PRINCIPLES OF DRUG DEVELOPMENT IN TRANSPLANTATION AND AUTOIMMUNITY

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CHAPTER 10.4

TACROLIMUS IN SOLID ORGAN TRANSPLANTATION

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PRECLINICAL DEVELOPMENT

Prograf™ (Fujisawa Pharmaceutical, Japan) (generic: tacrolimus), also known as FK506 in clinical trials and as FK900506 in preclinical trials, is the first baseline immunosuppressive agent approved by the Food and Drug Administration in over 10 years. In 1982, Fujisawa Pharmaceutical Company instituted a drug screening program to search for new immunosuppressive agents. In May 1984, Goto and Kino described the in vitro immunosuppressive qualities of a fermentation extract of strain 993 of *Streptomyces (Streptomyces tsukubaensis).*[1-3] As reported by Ochiai in August, 1986 at the 11th International Congress of the Transplantation Society (Helsinki, Finland), FK900506 was described as a new macrolide antibiotic with potent in vivo immunosuppressive activity.[4] Further preclinical data on FK900506 was presented the following year (June, 1987) at the European Society of Organ Transplantation in Sweden.[5]

The molecular structure of FK506 is unrelated to cyclosporine, and the two drugs have different cytosolic binding sites.[6] Although both drugs inhibit T-lymphocyte activation and subsequent cytokine production, there is a practical difference in the clinical utility of the two agents. Murase et al demonstrated the ability of FK506 to be used to reverse ongoing, established rejection.[7] The ability of FK506 to be used to reverse ongoing, established rejection, is not a characteristic of cyclosporine. It was this property that was utilized in the first clinical trials of FK506.[8,9]

CLINICAL TRIALS

The first clinical trial of FK506 began at the University of Pittsburgh in February of 1989, under an "Investigational New Drug" (IND) application maintained at the University of Pittsburgh. This followed lengthy discussions between the principal investigators, the Food and Drug Administration and the University of Pittsburgh Institutional Review Board (IRB). Dosing guidelines were developed by careful extrapolation of doses in animal models, normalizing dose to body surface area. A special committee of the IRB was formed to review protocols and results of those protocols.

Later trials for liver, kidney and intestinal transplantation were also initiated under separate IND applications sponsored by Fujisawa. A primary kidney transplant trial was started in Japan in 1990, and a multicenter primary liver transplant trial was started in both Europe and the United States in 1990.

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The initial doses of FK506 recommended for the early trials were the same as those in the initial University of Pittsburgh trials, although the suggestion of dose reduction by the Pittsburgh investigators were not incorporated until midway through the pivotal multicenter European and American trials. Subsequently, dose finding studies have been completed for both primary liver and kidney transplant, verifying the need for lower maintenance oral FK506 doses (Fujisawa, personal communications).

Liver Transplantation

The first clinical use of FK506 was for the indication of uncontrolled liver allograft rejection and were therefore considered treatment failures of conventional immunosuppression. The results of this experience revealed a marked ability to reverse ongoing rejection, even in cases where chronic changes were observed.[8,9-12] Between 50% and 70% of patients treated by conversion to FK506 had both clinical and histopathologic improvement. In a recent long term followup of 113 patients with chronic rejection, 75% of patients were still alive at 3 years following FK506 conversion, and 65% of liver allografts were still functioning (Fig. 10.4.1).[12] The United States Multicenter FK506 Liver Study Group has recently reported their analysis of the prognostic factors for successful conversion from cyclosporine to FK506 based immunosuppressive therapy for refractory liver rejection.[13] At one month after conversion, 54% of patients were noted to have complete or partial improvement. By 6 months after conversion, this benefit was noted in 67% of patients, and 86% at one year. In this series in which the most refractory rejections were converted from cyclosporine to FK506, the one year actuarial graft and patient survival after conversion was 50% and 72%, respectively.

The ability of FK506 to reverse ongoing rejection was the impetus to begin trials of FK506 as initial baseline immunosuppression following primary liver transplantation. Three randomized trials have been performed, comparing FK506 with cyclosporine in primary liver transplantation. While each trial had unique features regarding the immunosuppressive regimens and the characteristics of the patient groups, one of the common primary endpoints was the failure of the defined immunosuppressive treatment to prevent and control rejection. In the University of Pittsburgh series, low risk candidates were randomized to FK506 with steroids as compared to cyclosporine with steroids.[14] One hundred fifty-four patients were enrolled between February 17, 1990 and December 30, 1991. The 1 year patient and graft survival, as analyzed by intent to treat, was 92% and 88% respectively for FK506, compared to 85% and 79% respectively for cyclosporine. The followup at 2 years revealed that the modest increase in patient and graft survival in the FK506 limb was retained. The good results in the cyclosporine limb was due, in part, to the ability of FK506 to treat rejection in the cyclosporine group. The freedom from rejection was statistically greater in the FK506 treated group,

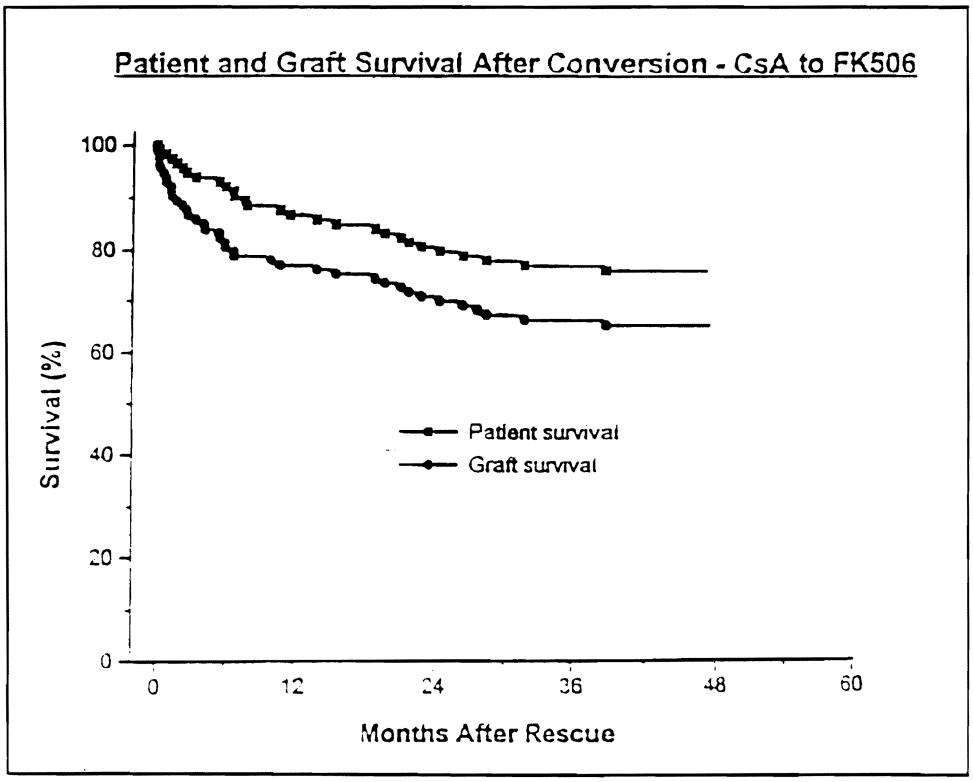

Fig. 10.4.1. Kaplan-Meier estimates of patient and graft survival of liver allograft in patients on cyclosporine who have been converted to FK506 for biopsy documented chronic rejection. Reprinted with permission of A. Jain.

as compared to the cyclosporine treated group. In addition, the freedom from steroid use, and the lower incidence of hypertension, were prominent features of patients treated with FK506.

The results in this single center study have been compared to two other randomized liver transplant trials conducted in the United States and in Europe (preliminary data presented at the International Liver Transplantation Society, Toronto, Canada, October, 1993 and at the Food and Drug Administration, December 1993). In each series, over 500 patients were entered into the combined limbs. Using a regimented FK506 treatment arm, the results obtained in these trials support the findings at the University of Pittsburgh. The European study demonstrated an enhanced patient and graft survival in the FK506 limb, when compared to the CsA limb. Although the patient survival was similar in both groups in the American study, there was a slight benefit in graft survival in the FK506 limb. In both series, approximately 10% of cyclosporine- treated patients required FK506 conversion to treat refractory rejection. This minimized the differences between survival in the FK506 and cyclosporine-treated groups. In both studies, there was less rejection in the FK506 limb, and the overall dosing of steroids was less in the FK506 limb. Lake et al[15] reported that FK506 based immunosuppression was found to lead to significantly lower overall hospital charges associated with liver transplantation, primarily due to less rejection, and the costs associated with treating rejection.

Todo et al described the impact of FK506 in liver transplantation at a single center with a large cohort of patients treated with FK506 as primary immunosuppression.[16] In spite of increasing medical severity and use of expanded donor pool liver allografts, the one year patient and graft survival over the past 13 years has shown a progressive improvement (see Table 10.4.1). In the FK506 era (beginning mid-1989), the 1 year patient (83%) and graft (72%) survival represent a significant improvement over the results obtained in the immediately preceding era of cyclosporine immunosuppression.

Kidney Transplantation

The application of FK506 rescue therapy to kidney transplantation was an extension of the experience gained in liver transplantation. The notable difference between the two organ systems is the predominance of arteriopathy and sclerosis of epithelial structures in the kidney allografts undergoing chronic rejection. This was found to limit the ability of FK506 to rescue kidney allografts with this pathologic finding. In a series of 35 patients, those with ongoing acute cellular rejection had a successful conversion rate of 71%, while those with stigmata of chronic rejection were not able to be rescued.[17] Those with living related kidney transplants had a higher rate of rescue (73%) as compared to those with cadaveric grafts (50%), probably related to an earlier referral for FK506 rescue therapy.

A randomized trial, utilizing FK506 in a double drug regimen (FK506 and steroids) versus a triple drug regimen (FK506, steroids and azathioprine), was performed in kidney transplantation at the University of Pittsburgh.[18] Two hundred four patients were enrolled; there were no specific exclusion criteria based on immunologic or transplant history. Thirty percent of the patients were undergoing a retransplant, with 17% of the recipients being sensitized

Table 10.4.1. Orthotopic liver transplantation—01/01/81 to 12/31/93

Year of Transplantation	Number of Patients	Patient Survival		Graft Survival	
		One Year	Five Years	One Year	Five Years
1981	26	65%	54%	57%	43%
1982	62	50%	44%	35%	29%
1983	75	68%	56%	52%	41%
1984	132	77%	70%	61%	50%
1985	178	67%	57%	51%	41%
1986	264	72%	59%	57%	44%
1987	295	75%	66%	59%	51%
1988	399	76%	65%	64%	51%
1989	395	80%	65%	65%	52%
1990	442	84%	-	71%	-
1991	368	81%	-	70%	-
1992	289	83%	-	72%	-
1993	304	83%	-	73%	-
Total	3229	78%	66%	64%	52%

Note: The survival rates were computed using the Kaplan-Meier method.

(PRA > 40%). The 1 year actuarial patient and graft survival for the two drug versus three drug regimen were: 95% and 90% versus 91% and 82%, respectively. No differences were noted in the kidney function with the mean serum creatinine of 1.8 ± 0.8 mg/dl. The rejection rate in the three drug regimen was less than that for the two drug regimen (37% vs 51%), although this was not statistically significant (p = 0.07). Crossover from one group to another was not uncommon; 25% of the two drug group were given azathioprine, principally due to rejection, while 45% of the three drug group required discontinuation of azathioprine because of leucopenia or hepatic dysfunction.

At the University of Pittsburgh, the impact of FK506 in kidney transplantation is shown in Table 10.4.2. Since mid-1990, the routine use of FK506 for kidney transplantation has improved the one year graft survival to 86%, which represents a significant improvement in graft survival as compared to the immediately preceding era of cyclosporine immunosuppression.

Heart Transplantation

Armitage and co-workers at the University of Pittsburgh have reported their experience with FK506-based immunosuppression following heart transplantation.[19,20] In 8 patients, FK506 was utilized as rescue therapy for patients suffering from persistent, refractory cardiac rejection on cyclosporine, azathioprine and steroids. All of these 8 patients had received and failed one or more courses of antilymphocyte therapy while on cyclosporine therapy. In keeping with the previous experiences with rescue therapy, all of these patients had demonstrated improvement in the histopathology after FK506 conversion.

Seventy-two adult patients were given FK506 as primary immunosuppression following heart transplantation.[20] The one year patient and graft survival was 92%. The freedom from rejection at 90 days was 41% and at 180 days was 34%. Renal dysfunction was frequently noted, and the mean serum creatinine at 6 months following transplantation was 2.2 mg/dl. The incidence of diastolic hypertension was 54%, but was considered mild, as treatment consisted of a single agent in all cases. The incidence of new onset diabetes was 20% in this group of patients.

Lung Transplantation

A small experience of pediatric lung transplantation under FK506 has been reported by Armitage and co-workers.[21] Eleven patients received FK506, two of whom have died because of early infectious complications. While rejection episodes were no different between FK506 and cyclosporine, unlike the cyclosporine experience, antilymphocyte preparations have not been required to reverse rejections on FK506. In addition, hypertension was not seen in the FK506 group, as compared to an incidence of 50% in the cyclosporine group.

A prospective randomized trial of primary adult pulmonary transplantation was conducted at the University of Pittsburgh.[22] Azathioprine was combined with either FK506 or cyclosporine, resorting to using steroids only if the recipient encountered more than one episode of rejection. Twenty eight patients were randomized to FK506, 29 patients were ran-

Table 10.4.2. Kidney transplantation—01/01/81 to 12/31/93

Year of Transplantation	Number of Grafts	Patient Survival		Graft Survival	
		One Year	Five Years	One Year	Five Years
1981	103	96%	86%	71%	47%
1982	119	87%	82%	73%	55%
1983	160	97%	91%	73%	56%
1984	204	90%	79%	68%	49%
1985	175	94%	86%	76%	54%
1986	264	90%	78%	69%	50%
1987	188	95%	85%	73%	55%
1988	209	95%	86%	78%	59%
1989	226	92%	83%	76%	55%
1990	203	93%	-	78%	-
1991	160	95%	-	84%	-
1992	201	93%	-	87%	-
1993	189	98%	-	90%	-
Total	2401	93%	84%	77%	57%

Note: The survival rates were computed using the Kaplan-Meier method.

domized to cyclosporine. The 6 month graft survival was statistically better in the FK506 group as compared to the cyclosporine group (86% vs 69% respectively, $p < 0.05$). Twenty-one percent of the FK506 patients were rejection free at 6 months, as compared to only 3% of the cyclosporine patients. At one year after lung transplantation, the rate of obliterative bronchiolitis, the manifestation of chronic rejection in lung allografts, was statistically less in the FK506 group as compared to the cyclosporine group (Dr. Robert Keenan, personal communication).

Intestinal Transplantation

One way to assess the impact of a new immunosuppressive agent in transplantation is the ability to successfully transplant organs which were not considered feasible with standard immunosuppression. Success with intestinal transplantation under cyclosporine immunosuppression has been sporadic. The use of FK506 for intestinal transplantation has allowed for better control of rejection, with resultant significant improvement in patient and graft survival.[23-25]

Forty-three patients were transplanted between May 2, 1990 and April 15, 1993. A total of 45 grafts were transplanted into the 43 recipients. Fifteen of these patients received an isolated intestinal graft, 21 received both a liver and intestine and 7 received a multivisceral graft. Two retransplantations were performed, one as an isolated intestinal graft, and the other a combined liver/intestine.

There were 22 children and 21 adults, with a preponderance of children requiring liver/intestine transplantation and adults receiving intestinal transplants alone. The sex distribution was similar between the three different types of intestinal transplants. The mean age for the pediatric population was 3.5 ± 3.7 years of age, and for the adults, 33.3 ± 10.2 years of age.

Short gut syndrome due to Crohn's disease was the most common reason for isolated intestinal transplantation, while neonatal intestinal complications with resulting TPN-induced liver failure was the most common reason for combined liver and intestinal transplantation. Mesenteric vascular occlusion, including complications due to inherent hypercoagulopathy, was the most common reason for multivisceral transplantation.

The mean followup of the 30 survivors following intestinal transplantation was 17 ± 9 months (range 6-39 months). The Kaplan-Meier estimates of actuarial survival for the 43 patients revealed an overall 3, 6, 12, and 24 month patient survival of 88%, 84%, 81% and 74%, respectively. When analyzed by cohorts corresponding to intestine alone, liver/intestine and multivisceral, the one year survival was 93%, 71% and 86%, respectively. The corresponding two year actuarial survival was 83%, 65% and 86%. Thirteen patients in all groups died. Four deaths occurred in the isolated small bowel transplant group, eight in the combined liver/intestine group and one in the multivisceral group. Generally the causes of death could be categorized into technical complications (3), opportunistic infections (2), posttransplant lymphoproliferative disease (2), persistent rejection (2), sepsis following graft removal (2) and others (2). In one intestine transplant recipient, death was due to a catheter-related pulmonary embolism which occurred 8 months following allograft enterectomy.

Graft survival was determined for 45 grafts, which included 43 primary and 2 retransplants. The estimated actuarial survival of all grafts was 80%, 78%, 72% and 59% at 3, 6, 12 and 24 months, respectively. Because of the ability to maintain recipients of small bowel allografts on TPN, following removal of the transplanted graft, the discrepancy between the patient and graft survival was greater in this group, as compared to the combined liver/intestine and multivisceral groups. Sixteen of the 45 implanted grafts were lost, either due to patient death[10] or following enterectomy.[6] The indication for allograft enterectomy was refractory rejection in all 6 patients.

DOSING OF FK506

Specific guidelines for the use of FK506 baseline immunosuppression will depend on the organ transplanted. Generally, orally administered FK506 is preferred over the intravenous route. However, when absorption of oral FK506 is not reliable, intravenous administration may be mandatory.

At the University of Pittsburgh, initiation of FK506 therapy is either intravenously as a constant 24 hour infusion, between doses of 0.05-0.15 mg/kg/d, or orally at 0.3 mg/kg/d (in two divided doses). Following liver transplants, lower initiating doses are given, while for kidney and heart transplants, intermediate doses are employed, and the highest levels are used for intestine and lung transplants. Abu-Elmagd et al have previously shown that hepatic dysfunction significantly impacts on FK506 metabolism, and that lower doses of FK506 are needed to achieve similar FK506 plasma concentrations, when compared to good hepatic function.[26] Overlap of intravenous and oral administration is *not* the general rule, except with intestinal transplantation, in which a transition period of approximately 3-5 days is used.

At the University of Pittsburgh, FK506 levels are determined by plasma concentrations using an ELISA assay, as previously reported (see below). While on constant intravenous dosing of FK506, levels between 2-4 ng/ml are used to help determine dosing guidelines. Trough levels are determined immediately prior to administration of oral FK506, and levels of 1-2 ng/ml are used to help guide chronic FK506 administration. Dose reductions of FK506 are made if the

liver function was compromised (see above), or if there are complications of nephrotoxicity or neurotoxicity. Dose increases of FK506 are made if rejection is detected by biopsy. The dose adjustability of FK506 allows the physician to titrate the immunosuppressive baseline to the threshold of rejection, rather than to arbitrarily rely on target drug levels, which may represent overimmunosuppression for some, and underimmunosuppression for others.

Corticosteroids are also individualized for each organ system. Generally, methylprednisolone is given as a single bolus of 1g following reperfusion, and then as a steroid taper for 5 days, beginning at 200 mg/d and ending at 20 mg/d of methylprednisolone. A maintenance dose of 20 mg/d of prednisone or methylprednisolone is given and tapered slowly, as tolerated. In lung transplantation, the steroid taper is omitted in the early posttransplant period, because of concerns about bronchial healing.

Mild to moderate acute rejection is generally treated with a single bolus of methylprednisolone followed by a 5 day recycle of methylprednisolone starting at 200 mg/d and ending at 20 mg/d. In heart transplant recipients, grade III or IV rejections (modified Billingham criteria) are usually given three consecutive 1g boluses of methylprednisolone. We have found that augmented steroid dosing has been required in approximately 50% of patients, who have not respond to increasing doses of FK506. Azathioprine can be added at doses of 0.5-1.0 mg/kg/d if there is persistent rejection, and when the white blood cell count is maintained above 3,500/mm^3. OKT3 (ORTHO, Raritan, NJ) is given at 5-10 cc/d over a 7-10 day course for severe rejection. In our experience, OKT3 was required in approximately 15-20% of transplant recipients.

MONITORING FK506

The results of clinical studies suggest that FK506 is effective for solid organ transplantation, but that toxicity is associated with high plasma or whole blood levels of FK506.[27] Thus the dosing schedule and monitoring techniques for FK506 continue to be modified, at different centers, with different organs. One of the principle issues regarding the use of FK506 has been with monitoring of drug levels. In Europe and at other centers in the United States, whole blood levels have been utilized. This measures not only plasma levels, but also cell bound FK506. The principle advantage of whole blood levels are the higher FK506 levels which can be detected, making adjustments in FK506 dosing potentially easier. Recommended therapeutic whole blood levels are between 10-20 ng/ml with the same caveats regarding dose adjustments for rejection or toxicity. In addition, whole blood monitoring offers the advantage of faster analysis times and less variability of sample storage and preparation disparity. The principal limitations of whole blood monitoring using automated systems (Abbott), has been the insensitivity of the assay at the lower end of detection (< 5 ng/ml).

The issue of appropriate monitoring either whole blood FK506 levels and/or plasma FK506 levels, is reminiscent of the disparity in cyclosporine monitoring techniques.[28]

TOXICITY

FK506 is not without its limitations.[27,29] Toxicity profiles for FK506 are similar to that of cyclosporine, perhaps because of similar mechanisms of action. Both FK506 and cyclosporine administration have been associated with side effects, many of which are similar, and some of which are peculiar to a given organ transplant. Adverse reactions requiring treatment or adjustment of FK506 doses can be categorized into four primary areas. These are: (1) alterations in kidney function; (2) alterations in glucose metabolism; (3) neurotoxicity; and (4) susceptibility to infection or malignancy.

One of the major benefits of FK506 appears to be a relative lack of some of the side effects of cyclosporine. Some of these are cosmetic, such as hirsutism and gingival hyperplasia, which has not been seen with FK506. Other more significant side effects, such as hypertension, appear to be less in the FK506 patients than for those on cyclosporine. The ability to use less steroids in patients with FK506, when compared to cyclosporine, may result in less complications ascribed to chronic steroid use. In one study where FK506 was compared to cyclosporine in pediatric renal transplant recipients, 78% of all FK506 recipients were off steroids.[30] Significant improvement in the growth pattern of preadolescents on FK506 monotherapy was noted, when compared to those on FK506 and steroids, or cyclosporine and steroids.

A clearer profile of adverse reactions and side effects of FK506 has been accumulated. In humans, the principal side effects of FK506, in order of decreasing frequency, are: insomnia, tremors, headaches, muscle aches, itching, fatigue, visual sensitivity to light and GI symptoms.[29] In a prospective, randomized study, the side effects of FK506 were compared to that of cyclosporine. The only differences between the two drugs were an increased incidence of headaches and insomnia in the FK506 group, while those on cyclosporine had an increased incidence of hair growth.[14]

The treatment for the four areas of toxicity varies to a large extent to the degree of toxicity. Generally, toxicity improves with lowering the doses of FK506. Some reactions, such as the development of dysarthrias, may be idiosyncratic or require multiple factors in order to become apparent, and therefore may not respond to decreased doses of FK506. Spe-

cific therapy or supportive measures are indicated until the toxicity resolves. The requirement for hemodialysis in liver transplant patients is such an example. Renal dysfunction in these cases is generally reversible.

The effect of FK506 on fertility and on a fetus is not fully known. Of the 12 female patients treated with FK506 who became pregnant, there were two fetal losses, one from CMV infection and the other from premature delivery (24 weeks). The 10 other babies are healthy and without birth defects.[31]

CONCLUSIONS

While impressive gains in patient and graft survival have been obtained in solid organ transplantation, rejection continues to play a significant role in morbidity and mortality. A search for new immunosuppressive agents must be able to impact on the rates and severity of rejection. Ample clinical reports correlate the adverse impact of rejection on long term graft survival. In the clinical trials presented here, FK506 appears to provide a baseline immunosuppression which is more potent than cyclosporine. At our institution, this is correlated with enhanced patient and graft survival at one year and beyond. In addition, the impact of FK506 on previously "forbidden" transplantation, has been dramatic, with the introduction of a successful intestinal transplantation program and enhanced results of lung transplantation.

Given the effectiveness of current polypharmacy in minimizing graft loss from rejection, improvement in the classical primary endpoint of patient and graft survival will be difficult, taking into consideration the complexity of factors which impact on this endpoint. Secondary endpoints, such as morbidity and quality of life issues, are areas in which newer immunosuppressive agents will have a noticeable impact.

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REFERENCES

1. Kino T, Hatanaka H, Hashimoto M, Nishiyama M, Goto T, Okmuhara M, Kohsaka M, Aoki H, Imanaka H. FK-506, a novel immunosuppressant isolated for a Streptomyces. I. Fermentation, isolation and physiochemical and biological characteristics. J Antibiot 1987; 40:1249-1255.
2. Goto T, Kino T, Hatanaka H, Nishiyama M, Okuhara M, Kohsaka M, Aoki H, Imanaka H. Discovery of FK-506, a novel immunosuppressant isolated from Streptomyces tsukubaensis. Transplant Proc 1987; 19:4-8.
3. Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, Yajima T, Goto T, Okuhara M, Kohsaka M, Aoki H, Ochiai T. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effects of FK-506 in vitro. J Antibiot 1987; 40:1256.
4. Ochiai T, Nakajima K, Nagata M, Suzuki T, Asano T, Uematsu T, Goto T, Hori S, Kenmachi T, Nakagori T, Isono K. Effect of a new immunosuppressive agent, FK-506, on heterotopic allotransplantation in the rat. Transplant Proc 1987; 19:1284-1286.
5. Starzl TE, Makowka L, Groth K, eds. FK-506: A Potential Breakthrough in Immunosuppression. Proceedings of the European Society of Organ Transplantation Satellite Symposium. Transplant Proc 1987; vol 19(Suppl 6).
6. Siekierka JJ, Hung SHY, Poe M et al. A cytosolic binding protein for the immunosuppressant FK-506 has peptidyl-prolyl isomerase activity but is distinct from cyclophillin. Nature 1989; 341:755-757.
7. Murase N, Kim DG, Todo S et al. Suppression of allograft rejection with FK-506. I. Prolonged cardiac and liver survival following short course therapy. Transplantation 1990; 50:186-189.
8. Fung JJ, Todo S, Tzakis A, Demetris A, Jain A, Abu-Elmagd K, Alessiani M, Starzl TE. Conversion of liver allograft recipients from cyclosporine to FK-506 based immunosuppression: Benefits and pitfalls. Transplant Proc 1991; 23:14-21.
9. Starzl TE, Fung J, Venkataramman R, Todo S, Demetris AJ, Jain A. FK-506 for liver, kidney and pancreas transplantation. Lancet 1989; ii:1000-1004.
10. Winkler M, Ringe B, Gerstenkorn C et al. Use of FK-506 for treatment of chronic rejection after liver transplantation. Transplant Proc 1991; 23:2984.
11. Holland R, Sorrell M, Langnas A et al. Chronic rejection in liver transplant recipients: Does conversion to FK-506 confer a survival benefit? Hepatology 1993; 18:74A.
12. Fung JJ, Jain A, Hamad I et al. Long term effects of FK-506 following conversion from cyclosporine to FK-506 for chronic rejection in liver transplant recipients. Hepatology 1993; 18:74A.
13. McDiarmid SV, Klintmalm G, Busuttil RW. FK-506 rescue therapy in liver transplantation: outcome and complications. Transplant Proc 1991; 23:2996.
14. Fung J, Todo S, Abu-Elmagd K et al. A randomized trial in primary liver transplantation under immunosuppression with FK-506 or cyclosporine. Transplant Proc 1993; 25:1130.
15. Lake J, Gorman K, Emond J, Gordan J. Reduced incidence of acute rejection in FK-506 treated patients results in lower overall average cost to treat rejection in liver transplant recipients. Presented at the American Society of Transplant Surgeons, May, 1994.
16. Todo S, Fung JJ, Starzl TE, Tzakis A, Doyle H, Abu-Elmagd K, Jain A, Selby R, Bronsther O, Marsh W, Ramos H, Reyes J, Gayowski T, Casavilla A, Dodson F, Furukawa H, Marino I, Mazariegos G, McMichael J, Kusne S, Venkataramanan R, Warty V, Murase N,

Demetris AJ, Iwatsuki S. Single center experience with primary orthotopic liver transplantation under FK-506 immunosuppression. Ann Surg (in press).
17. Jordan ML, Shapiro R, Jensen CWB, Scantlebury V, Fung J, Tzakis A, McCauley J, Jain A, Demetris AJ, Randhawa P, Simmons RL, Hakala TR, Starzl TE. FK-506 conversion of renal allografts failing cyclosporine immunosuppression. Transplant Proc 1991; 23:3078-3081.
18. Shapiro R, Jordan J, Scantlebury V et al. Randomized trial of FK-506/prednisone vs FK-506/azathioprine/prednisone after renal transplantation: Preliminary report. Transplant Proc 1991; 25:669.
19. Armitage JM, Kormos RL, Fung J, Starzl TE. The clinical trial of FK-506 as primary immunosuppression in adult cardiac transplantation. Transplant Proc 1991; 23:3054-3057.
20. Armitage JM, Kormos RL, Morita S et al. Clinical trial of FK-506 immunosuppression in adult cardiac transplantation. Ann Thorac Surg 1992; 54:205-211.
21. Armitage JM, Fricker FJ, Kurland G et al. Pediatric lung transplantation: The years 1985 to 1992 and the clinical trial of FK-506. J Thorac Cardiovasc Surg (in press).
22. Griffith BP, Bando K, Hardesty RL et al. Prospective randomized trial of FK-506 versus cyclosporine after human pulmonary transplantation. Transplantation (in press).
23. Todo S, Tzakis AG, Abu-Elmagd K et al. Cadaveric small bowel and small bowel-liver transplantation in humans. Transplantation 1992; 53:369-376.
24. Todo S, Tzakis AG, Abu-Elmagd K et al. Intestinal transplantation in composite visceral grafts or alone. Ann Surg 1992; 216:223-234.
25. Todo S, Tzakis A, Reyes J et al. Small intestinal transplantation in humans with or without colon. Transplantation (in press).
26. Abu-Elmagd K, Fung JJ, Alessiani M, Jain A, Takaya S, Venkataramanan R, Warty VS, Shgannon W, Todo S, Tzakis A, Van Thiel D, Starzl TE. Strategy of FK-506 therapy in liver transplant patients: Effect of graft function. Transplant Proc 1991; 23:2771-2774.
27. Alessiani M, Cillo U, Fung JJ, Irish W, Abu-Elmagd K, Jain A, Takaya S, van Thiel D, Starzl TE. Adverse effects of FK-506 overdosage after liver transplantation. Transplant Proc 1993; 25:628-634.
28. Task Force on Cyclosporine Monitoring: Critical issues in cyclosporine monitoring: Report of the Task Force on Cyclosporine Monitoring. Clin Chem 1988; 33:1269-1288.
29. Fung JJ, Alessiani M, Abu-Elmagd K, Todo S, Shapiro R, Tzakis A, van Thile D, Armitage J, Jain A, McCauley J, Selby R, Starzl TE. Adverse effects associated with the use of FK-506. Transplant Proc 1991; 23:3105-3108.
30. Ellis D, Shapiro R, Jordan ML et al. Comparison of FK-506 and cyclosporine regimens in pediatric renal transplantation. Pediatric Nephrology (in press).
31. Jain A, Venkataramanan R, Lever J, Warty J, Fung JJ, Todo S, Starzl TE. FK-506 and pregnancy in liver transplant patients. Transplantation 1993; 56:751.