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What Have We Learned About Primary Liver Transplantation Under Tacrolimus Immunosuppression?

Long-Term Follow-up of the First 1000 Patients

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Objective
To summarize the long-term efficacy and safety of tacrolimus in orthotopic liver transplant (OLT) recipients, as well as to examine the factors that influence long-term morbidity and mortality rates.

Background
Tacrolimus (FK506, Prograf) was introduced as primary immunosuppression for primary liver transplantation in 1989; many subsquent trials have verified the association of tacrolimus with decreased rates of acute rejection and steroid-resistant rejection after OLT. Cumulative experience with tacrolimus has also defined its short- and intermediate-term toxicity.

Methods
One thousand consecutive patients undergoing primary OLT at a single center from August 1989 to December 1992, under tacrolimus immunosuppression, were followed until January 1999. Patients were categorized by age. Mean follow-up was 93.4 ± 11 months after OLT. Patient survival, graft survival (with corresponding causes of death and retransplantation), and rejection rates (and corresponding doses of immunosuppression) were examined as efficacy parameters. Hypertension, renal function, incidence of malignancies, incidence of diabetes, and other toxicities were examined as safety parameters.

Results
Actual 6-year overall patient survival rate was 68.1% and graft survival rate was 62.5%, with significant differences in the patterns of survival among the different age groups. After the first post-OLT year, infection, recurrence of disease, *de novo* malignancies, and cardiovascular events were the main causes of graft loss and death during the long-term follow-up. Graft loss related to either acute or chronic rejection was rare. The rate of acute rejection beyond 2 years was approximately 3% per year, and most were steroid-responsive. Approximately 70% of the patients were receiving tacrolimus monotherapy beyond year 1; at the latest follow-up, 74.2% were maintained on tacrolimus alone. In 6.1% of the survivors, end-stage renal disease developed during the follow-up period, requiring either dialysis or kidney transplantation. Hyperkalemia and hypertension was observed in approximately one third of the patients. Insulin-dependent diabetes mellitus (including patients who had diabetes before the transplant) was observed in 14% in year 1, dropping to 11% in year 7. In 82 patients, *de novo* malignancies developed; in 41 patients, lymphoproliferative disorders developed during the entire follow-up period.

Conclusions
Long-term patient and graft survival rates are excellent under tacrolimus immunosuppression. Pediatric patients have a better long-term outcome than adults, in part because of the limited recurrence of the original disease, which was the most common cause of late graft loss (other than patient death, most commonly the result of late *de novo* malignancies and cardiovascular events). Graft loss from late rejection was rare.

Tacrolimus (FK506, Prograf) is a macrolide antibiotic derived from the soil fungus *Streptomyces tsukubaensis*. It has potent *in vitro* and *in vivo* immunosuppressive qualities. Clinical trials with tacrolimus began at our institution in March 1989, initially as rescue treatment for failing liver allografts under cyclosporine (CsA).[1–5] Subsequently, its utility was demonstrated in primary liver transplantation (OLT).[6–8] One clear early benefit was the reduction in the number of episodes and severity of acute rejection, which was demonstrated by 1990.[7,9,10] In addition, the acute toxicity profile of tacrolimus was also delineated in these and subsequent reports.[11–24]

These observations were subsequently verified in three prospective randomized trials, which were conducted before FDA approval: the Pittsburgh single-center trial, in which tacrolimus was compared with CsA, both using low-dose steroids alone;[25,26] and the U.S. and European multicenter trials comparing tacrolimus with low-dose steroids with CsA as part of double, triple, or quadruple induction protocols.[27,28] In these trials, the immediate benefits and limitations of tacrolimus were delineated. Further follow-up of the multicenter trials has demonstrated excellent patient and graft survival rates, with a long-term toxicity profile that has been quite acceptable.[29]

The purpose of this study is to evaluate the long-term efficacy and side effect profile of tacrolimus in primary OLT recipients, using the principal determinants of patient survival, graft survival, rates of rejection, baseline immunosuppression, and physiologic abnormalities associated with the drug in this population of patients.

MATERIALS AND METHODS

The study subjects are the first 1000 consecutive patients who received a primary OLT under tacrolimus-based immunosuppression between August 1989 and December 1992. There were 600 male patients and 400 female patients, with a mean age of 42.6 ± 20.2 years. For purposes of this study, infants were defined as ≤ 2 years (n = 75), children were > 2 years, ≤ 18 years (n = 91), adults were >18, ≤ 60 years (n = 630), and seniors were > 60 years (n = 204). Eight hundred forty-one (84.1%) patients were hospital-bound at the time of OLT. All patients were followed until January 1999. The mean follow-up was 93.3 ± 11 months (range 72–113). The indications for liver transplantation are shown in Table 1. The details of the immunosuppressive protocol used in this group of patients has been described before.[30,31] However, this group of patients represents an earlier experience with tacrolimus, using a dosing schedule with higher doses of tacrolimus than are used currently.[32]

Profiles of rejection, safety parameters, levels of immunosuppression, evidence of disease recurrence, and physiologic disturbances were evaluated using a prospective-designed clinical database (Electronic Database Interface for Transplantation [EDIT], Thomas Starzl Transplantation Institute, Pittsburgh, PA).

Table 1. INDICATIONS

	n (%)
Postnecrotic cirrhosis	341 (34.1)
Alcoholic cirrhosis	188 (18.8)
Biliary atresia	86 (8.6)
Primary biliary cirrhosis	83 (8.3)
Primary sclerosing cholangitis	60 (6)
Primary hepatic malignancy	79 (7.9)
Acute fulminant failure	34 (3.4)
Miscellaneous	129 (12.9)
Total	1000 (100)

RESULTS

Survival

The actuarial Kaplan-Meier estimates of patient and graft survival are shown in Figures 1 and 2. The greatest number of deaths occurred in the first year; the overall mortality rate averaged 3% every year thereafter, with an actual overall patient survival rate of 68.1% and a graft survival rate of 62.5% at 6 years and a actuarial survival rate at 9 years of 61.7% (patient) and 56.4% (graft). Of interest is the divergence of survival seen in the different age groups, with the best long-term survival rate seen in the pediatric group and the worst in the elderly.

The causes of death are shown in Table 2. Overall, infectious complications were the most common cause of death, particularly in the first year. Thereafter, recurrence of disease (including malignancy), *de novo* malignancies, and cardiovascular events constituted the main causes of death. As expected, these events were predominantly seen in the adult and elderly groups.

Causes for Retransplantation

During the entire follow-up period, 170 retransplantations were performed in the 1000 patients. One hundred forty-four patients lost their first graft and underwent a second transplant, 22 patients required a third transplant, and 4 patients needed a fourth transplant. As shown in Table 3, primary nonfunction, hepatic artery thrombosis, and recurrence of disease were the most common reasons for retransplantation. As expected, the greatest number of retransplantations were performed in the first year (n = 126—

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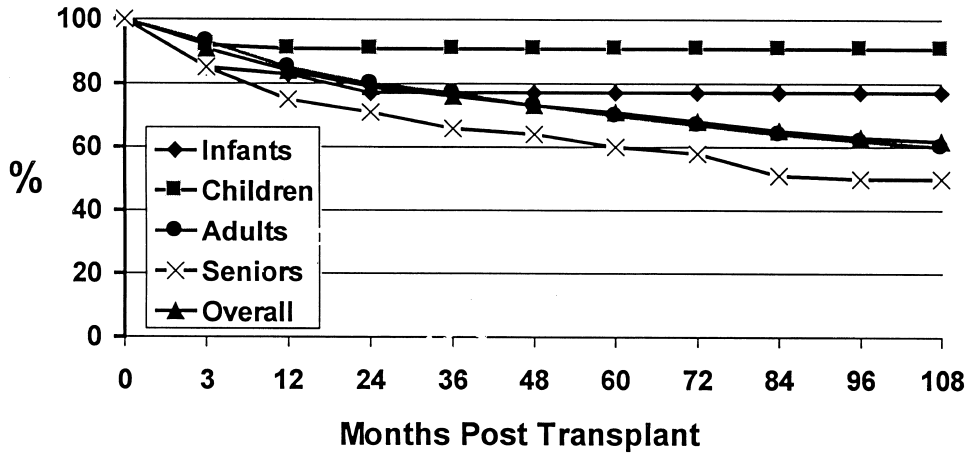

Figure 1. Kaplan-Meier patient survival rates for the 1000 primary liver transplant patients. Survival figures up to year 6 are actual; those after year 6 are actuarial survival.

74.1% of total retransplantations); it became a less frequent event after that point. In addition, late retransplantations were principally for recurrent disease and were mostly confined to the adult group.

Maintenance Immunosuppression

Tacrolimus

The mean tacrolimus dose was lower each year after OLT, starting at 8.5 mg/kg/day at year 1 and dropping to 4.8 mg/kg/day by year 5. Thereafter, doses of tacrolimus tended to remain constant. Similarly, the mean plasma tacrolimus trough concentration was 0.8 ng/ml at year 1 and 0.7 ng/ml at year 4.[33] After conversion of the monitoring assay for tacrolimus to trough whole blood concentration, the mean tacrolimus level was 9.7 ng/ml at year 5 (tacrolimus levels <5 ng/ml were considered as 5 ng/ml for purposes of calculating levels)[34] (Table 4).

Cyclosporine

Thirty-seven (4.4%) adults and seniors were converted to CsA because of neurologic events (n = 20), lack of appetite and failure to thrive (n = 6), hematologic disorders (n = 5), and other infrequent causes (n = 6). Currently, eight adults and seniors (1.6%) are receiving CsA. None of the infants or children require CsA, and none of the patients received CsA with tacrolimus simultaneously.

Corticosteroids

Approximately 70% of the patients were maintained without prednisone. Even the patients who required prednisone at 1 year (13%) were able to take lower doses of prednisone in subsequent years. Thus, although 15% of the patients were receiving >5 mg/day of prednisone at 1 year, this declined to 5% at year 5 (see Table 4).

Adjunctive Immunosuppressive Agents

Azathioprine was not used routinely after OLT, and mycophenolate mofetil was not available during this period. In only selected patients was azathioprine (and since 1995 mycophenolate mofetil) used, generally to minimize nephrotoxicity ascribed to tacrolimus. Currently, 27 adults/seniors and 4 infants/children are receiving azathioprine, whereas 9 adults/seniors and 5 infants/children are receiving mycophenolate mofetil. The azathioprine doses used were usually <50 mg/day; no patient received >100 mg/day. Mycophenolate mofetil doses averaged <2 g/day.

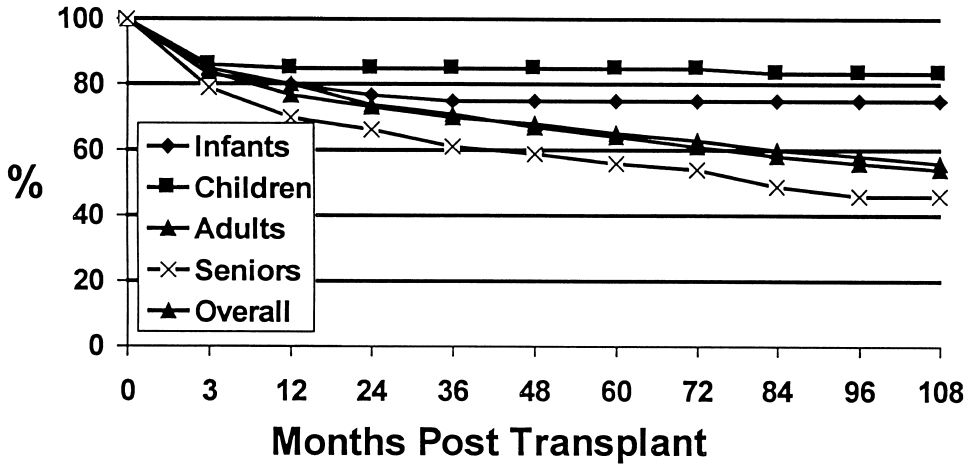

Figure 2. Corresponding Kaplan-Meier graft survival rates for the 1000 primary liver allografts. Survival figures up to year 6 are actual; those after year 6 are actuarial survival.

Table 2. CAUSES OF DEATH

	n (%)
Infection	123 (34)
Cardiopulmonary	57 (16)
Recurrent + de novo cancer	53 (15)
Recurrent disease	43 (12)
Multisystem organ failure	18 (5)
Cerebrovascular	16 (4)
Miscellaneous	50 (14)
Total	360 (100)

Adverse Events

Nephrotoxicity

Nephrotoxicity was the most common complication of tacrolimus use. However, after OLT, there was little change in the mean serum creatinine level or blood urea nitrogen over the follow-up period (Table 5). More than 80% of the patients had a serum creatinine level ≤2.0 mg/dl. Currently, 20 patients are surviving with kidney transplants performed for end-stage kidney disease, whereas 19 patients are currently receiving hemodialysis. The greatest incidence of kidney failure requiring long-term dialysis or kidney transplantation was seen in the adult and senior groups (n = 36, 7.2% survivors) compared with three in the infant and pediatric groups (2.1% survivors).

Hyperkalemia

As shown in Table 6, approximately 40% of patients in the first 3 years had hyperkalemia (defined as K^+ >5.0 requiring treatment). This was observed in approximately 18% of the study population currently (see Table 6). In all the cases it was readily controlled with fludrocortisone.

Hypertension

Thirty-five percent of the patients are currently receiving antihypertensive medications. In >75% of the patients, this hypertension was controlled with a small dose of a single antihypertensive agent, usually a calcium channel blocker (see Table 6).

Table 3. CAUSES OF RETRANSPLANTATION

Causes of first retransplant	n (%)
Primary nonfunction	63 (6.3)
Hepatic artery thrombosis	45 (4.5)
Recurrent hepatitis	11 (1.1)
Acute + chronic rejection	11 (1.1)
Biliary complications	8 (0.8)
Other	6 (0.6)
Total	144

II: 144 (14%); III: 22 (2%); IV: 4 (0.4%).

Table 4. IMMUNOSUPPRESSION

Months Post-OLT	3	6	12	24	36	48	60
Tacrolimus dose mg/d*	11.0	10.5	8.5	6.9	5.9	6.0	4.8
Tacrolimus level ng/ml*	1.0	0.9	0.8	0.7	0.6	0.7	9.7†
Prednisone							
0 mg/day (%)	53	57	67	72	71	66	67
1–9 mg/day (%)	37	35	30	24	25	29	28
≥10 mg/day (%)	10.0	8.0	3.0	4.0	4.0	5.0	5.0

* Mean value.
† Whole blood trough levels (others are plasma level).

New-Onset Diabetes

Fourteen percent of the patients at year 1 and 11% of the patients currently are receiving insulin, as shown in Table 6.

De Novo Malignancies

In 82 patients, *de novo* malignancies developed, 33 of which were skin cancers, including 2 melanomas. The remaining were gastrointestinal (n = 11), genitourinary (n = 9) pulmonary (n = 8), oropharyngeal (n = 7), breast (n = 3), leukemia (n = 3), Kaposi sarcoma (n = 2), thyroid (n = 2), unknown primary (n = 2), brain (n = 1), conjunctiva (n = 1), and *de novo* hepatocellular carcinoma (n = 1) (Table 7).

Lymphoproliferative Disorders

Lymphoproliferative disorders developed in 18 (10.8%) children or infants and in 23 (2.8%) adults or seniors. Sites are shown in Table 8.

Technical Complications

A total of 21 (12.7%) bile duct complications were observed in infants/children and 177 (21.2%) in adults/seniors during the entire study. In the pediatric population, these consisted of extrahepatic strictures in 11, intrahepatic strictures in 8, and bile duct leaks in 2 in the pediatric popula-

Table 5. NEPHROTOXICITY*

Months	0	3	6	12	24	36	48	60
BUN	22	31	30	29	27	27	26	25
Creatinine	1.1	1.7	1.7	1.7	1.6	1.6	1.7	1.7

* Values (mg/dl) determined in survivors.

Table 6. PHYSIOLOGIC DERANGEMENTS

Months	3	6	12	24	36	48	60	Current
Hypertension (%)	29	29	31	37	36	41	46	35
Diabetes mellitus (%)	24	13	14	15	16	17	18	11
Hyperkalemia (%)	39	44	47	46	40	38	35	18
Chol. (Mean:mg/dl)	102	159	165	172	176	179	178	179

tion. In the adult/senior population, they consisted of 119 extrahepatic strictures, 29 intrahepatic strictures, 28 ampullary dysfunctions, and 28 bile leaks.

Seventy-six patients had hepatic artery complications (7.6%)—64 (7.6%) in the adult/senior group and 12 (7.2%) in the pediatric population. These complications consisted of thrombosis in 44 patients, strictures in 28, and aneurysms in 4. Although most of the arterial complications in infants/children occurred within the first postoperative month, in adults/seniors these complications occurred early and late after OLT in equal proportions.

DISCUSSION

The introduction of CsA led to significant improvements in OLT patient survival and graft survival rates, with reduction in the incidence and severity of rejection.[35,36] In addition, the safety profile of CsA was determined early, with nephrotoxicity, hepatotoxicity, and neurotoxicity being particularly notable.[37,38] However, even after a decade of use in liver transplantation, many questions still remained.[39]

The use of the more potent immunosuppressive agent tacrolimus has further reduced the frequency and severity of rejection in OLT.[25,27,28,32] As demonstrated in randomized OLT trials comparing a simplified tacrolimus/low-dose steroid protocol with more complex CsA-based immunosuppressive regimens, patient and graft survival rates in the tacrolimus groups at 1 year were equal to or better than that for CsA, despite the artifact introduced by the high rate of successful conversions of CsA patients to tacrolimus for treatment of rejection.[32] The lower rate of rejection under tacrolimus may be in part due to the ability of increasing tacrolimus levels to reverse ongoing rejection.[2,40] In addition, other metabolic benefits were seen, such as lowered incidence of hypercholesterolemia and hypertriglyceridemia.[41] This survival difference has been magnified in follow-up reports of patients entered in this trial, and the benefits of tacrolimus therapy appear to be sustained at 5 years after OLT.[42] The rate of late acute rejection remains low and is often associated with noncompliance. The incidence of chronic rejection also appears less than that reported under historic CsA experiences (Demetris AJ, manuscript in preparation).

Table 7. DE NOVO NONLYMPHOID MALIGNANCIES*

Skin	33
Melanoma (2)	
Other (31)	
Gastrointestinal	10
Genitourinary	9
Lung	8
Oropharyngeal	7†
Miscellaneous	15
Breast (3)	
Leukemia (3)	
Unknown primary (2)	
Kaposi's (2)	
Thyroid (2)	
Brain (1)	
Eye (1)	
Liver (1)	

* Total: 82 (8.2%)
† Incidence significantly higher than expected.

Table 8. LYMPHOID MALIGNANCIES

	Adults/Seniors	Children/Infants
Site	n (%)	n (%)
Lymph node	10 (43)	6 (33)
Gastrointestinal	5 (22)	5 (28)
Liver	4 (19)	3 (17)
Lung	2 (9)	2 (11)
Tonsil	2 (9)	1 (6)
Skin	0	1 (5)
Total	23 (2.8)	18 (10.8)

Both patient and graft survival rates were statistically significantly better in the pediatric population (infants and children) than in the adult population (adults and seniors).[43] In this analysis, the pediatric population enjoyed an overall 6-year actual survival rate of >85%. This compares very favorably with contemporaneous reports on pediatric transplantation under CsA.[44] Most of the diseases likely to recur (*e.g.*, hepatitis C, primary sclerosing cholangitis, and alcoholic liver disease) are seen in adult men; thus, recurrence of disease is likely to lead to diminished long-term patient and graft survival rates.[45]

In this large series of comprehensively followed OLT patients receiving tacrolimus, with the longest follow-up available, we sought to profile the factors that affect long-term morbidity and mortality rates. This is particularly important because there have been many concerns about the durability of CsA and long-term toxicity, including renal failure, development of malignancies, and increased risks of cardiovascular complications.[46] The patients in this study received an initial tacrolimus dose three to five times higher than those currently used, although the doses at year 7 are similar to maintenance doses for more recently transplanted patients.[47] These results have been achieved using low-dose maintenance corticosteroids, and in nearly 70% of patients

corticosteroids have been weaned entirely. Late-term graft loss and death are related to recurrence of disease, such as malignancies,[48] hepatitis B,[49] hepatitis C,[50] and primary sclerosing cholangitis. Other have reported no differences in the incidence of recurrent disease with one immunosuppressive agent *versus* another,[51] whereas others have suggested differences.[52] Recurrent primary biliary cirrhosis and recurrent autoimmune hepatitis are generally associated with allograft dysfunction but not graft loss.[53]

Despite the concern of long-term chronic nephrotoxicity, end-stage renal disease requiring dialysis or kidney transplantation occurred in approximately 6% of patients in this long-term follow-up study. This figure is consistent with the findings of comparable early nephrotoxicity after OLT using either CsA or tacrolimus,[27,28,54,55] and longer-term follow-up.[42,56] The cumulative increase in the development of end-stage renal failure in tacrolimus-treated patients is consistent with other reports in CsA-treated patients.[57] The incidence and management of hyperkalemia and hypertension appear to be stable with long-term follow-up. The diabetogenicity of tacrolimus appears to be limited: there was no significant difference in the incidence at year 1 (14%) and at year 6 (11%). The lower levels of tacrolimus late after OLT may account for a decreased incidence of diabetes, as has been shown for other adverse events.[58,59] This study also suggests that cardiovascular risk factors, such as hypertension and altered lipid profiles, favor the use of tacrolimus,[60] although the increasing use of steroid weaning in CsA-treated patients may improve the profile of these cardiovascular risk factors.[61–64] The cardiovascular risk profile of the newer CsA formulation Neoral does not appear to be much different than the standard formulation in OLT patients.[65]

The enhanced immunosuppressive potency of tacrolimus does not appear to be associated with a late risk for development of malignancy. Lymphoproliferative disorders developed in 41 patients in this series, in which higher doses of tacrolimus were initially used; the predominance in the pediatric population (12.7%) was significantly higher than in adults (2.8%). The risk factors for the higher incidence in pediatric patients has been previously reported.[66] Fortunately, in both populations, the majority (>70%) of patients survived with resolution of the lymphoproliferative disorder (data not shown). Finally, the risk of nonlymphoid tumors does not appear to be higher than for CsA, with a wide variety of solid and hematologic malignancies.[67] The age-adjusted risk appears increased for the aerodigestive system only.[68] We have previously suggested that the increased incidence in aerodigestive malignancies may be associated with the risk factors of a long smoking history and chronic alcohol use. However, *de novo* malignancies are a risk factor for long-term survival in adult and senior OLT recipients, whether the incidence is increased or not, compared with age-adjusted general population cohorts.[69,70]

In conclusion, the principal limitations of long-term OLT survival no longer include rejection. Recurrent diseases, in particular recurrent malignancy and recurrent viral hepatitis, as well as cardiovascular events, are the major causes of graft and patient loss. The development of *de novo* malignancies may be prevented by careful screening, and effective treatment can be instituted with earlier detection. Long-term survival in pediatric patients is excellent; the risk of lymphoproliferative disease development is higher, but the impact on survival is minimal.

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Discussion

DR. DAVID L. DUNN (Minneapolis, Minnesota): I rise to congratulate Drs. Fung, Starzl, and colleagues for an excellent presentation providing us with much more than an incremental advance in the immunosuppressive drug therapy for transplantation. This is a very interesting study, and it follows on the heels of several prospective randomized trials comparing other immunosuppressive drugs, particularly cyclosporine, to FK506 or tacrolimus.

It is an interesting study from an historical perspective because the Pittsburgh group very clearly resurrected tacrolimus. The initial studies with FK506 experimentally and clinically indicated to us that it was diabetogenic, that there were problems associated with arteritis. Yet they persevered and very clearly have demonstrated enhanced graft survival, and therefore patient survival, in this liver transplant patient population.

Having said that, I have several questions for you, Dr. Fung.

First, cyclosporine and FK506 are so similar from an immunologic standpoint. Why is it, do you think, that this is a superior drug? Is there something different about the liver immunologically that lends itself to FK506 immunosuppressive drug therapy?

Secondly, it did seem as though initially you observed a slightly higher incidence of diabetogenic properties. Have you seen that in this trial now showing us long-term results?

Thirdly, looking at the steroid withdrawal in your patients, was this done by protocol in the FK506 patients, and was it similar to the previously studied cyclosporine patients? It was not clear to me in the manuscript whether this was the case, as certainly that would influence your overall allograft and therefore patient survival.

Lastly, you have once again shown us a relatively high incidence of PTLD in your pediatric liver transplantations. Is that similar to your previous studies with cyclosporine or is it different with FK506?

PRESENTER DR. JOHN J. FUNG (Pittsburgh, Pennsylvania): The mechanism of action of FK506 and cyclosporine share a common pathway of inhibiting calcineurin-mediated activation of T cells. However, there may be a biologic difference in the induction of drug resistance by these two agents.

One hypothesis has been that p-glycoprotein, which is responsible for modulating cellular efflux of both cyclosporine and tacrolimus, is differentially induced by these agents. Tacrolimus appears to be associated with decreased induction of this pathway of drug resistance. Outside of this mechanism, and pharmacokinetic differences between these agents, there isn't a clear explanation. There has also been some speculation that TGF-β expression is enhanced with tacrolimus, which may account for some immunosuppressive benefit.

In addressing some of the adverse events associated with tacrolimus, particularly diabetes, there was an early learning curve. Currently, we are using tacrolimus doses that are between 50% and 75% lower than was used during the period in question. Having said that, I do believe that tacrolimus alone does have a diabetogenic potential, but not substantially greater than cyclosporine/corticosteroids.

Recently, patients who develop complications, such as nephrotoxicity and diabetes, will be converted to a triple drug regimen with mycophenolate. And Ron Shapiro's data from our kidney transplant group shows a significant reduction of diabetes with tacrolimus steroids and mycophenolate. Thus, the polypharmacy approach can be of benefit to a certain group of patients.

This study demonstrated to us that steroid weaning could be achieved easily, and altered our approach to management of patients on both tacrolimus and cyclosporine. I feel that most of our clinicians are more willing to wean steroids in all transplant patients. This was not a comparative study. However, both the American and European multicenter tacrolimus and cyclosporine trials did show a benefit of tacrolimus in terms of lowering baseline corticosteroid requirements.

Lastly, the incidence of PTLD with the use of tacrolimus is within the range that has been previously described with cyclosporine. But I think one of the things that has been different is that our understanding of PTLD—what we consider PTLD is—continues to evolve. We are currently now using quantitative EBV-PCR to detect EBV and have introduced both with the introductive preemptive ganciclovir therapy. These modifications appear to have diminished the frequency and severity of PTLD.

DR. DONALD C. DAFOE (Palo Alto, California): Dr. Fung, this is an impressive experience. A thousand procedures of any sort is a remarkable accomplishment: 1000 liver transplants, truly awe-inspiring. Through all-inclusive long-term reports like this one where all the data are laid out, the Pittsburgh group, Drs. Starzl, Fung, and colleagues, continue to establish the benchmarks for other programs.

It is clear that tacrolimus monotherapy provides excellent patient and graft survival. But the downside of tacrolimus and any other effective regimen is infection, oncogenicity, recurrent disease, hypertension, diabetes, and accelerated cardiovascular disease. I suspect this paper sets the stage for the next phase at Pittsburgh of tolerance induction through the establishment of stable chimerism, as suggested by the paper we heard yesterday from Dr. Corry. Until that day arrives, I have a few questions.

Due to the incidence of diabetes and other tacrolimus-related problems, should the dose be lower and should other agents such as mycophenolate be added back?

Although some of the causes of graft and patient loss such as cancer can be addressed through vigilant follow-up, recurrent

hepatitis is less amenable to early diagnosis and treatment. I would like to ask whether lamivudine has had an impact on your recurrent hepatitis B. And given the impending epidemic of hepatitis C leading to end-stage liver disease, what is your current strategy to prevent recurrent hepatitis C?

DR. FUNG: All of us who deal with liver transplantation are concerned with recurrent disease, particularly hepatitis C.

We know that almost 100% of patients that are transplanted for hepatitis C have recurrent infection, although the timing and incidence of developing complications of this recurrent disease are quite variable. Unfortunately, we have seen patients that have had recurrent hepatitis C destroy their livers within a short period of time, 6 months. Unfortunately, the treatment that is available is very limited. Ribavirin and interferon protocols at best clear virus in about 5 to 10% of patients. So we would say that this represents a dismal outlook.

There is a definite trend toward using lower-dose tacrolimus with a lower threshold to adding other agents like mycophenolate. We completed a tacrolimus and steroid *versus* tacrolimus, steroid, and mycophenolate randomized trial. Dr. Jain reported this at the ASTS last year. There is a clear decreased incidence of nephrotoxicity and diabetogenicity.

It will be of interest to determine a variety of new immunosuppressive agents, *e.g.*, IL-2 receptor monoclonals and rapamycin in future trials.

DR. MARLON LEVY (Dallas, Texas): Dr. Fung, thank you for making your manuscript available for review. I also congratulate you on spectacular results. Really, no one in the transplant world can match Pittsburgh's numbers and longevity and the ability to report series such as these. I think both your paper and your presentation were interesting and odd in a way. There is sort of a shadow-boxing going on with comparing cyclosporine, and yet you don't really ever give any data on cyclosporine. So it is sort of an odd way to present this.

My questions are related to specific immunosuppressive strategies. You cite an extremely low incidence of chronic rejection, extremely low incidence of graft loss due to chronic rejection, but all except one of the primary advantages of FK is its ability to modulate both the key rejection and chronic rejection. So what is your strategy for chronic rejection, specifically as it relates to FK, and what is your incidence of chronic rejection?

The second question is about how you are now integrating Prograf with some of the newer immunosuppressants. You have talked a little bit about CellCept. What about perhaps rapamycin and some of the new generation monoclonals, including either the humanized or the chimeric monoclonals?

DR. FUNG: Baylor was a key institution in the multicenter tacrolimus trial in liver transplantation and appreciated some the problems with the early learning phase. We have not had the opportunity of using any of the anti–IL-2 receptor monoclonal antibody preparations.

The NIDDK study clearly showed that acute rejection *per se* does not necessarily portend a poor outcome in liver transplant. So the prevention of acute early rejection in livers doesn't have the same kind of impact that has been reported in kidney transplantation. We have not tried to further reduce acute rejection episodes, which under tacrolimus and mycophenolate are running about 20%. I think a zero incidence of rejection would increase the risk of infection, as Dr. Diethelm had suggested.

I think there should be better individualization of patients for immunosuppressive agents and more thoughtfulness given to agents that are selected. For example, somebody with alcoholic liver disease doesn't have the same risk of rejection as, say, someone with autoimmune hepatitis. This patient might benefit from less immunosuppression.

With *de novo* malignancy, particularly the aerodigestive tract, malignancies were seen predominantly in the alcoholic group. These patients have risk factors for aerodigestive cancers based on their smoking and drinking history. Once we identified this as being a risk in this particular group, we increased our screening, so all of our patients that had a history of smoking are subjected to very careful ENT examination. We have picked up a number of severe dysplasias and vocal cord and laryngeal malignancies. These types of high-risk patients may be better off on rapamycin, since rapamycin has antiproliferative potential in malignancies.

Lastly, this was not a study that was designed to compare tacrolimus with cyclosporine. As you know, this data was being accumulated during the period the U.S. and European multicenter trials were being conducted. This single-center experience allowed us to get a very good handle on limitations of FK. Since Dr. Todo's initial presentation at this meeting 6 years ago, we much better understand the side-effect profile. As this experience has evolved, we have been able to maximize the benefits of tacrolimus while minimizing its toxicities.