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Annual Update 2003 Transplantation

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Abstract

The Annual Update 2003 of Transplantation is comprised of a Compendium of current therapy to prevent and treat organ rejection after heart, kidney, liver and bone marrow/stem cell transplantation, and includes 19 compounds from the following drug classes: antimetabolites, mTOR inhibitors, calcineurin inhibitors, T-cell targeted therapy, complement system regulators, cytokine and chemokine modulators and antiinflammatory agents. The section on monographs offers updated information on the following drugs that were published in previous issues of the journal: pentostatin, visilizumab, everolimus, FK-778 and FTY-720. A table listing the drugs, their manufacturers, indications and developmental phases is also featured.

Introduction

The Annual Update 2003 of Transplantation is comprised of a Compendium of current therapy to prevent and treat organ rejection after heart, kidney, liver and bone marrow/stem cell transplantation, and includes 19 compounds from the following drug classes: antimetabolites, mTOR inhibitors, calcineurin inhibitors, T-cell targeted therapy, complement system regulators, cytokine and chemokine modulators and antiinflammatory agents. The section on monographs offers updated information on the following drugs that were published in previous issues of the journal: pentostatin, visilizumab, everolimus, FK-778 and FTY-720. A table listing the drugs, their manufacturers, indications and developmental phases is also featured.

Annual Update 2003: Transplantation

Drug	Source	Condition	Phase
AP-1903/Fas System	Ariad	Graft-*versus*-host disease	II
Denileukin Diftitox[1]	Ligand	Graft-*versus*-host disease	I/II
Inolimomab	Orphan Pharma International/Diaclone	Graft-*versus*-host disease	II
Pentostatin[1,2]	SuperGen	Graft-*versus*-host disease	II/III
Visilizumab[2]	Protein Design Labs	Graft-*versus*-host disease	II
Repertaxin	Dompé	Transplant rejection	I
AGI-1096	AtheroGenics	Transplant rejection prophylaxis	I
Mycophenolic Acid Sodium Salt	Novartis	Transplant rejection prophylaxis	R-2002
TRX-1	TolerRx	Transplant rejection prophylaxis	I
Everolimus[2]	Novartis	Transplant rejection, heart	Prereg
APT-070C	AdProTech	Transplant rejection, kidney	II
Everolimus[2]	Novartis	Transplant rejection, kidney	Prereg
FK-778[2]	Fujisawa/Aventis	Transplant rejection, kidney	II
FTY-720[2]	Novartis/Mitsubishi Pharma	Transplant rejection, kidney	II
ISAtx-247	Isotechnika	Transplant rejection, kidney	II
LEA29Y	Bristol-Myers Squibb/Novartis	Transplant rejection, kidney	II
FK-778[2]	Fujisawa/Aventis	Transplant rejection, liver	II

[1]Launched for another indication. [2]Monograph previously published in Drugs of the Future.

Compendium of Transplantation

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Background information

Transplantation is a life-saving procedure for many patients and is often the only viable treatment available. Each year more than 19,000 transplants are performed in the U.S. An additional 56,000 critically ill people in the U.S. are waiting to receive an organ transplant that could forestall further disability or death from conditions such as heart disease, kidney failure, liver disease and diabetes. In 1992, more than 32,000 organ transplants were performed worldwide and nearly half of those were done in the U.S. These transplants, collectively, involved every major organ. Success rates vary for the different types of organs transplanted and can be as high as 90% or as low as 50% for 1 year. Immunologic rejection, waiting lists and the increasing number of patients in need of transplants are among the obstacles preventing successful transplantations.

Except for transplants between identical twins, all transplant donors and recipients are immunologically incompatible. This biologic incompatibility is an obstacle that causes the recipient to try to destroy or reject the new organ, tissue or cells. In graft-*versus*-host disease (GVHD), rejection occurs when the transplanted donor cells try to destroy or reject recipient tissues. Graft survival is poorer in African-Americans, in patients who have had a previous transplant and in children.

Although advances derived from transplantation research have improved rates of transplant success and patient survival, problems such as complications from immunosuppressive therapy remain to be solved. Immunosuppression is the current therapy to prevent and treat rejection, but may increase the incidence of infection and cancer. Ongoing evaluation of several new immunosuppressive agents in development may lead to more effective therapy while causing fewer undesirable side effects.

Heart transplant

When the heart can no longer adequately work and a person is at risk of dying, a heart transplant may be indicated. It involves removing a diseased heart and replacing it with a healthy human heart. Cardiac transplantation is recognized as a proven procedure in appropriately selected patients. There were 2,202 heart transplants performed in the U.S. in 2000 and 2,184 in 1999. In the U.S., about 73% of heart transplant patients are male, 74% are Caucasian, 49% are 50-64 years of age and 21% are 35-49 years of age. The 1-year survival rate is 85%, the 3-year survival rate is approximately 77% and the 5-year survival rate is 71%.

New immunosuppressive therapies have reduced the risk of rejection in recent years. Ciclosporin-treated recipients have a 1-year survival rate of approximately 80%. Symptoms of rejection in the initial postoperative period include fever, malaise, tachycardia and hypotension. Corticosteroids, anti-thymocyte globulin (ATG) and monoclonal antibodies (OKT3) are the preferred treatment for severe rejection. Graft arteriosclerosis is a common side effect of ciclosporin in 25% of all heart transplantations because of increased incidence of hypertension and direct vascular toxicity. About 70% of deaths after transplantation are due to infection and malignancies.

Kidney transplant

More than 200,000 people in the U.S. suffer from kidney failure. Treatment of kidney failure incurs medical payments of more than USD 7 billion annually. The most common cause of end-stage renal disease is diabetic nephropathy, followed by hypertensive nephroangiosclerosis and various primary and secondary glomerulopathies. Patient survival 1 year after transplantation from a living related donor is 95% and slightly less if the organ comes from a cadaveric donor. Graft survival ranges between 70-90% at 3-5 years.

Newly developed immunosuppressive drugs and immunologic monitoring have led to the expansion of recipient age from 5 months up to 75 years. The major complication after transplantation is rejection of the organ, and most rejection episodes occur in the first 4 months after transplantation. In spite of primary immunosuppressive therapy, patients can suffer from

multiple acute rejection episodes. If treatment does not reverse the rejection, the recipient has to return to hemodialysis and wait for another chance. Other later complications are drug toxicity, especially nephrotoxicity of ciclosporin, infection, kidney-related diseases and malignancies. The risk of epithelial carcinoma and lymphoma increases to 30 times higher than in the normal population. Aggressive tumors and lymphomas are treated by reduction or interruption of the current immunosuppressive therapy.

Liver transplant

The most frequent indications for liver transplantation are end-stage chronic hepatitis and biliary cirrhosis in adults, and biliary atresia and inborn metabolic deficiencies in children. More than 60,000 Americans die each year from liver failure. Liver transplantation is usually done when other medical treatment cannot keep a damaged liver functioning. About 80-90% of people survive liver transplantation. Survival rates have improved over the past several years because of drugs like ciclosporin and tacrolimus, which suppress the immune system and keep it from attacking and damaging the new liver.

Acute and chronic rejection occurs occasionally after liver transplantation. Intensive immunosuppressive therapy is attempted, but fulminant rejection or even chronicle symptoms are refractory to drug management. Retransplantation is the mandatory treatment. Immunosuppressive therapy includes ciclosporin, corticosteroids and azathioprine. Due to surgery, postsurgical blood transfusions and immunosuppressive therapy, renal function often declines in the postoperative period and, therefore, hemodialysis may be essential.

Lung transplant

Lung transplants may involve two lungs, a single lung or a lobe of a lung. Most lung lobe transplants have been performed in patients with cystic fibrosis and have involved the donation of a lung lobe from each of two living donors. The 1-year patient survival is more than 70%.

Acute rejection is quite common, but can be reduced by intensive treatment with high-dose corticosteroids and ATG or OKT3. Decreased lung function parameters demonstrate acute rejection in the early transplant period. Bronchial stenosis is a common complication of the late transplant period and can be treated with dilatation or stent placement. Chronic rejection is characterized by slowly progressive airway constriction and a decline in lung function parameters.

Pancreas transplant

Pancreas transplantation is indicated if the complications of type 1 diabetes (*e.g.*, nephropathy, retinopathy, neuropathy) increase and if other regimens cannot stabilize progression of secondary processes. The success of pancreas transplantation depends on adequate immunosuppressive therapy and optimal surgical procedures. The success of transplantation as measured by the return of patients to normal glycemia ranges from 40-80%.

Rejection treatment is similar to that for kidney transplant immunosuppression and the addition of ATG or OKT3. Graft pancreatitis and general infections are secondary complications.

Bone marrow/stem cell transplant

Bone marrow, the spongy tissue found in the cavities of the bones, is where all of the body's blood cells are produced. Every type of blood cell in the marrow begins its life as a stem cell, which forms the different cells that make up your blood and the immune system. These include leukocytes, erythrocytes and platelets. The goal of a bone marrow transplant is to cure some types of cancer and diseases by replacing unhealthy cells with healthy ones. Bone marrow/stem cell transplant is different than other transplants in that it does not involve surgery, but is performed intravenously, like a blood transfusion. High doses of chemotherapy or radiation are given beforehand to kill off diseased cells. The stem cells generally begin to produce life-sustaining blood cells within several weeks, depending on the type of transplant.

Complications include infection, hemorrhage, organ damage from chemotherapy or radiation and GVHD.

Treatment

The total transplantation market is worth more than USD 2.5 billion per year. Drug sales in the therapeutic area of transplantation accounted for 0.9% of the total drug sales worldwide in 2002; this tendency is expected to remain constant or vary only slightly through 2005. Ciclosporin (Sandimmune/Neoral®; Novartis) has become the drug of choice in its class worldwide since its launch in 1983. Other widely used transplant drugs include tacrolimus (Prograf®; Fujisawa), mycophenolate mofetil (CellCept®; Roche) and sirolimus (Rapamune®; Wyeth).

Antimetabolites

The Swiss agency for therapeutic products, Swissmedic, last year approved for marketing Novartis's **mycophenolic acid sodium salt** (Myfortic®). The product is indicated for use in combination with ciclosporin and corticosteroids for the prevention of acute transplant rejection in adult patients receiving allogeneic renal transplantation. Myfortic® is an advanced, enteric-coated tablet containing mycophenolic acid (MPA) sodium that was developed to protect the upper gastrointestinal tract from

the side effects of MPA. In contrast to the previously marketed product mycophenolate mofetil, which is a prodrug and must be converted to MPA, Myfortic® contains MPA itself as the active component. The drug is a highly specific inhibitor of T- and B-lymphocyte proliferation and is effective in the prevention of acute graft rejection. MPA is a potent, selective, noncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase, upon which active lymphocytes are dependent for their proliferation. In two major pivotal trials in 748 patients, enteric-coated Myfortic® was demonstrated to be a highly potent and well-tolerated immunosuppressant for new renal transplant patients. Transplant patients taking other MPA drugs could be safely switched to Myfortic®. A trend was seen towards fewer dose reductions due to gastrointestinal intolerability and less serious infections. The first launch for Myfortic® is scheduled to take place in Switzerland in April 2003.

Pentostatin inhibits a key enzyme, adenosine deaminase (ADA), found in all lymphocytes. ADA is a key enzyme involved in DNA synthesis and is expressed in particularly high levels in rapidly dividing cells such as those involved in the leukemias and lymphomas. Pentostatin is approved in the U.S. for the treatment of hairy cell leukemia. SuperGen is supporting a growing body of clinical investigation around the country using pentostatin for a wide array of malignancies including GVHD, prolymphocytic leukemia, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma and non-myeloblative transplant conditioning. Phase II/III trials are currently under way for the GVHD indication.

Under license from Aventis, Fujisawa is evaluating the immunosuppressive compound **FK-778** (formerly HMR-1715) in European phase II trials for the prevention of liver and kidney transplant rejection. A dihydroorotate dehydrogenase inhibitor, FK-778 is expected to have synergistic effects in combination with tacrolimus, and clinical studies are evaluating the two in combination.

mTOR inhibitors

The rapamycin compound **everolimus** (Certican™, SDZ-RAD) is undergoing regulatory review in the U.S. and European Union. Developed by Novartis, everolimus is targeted for the prevention, in combination with ciclosporin, of heart and kidney transplant rejection. In April 2002, Novartis researchers reported the first-ever clinical evidence of an immunosuppressant drug with significant potential to reduce graft vasculopathy, a surrogate marker for chronic rejection. Vasculopathy is defined as the thickening of the intima (innermost wall) of the graft vessel. This leads to the gradual narrowing of the vessel, restricting blood supply. This can compromise the stability and longevity of the transplanted organ. At 1 year post-transplant, vasculopathy is the most serious risk factor for long-term graft loss. The results reported last April were from a 12-month, double-blind, international study of 634 heart transplant patients all receiving the standard regimen of ciclosporin and steroids. The addition of everolimus to the standard regimen appeared to significantly lower the incidence of acute rejection episodes and reduce the potential for graft vasculopathy, when compared to the standard add-on immunosuppressant therapy azathioprine. The study was planned to run for another year.

Calcineurin inhibitors

Isotechnika and codevelopment partner Roche are developing the novel immunosuppressive compound **ISAtx-247** in phase IIa trials for the treatment of renal transplant recipients. ISAtx-247, a ciclosporin analogue and next-generation calcineurin inhibitor, is more potent and less toxic than other drugs in its class. In the ongoing trial, 130 stable renal transplant recipients who are at least 6 months postoperative will be treated with ISAtx-247 in substitution for ciclosporin for a period of 3 months. The study is designed to evaluate the renal side effects of the calcineurin inhibitor as well as its efficacy in preventing organ rejection.

T-cell-targeted therapy

Strategies to block the undesirable immune response in transplant rejection and GVHD have centered around the pathways required by the immune system's T-cells to reject invading microorganisms as well as transplanted tissues. At least two immune system signals are required for optimal T-cell activation. One of these is the costimulatory pathway. Manipulation of the costimulatory pathway involving the interaction between CD28 and B7 antigens prevents T-cells from getting the necessary second signals, and has been shown to hold great promise in the treatment of autoimmune diseases and prevention of transplant rejection.

Although CTLA4-Ig is a potent inhibitor of T-cell responses, scientists have questioned its long-term ability to block transplant rejection and thus have developed an improved form of this fusion protein. **LEA29Y** is a modified form of the CTLA4-Ig fusion protein that has been developed for the treatment of solid organ transplant rejection. The subject of a codevelopment agreement between Bristol-Myers Squibb and Novartis, it is in phase II testing for renal transplant rejection. This potent immunosuppressive agent was also shown by independent researchers to be effective in a nonhuman primate model of islet cell transplantation.

Visiluzumab (Nuvion™) is a humanized monoclonal antibody from Protein Design Labs that specifically recognizes the CD3 receptor on the surface of T-cells. Visilizumab binds to the surface of activated T-cells, which are believed to be involved in GVHD, and causes them to be removed from the circulation. The company is conducting phase II trials evaluating the product in steroid-refractory, acute GVHD, as well as a phase I/II

trial evaluating its efficacy as primary therapy for acute GVHD following hematopoietic stem cell transplantation.

TolerRx has designed **TRX-1**, a humanized monoclonal antibody to the human T-cell receptor CD4, as a potential agent for preventing transplant rejection. TRX-1 will be used to induce immunological tolerance in autoimmune disease settings, transplantation and in settings requiring the chronic administration of biological therapeutic agents. In November 2002, TolerRx initiated a single dose, placebo-controlled, double-blind, dose-escalating, phase I study evaluating the safety and pharmacokinetics of TRX-1. The antibody is being developed in collaboration with Genentech.

Ariad is developing **AP-1903/Fas system**, a nonimmunosuppressive treatment for GVHD that will target and eliminate the T-cells that are causing the disease (*i.e.*, the donor's T-cells), if those T-cells attack the patient's own tissues, while preserving the immune cells that are being produced by the bone marrow transplantation (BMT). In the Ariad approach, donor T-cells are modified using a gene transfer vector to make them susceptible to the drug candidate, AP-1903. This drug candidate may be administered if GVHD occurs, potentially killing the disease-causing donor T-cells. Specifically, donor T-cells are genetically engineered to introduce an inactive form of the apoptosis gene *Fas*, thereby making them potentially susceptible to the effects of AP-1903, which is administered only if and when rejection occurs. AP-1903 activates *Fas* cell signaling and would rapidly cause the donor's T-cells to die, leaving the underlying bone marrow and immune system unaffected and thus potentially treating the primary cause of GVHD. The product is currently in phase II development.

Complement system regulators

AdProTech's **APT-070C** is designed to protect transplanted organs from reperfusion injury and hence minimize chronic organ rejection and graft dysfunction in kidney transplantation. APT-070C is based on part of a naturally occurring complement inhibitor protein which, when attached to cells, identifies them as nonforeign and thereby protects them from complement system attack. Adprotech has identified that part of the natural protein which is central to the protective effect. Company scientists have modified this protein to improve its performance and enhanced its ability to bind to target cells. APT-070C differs from other medicines in that it is added to the organ perfusion solution prior to transplantation in order to prevent ischemia/reperfusion injury to the organ before it is transplanted; the product is thus designed to be used in combination with other antirejection therapies. Preclinical research has shown that APT-070C helps preserve kidney function and that it is retained and active within transplanted kidneys. The product, which has orphan drug status in Europe, is in phase II clinical testing.

Cytokine and chemokine modulators

Inolimonab (Leukotac®), an anti-interleukin 2 receptor (CD25) monoclonal antibody, is being developed by Orphan Pharma International for the treatment of acute GVHD following hematopoietic stem cell transplantation or BMT. Acute GVHD is the major complication of BMT and is responsible for approximately 30% of deaths following the procedure. Preliminary phase II results indicate that inolimonab reverses 80% of acute GVHD, and achieves patient survival of 65%. The product has orphan drug status in the E.U. and the U.S. and is being codeveloped with Diaclone.

The selective immunosuppressant **FTY-720**, discovered by Mitsubishi Pharma and licensed to Novartis, is in phase II testing in the U.S. and Europe for the prevention of acute rejection and graft loss in kidney transplant patients. FTY-720 was synthesized using a component of the *Isaria sinclairii* fungus and has a novel mechanism of action, via which it protects transplanted organs against T-cells without affecting the host's ability to respond to antigens. FTY-720 prevents the recirculation of lymphocytes, a process that is regulated by chemokines, reportedly by interfering with the effects of chemokines. Acute rejection rates with FTY-720 in clinical trials to date have been very low as compared to low-dose ciclosporin.

Independent investigators reported in February 2003 the results of a small phase I/II study evaluating **denileukin diftitox** (Ontak®) in the treatment of GVHD. Denileukin diftitox is being evaluated as a potential treatment for acute GVHD based on the product's ability to kill selectively those activated immune cells that bear the high-affinity IL-2 receptor and that contribute to the condition. In this study, reported at the combined annual meeting of the American Society for Blood and Marrow Transplantation and the International Bone Marrow Transplant Registry/Autologous Blood and Marrow Transplant Registry, 11 recipients of allogeneic stem cell transplants suffering acute steroid-resistant GVHD were treated with denileukin diftitox (4.5 μg/kg/day for 5 days, followed by 4.5 μg/kg once weekly for 4 weeks). Five of the 11 patients treated had complete remission of acute GVHD, and 2 other patients achieved partial remission. Complete overall response was defined as complete resolution of rash and absence of GVHD diarrhea. Partial response was defined as improvement by at least one GVHD grade in any organ system without worsening in others. In addition, 10 patients improved by at least one GVHD grade in one or more affected organs. Six of the 11 patients were alive after 100 days of follow-up. Two of the deaths were caused by GVHD and 3 by infections.

The understanding of the pathophysiological mechanism of neutrophil-mediated tissue injury by Dompé researchers has led to the discovery and characterization of **repertaxin**, a potent interleukin-8 (IL-8) inhibitor that acts through the inhibition of intracellular mediators activated by IL-8 receptors. *In vitro* studies have demonstrated that repertaxin is a potent inhibitor of human neutrophil

chemotaxis induced by IL-8. In *in vivo* models, repertaxin prevented tissue damage induced by postischemia reperfusion injury. In a rat model of delayed graft function, repertaxin reduced kidney damage assessed by the serum creatinine increase. Preclinical toxicology and safety studies confirm the good safety profile of this compound, which has entered clinical phase I.

Antiinflammatory agents

Solid organ transplant rejection is a condition characterized by a chronic, harmful inflammatory response. Chronically inflamed tissues continuously generate signals that attract leukocytes from the bloodstream. When leukocytes migrate from the bloodstream into the tissue, they amplify the inflammatory response. This chronic inflammatory response can break down healthy tissue in a misdirected attempt at repair and healing.

AGI-1096 is a novel antioxidant and selective antiinflammatory agent which is being developed to address the accelerated inflammation of grafted blood vessels, known as transplant arteritis, common in chronic organ transplant rejection. AtheroGenics commenced a phase I clinical trial in February 2002 to assess the safety and tolerability of AGI-1096 in healthy volunteers. The results of the AGI-1096 clinical trial data demonstrated that the drug was well tolerated at all oral doses, with no drug-related adverse events.

Information sources on the internet

American Society of Transplantation
http://www.a-s-t.org/

Eurotransplant
http://www.transplant.org/

National Bone Marrow Transplant Link
http://comnet.org/nbmtlink/

Transplantation
http://www.niaid.nih.gov/publications/transplant/default.htm

Transweb
http://www.transweb.org/

United Network for Organ Sharing
http://www.unos.org/

ustransplant.org
http://www.ustransplant.org/index.html

Monograph Updates of Transplantation

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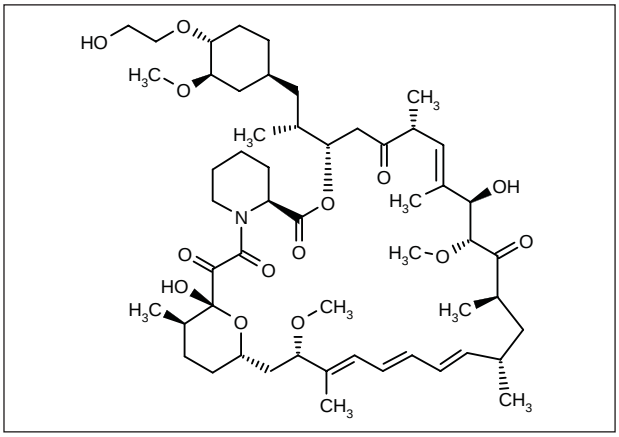
Everolimus



Everolimus (SDZ-RAD, RAD-001, NVP-RAD-001, Certican™) is a potent proliferation inhibitor with immunosuppressive properties. Novartis has submitted an NDA to the FDA and an MAA in the E.U. for everolimus for the prevention of rejection following heart and kidney transplantation. Novartis has a license agreement granting Guidant exclusive worldwide rights to use everolimus in drug-eluting stents for the prevention and treatment of coronary and peripheral vascular diseases. Pending regulatory approvals, Guidant expects to initiate clinical trials of everolimus-eluting coronary stents this quarter.

Regulatory filings included results from clinical trials in more than 1,500 heart and kidney transplant recipients for up to more than 36 months showing that, when administered concurrently with ciclosporin and corticosteroids, everolimus effectively prevents graft rejection. In a 2-year, international phase III study, 2 doses of everolimus (1.5 and 3 mg/day) were compared to azathioprine as part of triple immunosuppressive therapy in *de novo* adult heart transplant recipients. At 6 and 12 months after transplantation, everolimus at both doses was statistically significantly superior to azathioprine in the primary composite endpoint, which included the incidence of acute rejection greater than ISHLT grade 3A, acute rejection associated with hemodynamic compromise, graft loss, patient death and loss to follow-up. The incidence of biopsy-proven acute rejection of ISHLT grade 3A or more at month 12 was 30.6% for the 1.5 mg/day group, 21.3% for the 3 mg/day group and 45.8% for the azathioprine group. Both everolimus doses were associated with a significantly lower incidence of allograft vasculopathy. For kidney transplantation, 2 international, double-blind, randomized, parallel-group phase III studies compared everolimus (1.5 and 3 mg/day) with mycophenolate mofetil (MMF; 2 mg/day) as part of a triple immunosuppressive regimen with ciclosporin and corticosteroids. In terms of primary composite endpoints, there were no significant between-group differences for efficacy at 6 or 12 months posttransplant. In the first study (B201), the incidence of biopsy-proven acute rejection at 12 months was 23.2%, 19.7% and 24.0% for the everolimus 1.5 mg/day, everolimus 3 mg/day and MMF groups, respectively. In the second study (B251), the incidence of biopsy-proven acute rejection was 19.2%, 22.2% and 24.0%, respectively (1).

The comparative activities of everolimus (1.5 and 0.5 ng/ml) and ciclosporin (1 and 3 μg/ml) on TNF-α-induced expression of human endothelial tissue factor and adhesion molecule expression were assessed in human umbilical vein endothelial cells (HUVEC). In contrast to the procoagulant activity of ciclosporin, everolimus lowered tissue factor activity and did not alter the expression of VCAM-1 and ELAM-1. The potential synergy between the drugs in promoting immunosuppression, preventing

chronic rejection and extending graft survival following transplantation requires further study (2).

The effect of combining chloroquine with either tacrolimus or everolimus on T-cell responses to minor histocompatibility antigens and antigen-presenting cell (APC) function was evaluated *in vitro*. T-cell responses were evaluated in both an APC-dependent C57BL/6 anti-BALB.B model and an APC-independent anti-CD3ε antibody-mediated system. Chloroquine was found to synergistically reduce APC-dependent and APC-independent T-cell responses when combined with everolimus or tacrolimus. The tacrolimus/chloroquine combination affected both APC and T-cell function, while the everolimus/chloroquine combination primarily affected T-cell proliferation and cytokine production (3).

A model of bleomycin-induced pulmonary fibrosis in rats was used to evaluate the ability of everolimus (2.5 mg/kg/day via oral gavage) to prevent the build-up of lung collagen. Everolimus reduced collagen aggregation and lung weight by 75 ± 12% and 56 ± 6%, respectively, indicating its potential application in the treatment of conditions characterized by elevated production of extracellular matrix, such as fibrotic pulmonary disease (4).

A rat model of chronic renal allograft rejection was used to assess the efficacy of everolimus. Two groups of animals were treated with everolimus (0.5 or 1 mg/kg/day) and control groups received vehicle. Everolimus significantly lowered proteinuria 4 weeks after treatment and glomerular sclerosis, vascular intimal thickening and the infiltration of macrophages and lymphocytes after 16 weeks, consistent with lower TGF-β1 and PDGF-AA mRNA expression. The putative mechanisms of action of everolimus may therefore involve the reduction of protein in urine and growth factor expression (5).

The ability of everolimus to inhibit in-stent neointimal growth was evaluated in rabbit iliac arteries. Group 1 animals received 1.5 mg/kg/day starting 3 days before stenting, which was reduced to 1 mg/kg/day from days 14-28, while animals in group 2 received 1.5 mg/kg given 1 day before stenting, followed by 0.75 mg/kg/day for 28 days. Everolimus was better tolerated by group 2 animals and both groups had significant reductions in in-stent neointimal growth (46% and 42% reduction in intimal thickness in groups 1 and 2, respectively). Animals receiving 1.5 mg/kg followed by 0.75 mg/kg/day also showed a 24% reduction in intimal area and a 25% reduction in stent stenosis, and greater than 80% of stents from these animals were endothelialized. It was concluded that everolimus may be useful as a stent coating and/or oral adjunct to drug-eluting stents (6, 7).

Everolimus pharmacokinetics were determined as part of a multicenter, randomized, double-blind trial in 634 *de novo* heart transplant recipients. The patients were given everolimus 0.75 or 1.5 mg b.i.d. or azathioprine plus ciclosporin and corticosteroids. During the 6 months following transplantation, everolimus exposure was proportional to dose. Everolimus C_{min} also correlated well with AUC. Ciclosporin C_{min} values were similar in the treatment groups, although ciclosporin doses were lower in patients treated with everolimus (8).

Researchers evaluated steady-state everolimus pharmacokinetics over 6 months in 19 pediatric *de novo* kidney allograft recipients. Everolimus 0.8 mg/m^2 b.i.d. was administered as a dispersible tablet in water along with ciclosporin and corticosteroids. The results indicated that body surface area-adjusted dosing was appropriate in pediatric patients. Everolimus exposure was determined to be stable over time, although therapeutic monitoring was suggested for pediatric patients (9).

The effect of food and the bioavailability of a dispersible tablet formulation of everolimus were compared with the adult tablet in a 3-way crossover trial involving 24 adult volunteers. The C_{max} of the dispersible tablet was on average 24% lower than the conventional tablet (12.3 ± 4.8, 9.3 ± 3.3 and 4.6 ± 1.8 ng/ml for the tablet, dispersible tablet in a fasted and fed state, respectively), and the AUC was 10% lower (99 ± 28, 92 ± 37 and 94 ± 39 ng·h/ml, respectively), as was everolimus bioavailability. The t_{max} was delayed by 2.5 h and the C_{max} attenuated by 50% following a high-fat meal, although overall absorption was unaffected; the AUC fulfilled fasting and fed state equivalence criteria. Everolimus (0.8 mg/m^2 p.o. b.i.d.) steady-state pharmacokinetic parameters in pediatric kidney allograft patients were: C_{min}, 4.4 ± 1.7 ng/ml; C_{max}, 13.6 ± 4.2 ng/ml; and AUC, 87 ± 27 ng·h/ml. Steady-state concentration-time profiles in pediatric patients receiving the dispersible tablet were comparable to adult patients receiving the conventional tablet when dosed to yield similar trough concentrations (10, 11).

Data from a phase III trial in which *de novo* heart transplant patients received everolimus 0.75 or 1.5 mg b.i.d. plus prednisone and ciclosporin were analyzed to determine therapeutic everolimus concentrations. The lower limit of the therapeutic range of everolimus plasma levels when administered with full-dose ciclosporin appeared to be 3 ng/ml. The rates of hyperlipidemia were not related to increasing everolimus C_{min} values, whereas thrombocytopenia was related to everolimus C_{min}, although the overall incidence was below 10%. Everolimus was safely administered up to a C_{min} of 22 ng/ml (12).

The combination of everolimus (0.8 mg/m^2 b.i.d.), ciclosporin (100-300 ng/ml trough levels) and prednisone in pediatric renal transplant patients was evaluated in an open, multicenter trial. At 3 months, no episodes of rejection, graft failures, decline in graft function or deaths occurred. This dose of everolimus demonstrated predictable drug exposure. Although 4 of 10 patients experienced serious side effects, no cytomegalovirus or *Pneumocystis* infections or malignancies were seen (13).

Everolimus combined with ciclosporin, corticosteroids and an anti-CD25 monoclonal antibody was administered to 19 pediatric *de novo* renal transplant patients in a multicenter, open-label, 1-year study. Patients were given oral everolimus 0.8 mg/m^2 b.i.d. At 6 months, 3 mild or moderate acute rejections were seen but there were no deaths or graft losses. Lipids increased but were

decreasing by 6 months. With the regimen used, steady-state everolimus trough levels were above the therapeutic threshold identified in adults in most of these pediatric patients (14).

An economic analysis was undertaken to compare the costs of everolimus 1.5 or 3 mg/day and mycophenolate mofetil 2 g/day in 588 renal transplant patients in 14 countries. Ciclosporin and steroids were also administered. No differences in the incidences of acute rejection, graft loss or death were found during the first 6 months posttransplant. Also, no significant differences in the mean cost of treatment were seen among the treatment groups (15).

Renal transplant patients enrolled in 2 phase III trials of everolimus 1.5 or 3 mg/day compared to mycophenolate mofetil 2 g/day were allowed to select a protocol amendment to lower ciclosporin exposure. In 88 patients in whom the ciclosporin dose was reduced by 25%, 33% had an improvement of at least 10% in creatinine clearance and 50% had a change in creatinine clearance of 10% (16).

A study enrolled 56 patients with first or second cadaveric renal allografts at increased risk of delayed graft function. Patients were treated with FTY-720 (5-mg loading dose pretransplant followed by 2.5 mg once daily), everolimus (4-mg loading dose pretransplant followed by 2 mg b.i.d., adjusted to trough levels of 4-8 ng/ml) and corticosteroids. Immunoprophylaxis with this regimen was determined to be adequate in 52 patients after a mean follow-up of 224 days. The frequency of adverse events was low (17).

1. *Novartis files NDA for Certican for prevention of kidney and heart transplant rejection*. DailyDrugNews.com (Daily Essentials) Dec 30, 2002.

2. Haubitz, M., Brunhorst, R. *Influence of a novel rapamycin analog SDZ RAD on endothelial tissue factor and adhesion molecule expression*. Transplant Proc 2002, 34(4): 1124.

3. Hsiao, C.C., Su, W.N., Forooghian, F., Bader, S., Rempel, J., Hay-Glass, K.T., Gilman, A., Schultz, K.R. *Evaluation for synergistic suppression of T cell responses to minor histocompatibility antigens by chloroquine in combination with tacrolimus and a rapamycin derivative, SDZ-RAD*. Bone Marrow Transplant 2002, 30(12): 905.

4. Simler, N.R., Howell, D.C.J., Marshall, R.P., Goldsack, N.R., Hasleton, P.S., Laurent, G.J., Chambers, R.C., Egan, J.J. *The rapamycin analogue SDZ RAD attenuates bleomycin-induced pulmonary fibrosis in rats*. Eur Respir J 2002, 19(6): 1124.

5. Zou, H., Lai, D., Xiong, C., Huang, Z., Yao, J. *Efficacy of 40-O-(2-hydroxyethyl)-rapamycin in treating chronic renal allograft rejection in rats*. J Am Soc Nephrol 2002, 13(Abstracts issue): Abst PUB481.

6. Farb, A., John, M., Acampado, E. , Kolodgie, F.D., Prescott, M.F., Virmani, R. *Oral everolimus inhibits in-stent neointimal growth*. Circulation 2002, 106(18): 2379.

7. Farb, A., John, M., Acampado, E., Kolodgie, F.K., Weber, D.K., Prescott, M.F., Virmani, R. *Oral administration of everolimus (RAD) reduces in-stent restenosis in the NZW rabbit*. Circulation 2002, 106(19, Suppl. 2): Abst 2930.

8. Pirron, U., Kovarik, J.M., Eisen, H., Dorent, R., Mancini, D., Rordorf, C., Bernhardt, P. *Longitudinal pharmacokinetics of everolimus in de novo heart transplant patients and its influence on cyclosporine*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0626.

9. Hoyer, P.F., Ettenger, R., Lemire, J., Webb, N., McMahon, L., Kovarik, J.M. *Everolimus steady-state pharmacokinetics in pediatric de novo renal transplant patients*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0650.

10. Noe, A., Kovarik, J.M., Berthier, S., Langholff, W.K., Moraczewski, S.K., Rordorf, C. *Characterizing a pediatric dispersible tablet formulation of everolimus: Relative bioavailability to the conventional tablet and food effect*. J Clin Pharmacol 2002, 42(9): Abst 14.

11. Kovarik, J.M., Noe, A., Berthier, S., McMahon, L., Langholff, W.K., Marion, A.S., Hoyer, P.F., Ettenger, R., Rordorf, C. *Clinical development of an everolimus pediatric formulation: Relative bioavailability, food effect, and steady-state pharmacokinetics*. J Clin Pharmacol 2003, 43(2): 141.

12. Pirron, U., Kovarik, J.M., Eisen, H., Dorent, R., Mancini, D., Rordorf, C., Bernhardt, P. *Exposure-response relationships to define a therapeutic range for everolimus in de novo heart transplantation*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 2142.

13. Vester, U., Kranz, B., Wehr, S., Boger, R., Hoyer, P.F. *Everolimus (Certican) in combination with Neoral in pediatric renal transplant recipients: Interim analysis after 3 months*. Transplant Proc 2002, 34(6): 2209.

14. Ettenger, R., Hoyer, P.F., Lemire, J., Webb, N.J.A., Mayer, H. *Six-month results in pediatric de novo renal transplant patients treated with everolimus in combination with cyclosporine: Excellent efficacy in prevention of acute rejection and no occurrence of graft loss or death*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0649.

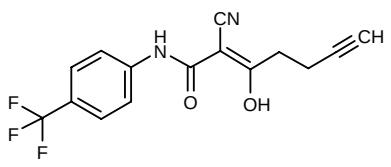
15. Holmes, M.W., Chilcott, J.B., Walters, S.J., Whitfield, M.D., Whitby, S., Akehurst, R.L. *Economic evaluation of everolimus (Certican™) versus mycophenolate mofetil as part of triple immunosuppressive therapy in de novo renal transplant recipients*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 2148.

16. Tedesco, H., Ambühl, P., Lorber, M., Oeyen, O., Pirron, U. *Cyclosporine dose reduction in kidney transplant recipients taking everolimus (Certican™) improves renal function*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0258.

17. Tedesco-Silva, H., Rosso-Felipe, C., Lorber, M. et al. *FTY720, RAD and corticosteroids for the prevention of graft loss and acute rejection in renal allograft recipients at increased risk of delayed graft function*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0050.

Original monograph - Drugs Fut 1999, 24(1): 22.

FK-778



FK-778 (MNA-715, X-920715, HMR-1715), a short-acting malononitrilamide leflunomide analogue, is a low-molecular-weight immunosuppressant that interacts directly with B-cell and T-cell function by inhibiting a critical enzyme in *de novo* pyrimidine synthesis. The compound is currently in phase II clinical trials for transplant rejection at Fujisawa under license from Aventis.

When tested for effects on mouse, monkey and human B-cell proliferation, 42 of 59 combinations of FK-778 or the related malononitrilamide FK-779 plus tacrolimus demonstrated additive to synergistic effects. Of the 59 combinations, 50 demonstrated antagonistic effects in the blockade of T-cell proliferation. In rats undergoing orthotopic kidney transplantation, the combination of FK-778 and tacrolimus was more effective in prolonging recipient survival than monotherapy with either agent, particularly when the agents were not administered simultaneously (1).

Tacrolimus, FK-778 and FK-779 were administered orally, alone and in combination, in an evaluation of the prevention of acute heart and kidney allograft rejection and in the reversal of ongoing heart allograft rejection in rats. Median survival time in the acute heart model was dose-dependently lengthened when FK-778 or FK-779 was administered with tacrolimus. The effect was additive to synergistic compared to monotherapies. The results were comparable in the acute kidney model. The combination of tacrolimus and FK-778 was also strongly synergistic in the reversal of ongoing acute heart allograft rejection (2).

Renal allograft survival was assessed in dogs treated with FK-778 or ciclosporin alone or in combination. Median survival was 14.5, 7 and 36 days for animals receiving ciclosporin, FK-778 and the combination treatment, respectively (3).

Immunosuppression, adverse effects and pharmacokinetics of FK-788 were evaluated in dogs undergoing kidney transplantation. FK-778 was administered alone or in combination with tacrolimus or ciclosporin orally for 90 days following the procedure. Median survival was 10, 30.5, 75.5 and 50.5 days for controls, FK-778 4 mg/kg alone, FK-778 4 mg/kg plus tacrolimus 0.3 mg/kg and FK-778 plus ciclosporin 10 mg/kg, respectively. Vomiting and diarrhea were common side effects (4).

Rats receiving renal allografts were treated with oral FK-778 10 or 20 mg/kg/day for 10 days posttransplant or FK-778 3 mg/kg/day plus tacrolimus 1 mg/kg/day for 90 days. Analysis of harvested kidney grafts showed that FK-778-treated animals maintained normal serum creatinine and lower proteinuria for 90 days as compared to untreated controls. Chronic pathological changes were also dose-dependently reduced. In addition, intragraft CD8 T-cells, NK cells, ED1 macrophage infiltration and TGF-β mRNA expression were reduced (5).

Treatment with FK-778 and imatinib was compared in a rat model of endothelial injury. Male Wistar rats were used for aorta denudation and aortic arteries were harvested for morphometric analysis. Both agents were found to equally and dose-dependently inhibit the intimal proliferation seen in untreated controls. Exogenous uridine reversed the antiproliferative effect of imatinib, but not of FK-778 (6, 7).

The antipolyomavirus activities of FK-778 and FK-779 were assessed using a cytopathic reduction assay and a virus yield assay. In UC1-B cells, IC_{50} values for FK-778 and FK-779 for inhibition of the replication of 4 different strains of murine polyomavirus were 0.95-2.3 and 1.8-24.4 μg/ml, respectively, with a minimum cytotoxic concentration (MCC) of 20 μg/ml. Mean IC_{50} values of FK-778 and FK-779 against 3 simian polyomavirus strains in Vero cells were 1-2.5 and 4.6-6.7 μg/ml, respectively, with an MCC value of 5 μg/ml (8).

Combination therapy for the treatment of immunological disorders and degenerative processes has been claimed, comprising an antigen involved in an unwanted immune reaction or in regenerative processes, a protein synthesis inhibitor and, if necessary, an active substance which suppresses acute inflammatory reactions. Candidate agents include the dihydroorotate dehydrogenase inhibitors leflunomide, HMR-279 and FK-778 (9).

1. Vu, M.D., Qi, S., Wang, X., Jiang, W., Xu, D., Wu, J., Beckersky, I., Fitzsimmons, W.E., Chen, H. *Combination therapy of tacrolimus with malononitrilamides in cell proliferation assays and in rats receiving renal allografts*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0027.

2. Bilolo, K.K., Ouyang, J., Wang, X. et al. *Synergistic effect of FK778 and FK779 with tacrolimus in prevention of acute heart and kidney allograft rejection and in reversal of ongoing heart allograft rejection in the rat*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 2034.

3. Kyles, A.E., Gregory, C.R., Griffey, S.M., Bernsteen, L., Pierce, J., Lilja, H., Morris, R.E. *Combined immunosuppression with FK778 and cyclosporine prolongs renal allograft survival in mismatched mongrel dogs*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0026.

4. Jin, M.B., Nakayama, M., Ogata, T., Fujita, M., Mino, K., Taniguchi, M., Suzuki, T., Shimamura, T., Furukawa, H., Todo, S. *A novel leflunomide derivative, FK778, for immunosuppression after kidney transplantation in dogs*. Surgery 2002, 132(1): 72.

5. Jiang, H., Pan, F., Ebbs, A., Wynn, C., Erickson, L., Jang, M., Crews, G., Kobayashi, M., Paul, L., Benediktsson, H. *FK778, a powerful new immunosuppressant, effectively prevents chronic rejection in rat renal allografts*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0601.

6. Savikko, J., von Willebrand, E., Haäyry, P. *Leflunomide analogue FK778 is vasculoprotective independent of its immunosuppressive effect. Potential applications for restenosis and chronic rejection*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 2033.

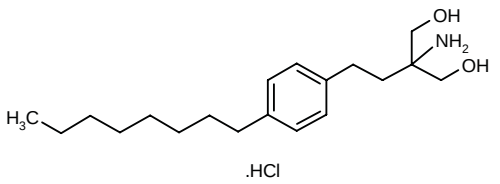
7. Savikko, J., von Willebrand, E., Hayry, P. *Leflunomide analogue FK778 is vasculoprotective independent of its immunosuppressive effect: Potential applications for restenosis and chronic rejection*. 5th Int Conf New Trends Clin Exp Immunosuppr (Feb 7-10, Geneva) 2002, Abst 73.

8. Snoeck, R., Andrei, G., Lilja, H.S., Fitzsimmons, W., De Clercq, E. *Activity of malononitrilamide compounds against murine and simian polyomavirus*. 5th Int Conf New Trends Clin Exp Immunosuppr (Feb 7-10, Geneva) 2002, Abst 67.

9. Lindner, J. (Aventis Behring GmbH). *Combination therapy for the treatment of immunological disorders*. EP 1275638, DE 10132308.

Original monograph - Drugs Fut 2002, 27(8): 733.

FTY-720



FTY-720 is a novel immunosuppressant currently undergoing phase II clinical trials for organ transplantation. The compound represents a novel concept in immunosuppression, protecting the transplant against T-cells without affecting the host's ability to respond to antigens. Originally developed by Mitsubishi Pharma and Taito, it is licensed to Novartis for the E.U. and the U.S.

Bioactive sphingolipids such as sphingosine-1-phosphate (S1P), sphingosine and their precursor ceramide are messengers involved in cell growth, survival and death that are derived from the catabolism of sphingomyelin in the plasma membrane. Ceramide often has antiproliferative and proapoptotic effects, but S1P promotes cell proliferation and survival; it has been suggested that the balance between ceramide and S1P may be important in determining survival or death of mammalian cells. Ceramide-based strategies are currently being developed to improve cancer therapies through promoting apoptosis or blocking proliferation of tumor cells. Similarly, pharmacological manipulation of S1P may provide therapeutic benefits in transplantation and the treatment of autoimmune disorders. FTY-720 is a strong immunosuppressant that alters lymphocyte trafficking and protects solid organ allografts. When activated by phosphorylation, FTY-720 becomes a structural analogue of S1P and shows the same affinity for S1P receptors, and early clinical trials conducted in renal transplant patients have shown that FTY-720 is both safe and well tolerated. Sphingolipid analogs like FTY-720 may represent a new class of therapeutic agents to use in transplantation and against autoimmune diseases (1).

Experiments with FTY-720 *in vivo* and *ex vivo* indicated that the phosphorylated form FTY-720-P is the active principle *in vivo*. FTY-720-P was found to bind and signal at the G-protein-coupled sphingosine-1-phosphate receptor 1 (S1P1/edg1) and sphingosine-1-phosphate receptor 4 (S1P4/edg6) (2).

A study by Japanese researchers confirmed that FTY-720 induced T cell-selective apoptosis in lymphoma cell lines *in vitro*. After exposure to FTY-720, there were significant signs of apoptosis (loss of viability, DNA fragmentation, annexin V binding and caspase activation) in T-lymphoma cells, but not in B-lymphoma cells. FTY-720 also released cytochrome *c* in T-lymphoma cells, but not in B-lymphoma cells. Furthermore, B-lymphoma cells and B-cells had much higher levels of Bcl-2 than did T-lymphoma cells and T-cells. FTY-720-induced apoptosis was inhibited by the overexpression of Bcl-2, suggesting that cellular Bcl-2 levels regulated the sensitivity to FTY-720 (3).

The potential insulinotropic effects of FTY-720 were evaluated in streptozotocin-induced diabetic mice and autoimmune nonobese diabetic mice following allogeneic islet transplant. Treatment with FTY-720 resulted in long-term normoglycemia (more than 100 days) in 100% and 50% of animals, respectively, compared with 11 and 7 days of normoglycemia in untreated mice. These results suggest that the compound may have potential in preventing type 1 diabetes (4).

The ability of FTY-720 (1 or 5 mg/kg) and CTLA4IgG (299 or 500 μg/head), either alone or in combination, to preserve the respiratory epithelium and inhibit the development of obliterative airways disease, was evaluated in BALB/c mice transplanted with trachea from C3H/He mice. Either drug alone significantly inhibited the development of obliterative airways disease, but normal ciliated columnar respiratory epithelial cells were lost in the allografts. In contrast, combination therapy preserved the respiratory epithelium of the allografted trachea. High tissue concentrations of FTY-720 inhibited mixed lymphocyte reactions and augmented T-cell apoptosis (5).

FTY-720 3 mg/kg/day by oral gavage was given as pretreatment to mice receiving cardiac allografts. Three different time courses of FTY-720 administration were tested. Compared to untreated controls and animals treated with ciclosporin or everolimus, FTY-720-pretreated animals had prolonged graft survival (median survival time 14- > 55 days compared with 7-8 days for nontreated controls). Further *in vitro* investigations revealed that FTY-720 induced apoptosis of T-cells and downregulated CD25 expression on T-cell membranes (6).

FTY-720 (1 or 3 mg/kg) with or without ciclosporin (15 mg/kg) was administered to rats receiving transplants of fetal porcine islet-like cell clusters. Although FTY-720 and ciclosporin alone had no effect, combination of the agents inhibited xenograft rejection for up to 24 days. In an additional study, FTY-720 (1 mg/kg) and ciclosporin (15 mg/kg) were administered to diabetic rats receiving adult porcine islet transplants. In these animals, graft survival improved to over 50 days from 5.5 ± 0.3 days in untreated controls (7).

NOD mice were administered FTY-720 0.5 mg/kg/day by oral gavage 5 days a week from the age of 4-35 weeks, and the treatment was found to prevent the spontaneous or cyclophosphamide-induced development of diabetes. FTY-720, therefore, is suggested to have potential in the prevention of diabetes in prediabetic subjects (8).

The mechanism by which FTY-720 mediates T-cell migration and lymph node homing was investigated in a series of experiments using T-cells isolated from wild-type and 5-lipoxygenase, Mdr1 and Mrp1 knockout mice. Using a comparison model with the effects of sphingosine-1-phosphate, the results suggest that FTY-720 enhances T-cell migration and homing through the sequential engagement of the sphingosine transporter Abcb1 (Mdr1), Sp1 receptors, 5-lipoxygenase and the LTC4 transporter Abcc1 (Mrp1) (9).

The development of autoimmune diabetes in nonobese diabetic mice was assessed with and without treatment with FTY-720. Treatment of mice with FTY-720 (0.5 mg/kg p.o. 5 times a week beginning at 4 weeks of age) blocked the development of diabetes in 94% (15 of 16) of the animals, while 70% of untreated animals developed diabetes by 35 weeks of age. Five mice developed diabetes within 2 weeks upon withdrawal of treatment at 35 weeks of age, while the other treated mice remained without diabetes for as long as 9 weeks. The treatment was safe and further experiments showed that it could also prevent the development of diabetes induced by cyclophosphamide in nonobese diabetic mice (10).

The effects of lymphocyte depletion with FTY-720 were investigated in an animal model of warm hepatic ischemia and reperfusion injury. Rats undergoing 60-min partial warm hepatic ischemia/reperfusion were left untreated (sham) or treated with water (controls) or FTY-720 p.o. 3 days before ischemia. Serum glutamic pyruvic transaminase and peripheral blood lymphocytes were significantly reduced in FTY-720-treated animals compared to controls, and FTY-720-treated livers had significantly improved histological grade *versus* controls. T-cell infiltration was also significantly reduced in FTY-720-treated livers. Survival at 7 days was significantly better in treated animals versus controls after 150-min total ischemia/reperfusion with portojugular shunt, pointing to the importance of T-lymphocytes in hepatic ischemia/reperfusion injury and suggesting potential important implications for FTY-720 and its lymphocyte-homing action in liver transplantation (11, 12).

FTY-720 was investigated alone or in combination with ciclosporin or everolimus as immunosuppression in cynomolgus monkeys receiving kidney allografts. FTY-720 3 mg/kg/day, but not 0.3 mg/kg/day, increased rejection-free survival, as did ciclosporin 30 mg/kg/day and everolimus 0.75 mg/kg/day. FTY-720 0.1-0.3 mg/kg/day combined with ciclosporin 10-30 mg/kg/day and everolimus 0.25-0.50 mg/kg/day was synergistic in preventing rejection (13).

In a rat heart transplant model, the effect of mycophenolate sodium (MPS) as monotherapy or combined with FTY-720 was evaluated on graft survival. Animals treated with 3, 10 and 30 mg/kg/day MPS survived a median of 6, 14.5 and >56 days, respectively; animals who received combined treatment with 10 mg MPS and 0.03 or 0.1 mg/kg/day p.o. FTY-720 survived a median of 41 and 43.5 days, respectively. Monotherapy with MPS was associated with severe side effects including diarrhea, weight loss and lymphopenia, but when MPS (10 mg) was administered with FTY-720, the regimen was well tolerated. It was concluded that combination regimens may represent an alternative treatment option for transplant patients (14, 15).

FTY-720 0.1, 1, 3 or 6 mg/kg was administered to mice undergoing bone marrow transplants to see if the agent could prevent graft-*versus*-host disease (GVHD) while preserving graft-*versus*-leukemia/lymphoma effects. FTY-720 was given from day 0 to day 28 in lethally irradiated mice which received either syngeneic marrow or haplotype-mismatched GVHD-inducing allogeneic marrow and spleen cell inoculum. Survival was increased in animals receiving FTY-720, which experienced only mild, transient GVHD. The effect of FTY-720 appeared to be dose-dependent. Further experiments revealed no significant differences between the allogeneic groups treated with FTY-720 or placebo following inoculation with EL4 (T-cell leukemia/lymphoma) cells, with both groups demonstrating a graft-*versus*-lymphoma/leukemia effect. FTY-720 may therefore represent a novel, clinically relevant strategy for the separation of graft-*versus*-leukemia/lymphoma and graft-*versus*-host reactions (16, 17).

The effect of acute and repeated administration of therapeutically applicable doses of FTY-720 (0.3, 1 or 5 mg/kg/day p.o. and i.v.) was explored using anesthetized male rats. No significant alteration of systemic, renal or hepatic hemodynamics or glomerular perfusion was observed via either route, and although repeated oral dosing may result in reduced sodium excretion, FTY-720 appears to offer a potential safeguard against calcineurin inhibitor-induced nephrotoxicity (18).

Pretreatment with FTY-720 (3 mg/kg/day p.o.) was assessed in naive and presensitized mice undergoing cardiac transplantation. Presensitized mice received donor-type skin 21 days before cardiac transplantation. It was found that FTY-720 administered from day -3 to day 11 significantly improved cardiac allograft survival in naive but not presensitized animals (median survival >100 and 8 days, respectively). High Bcl-xL expression in the spleen and rapid recovery to normal levels of peripheral lymphocytes in presensitized animals indicated that memory lymphocytes were resistant to FTY-720-induced apoptosis (19).

In rats undergoing renal transplantation, investigators assessed treatment with FTY-720 (1 mg/kg i.v.) alone, antisense oligonucleotides designed to inhibit ICAM-1 alone (1 mg/kg) or FTY-720 plus antisense oligonucleotides. Both agents given separately inhibited acute renal failure resulting from ischemia/reperfusion injury to a similar degree, and a small additive effect was seen in the combination treatment group (20).

Human cytomegalovirus (HCMV)-specific effector T-cell frequencies were evaluated in 6 renal transplant recipients who received FTY-720 (5 mg/day) plus steroids and ciclosporin (8-10 mg/kg/day or 2-3 mg/kg/day). While patients receiving FTY-720 and low-dose ciclosporin did not have a greatly decreased HCMV-specific memory T-cell response, those given FTY-720 and standard-dose ciclosporin demonstrated loss of HCMV-specific T-cell

response after transplantation, which may lead to a higher incidence of HCMV-related infection (21).

A study enrolled 56 patients with first or second cadaveric renal allografts at increased risk of delayed graft function. Patients were treated with FTY-720 (5-mg loading dose pretransplant followed by 2.5 mg once daily), everolimus (4-mg loading dose pretransplant followed by 2 mg b.i.d., adjusted to trough levels of 4-8 ng/ml) and corticosteroids. Immunoprophylaxis with this regimen was determined to be adequate in 52 patients after a mean follow-up of 224 days. The frequency of adverse events was low (22).

Stable renal transplant patients (n=10) were given single oral doses of FTY-720 0.25-3.5 mg in a double-blind, placebo-controlled phase I study of the drug's effects on T-lymphocyte subpopulations in peripheral blood. FTY-720 significantly reduced peripheral lymphocyte counts. Doses between 0.25 and 2.0 mg significantly increased the percentage of CCR5-positive T-lymphocytes, while the percentage of CD62L-positive T-lymphocytes was significantly decreased (23).

The combination of FTY-720 and reduced-exposure ciclosporin was investigated in a multicenter, randomized, dose-finding safety, tolerability and efficacy study in 266 renal transplant patients. The regimens evaluated were: FTY-720 as a 5-mg loading dose and then 5 mg once daily plus reduced-exposure ciclosporin; FTY-720 as a 5-mg loading dose and then 2.5 mg once daily plus reduced-exposure ciclosporin; FTY-720 as a 5-mg loading dose and then 2.5 mg once daily plus full-exposure ciclosporin; or mycophenolate mofetil as a 1-g loading dose and then 2-3 g daily in divided doses plus full-exposure ciclosporin. Rejection prophylaxis with FTY-720 and ciclosporin was considered adequate. Preliminary results show that most acute rejection episodes were mild and that the combination of FTY-720 with reduced- or full-exposure ciclosporin was well tolerated (24).

1. Kolesnick, R. *The therapeutic potential of modulating the ceramide/sphingomyelin pathway*. J Clin Invest 2002, 110(1): 3.

2. Brinkmann, V., Davis, M.D., Hesise, C.E. et al. *FTY720, a novel therapeutic for transplantation and autoimmunity, targets G-protein-coupled receptors for sphingosine-1-phosphate*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0116.

3. Nagahara, Y., Ikekita, M., Shinomiya, T. *T cell selective apoptosis by a novel immunosuppressant, FTY720, is closely regulated with Bcl-2*. Br J Pharmacol 2002, 137(7): 953.

4. Fu, F., Hu, S., Deleo, J., Li, S., Hopf, C., Hoover, J., Wang, S., Brinkmann, V., Lake, P., Shi, V.C. *Long-term islet graft survival in streptozotocin- and autoimmune-induced diabetes models by immunosuppressive and potential insulinotropic agent FTY720*. Transplantation 2002, 73(9): 1425.

5. Konishi, K., Inobe, M., Yamada, A., Murakami, M., Todo, S., Uede, T. *Combination treatment with FTY-720 and CTLA-4 IgG preserves the respiratory epithelium and prevents obliterative disease in a murine airway model*. J Heart Lung Transplant 2002, 21(6): 692.

6. Zhang, L., Zhu, T., Sun, E.W., Shen, S.Q., Guo, H., Chen, S., Min, Z.L., Chen, Z.K. *Pretreatment with FTY720 alone induced tolerance to cardiac allograft in mouse*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0602.

7. Maeda, A., Goto, M., Elfman, L., Groth, C.G., Korsgren, O., Wennberg, L. *Immunosuppression with FTY720 and cyclosporine A inhibits rejection of adult porcine islet xenografts in rats*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0249.

8. Maki, T., Gottschalk, R. *Prevention of autoimmune diabetes by FTY720*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0465.

9. Honig, S.M., Fu, S., Mao, X., Yopp, A., Gunn, M.D., Randolph, G.J., Bromberg, J.S. *FTY720 stimulates multidrug transporter- and cysteinyl leukotriene-dependent T cell chemotaxis to lymph nodes*. J Clin Invest 2003, 111(5): 627.

10. Maki, T., Gottschalk, R., Monaco, A.P. *Prevention of autoimmune diabetes by FTY720 in nonobese diabetic mice*. Transplantation 2002, 74(12): 1684.

11. Anselmo, D.M., Amersi, F.F., Shen, X.-D. et al. *FTY720: A novel approach to the treatment of hepatic ischemia-reperfusion injury*. Transplant Proc 2002, 34(5): 1467.

12. Anselmo, D.M., Amersi, F.F., Shen, X.-D. et al. *FTY720 pretreatment reduces warm hepatic ischemia reperfusion injury through inhibition of T-lymphocyte infiltration*. Am J Transplant 2002, 2(9): 843.

13. Schuurman, H.-J., Menninger, K., Audet, M. et al. *Oral efficacy of the new immunomodulator FTY720 in cynomolgus monkey kidney allotransplantation, given alone or in combination with cyclosporine or RAD*. Transplantation 2002, 74(7): 951.

14. Matsumoto, Y., Hof, A., Baumlin, Y., Hof, R.P. *Effects of mycophenolate sodium (MPS) with or without FTY720 in a DA to Lewis rat heart transplantation model*. 5th Int Conf New Trends Clin Exp Immunosuppr (Feb 7-10, Geneva) 2002, Abst 48.

15. Matsumoto, Y., Hof, R.P. *Effects of mycophenolate sodium with or without FTY720 in a DA-to-Lewis rat heart transplantation model*. Transplant Proc 2002, 34(7): 2891.

16. Kim, Y.M., Sachs, T., Sykes, M. *FTY720 inhibits graft-versus-host disease (GVHD) in a murine haplotype-mismatched bone marrow transplantation (BMT) model*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0603.

17. Kim, Y.-M., Sachs, T., Asavaroengchai, W., Bronson, R., Sykes, M. *Graft-versus-host disease can be separated from graft-versus-lymphoma effects by control of lymphocyte trafficking with FTY720*. J Clin Invest 2003, 111(5): 659.

18. Tawadrous, M.N., Mabuchi, A., Zimmermann, A., Wheatley, A.M. *Effects of immunosuppressant FTY720 on renal and hepatic hemodynamics in the rat*. Transplantation 2002, 74(5): 602.

19. Zhu, T., Zhang, L., Shen, S.Q., Zuo, L.Q., Xia, S.S., Chen, Z.K. *Pretreatment with FTY720 induces long-term survival of cardiac allograft in naive but not presensitized mice*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 1141.

20. Troncoso, P., Ortiz, M., Kahan, B.D. *Prevention of ischemia-reperfusion injury using FTY 720 in combination with ICAM-1 antisense oligonucleotides*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 1750.

21. Nickel, P., Biti, D., Bold, G., Presber, F., Neuhaus, P., Kern, F., Volk, H.D., Reinke, P. *Effect of FTY720-treatment on HCMV-specific T cell frequencies in renal allograft recipients*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0051.

22. Tedesco-Silva, H., Rosso-Felipe, C., Lorber, M. et al. *FTY720, RAD and corticosteroids for the prevention of graft loss and acute rejection in renal allograft recipients at increased risk of delayed graft function*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0050.

23. Boehler, T., Schuetz, M., Budde, K., Neumayer, H.-H., Waiser, J. *FTY720 alters the composition of T-lymphocyte subpopulations in the peripheral blood compartment of renal transplant patients*. Transplant Proc 2002, 34(6): 2242.

24. Ferguson, R.M., Mulgaonkar, S., Tedesco, H., Oppenheimer, F., Walker, R., Kunzendor, U., Binswanger, U., Mullon, C., Fornairon, S. *FTY720 with reduced-exposure Neoral provides adequate rejection prophylaxis in de novo renal transplant recipients. Interim results*. 19th Int Congr Transplant Soc (Aug 25-30, Miami) 2002, Abst 0049.

Original monograph - Drugs Fut 1997, 22(1): 18.