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FK778, A Synthetic Malononitrilamide

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FK778 is a synthetic malononitrilamide (MNA) that has been demonstrated to have both both immunosuppressive and anti-proliferative activities. The MNAs inhibit both T-cell and B-cell function by blocking de novo pyrimidine synthesis, through blockade of the pivotal mitochondrial enzyme dihyroorotic acid dehydrogenase (DHODH), and the inhibition of tyrosine kinase activity. FK778 has been demonstrated to prevent acute allograft rejection in multiple experimental transplant models in rodents, dogs and primates and to be effective in the rat model of chronic renal allograft rejection. In addition, FK778 has been shown to prevent vascular remodeling after mechanical intimal injury via a mechanism which may be related to tyrosine kinase inhibitory activity in vascular smooth muscle cells. Another intriguing activity of the MNA family is the ability to block replication of members of the Herpes virus family with *in vitro* evidence that of efficacy against cytomegalovirus (CMV) and polyoma virus, important pathogens in the transplant recipient. FK778 is currently being explored in a number of trials in solid organ transplant recipients.

Key Words: FK778, malononitrilamides, acute rejection, chronic rejection, cytomegalovirus, polyoma virus

INTRODUCTION

The introduction of more potent immunosuppressive agents over the last two decades has resulted in a progressive improvement in 1-year graft survival rates after renal transplantation. While long-term graft survival has followed a similar trend,[1] graft loss after the first year post-transplantation continues to decline at an annual rate of 3-5%.[2] In the majority of cases, late graft loss is attributed to chronic allograft nephropathy or death with a functioning graft.[2,3] Death with a functioning graft is a leading cause of late renal allograft loss, and in the majority of cases the cause of death is related to cardiovascular events.[4,5] Candidates for renal transplantation often have a high cardiovascular risk profile due to pre-existing conditions. Furthermore, their risk of post-transplant cardiovascular disease is increased by hypertension, hyperlipidemia and diabetes, all of which can be exacerbated by immunosuppressive agents.[5,6] FK778 is a novel immunosuppressive agent that may have an impact on chronic allograft nephropathy and cardiovascular risk factors, as well as potential anti-viral activity.[7]

FK778

FK778, a synthetic malononitrilamide (MNA), is derived from A771726, the active metabolite of leflunomide. FK778 has a much shorter half-life than leflunomide, thereby making it potentially suitable for use in transplantation,[8] and has been demonstrated, both *in vitro* and *in vivo*, to have an equivalent or higher immunosuppressive activity.[9,10] FK778 has both immunosuppressive and anti-proliferative activities. The MNAs inhibit both T-cell and B-cell function by blocking de novo pyrimidine synthesis, through blockade of the pivotal mitochondrial enzyme dihyroorotic acid dehydrogenase (DHODH),[11-14] and the possible inhibition of tyrosine kinase activity.[14,15]

FK778 has been demonstrated to prevent acute allograft rejection in multiple experimental transplant models in rodents, dogs and primates.[10,16-18] In the rat model of chronic renal allograft rejection, recipients treated with FK778 for 10 days exhibited a dose-dependent decrease in proteinuria

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and serum creatinine levels during the 90-day follow-up period after transplantation when compared to allograft controls.[19] In the FK778-treated animals there was significantly fewer chronic allograft nephropathy changes (tubular atrophy, glomerulosclerosis, fibrointimal hyperplasia, and transplant glomerulopathy). In addition, FK778 treatment was associated with decreased intragraft mononuclear cell infiltration, as well as significantly lower serum allo-specific immunoglobulin production (both IgG and IgM), and intragraft transforming growth factor (TGF) beta messenger RNA expression.[19] Recently, FK778 has been shown to prevent vascular remodeling after mechanical intimal injury.[20] The drug was shown to block intimal expansion following non-immune vascular injury in rats. The authors speculate that the vasculoprotective effects are independent of its immunosuppressive effect, and may be linked to inhibition of tyrosine kinase and possibly platelet derived growth factor (PDGF). These findings support earlier work indicating that FK778 inhibits neointima formation via a mechanism which may be related to tyrosine kinase inhibitory activity in vascular smooth muscle cells.[21]

Another intriguing activity of the MNA family is the ability to block replication of members of the Herpes virus family.[22-24] In contrast to the antiviral drugs currently in use, it has been shown that leflunomide does not inhibit viral DNA synthesis, but rather appears to interfere with virion assembly at the level of nucleocapsid tegumentation, and is thus effective against multi- drug-resistant CMV isolates.[22,23] The recent observation that FK778 can inhibit polyoma virus replication *in vitro*[25] and the reports that leflunomide therapy may clear human polyoma BK nephropathy[26] provide provocative evidence that this drug family may also possess meaningful anti-viral activity.

Single and repeat dose Phase 1 studies with FK778 have been completed in Europe and the U.S. Phase 1 data indicate that there is no substantial effect of food on the oral bioavailability of FK778, no gender effect on the pharmacokinetics, and very little elimination of the unchanged drug in the urine. Dose limiting adverse effects were not seen despite escalation of single doses to 1100 mg and repeat doses to 200 mg/day. A Phase 2 concentration controlled trial in renal transplant patients has been completed in Europe. [27]In a double-blind manner, 149 patients were randomized to 12 weeks of therapy into one of three treatment arms: High level FK778/tacrolimus/steroids (n=49), low level FK778/tacrolimus/steroids (n= 54), or placebo/tacrolimus/steroids (n=46). Both active treatment arms showed efficacy, as expressed by a reduction in the incidence of biopsy proven acute rejection by 12.6% and 13.2%, respectively, for the high and low level groups compared to placebo (acute rejection incidence 26.5%, 25.9%, and 39.1% for the high level, low level, and placebo groups, respectively). For the subgroup of patients in which target levels of FK778 were reached by week 2, the respective rejection rates were 7.7%, 27.1%, and 39.1%. This illustrates the importance of of early attainment of target drug levels. Infections, gastrointestinal side effects, and hepatic and renal function were comparable between the groups. Anemia was the most frequently reported adverse event and was dose-related; similar observations have been reported after administration of leflunomide to renal transplant recipients.[28] During the treatment phase, mean total cholesterol and LDL-cholesterol were significantly lower in both FK778-treated patients compared to patients of the placebo group, and comparison of blood pressure measurements showed a trend towards lower systolic and diastolic blood pressure values in both FK778 arms.[27] Currently additional clinical studies are underway in Europe and the U.S. in liver and kidney transplant recipients. In these studies, the potential anti-viral properties of FK778 are being evaluated.

CONCLUSION

While improvement in short term results in solid organ transplant recipients has occurred in recent years, there has been little change in the long-term outcome. The development of chronic allograft nephropathy, and death due to cardiovascular and infectious causes are the main reasons for the continued allograft failure which occurs beyond the first year after transplantation. Data from pre-clinical and clinical studies with FK778 indicate the potential for improvement in

transplant outcomes based on the drug's ability to prevent acute and chronic allograft rejection in organ transplant models, a more favorable cardiovascular risk profile, and anti-viral activity.

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