in the interim and final analyses.

Thus, there are limitations to the reliability of the ascertainment of these secondary endpoints.

The written protocol called for the use of a an "Endpoint Evaluation Committee" to review the judgement of each individual investigator, that a patient had experienced a failure of immunosuppressive therapy. This procedure does not appear to have been implemented according to the study report submitted with the NDA.

8.1.3.4 Statistical considerations

The sample size was chosen to ensure that a difference of $\pm 10\%$ in the percent of surviving grafts at one year between the FK506 and CBIR treatment arms would be detected with a power of 80%, when a 5% level two-sided significance test was used. It was estimated that 207

patients per arm would be needed to achieve the required power. With an allowance of an additional 18 patients per arm to compensate for dropouts, the protocol was written to require that a total of 450 subjects be enrolled. The final number of patients enrolled in the study was determined by a closing date for enrollment chosen well in advance of the completion of the study.

The efficacy and safety parameters for patients (in each arm of the study) were to be evaluated in all enrolled patients over a 12 month period. Patients who prematurely discontinued were also to be followed for a total of 12 months.

All patients who were randomized and transplanted were included in both the safety and efficacy analyses, under the assumption that there was no "intent to treat" a patient who had not received a liver allograft. While the primary analysis was the intent-to-treat analysis, the sponsor did perform an evaluable patient analysis for patient and graft survival to assess the impact of crossovers.

Two interim analyses were allowed in the written protocol: the first within two months of enrollment of 150 patients and the second within two months of enrollment of 300 patients. In fact, three rather than two interim analyses were performed. The first two were done by the medical monitor as part of an ongoing safety assessment of the study, and did not generate formal reports, because continuous monitoring of patient and graft survival suggested that the treatment groups remained equivalent. A third interim analysis, not planned for in the original protocol, was performed by the sponsor when all patients had completed the 28 day visit, because it was believed by the investigators that most of the events were occurring early in the course of treatment. In addition, there was continuous monitoring of the data, which is itself a form of interim analysis. Thus, although the final analysis was adjusted for three interim looks, it is not clear what final p-value should have been chosen.

The primary study medications were started per protocol at different times with respect to transplantation in the two study arms. In particular, cyclosporine was given before transplantation in the CBIR arm while tacrolimus was started 6-24 hours after surgery, and could be delayed until 48 hours after surgery if renal or hepatic function were poor. Therefore, the sponsor chose to attribute events to the treatment beginning on the day after surgery, rather than beginning on the day study drug was started.

The date of transplantation, not the date of randomization was used to as the baseline date for time-to-event analyses. The assumption was that patients would be randomized within hours of their surgery.

Additional subset analyses were performed for pediatric (≤ 12 years) and adult patients (> 12 years) at the request of the FDA.

Please see the Statistical Review and Evaluation for additional details.

8.1.4 Results

8.1.4.1 Populations enrolled/analyzed

8.1.4.1.1 Patient Population Accountability

A total of 598 patients were screened, 555 patients were randomized and 529 patients were enrolled (transplanted) at 12 centers. Enrollment by principal investigator and center is shown in Table 7.

TABLE 7
Patient Accountability by Investigative Site

Investigator	Site	FK506	CBIR
		Randomized/Enrolled	Randomized/Enrolled
GB Klintmalm, MD	Baylor University	35 34	34 31
R Wiesner, MD	Mayo Clinic	21 20	22 19
B Shaw, MD	Univ Nebraska	33 27	34 31
J Roberts, MD	UCSF	26 26	27 27
R Busuttil, MD	UCLA	58 57	58 58
C Esquivel, MD	California Pacific	25 25	25 25
JW Marsh, MD	Barnes Hospital	10 10	11 10
M Kalayoglu, MD	Univ Wisconsin	12 12	12 12
C Miller, MD	Mt Sinai Medical Center	35 30	35 33
WD Lewis, MD	NE Deaconess Hospital	9 9	8 7
R Thistlethwaite, MD	Univ. Chicago	2 2	3 2
J Burdick, MD	Johns Hopkins Hospital	10 10	10 10
	TOTAL	276 263	279 266

One patient at Mt Sinai was randomized to CBIR, but was treated with Tacrolimus.

Twenty-six patients were randomized, but were not transplanted and therefore not enrolled (13 Tacrolimus and 13 CBIR); the reasons for this are listed in TABLE 8.

TABLE 8
Reasons not Transplanted (Enrolled)

Reason	Tacrolimus	CBIR
Error in following randomization	8	9
Unqualified due to cancer	2	1
Unqualified for other reason	1	0
Patient randomized but not transplanted before 10/15/91 enrollment cut-off date.	1	1
Unqualified, liver looked OK at surgery.	0	1
Transplant Cancelled	0	1
Reason not specified	1	0
TOTAL	13	13

There does not appear to have been an imbalance between the two treatment groups in reasons for not being transplanted after randomization. Thus, there were 263 tacrolimus patients and 266 CBIR patients for a total of 529 patients who were randomized, transplanted and supplied with drug (TABLE 9).

TABLE 9
Patient Accounting by Treatment Group

	Tacrolimus	CBIR	Total
Patients Screened			598
Patients Randomized	276	279	555
Patients Enrolled	263	266	529
Patients with Day 28 Visit	213	219	432
Patients Completing Study	180	164	344
Patients Discontinued	83	102	185

Following transplantation, 83 Tacrolimus patients and 103 CBIR patients were discontinued from the study. Reasons for these discontinuations are listed in Table 10. patients who remained alive following discontinuation were followed for up to one-year post-transplantation.

TABLE 10
Reasons for Discontinuation

Reason	Tacrolimus	CBIR	Total
Adverse Event	37	13	50
Lack of Efficacy	6	32	38
Death	14	16	30
Administrative Reasons	9	20	29
Retransplant - Technical Problems	17	21	38
Total	83	102	185

Of the 529 patients randomized and transplanted, 9/263 (3.5%) of tacrolimus-treated patients and 20/266 (7.5%) of CBIR-treated patients were discontinued for administrative reasons. Administrative reasons included patients discontinued because they were found not to meet the protocol selection criteria after transplantation (discovery of an exclusion criteria such as malignancy, ABO mismatch, preexisting renal failure), patients who refused to continue the study, and patients lost to follow up. These patients were not excluded from the intent-to-treat efficacy analyses. This matter is further addressed in the FDA's Statistical Review and Evaluation. In particular, the fact that these discontinuations were not clustered towards the beginning of the study suggests that patients did not withdraw immediately after learning their treatment assignment.

8.1.4.1.2 Demographics

Patient demographic characteristics are listed in Table 11. These characteristics were comparable across the two treatment groups. The mean ages were 44.0 years in the tacrolimus group and 44.0 years in the CBIR group; the number of males and females were approximately equal in both treatment groups. The majority of patients in both groups were caucasian.

TABLE 11
Patient Demographics

Characteristic	Tacrolimus	CBIR
Age (years)		
N	263	266
Mean	44.0	44.0
SD	18.0	16.4
Range		
Height (cm)		
N	253	262
Mean	161.0	163.3
SD	29.5	26.1
Range		
Weight		
N	263	266
Mean	66.0	69.1
SD	25.2	23.7
Range		
Gender		
Male	136 (51.7%)	140 (52.6%)
Female	127 (48.3%)	126 (47.4%)
Race		
White	208 (79.1%)	203 (76.3%)
Black	13 (4.9%)	14 (5.3%)
Asian	9 (3.4%)	3 (1.1%)
Hispanic	29 (11.0%)	41 (15.4%)
Other	4 (1.5%)	5 (1.9%)

Fifty-one pediatric patients ($\leq$ 12 years) were enrolled in this study, 30 in the Tacrolimus group and 21 in the CBIR group.

8.1.4.1.3 Baseline Characteristics

The primary etiologies of pre-study liver failure are shown in Table 12. The distribution of these etiologies was similar between the two groups.

TABLE 12
Primary Reasons for Pre-Study Liver Failure

Reason	Tacrolimus (N=261)	CBIR (N=262)
Hepatitis, Chronic	48 (18.4%)	53 (20.2%)
Cirrhosis, Laënnec's	48 (18.4%)	48 (18.3%)
Primary Biliary Cirrhosis	32 (12.3%)	32 (12.2%)
Primary Sclerosing Cholangitis	31 (11.9%)	31 (11.8%)
Cirrhosis, Cryptogenic	21 (8.0%0	22 (8.4%)
Biliary Atresia	20 (7.7%)	17 (6.5%)
Hepatitis, Cirrhosis	12 (4.6%)	13 (5.0%)
Multiple Reasons	11 (4.2%)	13 (5.0%)
Other	8 (3.1%)	13 (5.0%)
Autoimmune Hepatitis	9 (3.4%)	11 (4.2%)
Cirrhosis	6 (2.3%)	3 (1.1%)
Hepatitis, Fulminant	4 (1.5%)	1 (0.4%)
Alpha-1 Antitrypsin Deficiency	3 (1.1%)	2 (0.8%)
Post Necrotic Cirrhosis	4 (1.5%0	0 (0.0%)
Toxic Hepatitis	3 (1.1%)	1 (0.04%)
Wilson's Disease	1 (0.4%)	2 (0.8%)

Perioperative observations regarding the donor liver and recipient condition were assessed in up to 249 tacrolimus and 251 CBIR patients (Appendix B, Table B.2.5 of the Study Report). These parameters were comparable across the two study groups with the exception of the overall incidence of hypotension, and of hypotension requiring treatment with vasopressors which were more frequent in the tacrolimus group. The mean ($\pm$ SD) reperfusion time was also longer in the tacrolimus group compared to the CBIR group (14.0 ± 5.4 vs. 13.0 ± 5.8; $P = 0.035$). These are differences that would have favored the CBIR group.

8.1.4.1.4 Protocol Violations

Several known protocol violations occurred with respect to inclusion and exclusion criteria for entry into the study. These were reported to the sponsor and documented on the Case Report Forms and are listed in the study report (Appendix B, Table B.3 [total] and Appendix D. Listing D.3 [by center]). Table 13 lists those violations of the entry criteria which could affect patient

outcome.

TABLE 13
Violations of Entry Criteria to the Study

Protocol Violation	Tacrolimus (N=263)	CBIR (N=266)
Exception to Entry Criteria (total including all exceptions)	87	88
Entry Criteria not Available	5	3
GFR not Measured	59	55
GFR < 30 mL/mn	1	2
Serum Creatinine ≥ 2.1 mg/dL	3	8
Renal Failure	1	6
BUN ≥ 100 mg/dL	1	2
ABO - Incompatible Graft	1	2

8.1.4.2 Efficacy endpoint outcomes

This section of the review reflects discussion with the FDA biostatistical reviewer. Please refer to the FDA's Statistical Review and Evaluation for additional details.

The primary efficacy analysis was based on an intent-to-treat analysis of patient and graft survival at 12 months in the 529 patients who underwent transplantation after randomization (263 tacrolimus, 266 CBIR). Acute rejection was the principal secondary endpoint analyzed in the same population. This section will focus on these three endpoints. The distribution of endpoints among the treatment groups is presented in Table 14.

TABLE 14
Endpoints by Treatment Group

Endpoint	Tacrolimus (N=263)	CBIR (N=266)
Patient Deaths	31 (11.8%)	33 (12.4%)
Graft Loss (Retransplant or Patient Death)	48 (18.3%)	55 (20.7%)
Acute Rejections	154 (58.6%)	173 (65.0%)

There was complete ascertainment of patient and graft survival status at one year.

8.1.4.2.1 Patient Survival

The sponsor's Kaplan-Meier estimates of patient survival rates are presented here in TABLE 15 (Source: Vol 1.69, Tables B.6.1 through B.6.3; and FUSA Response to statistical questions from 10/18/93 facsimile).

TABLE 15
Patient Survival

	Tacrolimus	CBIR
Kaplan-Meier Estimate at 360 Days	88 %	88 %
95% (adjusted) Confidence Intervals*.	84%, 92%	83%, 92%

* Since three interim analyses were performed, the final analysis was conducted at the 0.035 significance level to ensure an over all significance level of 0.05 or less. All confidence intervals were computed by the sponsor at a 96.5% confidence level, but were reported as "95% (adjusted p) confidence intervals" (see Vol 1.70). The sponsor's P value for the Wilcoxon test for comparison of the survival curves was 0.85.

To assess whether Tacrolimus and CBIR were "equivalent" with respect to patient survival the sponsor presented 95% (adjusted) confidence intervals on the differences between the two treatment groups (Vol 1.69, Appendix B, Table B.6.2.1.; Response to statistical questions from 10/18/93 facsimile) (see Table 16).

When stratified by investigator (as was the randomization), the estimated differences and corresponding confidence intervals were consistent with the unstratified analysis (see Table 16).

TABLE 16
Difference in 12 month Patient Survival

	Estimate of Difference in Patient Survival (Tacrolimus - CBIR) at Day 360	95% (adjusted) CI
Sponsors Unstratified Analysis	0.6%	-5.4%, 6.6%
FDA's Analysis Stratified by Investigator	2.1%	-2.7, 6.9%

Because the 95% confidence intervals include zero and dwell within a $\pm 10\%$ zone around zero, tacrolimus based immunosuppression and CBIR appear equivalent with respect to patient survival.

The sponsor also presented an analysis of pediatric (≤ 12 years of age) patient survival in the two treatment groups (Vol. 1.69, Appendix B, Table B.6.2.PED). The survival estimates at day 360 were 80% and 81% for Tacrolimus and CBIR respectively. These estimates were lower than those in the overall population (see Table 15 above) or those in adults only: 89% and 88% (Vol 1.69, Appendix B, Table B.6.2.ADU).

8.1.4.2.2 Graft Survival

The sponsor's results for overall cumulative graft survival and the Kaplan-Meier estimates of graft survival rates in patients are presented in Vol 1.69, Appendix B, Tables B.6.4 through B.6.6. The one year rates of graft survival are summarized in Table 17.

TABLE 17
Graft Survival

	Tacrolimus	CBIR
Kaplan-Meier Estimate at 360 Days	82%	79%
95% (adjusted) Confidence Intervals*	77%, 87%	74%, 85%

* Since three interim analyses were performed, the final analysis was conducted at the 0.035 significance level to ensure an over all significance level of 0.05 or less. All confidence intervals were computed by the sponsor at a 96.5% confidence level, but were reported as "95% (adjusted p) confidence intervals" (see Vol 1.70). The sponsor's P value for the Wilcoxon test for comparison of the survival curves was 0.55.

To assess whether FK506 and CBIR were "equivalent" with respect to one year graft survival the sponsor presented 95% (adjusted) confidence intervals on the differences between the two treatment groups (Vol 1.69, Appendix B, Table B.6.5.1; Response to statistical questions from 10/18/93 facsimile) (see Table 18).

When stratified by investigator (as was the randomization), the estimated differences and corresponding confidence intervals were consistent with the unstratified analysis.

TABLE 18
Difference in 12 Month Graft Survival

	Estimate of Difference in Graft Survival (Tacrolimus - CBIR) at Day 360	95% (adjusted) CI
Unstratified Analysis (Sponsor's)	2.4%	-4.8%, 9.7%
Analysis Stratified by Investigator (FDA's)	4.6%	-2.1%, 11.3%

Because the 95% confidence intervals include zero and dwell within a ±10% zone around zero tacrolimus-based immunosuppression and CBIR appear equivalent with respect to graft survival.

The sponsor also presented an analysis of graft survival in pediatric (age ≤12 years) patients enrolled in this study (Vol 1.69, Appendix B, Table B.6.5.PED). No differences were seen in the Kaplan-Meier estimates of graft survival in the two treatment groups. The day 360 estimates of 70% and 71% for the tacrolimus group and CBIR group, respectively were lower than those for the overall population but are consistent with one year graft survival observed in the Pitt-UNOS Liver Transplant Registry (see Section 6.1 of this review).

8.1.4.2.2 Acute Rejection

The sponsor also presented an analysis of acute rejection. Kaplan- Meier estimates of the proportion of patients who experienced at least one episode of acute graft rejection (confirmed by biopsy) are presented in Vol 1.69, Appendix B, Table B.7.1 (see also Response to statistical questions from 10/17/93 facsimile) and summarized in Table 19 below.

TABLE 19
Acute Rejection

	Tacrolimus	CBIR
Kaplan-Meier Estimate at 360 Days	68%	76%
95% (adjusted) Confidence Intervals*	60%, 75%	70%, 82%

* Since three interim analyses were performed, the final analysis was conducted at the 0.035 significance level to ensure an over all significance level of 0.05 or less. All confidence intervals were computed by the sponsor at a 96.5% confidence level, but were reported as "95% (adjusted p) confidence intervals" (see Vol 1.70). The sponsor's P value for the Wilcoxon test for comparison of the survival curves was <.01.

The sponsor's conclusion was that the rate of acute rejection was significantly less for patients randomized to tacrolimus than for patients randomized to CBIR. To further assess this the sponsor presented 95% (adjusted) confidence intervals on the differences between the two groups (See Table 21).

Treatment by investigator interaction was not assessed for acute rejection at one year in the sponsor's analysis. Examination of the rate of rejection at day 360 by study center showed considerable variability (see Table 20 excerpted from the FDA's statistical review).

TABLE 20

Acute Rejection at Day 360

	FK506	CBIR	Difference
Center	*Percentage*	*Percentage*	In Proportions
Baylor Unversity	66.2%	77.1%	-0.109
Mayo Clinic	63.0%	43.0%	0.200
U of Nebraska	76.7%	82.9%	-0.062
UCSF	76.3%	64.4%	0.119
UCLA	36.9%	62.9%	-0.260
Pacific Presbyterian	79.2%	74.6%	0.046
Barnes	100.0%	100.0%	0.000
U of Wisconsin	80.0%	100.0%	-0.200
Mt. Sinai Medical Center	84.9%	96.8%	-0.119
New England Deaconess	87.5%	66.7%	0.208
U of Chicago	100.0%	100.0%	0.000
Johns Hopkins Hospitnl	66.7%	88.9%	-0.222

In the FDA's statistical evaluation (see Section 2.2 of statistical review) the assessment of homogeneity indicated a significant center by treatment interaction, suggesting that the estimated differences in one-year acute rejection rates varied across investigator. UCLA (the largest center) and the Mayo Clinic stand out as extremes. The one year rate of acute rejection at UCLA was much lower among tacrolimus patients (36.9%) than the rates at the other centers, while the rate among CBIR-treated patients was comparable to what was observed in the other centers. At the Mayo Clinic (which enrolled 39 patients) the one-year rate of acute rejection among CBIR-treated patients was lower than the rates at the other centers, while the rate among tacrolimus treated patients was comparable to what was observed in other centers.

TABLE 21
Difference in Rate of Acute Rejection

	Estimate of Difference (Tacrolimus - CBIR) at Day 360	95% (adjusted) CI
Sponsors Unstratified Analysis	-8.0%	-17.0%, 1.1%
FDA's Analysis Stratified by Investigator *	-8.5%	-12.3%, -4.3%
FDA's Analysis Stratified by Investigator **	-7.4%	-12.0%, -2.8%

* Excluding Barnes and U of Chicago, for whom all patients experienced acute rejection.
** Excluding Barnes, U Chicago, UCLA and the Mayo Clinic.

Although the 95% confidence intervals include zero, the sponsor's analysis found that the rate of acute rejection was significantly less for patients randomized to tacrolimus than for patients randomized to CBIR. The adjusted confidence intervals for acute rejection exclude zero in the FDA's analyses, indicating that tacrolimus-treated patients experience a statistically significant lower rate of acute rejections. However, in both analyses the intervals fall primarily within the equivalence zone of $\pm 10\%$. This indicates that the evidence is insufficient to support the hypothesis that tacrolimus-based therapy is clinically superior to CBIR with respect to acute rejection, all the more that this small difference in rate of acute rejection did not result in a significant difference in patient or graft survival at 12 months.

The sponsor's "evaluable patient" analyses were consistent with the intent-to-treat analyses.

Although multiple analyses of other secondary endpoints were included in the study report, they were not considered for a detailed review by the FDA statistical and clinical reviewers,

because of shared concerns over the reliability of their ascertainment, and the changes in definitions of endpoints resulting from protocol amendments modifying the antirejection regimens. These secondary endpoints included acute rejection, steroid resistant rejection (both included in the written protocol), the use of OKT3 (not included in the written protocol but part of the interim and final analyses), and treatment failure. The F.D.A.'s concerns, regarding these secondary endpoints, were detailed in sections 8.1.3.2.6 and 8.1.3.3 of this review. Finally, as mentioned in section 6.5 of this review, evaluation and assessment of efficacy will be based on one year patient and graft survival.

8.1.4.3 Safety outcomes

Adverse events were coded by the sponsor according to the COSTART system using the preferred term and body system. The overall incidence rates for both treatment groups are presented in Vol 1.69, Appendix B, Table B.8.1 and the incidence rates of serious adverse events by treatment group are presented in Vol 1.69, Appendix B, Table B.8.2 (overall) an Table B.8.3 (by site).

As expected in this clinical setting, adverse events were frequently reported in both treatment groups. This review will focus on those events which were high (generally greater than 3%) in incidence, differed between the two treatment arms, or were clinically significant. Because of the study design, one cannot fully evaluate the relative contribution of tacrolimus to adverse events.

8.1.4.3.1 Discontinuation from Study

Thirty-seven patients in the tacrolimus group and 13 patients in the CBIR group were discontinued from the study for serious adverse events. Table 22 summarizes the serious or life threatening adverse events in patients who discontinued the study medication for adverse events (Source: Vol 1.69, Appendix B, Table B.8.5). Serious adverse events related to the nervous system, the urogenital system the respiratory system and the cardiovascular system were more frequent in patients who discontinued in the tacrolimus arm than in those who discontinued in the CBIR arm.

TABLE 22
Severe or Life Threatening Adverse Events in Patients
Who Discontinued for Adverse Events
By Body System and Treatment Group

Body System	Tacrolimus (N = 37) N (%)	CBIR (N = 13) N (%)
Body as a Whole	4 (10.8)	1 (7.7)
Cardiovascular	11 (29.7)	3 (23.1)
Digestive System	5 (13.5)	1 (7.7)
Hemic and Lymphatic	6 (16.2)	1 (7.7)
Metabolic and Nutritional	4 (10.8)	1 (7.7)
Musculoskeletal	1 (2.7)	1 (7.7)
Nervous System	12 (32.4)	1 (7.7)
Respiratory	8 (21.6)	1 (7.7)
Skin and Appendages	3 (8.1)	0 (0.0)
Urogenital	8 (21.6)	2 (15.4)

Table 23 summarizes the adverse events which led to discontinuation. Nephrotoxicity [including kidney function abnormal (8), kidney failure (2), oliguria (2), creatinine increased (1) and toxic nephropathy (1)] was the most common adverse event leading to discontinuation in the tacrolimus arm (14). Neurotoxicity [including encephalopathy (4), convulsion (3), neuropathy (3), headache (1) and psychosis (1)] was also a common reason for discontinuation in the tacrolimus arm.

TABLE 23
Adverse Events Leading to Discontinuation by Body System and Treatment Group

ADVERSE EVENT BODY SYSTEM	Tacrolimus N=255 N (%)	CBIR N=253 N (%)
NEPHROTOXICITY	14 (5.5)	5 (2.0)
NEUROTOXICITY	12 (4.7)	4 (1.6)
INFECTION	1 (0.4)	2 (0.8)
RASH	4 (1.6)	0 (0.0)
GI TOXICITY/WT LOSS	3 (1.2)	0 (0.0)
OTHER	3 (1.2)	2 (0.8)

8.1.4.3.2 Deaths

There were 31 deaths in the tacrolimus treatment group and 33 in the CBIR treatment groups. Fourteen and 16 of these occurred while still on the treatment assigned by the randomization in the tacrolimus and CBIR groups, respectively (See Table 10 above).

Table 24 represents the causes of death by treatment group counting a single cause per patient death (excerpted from Vol 1.68, Appendix B, Table B.1.8; see also Vol 1.70, Appendix D, Table D.1.2 and Vol 1.87, Appendix F). As expected in this clinical setting, infection and multisystem failure were common causes of death in this study. Examination of the individual summaries of patients who died (Vol 1.87, Appendix F) reveals that manifestations of multisystem failure, sepsis, cardiovascular events and central nervous system event were often present in the same patient prior to death. Most of the deaths occurred early during the first 28 days post transplant. While immunosuppression may have contributed to some of these deaths, tacrolimus-based immunosuppression does not appear to have been associated with an excess in deaths compared to CBIR.

TABLE 24
Causes of Death by Treatment Group

	FK506	CBIR
MULTISYSTEM FAILURE	7	4
INFECTION/SEPSIS	10	7
LIVER FAILURE	3	3
NEOPLASM	0	3
CNS EVENT	5	2
CV EVENT	5	7
LPD	0	1
PULMONARY EVENT	1	3
OTHER	0	3
TOTAL	31	33

8.1.4.3.3 Adverse Events by Body System

8.1.4.3.3.1 Urogenital System

The nephrotoxic potential of cyclosporine used in the active control regimen is well recognized (See official labeling for Sandimmune[R] in effect on August 1, 1992). However, adverse events involving impairment of renal function were reported more frequently in the tacrolimus group than in the CBiR group (Table 25, Source: Vol 1.67, Study Report: FPC-FK506-7, Table 26). In particular, hyperkalemia, abnormal kidney function, increased creatinine, increased BUN and oliguria were events more frequently reported in the tacrolimus group.

TABLE 25
Overall Adverse Events Related to Nephrotoxicity

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Kidney Function Abnormal	101 (40)	69 (27)
Hyperkalemia	112 (44)	66 (26)
BUN Increased	75 (29)	55 (22)
Creatinine Increased	100 (39)	62 (25)
Oliguria	45 (18)	37 (15)
Kidney Failure	10 (4)	10 (4)
Kidney Tubular Necrosis	6 (2)	0 (0.0)
Anuria	4 (2)	3 (1)

Serious adverse events involving nephrotoxicity were also reported more frequently in the tacrolimus group (Table 26, Source: Vol 1.67, Study Report: FPC-FK506-7, Table 35).

TABLE 26
Serious Adverse Events Relative to Nephrotoxicity

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Kidney Function Abnormal	14 (5.5)	12 (4.7)
Creatinine Increased	10 (3.9)	4 (1.6)
Kidney Failure	9 (3.5)	7 (2.8)
Oliguria	8 (3.1)	3 (1 2)
BUN Increased	7 (2.7)	3 (1.2)
Hyperkalemia	6 (2.4)	8 (3.2)
Kidney Tubular Necrosis	2 (0.8)	0 (0.0)
Anuria	1 (0.4)	2 (0.8)
Toxic Nephropathy	1 (0.4)	0 (0.0)

To better delineate the severity of renal dysfunction, the sponsor has examined the numbers of patients in study -7 requiring dialysis or ultrafiltration during the first month post

transplant (Table 27, Source: Vol. 1.69, Appendix B, Table B.11.10). This data was volunteered in comments and not specifically requested by the case report forms.

A total of 27 Tacrolimus and 17 CBIR patients required dialysis or ultrafiltration during the study, 25 and 15 respectively, for the first time following transplantation. In the Tacrolimus group, 8 patients were subsequently discontinued from Tacrolimus, 10 patients expired, and 7 patients were retransplanted. In the CBIR group, 5 patients were subsequently discontinued from CBIR, 5 patients expired, and 3 patients were retransplanted.

TABLE 27
Patients Requiring Dialysis

	Tacrolimus	CBIR
Required dialysis	27/263 (10.3%)	17/266 (6.4%)
Required dialysis after transplantation and after start of study drug	25/251 (10.0%)	15/256 (5.9%)
Study drug stopped or interrupted	8/251 (3.2%)	5/256 (2.0%)
Died	10/251 (4.0%)	5/256 (2.0%)
Retransplanted	7/251 (2.8%)	3/256 (1.2%)

The sponsor presented an analysis of adverse events by whole blood trough levels and by intravenous dose. Increased BUN and creatinine showed an apparent relationship with regard to initial IV dose and to higher whole blood trough levels (Tables 28 and 29, Source: Sponsor's Slide Presentation to the Advisory Committee on 11/22/93).

TABLE 28
Incidence (%) of Adverse Events by IV Dose

IV Dose (mg/kg/day)	< 0.05	0.05 - 0.10	> 0.10
BUN Increased	3.9	5.5	7.8
Creatinine Increased	3.6	6.2	9.6

TABLE 29
Incidence (%) of Adverse Events by Whole Blood Trough Levels (ng/mL)

Whole Blood Trough Level	≤ 10 ng/mL	>10-20 ng/mL	>20-30 ng/mL	>30 ng/mL
BUN Increased	0.6	3.0	5.5	8.5
Creatinine Increased	0.6	3.0	7.3	16.0

Spironolactone, a potassium-sparing diuretic, is commonly used to treat edema and ascites seen in patients with liver disease. The sponsor has also explored the association of spironolactone use and hyperkalemia during the trial (Table 30, Source: Vol 1.69, Appendix B, Table B.11.7). Spironolactone was used in a small proportion of patients in this study, and was not associated with a greater incidence of hyperkalemia than observed overall. Although spironolactone was used more frequently in the tacrolimus group than in the CBIR group, this difference cannot account for the higher incidence of hyperkalemia observed with tacrolimus compared to CBIR.

TABLE 30
Association of Spironolactone and Hyperkalemia

	Tacrolimus Patients Taking Spironolactone While on Study Drug	All Tacrolimus Patients	CBIR Patients Taking Spironolactone While on Study Drug	All CBIR Patients
Total Number	28	255	17	253
Patients Developing Hyperkalemia while on Spironolactone and Study Drug	10 (36%)	-	4 (24%)	-
Total Patients Developing Hyperkalemia	10 (36%)	112 (44%)	4 (24%)	66 (26%)

8.1.4.3.3.2 Nervous System

Selected adverse events related to neurotoxicity are shown in Table 31 (Source: Vol 1.67, Study Report: FPC-FK506-7, Table 22). Nervous system adverse events were commonly reported in both treatment groups. Tremor is a known adverse effect of cyclosporine, but was more frequently reported in the tacrolimus group (55% versus 46%). Headaches were also common and were reported more frequently in the tacrolimus group than in the CBIR group (63% versus 59%) as were paresthesias (39% versus 30%).

TABLE 31
Overall Adverse Events Relative to Neurotoxicity

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Headache	160 (63)	149 (59)
Tremor	139 (55)	116 (46)
Paresthesia	99 (39)	77 (30)
Dizziness	53 (21)	54 (21)
Neuropathy	23 (9)	24 (9)
Convulsion	16 (6)	17 (7)
Encephalopathy	13 (5)	15 (6)
Grand Mal Convulsion	6 (2)	3 (1)

The sponsor presented an analysis of whole blood tacrolimus levels and neurological adverse events. Tremor correlated ($P < 0.10$), using a step-wise logistic regression with whole blood trough levels of tacrolimus during days 1-7 (Vol 1.69, Appendix B, Table B.11.13). Headache and tremor correlated ($P < 0.10$) using the Cox proportional hazards method with whole blood levels of tacrolimus over the first 90 days (Vol 1.69, Appendix B, Table B.11.14).

Selected adverse neurological adverse events that were considered serious (grade 3 or 4 or requiring an IND Safety Report) are listed in Table 32 (Source: Vol 1.67, Study Report: FPC-FK506-7, Table 32). These events were more frequent in the tacrolimus group than in the CBIR group. As noted in Table 23 above, nervous system adverse events led to discontinuation from study drug for 12 (4.7%) of the tacrolimus patients compared to 4 (1.6%) of the CBIR patients.

TABLE 32
Serious Nervous System Adverse Events

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Headache	15 (6)	7 (3)
Convulsion	11 (4)	10 (4)
Encephalopathy	8 (3)	3 (1)
Neuropathy	8 (3)	2 (1)
Tremor	6 (2)	1 (0.4)
Grand Mal Convulsion	5 (2)	3 (1)

Selected nervous system adverse events related to cognitive function are shown in Table 33 (Source: Vol 1.67, Study Report: FPC-FK506-7, Table 23). Agitation, Confusion and Nervousness were more frequently reported in the tacrolimus group than in the CBIR group.

TABLE 33
Nervous System Adverse Events Related to Cognitive Function

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Insomnia	161 (63)	171 (68)
Depression	53 (21)	63 (25)
Confusion	42 (17)	25 (10)
Agitation	39 (15)	32 (13)
Somnolence	33 (13)	22 (9)
Nervousness	32 (13)	12 (5)
Thinking Abnormal	23 (9)	19 (8)

Serious adverse events involving cognitive function are shown in Table 34 (Source: Vol 1.67, Study Report: FPC-FK506-7, Table 32). Serious agitation and confusion (grade ≥ 3, or requiring an IND Safety Report) were more frequently reported in the tacrolimus group than in the CBIR group.

TABLE 34
Serious Nervous System Cognitive Function Adverse Events

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Confusion	9 (3.5)	1 (0.4)
Thinking Abnormal	6 (2.4)	4 (1.6)
Agitation	5 (2.0)	0 (0.0)
Psychosis	1 (0.4)	4 (1.6)

8.1.4.3.3.3 Cardiovascular System

Hypertension was the most commonly reported cardiovascular adverse event in 47.1% and 56.1% of the tacrolimus and CBIR patients respectively. Other adverse events related to the cardiovascular system, including abnormal ECG, hemorrhage, and hypotension were reported with similar frequency in both groups, while chest pain, tachycardia, and vasodilatation were more common in tacrolimus patients.

Table 35 shows serious adverse events involving the cardiovascular system (excerpted from Vol 1.67 Study Report: FPC-FK506-7, Table 29).

TABLE 35
Selected Serious Cardiovascular Adverse Events

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Bradycardia	5 (2.0)	0 (0.0)
Heart Arrest	9 (3.5)	3 (1.2)
Hemorrhage	15 (5.9)	8 (3.2)
Hypertension	4 (1.6)	12 (4.7)
Hypotension	15 (5.9)	14 (5.5)
Thrombophlebitis	5 (2.0)	0 (0.0)
Thrombosis	3 (1.2)	1 (0.4)

8.1.4.3.3.4 Digestive System

The most frequently reported adverse events related to the gastrointestinal system are displayed in Table 36 (Vol 1.69, Appendix B. Table B.8.7). Diarrhea, nausea and/or

vomiting, anorexia and dyspepsia were more frequently reported in the tacrolimus group. The incidence of serious adverse events involving the digestive tract were similar in frequency between the two treatment groups.

TABLE 36
Selected Adverse Events Related to Gastrointestinal Disturbances

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Diarrhea	180 (71)	121 (49)
Nausea	116 (46)	93 (37)
LFT Abnormal	90 (35.5)	78 (31)
Anorexia	86 (34)	59 (23)
Vomiting	67 (26)	37 (15)
Jaundice	64 (25)	49 (19)
Constipation	61 (24)	68 (26.9)
Dyspepsia	52 (20)	35 (14)
Nausea and Vomiting	49 (19)	35 (13/8)

8.1.4.3.3.5 Abnormalities in Glucose Metabolism and Diabetes Mellitus

The most commonly occurring adverse event was hyperglycemia which was reported more frequently in FK506-treated patients than in CBIR-patients (Table 37; Source: Vol 1.67, Study Report: FPC-FK506-7 Table 20). Diabetes mellitus was also more frequent in FK506-treated patients.

TABLE 37
Overall Adverse Events Relative to Impaired Glucose Metabolism

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Diabetes Mellitus	9 (3.5)	3 (1.2)
Hyperglycemia	118 (46.3)	100 (39.5)
Hypoglycemia	10 (3.9)	9 (3.6)
Glycosuria	4 (1.6)	7 (2.8)

8.1.4.3.3.6 Hemic and Lymphatic System

Anemia, thrombocytopenia and coagulation disorders occurred frequently in both treatment groups. These are considered expected consequences of liver transplantation surgery, and the differences between treatment groups in incidence rates for these events were small (Table 21, Source: Vol 1.67, Study Report: FPC-FK506-7, Table 21).

TABLE 38
Selected Hemic and Lymphatic Adverse Events

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Anemia	119 (470	98 (39)
Thrombocytopenia	59 (23)	56 (22)
Coagulation Disorder	30 (12)	26 (10)
Prothrombin Decreased	19 (7)	14 (5)
Thromboplastin Decreased	12 (5)	12 (5)

8.1.4.3.3.7 Metabolic and Nutritional Disorders

This category contains classifications which are better described under other body systems. Elevated serum creatinine and hyperkalemia are discussed under kidney disfunction (see section 8.1.4.3.3.1 of this review). Table 39 (Source: Vol 1.67, Study Report: FPC-FK506-7, Table 22) describes selected metabolic adverse events. As expected in this clinical setting, metabolic disturbances were common in both treatment arms. Few of these were judges to be serious. There was slightly more acidosis reported in the tacrolimus patients and more alkalosis in the CBIR patients. For other metabolic events the differences between treatment groups were small.

TABLE 39
Selected Metabolic Adverse Events

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Acidosis	33 (13)	22 (9)
Alkalosis	16 (6)	25 (10)
Amylase Increased	13 (5)	16 (6)
Edema	53 (21)	65 (28)
Hypocalcemia	55 (22)	53 (21)
Hypokalemia	74 (29)	85 (34)
Hypomagnesemia	119 (47)	115 (46)
Hyponatremia	38 (15)	23 (9)
Hypophosphatemia	49 (19)	44 (17)

8.1.4.3.3.8 Skin and Appendages

Hypersensitivity-type reactions including pruritus, rash and urticaria were more commonly reported in tacrolimus patients than CBIR patients (Table 40; Source: Vol 1.67, Study Report: FPC-FK506-7, Table 25). In the skin the most common serious adverse event was rash which was reported in 5 tacrolimus patients and 1 CBIR patient. As noted above in Table 23, rash led to discontinuation from study drug for 4 patients in the tacrolimus group and none in the CBIR group.

TABLE 40
Hypersensitivity-Type Reactions

Adverse Event	Tacrolimus N (%)	CBIR N (%)
Pruritus	90 (35)	51 (20)
Rash	60 (23)	47 (19)
Maculopapular Rash	7 (3)	0 (0.0)
Urticaria	6 (2)	2 (1)
Vesiculobullous Rash	3 (1)	5 (2)

8.1.5 Conclusions Regarding Efficacy and Safety Data

The following conclusions reflect discussion with the reviewers who conducted the FDA Statistical Review and Evaluation of this study.

Tacrolimus and CBIR appear equivalent with respect to 12 month patient and graft survival. The results of the FDA's analyses that were stratified by study investigator were consistent with the sponsor's unstratified analyses. The adjusted 95% confidence intervals from the FDA's stratified analyses suggested that the one-year rate of patient survival could be as much as 6.9% higher or as much as 2.7% lower among patients randomized to tacrolimus; the one-year rate of graft survival could be as much as 11.3% higher or as much as 2.1% lower among patients randomized to CBIR.

A strength of the study is the complete ascertainment of patient and graft status at one year. The major weakness of the study is its open-label design. Analyses performed by the sponsor and by the FDA to assess the influence of bias suggest that the impact was probably minimal on the estimates of the treatment effects reflected in the primary efficacy endpoints (12 month patient and graft survival).

One of the difficulties resulting from the open label design was the ascertainment of secondary endpoints, especially acute rejection. At best, the evidence is insufficient to support that tacrolimus was therapeutically superior to CBIR with respect to acute rejection. The 95% confidence intervals for both the sponsor's unstratified analysis and the FDA's stratified analysis of the difference between treatment groups in one-year rates of acute rejection fell within the $\pm 10\%$ zone used to define equivalence for the primary endpoints

The pattern of toxicities observed in the tacrolimus group was similar overall to that observed in the CBIR. However, at the doses used in this study, the tacrolimus-based regimen was associated with a greater incidence of gastrointestinal, neurologic, and renal adverse events than was CBIR. Most of these events were not serious. They also appeared to be related to dose and/or blood levels, to be reversible, and to respond to dose adjustments.

Hyperglycemia, diabetes mellitus and hyperkalemia were also more frequently reported in the tacrolimus group. These events were reversible and did not appear related to tacrolimus dose or blood levels.

Overall, there is insufficient evidence to support the claim that tacrolimus-based immunosuppressive therapy was safer than cyclosporine-based therapy when used to prevent organ rejection in patients who received liver transplants.

8.2 Reviewer's Trial # 2 Sponsor's protocol # GHBA-157

8.2.1 Objective/Rationale

The primary objectives of the study were to evaluate the safety and efficacy of tacrolimus combined with corticosteroids as prophylactic immunosuppressive therapy compared with conventional cyclosporine-based immunosuppressive regimens (CBIR) in patients receiving a primary liver allograft.

8.2.2 Design

This was a Phase II/III, international, multicenter, open-label, prospectively-randomized parallel group study, conducted in four European countries. Patients were randomized to receive treatment with either a tacrolimus based regimen or a site-specific CBIR as the active control. The trial was conducted in 8 centers: Huddings Hospital, Huddings, Sweden; Medizinische Hochschule, Hannover, Hannover, Germany; Universitätsklinikum Rodolf Virchow, Berlin, Germany; Hôpital Paul Brousse, Villejuif, France; Chirurgische Klinik der Universität Heidelberg, Heidelberg, Germany; Kings College Hospital, London, U.K.; Addenbrookes Hospital, Cambridge, U.K.; Queen Elizabeth Hospital, Birmingham, U.K.

The primary endpoints were 12 month patient and 12 month graft survival. The sample size estimates were based on ruling out a difference in 12 month survival of more than 10% between tacrolimus and CBIR. Secondary endpoints included time to first acute rejection, proportion with one or more episodes of rejection, and intractable rejection.

The active control arm included a combination of immunosuppressive drugs that the patients would have received after liver transplantation had they not been enrolled in this study. Each center used their own CBIP which corresponded to the "most effective conventional immunosuppressive treatment" used at that center. This treatment consisted of cyclosporine, azathioprine, corticosteroids and, in some institutions, anti-lymphocyte globulin (ALG). Because of the variety of regimens used at each site and differences between study arms in use of corticosteroids and azathioprine, the sponsor and investigators felt it would not be feasible to conduct a double blind study.

The open-label study design has limitations. This design may have allowed for biased estimates of the treatment effects. Precautions taken included randomization before surgery and keeping the assignment blinded until the administration of the first dose of immunosuppressive drug. Additionally, the primary analysis of 12 month patient and graft survival was conducted according to intent-to-treat.

Like the design of Study FPC-FK506-7, the design of GHBA-157 did not allow one to assess the relative contribution of tacrolimus to the safety and efficacy outcomes. The assumption was made that if equivalence between a combination of tacrolimus plus a

moderate dose of corticosteroids and cyclosporine-based combinations could be supported by this study, it could not be accounted for by the use of corticosteroids alone.

Patients who were diagnosed as having intractable rejection (in both the tacrolimus and control arms) were eligible to enter a rescue protocol GHBA-159 which would allow them to receive tacrolimus-based immunosuppressive therapy. [Reviewer's note: Protocol GHBA-159 has not been submitted to the sponsor's US IND, nor is it included in this NDA.]

8.2.3 Protocol

8.2.3.1 Population

Male or female patients, aged 18-70 years, with end-stage liver disease who were considered suitable for liver allograft transplantation and were about to undergo liver transplant surgery were eligible for entry.

Significant exclusion criteria were a diagnosis of vasculitis or arteritis, multiple organ transplants, a previous liver transplant failure, primary liver cancers with any evidence of metastases or a diagnosis of any other active neoplastic disease.

Unlike Protocol FPC-FK506-7, patients with fulminant hepatic failure were allowed to enroll and the randomization was stratified according to this condition. Fulminant hepatic failure was defined as: liver failure with stage III or IV encephalopathy (see Attachment 3 to protocol GHBA-175, in Vol 1.94, Appendix S3) developing in less than eight weeks in a patient without pre-existing liver disease.

8.2.3.2 Procedures

8.2.3.2.1 Tacrolimus Dosing

In the original protocol, dated 4/23/90, patients randomized to treatment with tacrolimus received an initial dose of 0.075 mg/kg given as a four-hour intravenous infusion every 12 hours for three days. The patient was then converted to oral tacrolimus therapy at a dose of 0.15 mg/kg twice daily.

This regimen was modified in protocol Amendment III, dated 2/20/91 (Vol 1.94, Appendix S3) because the investigators believed that the initial four-hour intravenous infusion led to an acute psychotic episode in some patients. The regimen amendment is summarized below:

Study Day	Dosage Regimen
Day 0	0.03-0.05 mg/kg intravenous infusion with normal saline or 5% dextrose in water over 12 hours. Initial dose could be reduced below 0.03 mg/kg over 12 hours in patients with renal dysfunction, graft dysfunction, or any other factor that could significantly influence the tolerability of FK506. Subsequently, intravenous infusions were given over 12-hour periods; however treatment was switched to oral administration as soon as possible.
Day 1 onwards	A daily oral dose of 0.03 mg/kg, administered twice daily in divided doses, unless the last intravenous dose was less than 0.05 mg/kg per 12 hours. If this was the case, then the maximal oral dose was not to exceed three times the last 12-hour intravenous dose. It was permissible to administer oral doses via the nasogastric tube.

The initial intravenous dose of tacrolimus was to be administered within six hours of surgery (i.e. after closure of the abdominal wall). However, if renal function was significantly impaired (i.e. urine output less than or equal to 40 mL/hour and/or a serum creatinine concentration greater than or equal to 140 μmol/L), tacrolimus could be withheld until renal function improved.

No specific recommendations were made as to administering oral tacrolimus in a fed or fasting state.

8.2.3.2.2 Changes in Tacrolimus Dosing

The original protocol, dated 4/23/90, allowed for tacrolimus dose adjustment in steps of 25% of the current dose. Amendment III, dated 2/20/91 (Vol 1.94, Appendix S3) allowed investigators more discretion in adjusting dose according to the patient's overall status including graft function, rejection status, degree of toxicity, and plasma concentrations. Clinical status took precedent over plasma concentrations.

8.2.3.2.3 Corticosteroid Dosing in Tacrolimus Patients

The corticosteroid regimen to be administered with tacrolimus as defined in the original protocol is summarized in Table 41. The corticosteroid dosage was amended on 2/20/91 at the investigators' request to allow more accurate corticosteroid administration in patients of

small body mass, as detailed in table 41.

TABLE 41
Corticosteroid Dosing in Tacrolimus Patients

Study Day	Original Protocol 4/23/90	Amendment III 2/20/91
Day 0	1 g methylprednisolone intravenously	10 mg/kg intravenous methylprednisolone given intra- or post-operatively
Day 1	20 mg/day oral prednisolone or an equivalent dose of methylprednisolone given parenterally if the patient could not tolerate oral administration	No Change

Corticosteroid dosing was tapered as clinically indicated and it was acceptable to discontinue prophylactic corticosteroid treatment completely.

8.2.3.2.4 Use of Azathioprine in the Tacrolimus Treatment Arm

The use of azathioprine was allowed in patients experiencing post-operative renal impairment or adverse experiences which required the interruption of tacrolimus therapy. Azathioprine was discontinued when tacrolimus therapy was initiated/reinitiated. Except for these cases, azathioprine was not to be given to patients in the tacrolimus treatment arm.

8.2.3.2.5 CBIR Dosing

The details of each investigational site's CBIR are summarized in Table 42 (Source: Vol 1.88, Report Number GHBA-157, Table 1). All treatment regimens included cyclosporine, azathioprine and corticosteroids. In the three German centers, Anti-Thymocyte Globulin (ATG, Fresenius) was also administered. The initial dose of each component of the regimen varied across the centers: cyclosporine, 1-15 mg/kg/day; azathioprine, 1-3 mg/kg/day; corticosteroids, 0.5-2 mg/kg/day. ATG was administered at a dose of 5 mg/kg/day for at least seven days post transplant.

TABLE 42
Composition of CBIR Dosing

Center	Cyclosporine	Azathioprine	Steroids	ATG
Cambridge Birmingham London France Sweden	1-6 mg/kg/day IV; then adjust by levels; then 8-15mg/kg PO adjusted by levels	1-3 mg/kg per day	Yes, tapered	No
Berlin* Heidelberg** Hannover***	2-4 mg/kg/day IV; then adjust by levels; 10-15 mg/kg/day PO or adjusted by levels	2-5 mg/kg/day* 2 mg/kg/ day** 1-1.5 mg/kg/day***	Yes tapered	5 mg/kg x 7 days

No two centers used the same regimen of cyclosporine, corticosteroids or azathioprine. Therefore, a great variety of immunosuppressive regimens were represented in the control arm. CBIR therapy was standardized, for each center, for the duration of the study. Cyclosporine concentrations were determined according to local hospital practice and the dose was adjusted accordingly.

8.2.3.2.6 Management of Rejection Episodes

The protocol required that the diagnosis of acute rejection be made on clinical and biochemical evidence and be confirmed by histology. If there was evidence of rejection on scheduled liver biopsies, but no clinical signs of rejection, treatment of rejection was not to be initiated.

Patients who experienced clinical acute rejection confirmed by histology, were to be treated similarly in the tacrolimus and the CBIR treatment arms. Each investigational site had a specific antirejection regimen which was to be utilized for both treatment arms. A broad variety of regimens were used across centers.

Each antirejection regimen usually included 3 to 5 days of methylprednisolone or hydrocortisone (500mg to 1g by bolus injection) as first-line treatment. One site (Cambridge) used Anti-Lymphocyte Globulin (ALG) or OKT3 for 7 to 10 days instead of steroids as first-line treatment for severe rejection.

As second-line treatment for steroid resistant and/or recurrent rejection all but one center used ALG or OKT3 for at least 7 to 10 days (in Berlin for up to 14 days). One center (London) used Methylprednisolone 1g/day x 3 days instead of anti-lymphocyte immunotherapy as second-line treatment of rejection.

If clinical signs and symptoms and laboratory evidence of rejection were still present after the "second cycle" of steroid administration or ALG treatment, a liver biopsy was to be obtained. If the liver biopsy revealed no histological improvement or worsening and these

findings were associated with clinical manifestations of rejection, the diagnosis of intractable rejection was to be made and the protocol required that the patient be withdrawn from the study treatment.

8.2.3.2.7 Liver Biopsy

The written protocol required three scheduled biopsies, in addition to biopsies that were necessary to confirm the diagnosis of rejection. A liver biopsy was to be obtained either prior to or after reperfusion of the liver (ie prior to closure of the abdominal wall). It was preferred that the biopsy be obtained after reperfusion of the liver. A liver biopsy was also obtained on day 7 of treatment u[illegible]ss the patient's clinical condition contraindicated the biopsy procedure (eg coagulopathy). If this situation occurred a biopsy was then to be obtained as soon as the patient's condition permitted.

A liver biopsy was also to be obtained after 12 months of treatment (Visit 24).

If a patient withdrew from the study prior to 12 months participation or died a liver biopsy was to obtained if possible.

8.2.3.3 Endpoints

Data on the primary endpoints, patient and graft survival, were collected through 1 year. Acute rejection data was collected over a 6 month time period.

Patients were followed until death, or their survival data were censored either at day 365 or their last date of follow-up if this occurred before day 365.

Graft survival included all patients that did not die or require retransplantation. The date of graft failure was taken to be the date of death or retransplantation, whichever occurred earlier. For those patients who neither died nor required retransplantation, patient data were censored at day 365.

A diagnosis of acute rejection was made based on clinical and biochemical evidence which was subsequently confirmed by histology.

The written protocol listed liver function tests (LFTs), steroid dose, histology (scheduled biopsies) and number of infections as additional measures of efficacy. These measures were well defined except for steroid dose. LFTs included serum transaminases (AST and ALT), total bilirubin, gammaglutamyl transferase and alkaline phosphatase which were measures as outlined in the protocol's flow chart.

Histology referred to the scheduled liver biopsies on day zero, day 7 and at 12 months (see 8.2.3.2.7 above). The protocol did not require that the pathologist reading the biopsy be blinded as to the patient's treatment assignment, chronology, rejection treatment or clinical

status.

For the purpose of the study the protocol stated that an infection was deemed to be present if, in response to clinical signs and symptoms, the investigator prescribed an antimicrobial medication, even if the infection was not confirmed microbiologically or by virology. The evaluation of this endpoint is problematic in an open label study. The study report introduced the new endpoint of infection confirmed by appropriate technique (microbiology and/or virology) that had not been part of the original study protocol.

The study report includes an additional efficacy measure, intractable rejection, that was included in the written protocol. A diagnosis of intractable rejection was made if there was histological evidence of unchanged or worsening acute rejection after two discrete courses of antirejection therapy, or if there was histological evidence of chronic rejection after two discrete courses of antirejection therapy. Evaluation of this endpoint is problematic in an open label study, particularly in this study where a variety of anti-rejection regimens were used at the discretion of the investigators and where liver biopsies were read by pathologists who were not required to be blinded to the patient's assignment, chronology and anti-rejection therapy.

Thus, there are limitations to the reliability of the ascertainment of these secondary endpoints. The appropriate primary endpoints for evaluating the efficacy of the tacrolimus-based regimen remain 12-month patient survival and 12-month graft survival.

8.2.3.4 Statistical Considerations

The sample size was based on a one-year survival rate of 80% for patients receiving conventional treatment. In the original data analysis plan, the sponsor estimated that a total of 414 evaluable patients (207 per treatment group) would be required to detect a 10% improvement in survival at one year, with a power of at least 80%. Allowing for drop-outs a minimum of 450 (225 per treatment arm) would have to be enrolled to detect a significant difference in survival time. The final number of patients enrolled in the study was determined by a closing date for enrollment chosen well in advance of the completion of the study.

At a later time, the goal of the study was re-defined as therapeutic equivalence (within 10%) instead of superiority (as confirmed by the sponsor in an April 8, 1993 telephone conversation).

The study was originally initiated (September 1990) as a twelve month study. No interim analysis was planned for in the written protocol. This was maintained in protocol amendment V dated November 22, 1991. Some time after initiation of the study the sponsor decided to perform an analysis after all patients had been enrolled for approximately six months. The study report states that this analysis was the subject of a protocol amendment (Vol 1.88 page 008-15775) but this analysis is not mentioned in any of the six protocol

amendments included in Appendix S3 (Vol 1.94).

The final data analysis plan included a six-month analysis of the secondary efficacy measurements: acute rejection; intractable rejection; histology; hepatic function; corticosteroid administration; and infections. The six to twelve month analyses pertained to patient survival, graft survival and serious adverse experiences.

Please refer to the Statistical Review and Evaluation dated December 30, 1993 for additional details.

8.2.4 Results

8.2.4.1 Populations Enrolled/Analyzed

8.2.4.1.1 Patient Population Accountability

A total of 545 patients from eight participating centers were recruited to the study. All 545 patients were randomized to treatment (270 to tacrolimus and 275 to CBIR). Table 42 summarizes the patient accountability by treatment group. Five patients were misrandomized and were excluded by the sponsor from the efficacy evaluation

TABLE 42
Patient Accountability by Treatment Group

	Tacrolimus	CBIR	Total
Patients Randomized	270	275	545
Patients Misrandomized	3	2	5
Patients Discontinued Prematurely (Before 6 Months)	77	93	170
Patients not Receiving Study Medication	5	11	16
Patients not Transplanted	0	1	1

Following transplantation, 77 tacrolimus patients and 93 CBIR patients were discontinued from the study before completing six months. The reasons for these discontinuations are listed in Table 43. Table 44 lists the number of patients who had withdrawn from the study by one year post transplantation.

TABLE 43
Reasons for Discontinuation

Reason	Tacrolimus	CBIR	Total
Adverse Experience (includes death)	70	62	132
Intractable Rejection	3	22	25
Lost to Follow-up	0	2	2
Unsatisfactory Compliance	0	1	1
Withdrew Consent	2	0	2
Investigator Withdrawal	2	5	7
Total	77	93	170

TABLE 44
Patients Withdrawn by 12 Months

Reason	Tacrolimus	CBIR	Total
Died within 12 Months Post Transplant	50	68	118
Withdrew but Survived 12 Months	36	17	84
Withdrew for Miscellaneous Reasons	10	27	37
Total Withdrawn from Study at 12 Months	90	112	202

8.2.4.1.2 Populations for Analysis

The Intent-to-Treat Population was based on all 545 patients randomized in the study (270 to tacrolimus and 275 to CBIR). Baseline characteristics, and patient and graft survival were presented by the sponsor for this population.

The Efficacy Population was based on 540 patients (267 randomized to tacrolimus and 273 to CBIR). This population excluded 5 patients who received incorrect treatment following randomization. The sponsor based efficacy and safety analyses on this population.

8.2.4.1.3 Demographics

Patient demographic characteristics for the Intent-to-Treat Population are listed in Table 45 (Source: Vol 1.88, Report Number GHBA-157, Table E). These characteristics were comparable across the two treatment groups. The mean ages were 45.7 in the tacrolimus group and 45.6 in the CBIR group; the majority of patients in both groups were caucasian. There was

a higher proportion of male patents in the CBIR group, but this difference was not statistically significant according to the sponsor analysis.

The demographic data for the Efficacy Population (Vol 1.88 Study Report GHBA-157, Table 9) were similar to those for the Intent-to-Treat Population. The two treatment groups were well matched for age and ethnic origin.

TABLE 45
Patient Demographics

Characteristic	Tacrolimus	CBIR
Age (years)		
N	263	275
Mean	45.7	45.6
SD	12.23	11.52
Range		
Age Ranges		
<18	0	2
18-35	56	49
36-50	100	118
51-65	112	102
>65	2	4
Gender		
Male	136	158
Female	134	117
Race		
White	260	260
Black	2	2
Asian	5	6
Other	0	2
Unknown	3	5

8.2.4.1.4 Baseline Characteristics

The baseline disease characteristics are given in Table 46 (Source: Vol 1.88, Report Number GHBA-157, Table F). There was a higher proportion of patients with carcinoma randomized to receive CBIR than to receive tacrolimus. In the Intent-to-Treat Population, 42 patients with carcinoma were randomized to CBIR therapy (15.3%) compared with 27 patients randomized to treatment with FK506 (10.0%). The immediate outcome for these patients may be better than for other diagnoses, since they most often are not suffering from liver failure at the time of transplantation; however, long-term survival is expected to be poor in this population (See section 6.1 above).

TABLE 46
Primary Reasons for Pre-Study Liver Failure

Characteristic	Tacrolimus (N = 270)	CBIR (N = 275)
Cirrhosis, Post Hepatitic	64 (23.7%)	64 (23.3%)
Primary Biliary Cirrhosis	53 (19.6%0	48 (17.5%)
Sclerosing Cholangitis	23 (8.5%)	23 (8.4%)
Hepatocellular Carcinoma	22 (8.1%)	29 (10.5%)
Cirrhosis, Alcoholic	21 (7.8%)	29 (10.5%)
Cirrhosis, Cryptogenic	17 (6.3%)	16 (5.8%)
Hepatitis, Fulminant	17 (6.3%)	19 (6.9%)
Budd-Chiari	11 (4.1%0	3 (1.1%)
Cirrhosis, Other	12 (4.4%)	7 (2.5%)
Other	11 (4.1%)	7 (2.5%)
Cirrhosis, Autoimmune	4 (1.5%)	6 (2.2%)
Carcinoma	4 (1.5%)	5 (1.8%)
Secondary Biliary Cirrhosis	4 (1.5%)	0 (0.0%)
Fulminant, Other	3 (1.1%)	3 (1.1%)
Fulminant, Overdose	2 (0.7%)	6 (2.2%)
Cholangiocellular Carcinoma	1 (0.4%)	6 (2.2%)
Metabolic Disease	1 (0.4%)	2 (0.7%)
Carcinoma, Metastasis	0 (0.0%)	2 (0.7%)

Concurrent medical conditions were well balanced between the two treatment groups. The most frequently reported concurrent medical conditions (those occurring in at least 25% of patients in at least one treatment group) were jaundice, ascites, vascular disorders, splenomegaly, encephalopathy, spider angioma and cachexia.

Concurrent medications (prophylactic and treatment) taken in the week prior to transplantation were also well balanced between the two treatment groups.

8.2.4.1.5 Protocol Violations

Antilymphocyte globulin (ALG) and antithymocyte globulin (ATG) constituted part of the cyclosporine-based immunosuppressive regimen. Administration of ALG or ATG to patients

assigned to tacrolimus treatment was considered to be a violation of the study protocol. Nine patients treated with tacrolimus also received ALG immunosuppression. All nine patients were from a single center. The duration of ALG administration ranged from 5 to 11 days and in all but two of these patients, ALG administration coincided with an interruption in tacrolimus therapy.

In the tacrolimus group, 14 patients (5.2%) also received ATG immunosuppression for between one and fifteen days. In two of these, ATG was given during an interruption of tacrolimus therapy. Two patients received ATG for graft-versus-host disease and acute rejection, respectively. In the remaining 10 patients ATG was administered as routine post-transplant prophylactic immunosuppression. These violations occurred at the three German centers where ATG therapy was routinely used to treat patients randomized to CBIR therapy immediately post-transplant. These 10 patients received ATG for the first week post-transplant in error and were included in the efficacy and safety analyses.

Azathioprine was a constituent part of the cyclosporine-based immunosuppressive regimen. Azathioprine administration was also allowed in the tacrolimus treatment arm during interruption of tacrolimus administration as a result of experiencing an adverse event. The concurrent use of azathioprine with tacrolimus was considered to be a violation of the study protocol. Of the 267 patients who received treatment with tacrolimus, 66 (24.7%) also received at least one dose of either intravenous or oral azathioprine. In the majority of these patients, azathioprine was administered during an interruption in tacrolimus therapy.

It is unclear what influence these violations had on the evaluation of efficacy measures. The sponsor was asked to provide one year patient and graft survival (simple proportions) broken down by protocol violators versus non violators. Those patients in the tacrolimus group who received ATG or ALG had a higher incidence of death or graft loss at twelve months than those who did not receive ATG or ALG.

8.2.4.2 Efficacy Endpoint Outcomes

This section of the review reflects discussion with the FDA's primary statistical reviewer for this study. Please refer to the FDA's Statistical Review and Evaluation for additional details.

The primary efficacy analysis was based on an intent-to-treat analysis of patient and graft survival at 12 months in the 545 patients randomized in the study. Six-month acute rejection was the principal secondary endpoint analyzed in this population. This section will focus on these three endpoints. The distribution of endpoints among the treatment groups is presented in Table 47 (Source: Vol 1.88, Report Number GHBA-157, Tables 25, 27, 29).

TABLE 47
Endpoints by Treatment Group

Endpoint	Tacrolimus (N=270)	CBIR (N=275)
Patient Deaths at Day 365	50 (18.5%)	68 (24.7%)
Patient or Graft Deaths at Day 365	64 (23.7%)	83 (30.1%)
Acute Rejection up to Day 183	103 (38.6%)	134 (49.1%)

8.2.4.2.1 Patient Survival

The sponsor's results for overall cumulative survival and Kaplan-Meier estimates of patient survival rates at one year are presented here in Table 48 (Source: Vol 1.88, Table 25).

TABLE 48
Patient Survival

	Tacrolimus	CBIR
Kaplan-Meier Estimate at 365 Days	81%	75%
95% Confidence Intervals	77%, 86%	70%, 80%

Wilcoxon test for comparison of survival curves, p-value = 0.078

To assess whether tacrolimus and CBIR were "equivalent" with respect to patient survival the sponsor presented 96.5% Confidence intervals on the differences between the two treatment groups (Source: Response to statistical questions dated 11/9/93). This analysis took into account stratification by center, and was based upon a weighted average over centers of the Kaplan-Meier estimated one-year survival rates. Two different weighting schemes were used. The first used equal weights for each center and the second used weights based on the number of patients in each center. The results are presented in Table 49.

96.5% confidence intervals were provided by the sponsor to maintain consistency with the confidence intervals provided for Study FPC-FK506-7.