Drugs of the Future 1998, 23(8): 827-837

CCC: 0377-8282/98

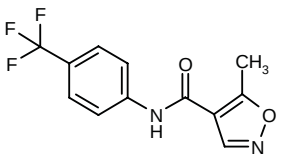
Leflunomide

Immunomodulator

Rec INN

HWA-486
SU-101
Arava™

5-Methyl-*N*-[4-(trifuoromethyl)phenyl]isoxazole-4-carboxamide



$C_{12}H_9F_3N_2O_2$ Mol wt: 270.21

CAS: 075706-12-6

EN: 116061

Synthesis

Leflunomide can be obtained by several related ways: Scheme 1.

1) The reaction of diketene (I) with 4-(trifluoromethyl)-aniline (II) in hot acetonitrile gives *N*-[4-(trifluoromethyl)phenyl]acetoacetamide (III) (1), which by reaction with triethyl orthoformate (IV) in refluxing acetic anhydride yields the corresponding ethoxymethylene derivative (V). Finally, this compound is cyclized with hydroxylamine in refluxing ethanol/water (1, 2).

2) The reaction of ethyl acetoacetate (VI) with triethyl orthoformate (IV) as before gives the corresponding ethoxymethylene derivative (VII), which by cyclization with hydroxylamine as before affords 5-methylisoxazole-4-carboxylic acid ethyl ester (VIII). The hydrolysis of (VIII) under acidic conditions yields the free acid (IX), which is converted into the acid chloride (X) by standard methods (1). Finally, this compound is condensed with 4-(trifluoromethyl)aniline (II) by means of triethylamine in acetonitrile (1, 3).

3) The formation of leflunomide from acid (IX) or its derivatives such as ethyl (VIII) or other esters can also be performed through other standard procedures of amide formation (1).

4) The *N*-[4-(trifluoromethyl)phenyl]acetoacetamide (III) can also be obtained by reaction of 4-(trifluoromethyl)aniline (II) with 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (XI) in refluxing xylene (2).

Description

Crystals, m.p. 166.5 °C (1); crystals, m.p. 165-6 °C (3); crystals, m.p. 166-7 °C (2).

Introduction

The isoxazole derivative leflunomide is a novel immunomodulating agent with a complex profile of activity. Its multiple mechanisms of action impart the compound with a wide range of potential uses, and leflunomide has been studied for various indications. The lion's share of attention, however, has been given to three potential therapeutic indications for leflunomide: rheumatoid arthritis, transplantation and cancer.

Mechanism of Action Studies

The exact mechanism of action of leflunomide remained unclear for many years, and even now cannot be explained in a simple, cut-and-dried fashion. Several mechanisms are involved in its therapeutic effects and have varying degrees of importance, depending upon the potential therapeutic use in question.

Several early studies were conducted with the dual objectives of demonstrating the therapeutic efficacy of leflunomide and uncovering its mechanism of action. Published summaries of these studies propose the following mechanisms of action as being responsible for the immunomodulating activity of lebflunomide: inhibition of tyrosine phosphorylation and pyrimidine nucleotide synthesis (4, 5); inhibition of interleukin-2-stimulated tyrosine kinase (p56lck) activity (6); inhibition of DNA replication, leading to inhibition of lymphocyte proliferation (7); inhibition of uridine biosynthesis and blockade of growth factor-stimulated proliferation of smooth muscle cells (8); inhibition of the production of immunostimulatory cytokines and promotion of the production of immunosuppressive cytokines (9); and inhibition of dihydroorotate dehydroge-

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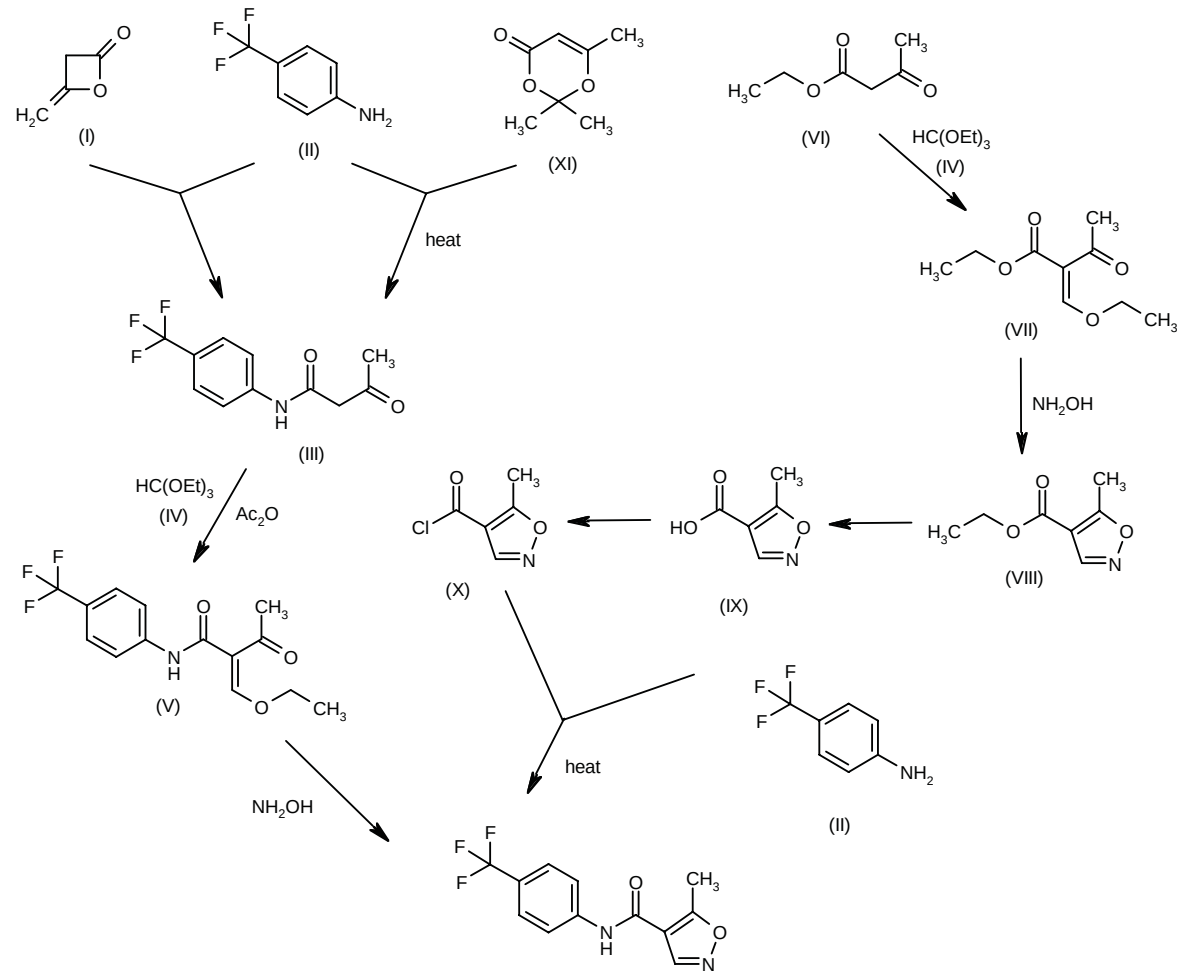
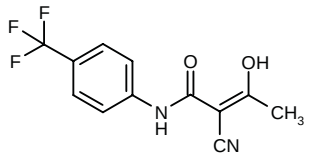
Scheme 1: Synthesis of Leflunomide



nase, an important enzyme in the *de novo* pyrimidine biosynthesis pathway (9, 10). The dihydroorotate dehydrogenase inhibitory activity of leflunomide has been demonstrated both *in vitro* and *in vivo* (11, 12).

Leflunomide is rapidly converted *in vivo* to the active metabolite A-771726 [I], and this metabolite has been shown to possess two important activities: inhibition of pyrimidine nucleotide synthesis and interference in tyrosine phosphorylation (13). A-771726 has also been shown to inhibit dihydroorotate dehydrogenase (11-13), a key enzyme in the synthesis of uridine monophosphate.



[I]

Pharmacokinetics, Pharmacodynamics and Metabolism

In a study of the blood distribution and pharmacokinetics of leflunomide following single-dose administration in rabbits, A-771726 was shown to be more than 95% bound to the lipoprotein-free fraction of plasma over the concentration range of 0.4-100 mg/l. Oral administration of leflunomide or i.v. administration of its metabolite to healthy white New Zealand rabbits yielded half-lives of 3.88 ± 2.3 and 3.18 ± 1.6 h, respectively. Volume of distribution values indicated that distribution into tissues was minimal following either oral or i.v. dosing. The mean residence time of A-771726 was greater following oral dosing of leflunomide than it was after i.v. dosing of the prodrug itself (mean residence time = 10.54 ± 2.6 and 6.76 ± 1.0 h, respectively). The area under the curve (AUC) was

statistically equivalent following i.v. dosing of the metabolite or oral administration of the prodrug, indicating 100% bioavailability (14).

A single- and multiple-dose study in rats was also conducted for purposes of pharmacokinetic/pharmacodynamic evaluation. Leflunomide was administered as single oral doses of 5, 10 or 20 mg/kg, leading to nearly complete inhibition of the incorporation of [^{3}H]-thymidine – a measure of lymphocyte proliferation – after just 2 h. Peak (C_{max}) and trough (C24) concentrations, AUC and clearance increased dose-dependently following single-dose administration. In the multiple-dose study, leflunomide was administered for 14 days at doses of 4 or 10 mg/kg, producing dose-dependent increases in peak (C_{max}) and trough (C24) drug concentrations and AUC. The elimination half-life of leflunomide was shorter and clearance values and volume of distribution were greater in the multiple-dose study than in the single-dose study. Pharmacokinetics and pharmacodynamics showed a high degree of interindividual variability at all doses tested; this variability was attributed to differences in degree of absorption, enzymatic induction and enterohepatic recirculation. Thus, clinical monitoring may be necessary until steady-state concentrations of the active metabolite are attained (15).

In a pharmacokinetic study in healthy volunteers, the parent drug leflunomide was not detected; only the active metabolite A-771726 was recovered. The majority (90%) of the administered dose was recovered in urine and feces. The terminal half-life of leflunomide was 7-8 days following a single dose, and averaged 11.1 days (range 3.7-28.4 days) following multiple dosing for 14 days. Clearance was approximately 51 ml/h. In healthy volunteers and in patients with rheumatoid arthritis, leflunomide was extensively metabolized to A-771726, and the metabolite was extensively protein-bound. Steady-state plasma concentrations were attained following 20 weeks of drug administration. Elimination of the active metabolite was very slow (6-40 days), and clearance of the metabolite decreased with increasing dose of leflunomide (16).

An HPLC method was developed for purposes of analyzing pharmacokinetics and plasma levels of leflunomide in rats. The analytical recovery using this method was 90% and the detection limit was 50 ng/ml. Leflunomide, administered at a dose of 5 mg/kg p.o., disappeared rapidly from the blood stream (C_{max} = 0.3 μg/ml; AUC = 0.5 μg.h/ml). Its active metabolite A-771726, however, had a C_{max} of 22.1 μg/ml and an AUC of 348 μg.h/ml, indicating that the immunosuppressive effects of leflunomide are indeed directly attributable to the pharmacokinetic behavior of its metabolite (17).

The development of an HPLC methodology for use in human pharmacokinetic studies has also been described. The method demonstrates good analytical recovery in human blood (78 ± 13.5-108 ± 4.8% for drug concentrations of 400-100,000 μg/l) (18).

A study designed to further investigate the metabolism of leflunomide has shown that the active pharmacophore responsible for the immunosuppressive effects of A-771726 is a beta-keto amide with the enolic hydroxy group fixed in a configuration cis to the amidic moiety (19).

Side Effects

Side effects of leflunomide in clinical studies have included dose-dependent rash, reversible alopecia and elevated liver function tests, as well as gastrointestinal side effects (16, 20). In general, gastrointestinal effects including anorexia, abdominal pain, diarrhea, nausea, vomiting, gastritis and gastroenteritis are the most frequently reported adverse events with leflunomide. No nephrotoxicity or myelotoxicity has been observed, and the drug is not associated with an increased incidence of opportunistic infections (24).

Leflunomide for Rheumatoid Arthritis

Several mechanisms of action appear to be involved in the antirheumatic activity of leflunomide. The compound significantly impairs the activity of various cytokines (IL-2, IL-3, IL-4, IL-5, TNF-α, GM-CSF), although it does not completely inhibit their production. It also inhibits the adhesion of inflammatory cells to the endothelium and inhibits the activation of tyrosine kinase. Finally, and perhaps most importantly, leflunomide inhibits the de novo biosynthesis of pyrimidine, resulting in direct antiproliferative effects in lymphocytes and B-cells. It has shown favorable activity in the adjuvant arthritis model in rats and in proteoglycan-induced arthritis in mice. In animal models of chronic rheumatoid arthritis, leflunomide did not simply suppress inflammation, it actually blocked the progression of disease to uninvolved joints (20).

In mice with proteoglycan-induced arthritis, treatment with leflunomide (35 mg/kg/day) for 12 weeks led to suppression of acute inflammatory events, reduced antibody titers and less joint stiffness. Further degradation of cartilage was also halted, and leflunomide-treated animals were protected against new inflammatory events as well as exacerbations of disease. The favorable effects of leflunomide in this model of arthritis were found to be primarily due to the suppression of autoreactive antibodies (21, 22).

In rats with established arthritis, treatment with leflunomide (2.5-10 mg/kg/day) was initiated on day 3 of disease, leading to effective inhibition of both acute and chronic phases of arthritis. Neither leflunomide nor ciclosporin (both at doses of 1 mg/kg/day), administered alone, had any measurable effect on disease severity or antibody levels in rats. Combination therapy with leflunomide plus ciclosporin at these subeffective doses, however, led to significant reductions in histopathological scores or chronic arthritis (23).

In a phase II placebo-controlled study in patients with severe rheumatoid arthritis, daily oral administration of leflunomide (5, 10 or 25 mg) for 6 weeks was well tolerated and resulted in objective and subjective clinical improvement, as well as improved immunologic parameters. Improvements were observed as early as 4 weeks after beginning treatment in all active treatment groups, as well as in the placebo group. Statistically significant improvements were seen in the 10-mg leflunomide group as compared to placebo. Efficacy did not increase with higher doses, although side effects became more problematic (20).

A prospective phase II trial, evaluating the safety and efficacy of leflunomide in treating active rheumatoid arthritis, was undertaken in 402 patients. Following a single loading dose of 50 or 100 mg, patients were treated with 5, 10 or 25 mg/day for 24 weeks. Both primary and secondary outcome parameters improved in a dose-dependent fashion, although adverse events (gastrointestinal symptoms, rash, alopecia, liver enzyme abnormalities and thrombocytopenia) also increased with increased doses (21, 22). More than 500 patients were treated during an open-label extension of the same study, some for as long as 2 years. Improvements were noted as early as 4 weeks after beginning treatment and continued through weeks 16-26, at which point most patients experienced disease stabilization. Concomitant treatment with corticosteroids or NSAIDs did not appear to adversely affect the activity of leflunomide in this study (24).

The antiarthritic activity of leflunomide was compared to that of other disease-modifying antirheumatic drugs (DMARDs) (methotrexate, ciclosporin and levamisole) in 168 patients with RA. Treatment consisted of 10-25 mg/day for 30 months in the case of leflunomide, 7.5-15 mg/week for 12-84 months in the case of methotrexate, 2.5-5 mg/kg/day for 6-12 months in the case of ciclosporin and 150 mg/week for 12 months or more in the case of levamisole. Full or partial clinical response rates for the four respective DMARDs were 87%, 85%, 68% and 63%. Adverse events were frequent with all four drugs, but were most pronounced on levamisole. In the case of the title compound, adverse events were generally mild and transient, and did not result in treatment withdrawal. Efficacy was most pronounced in RA patients in early stages of the disease (26).

Leflunomide has also been administered as a weekly dosing regimen (100 or 200 mg p.o./week x 24 weeks) in a small group of 47 patients with active RA. Although clinical response was generally good (54% and 65% for low- and high-dose leflunomide), compliance with the weekly regimen was problematic (27). Immune system activation, measured in terms of total number of T- and B-cells in peripheral blood, number of activated T-cells and memory T-cells, number of autoreactive B-cells and number of plasma ($CD38^+$) cells, decreased after 6 months of treatment with once-weekly leflunomide, and this decrease was correlated with clinical improvement in disease (28).

The FDA has approved leflunomide (Arava^{TM}) for the treatment of active rheumatoid arthritis in adults. The drug is indicated for reducing signs and symptoms and for retarding structural damage as evidenced by X-ray erosions and joint space narrowing, marking the first time a disease-modifying treatment has been indicated for retarding structural damage in rheumatoid arthritis based on X-ray analysis. The NDA was submitted by Hoechst Marion Roussel in March and in August an FDA advisory committee unanimously recommended approval. The company plans to begin shipping Arava^{TM} immediately and expects that the product will be available by mid-October. Arava^{TM} will be available as tablets containing 10, 20 and 100 mg (29). The company has also filed with the European Medicines Evaluation Agency for EU-wide approval (30).

Leflunomide for Transplantation

Leflunomide has also been extensively studied in animal models indicating its potential for use in transplant procedures. A recent study has shown that A-771726, the active metabolite of leflunomide, prolongs graft survival *in vivo* in rodents by decreasing the activity of dihydroorotate dehydrogenase (DHODH) in graft-infiltrating lymphocytes. Both the antiproliferative and immunosuppressant effects of leflunomide are antagonized by uridine supplementation, indicating that the immunosuppressive effects of A-771726 are based on interference with pyrimidine biosynthesis via DHODH inhibition (31). The suppression of nitric oxide release from activated macrophages has also been suggested to contribute to the immunosuppressive activity of leflunomide (32).

The inhibition of interleukin-2 (IL-2)-responsive lymphocytes is another mechanism that has been suggested to play a part in the immunosuppressive activity of leflunomide in transplantation. In a rat model of orthotopic small intestinal transplant (Brown Norway-to-Lewis), leflunomide (5 mg/kg/day by gavage for 7, 14 or 28 days) significantly prolonged the survival of allografts. When administered as the active metabolite A-771726 (5 mg/kg/day for 7 or 15 days), graft survival was prolonged even further. In a semiallogeneic model of acute graft-*vs.*-host disease and rejection, treatment with A-771726 resulted in virtually indefinite survival of grafts in all recipients. No significant toxicity was observed over the range of doses used in this study (33).

The effects of 21-day treatment with leflunomide (1, 2.5, 5 or 10 mg/kg once daily or 2.5 or 5 mg/kg b.i.d.) were evaluated in rat heart allograft recipients. Drug exposure was shown to correlate directly with dose and C_{max} and indirectly with histologic severity of transplant rejection. Repeat administration of leflunomide resulted in reduced drug exposure, probably as a result of induction of drug metabolism, suggesting that dose adjustment will be necessary in order to produce constant immunosuppression. Pharmacokinetics in this rat model correlated with pharmacodynamics, and degree of rejection severity correlated inversely with pharmacodynamics (34).

In a mouse model of graft-*vs.*-host disease, treatment with leflunomide restored abnormally suppressed lymphocyte responses to T-cell mitogens. Neither prednisolone nor indomethacin was effective in this model, and the activity of ciclosporin was dose-dependent and less potent; at some doses, ciclosporin actually inhibited the T-cell response further. In spite of this activity in graft-*vs.*-host disease, leflunomide appeared to be inactive in early models reflecting prevention of transplant rejection. Later studies proved, however, that leflunomide prolonged the survival of kidney allografts (BN-to-Lewis rats) from 8 days in untreated animals to >60 days in leflunomide-treated rats. The compound was also shown to be effective in preventing ongoing rejection of skin grafts in rats and, furthermore, demonstrated a significantly different mechanism of action from that of ciclosporin A in this model (35, 36).

In another study evaluating the immunosuppressive effects of leflunomide in skin graft allotransplantation models in rats, the title compound effectively increased survival time of transplanted skin in several different models. Long-term graft survival was obtained even after short courses of leflunomide therapy, and the compound was effective both in treating acute graft rejection and in inducing transplant tolerance. No side effects were reported. These findings are especially significant in light of the well-known, strong immunogenicity of the skin (37).

The efficacy and safety of leflunomide were demonstrated further in a rat cardiac allotransplantation model using two different strain combinations (DA-to-PVG and DA-to-Lewis). When administered to animals on day 2 of ongoing rejection (DA-to-PVG), title compound (5 mg/kg) was equipotent to tacrolimus (1 mg/kg) but less effective than ciclosporin. In the DA-to-Lewis model, however, leflunomide was equipotent to both tacrolimus (1 mg/kg) and ciclosporin (15 mg/kg). If administered on day 4 of ongoing rejection, leflunomide (10 mg/kg) improved graft survival in both strain combinations in a dose-dependent manner, and was as active as both reference drugs. The toxicity of the title compound (5-20 mg/kg) was less pronounced than that of the reference drugs at therapeutic doses (38). In another rat cardiac allotransplantation model (Brown Norway-to-Lewis), leflunomide prevented acute rejection and induced indefinite engraftment following 3 weeks of treatment. Its efficacy and toxicity were comparable to those of ciclosporin in this model, except that the ability of the title compound to reverse acute rejections and to produce significant allograft survival was superior to that of ciclosporin (39).

Due to the continued shortage of donor organs for transplantation, xenotransplantation is being investigated as an option. The problem of strong immunologic barriers when going from one species to another continues to exist, however, and improved rejection control is imperative if xenotransplantation is to become a reality. Leflunomide has been evaluated in models of hamster-to-rat xenotransplantation (40, 41) and in the so-called “rat-antihuman” xenogeneic model (42). In the former model, leflunomide monotherapy induced potent but imperfect immunosuppressive activity; when used in combination with ciclosporin, however, xenorejection and production of anti-xenoantibodies were suppressed entirely (40). Furthermore, induction therapy with the combination of leflunomide and ciclosporin enabled long-term survival of xenografts in rats on maintenance therapy with ciclosporin alone (41).

Other potential uses of leflunomide in transplantation have also been explored in preclinical models, including pancreatic islet transplantation in rats (43) and in murine recipients of porcine islet grafts (44), and in two different rat models of corneal allograft transplantation (45, 46).

Leflunomide in Combination Regimens for Transplantation

The activity of leflunomide was shown in several studies to be significantly enhanced when used in combination with other immunosuppressants, especially ciclosporin. In a canine model of renal transplantation, leflunomide was administered to 38 female dogs submitted to kidney transplant and bilateral nephrectomy. Dogs were either left untreated or administered leflunomide (2, 4, 8 or 16 mg/kg/day p.o.), alone or in combination with ciclosporin (10 mg/kg/day). For purposes of evaluating toxicity while maintaining constant low drug levels in blood, another group of 8 dogs was given the study drug by constant i.v. infusion (2, 4, 6 or 8 mg/kg/day). Graft survival duration in untreated controls averaged 9 days, while in the 2-, 4-, 8- and 16-mg/kg/day leflunomide groups mean survival was 9, 16, 28 and 21 days, respectively. Dogs treated with ciclosporin (10 mg/kg/day) monotherapy had a mean survival time of 13 days. In the leflunomide plus ciclosporin-treated group, mean graft survival time was 68 days. Animals administered leflunomide by constant i.v. infusion had mean survival times of 10, 20, 14 and 21 days in the respective dose groups. Acute allograft rejection was effectively prevented in dogs administered the highest oral dose of leflunomide, but animals died of inanition in spite of normal renal function. At a dose of 4 mg/kg/day, leflunomide was nontoxic but did not effectively prevent rejection. The most favorable results were thus obtained with the combination of suboptimal doses of leflunomide (4 mg/kg/day) and ciclosporin (10 mg/kg/day), which resulted in maintenance of normal renal function and weight for 30 days or more. These results imply interesting potential for clinical use of leflunomide in transplantation, alone and especially in combination with ciclosporin (47).

Synergistic immunosuppressive effects were observed with a combination, at low dosages, of leflunomide and ciclosporin in Brown Norway-to-Lewis rat cardiac transplantation. Pharmacokinetic evaluation demonstrated that treatment for 28 days did not alter trough levels or elimination of ciclosporin, indicating that synergy was not the result of decreased elimination. Toxicity of both drugs, whether administered alone or in combination, was negligible as judged by observation of general

appearance and testing for serum ALT, AST, bilirubin, creatinine, white blood cell counts and hemoglobin and appearance of gross necropsy. Evaluation of this combination in transplant procedures in human patients appears to be justified (48).

The mechanism underlying the synergy of leflunomide and ciclosporin was investigated in an *in vivo* study. Ciclosporin is known to act by specifically suppressing T-cell responses, thus interfering with TCR-induced signaling pathways, while leflunomide suppresses B-cell-mediated immune responses. Leflunomide also inhibits T-cell functions involved in clonal expansion but does not affect lymphokine secretion and therefore does not inhibit lymphokine-mediated immune functions. These significantly different and yet complementary mechanisms of immunosuppression lead to synergistic activity of the drugs when used in combination (49).

The use of leflunomide in other drug combinations has also been explored in preclinical transplantation models. The combination of leflunomide (5 or 10 mg/kg/day by gavage) and tacrolimus (0.2 or 0.5 mg/kg/day i.m.) was found to prolong the survival of pancreatic islet allografts in a rodent model, with more potent effects obtained with the combination of subtherapeutic doses of each drug than through use of either drug alone (50). The combination of leflunomide and tacrolimus was also evaluated in a rat model of cardiac allotransplantation. In this case, however, the results were not as clearly promising (51).

Leflunomide, administered in combination with another dihydroorotate dehydrogenase inhibitor, brequinar sodium, was evaluated in a rat cardiac allotransplantation model, and the effects were compared to those of brequinar plus tacrolimus. Promising results were obtained with both brequinar-containing combinations (52). Other combinations that have been evaluated include leflunomide plus rapamycin and leflunomide plus mycophenolate mofetil. Synergistic immunosuppressive effects have been observed in vitro in all cases (53). Interesting results were also obtained in an islet xenotransplantation model in rats using triple combination therapy with leflunomide, ciclosporin and mycophenolate mofetil (54).

Leflunomide for Other Immune Disorders

Based on its potent immunosuppressive and antiproliferative effects, leflunomide has also been evaluated in various preclinical models of immune diseases. Studies have been reported demonstrating the therapeutic effects of leflunomide and of its metabolite A-771726 in models of dermatologic disease (55), and a case report has described the beneficial effects obtained in a patient with severe, unstable plaque psoriasis treated with leflunomide for 12 weeks (56).

Leflunomide (30 mg/kg/day p.o.) prevented the development of parasite-induced lesions and the resulting immune response in genetically susceptible mice infected with Leishmania major, a model resembling human visceral leishmaniasis (57).

Title compound also demonstrated potent prophylactic and some therapeutic effects in a rat model of glomerulonephritis induced by antibasement membrane antibody (58, 59), as well as therapeutic efficacy in rats with acute and chronic relapsing experimental allergic encephalomyelitis, a model of human CNS disorders such as multiple sclerosis (60). Administration of leflunomide or A-771726 in a rodent model of toxin-induced autoimmune diabetes was shown to induce protective effects, with significant reduction of hyperglycemia and preservation of islet architecture in mice treated with the metabolite at doses of 5-25 mg/kg/day for 10 days, especially when treatment was initiated at the time of diabetes induction (61).

Finally, a series of canine models was employed to demonstrate the potential utility of leflunomide in treating naturally occurring immune-mediated and inflammatory diseases that are resistant to treatment with conventional drugs, including immune-mediated thrombocytopenia, immune-mediated hemolytic anemia and Evan's syndrome, multifocal nonsuppurative encephalitis/meningomyelitis and systemic histiocytosis (62).

Leflunomide for Cancer

Leflunomide has also been found to have significant therapeutic potential as an oncolytic agent, and Sugen has obtained rights from Hoechst Marion Roussel to develop the compound for this indication. SU-101, the designation used by Sugen, exerts its anticancer effects via a completely different mechanism: inhibition of the epidermal growth factor receptor (EGFR) tyrosine kinase. Sugen has been awarded various patents covering the use of the compound in treating cancer (2, 63), as well as a patent covering proprietary formulations (64).

SU-101 has been shown to inhibit PGDF-mediated signaling and cell proliferation *in vitro* and *in vivo* in rodent tumor xenografts. As stated above, the compound undergoes rapid metabolism in plasma to yield the butenamide metabolite, SU-0020 (Sugen's designation for A-771726). An *in vitro* study was conducted in order to determine whether SU-101 or SU-0020 is responsible for the antitumor activity of the compound. Both SU-101 and SU-65847 (a nonmetabolizable isomeric form of SU-101) inhibited PDGF-dependent cell cycle progression and PDGF receptor tyrosine autophosphorylation *in vitro* in tumor cells, and the addition of uridine had no effect on these activities. In contrast, SU-0020 did not inhibit PDGF receptor tyrosine autophosphorylation, and its cell-cycle progression-inhibitory and tumor growth-inhibitory activities were both reversed upon addition of uridine to the culture medium. The tumor growth-inhibitory activity of the metabolite was more pronounced in rodent cells than in human cells, which is consistent with previous reports describing species-dependent differences in the sensitivities of pyrimidine biosynthetic enzymes to the compound.

The *in vivo* antitumor activity of SU-101 plus uridine, both administered by the parenteral route, was equipotent to that of SU-101 alone; however, when administered by the oral route, uridine reversed the antitumor effects of SU-101. The results of these studies indicate that the antitumor effects of SU-101 are attributable to the compound itself and not to its major metabolite (65, 66). This is in contrast to the findings obtained with leflunomide, which exerts its immunomodulatory effects via its metabolite A-771726.

The human pharmacokinetics of SU-101 and SU-0020 have been evaluated in a phase I/II study involving 125 patients with advanced malignancies. Previously obtained pharmacokinetic data in animals did not adequately predict values in humans; for example, the half-life of the principal metabolite in rodents and monkeys was 8-16 h, whereas in humans it was 3-4 weeks. The drug appears to undergo enterohepatic recycling, and concomitant administration of cholestyramine reduced the elimination half-life of the metabolite significantly (to 24-48 h). Volume of distribution correlated negatively with dose, indicating that a higher infusion mass of SU-101 could yield increased tissue penetration (67).

Clinical trials have been conducted in patients with primary CNS malignancies, especially recurrent malignant glioma and advanced solid tumors (NSCLC, prostate, ovarian, sarcoma and others). In four separate phase I/II studies involving a total of 142 patients, SU-101 was administered either by a weekly-times-four regimen (consisting of 24-h infusions each week for 4 weeks, following by 2 weeks rest), or by a loading-dose regimen (dosing for 4 consecutive days, followed by weekly maintenance therapy). Doses ranged from 15-443 mg/m^2 in the prior regimen, and from 100-735 mg/m^2 in the latter. Volume of distribution of the parent drug SU-101 increased significantly with the loading-dose regimen's more rapid infusion rate. Fatal cerebral edema, the dose-limiting toxicity, occurred in 2 patients at a dose of 735 mg/m^2. Other grade III or higher toxicities reported were anemia, dyspnea, abdominal pain, asthenia, elevated liver transaminases and leukopenia; other mild to moderate toxicities of SU-101 included headache, asthenia, nausea, diarrhea, vomiting, anorexia, rash, constipation, anemia, arthralgia and peripheral edema. Use of the loading-dose regimen at a dose level of 440 mg/m^2 was recommended for phase II/III studies (68).

Results were recently reported from an ongoing phase II study in patients with hormone-refractory prostate cancer at a dose of 200 or 400 mg/m^2 for a 4-day induction period, followed by weekly infusions for 10 weeks. At time of reporting, 1 patient had completed a full cycle of treatment at the lower dose, and this patient had a 28% decrease in PSA at week 11. Another patient had completed 6 weeks of treatment at the higher dose and reported a significant decrease in bony pain and use of narcotic pain medication, although PSA count increased by 26% with respect to baseline. Tolerability was reported good at both dose levels, with mild asthenia, nausea, dyspepsia, anemia and moderate esophagitis as adverse effects. At least 30 patients are expected to be included in this ongoing study (69).

Another phase II study has been reported in 15 patients with recurrent malignant glioma administered a 4-day loading dose of 417-443 mg/m^2/day SU-101, followed by maintenance therapy every 7-14 days. Nine patients completed the first treatment cycle and 7 continued for a second cycle. One patient had a minor response (25% decrease in cross-sectional area) lasting for at least 32 weeks and 5 had stable disease lasting for 16-41 weeks. Toxicities were mainly grade I-II and were typical for this type of cytostatic agent. Grade III adverse effects included elevated ALT, asthenia and neutropenia, and 1 case of grade IV ALT elevation was reported in this study (possibly due to synergy with anticonvulsants). This dosing regimen was considered to provide optimized tolerability and anticancer activity in patients with malignant glioma (70).

Sugen recently initiated a new phase II trial evaluating SU-101 in patients with ovarian cancer. Thirty patients who have failed standard therapy will be enrolled in this study, which will evaluate efficacy as well as safety. The trial will take place at two U.S. centers, with two additional sites to be activated at a later date. The phase II trial is designed to assess the efficacy of SU-101 based on objective response and stabilization of disease. Secondary objectives include assessment of time to disease progression, effect on CA125 levels and safety. Clinical trials are also under way evaluating SU-101 in patients with glioblastoma as monotherapy in patients who have had a first relapse (phase III) and in combination with BCNU in newly diagnosed glioblastoma patients (phase II) (71).

Manufacturers

Hoechst Marion Roussel AG (DE); Sugen, Inc. (US).

References

1. Kämmerer, F.-J., Schleyerbach, R. (Hoechst AG). *Isoxazole deriv., process for its preparation, medicine containing it and intermediates required in the process.* DE 2854439, EP 013376, US 4284786, US 4351841.

2. Hirth, K.P., Schwartz, D.P., Mann, E. et al. (Sugen, Inc.; Biosignal, Ltd.; Yissum Res. Dev. Co., Hebrew Univ. Jerusalem; Max-Planck-Gessellschaft zur Förderung der Wissenschaften e.V.;Univ. California). *Treatment of platelet derived growth factor related disorders such as cancers using inhibitors of platelet derived growth receptor.* WO 9519169, US 5700823.

3. Fossa, P., Menozzi, G., Schenone, P., Filippelli, W., Russo, S., Lucarelli, C., Marmo, E. *5-Substd. 4-isoxazolecarboxamides with platelet antiaggregating and other activities.* Farmaco 1991, 46: 789-802.

4. Chong, A.S., Liu, W., Shen, J. et al. *In vivo mechanisms of action of leflunomide in allo-transplantation and xeno-transplantation.* Transplantation 1998, 65(8, Suppl.): Abst 678.

5. Cao, W.W., Kao, P.N., Chao, A.C., Gardner, P., Ng, J., Morris, R.E. *Mechanism of the antiproliferative action of leflunomide. A771726, the active metabolite of leflunomide, does not block T-cell receptor-mediated signal transduction but its antiproliferative effects are antagonized by pyrimidine nucleosides.* J Heart Lung Transplant 1995, 14: 1016-30.

6. Nikcevich, D.A., Finnegan, A., Chong, A.S.-F., Williams, J.W., Bremer, E.G. *Inhibition of interleukin 2 (IL-2)-stimulated tyrosine kinase activity by leflunomide.* Agents Actions 1994, 41: C279-82.

7. Cherwinski, H.M., McCarley, D., Schatzman, R., Devens, B., Ransom, J.T. *The immunosuppressant leflunomide inhibits lymphocyte progression through cell cycle by a novel mechanism.* J Pharmacol Exp Ther 1995, 272: 460-8.

8. Nair, R.V., Cao, W., Morris, R.E. *Inhibition of smooth muscle cell proliferation in vitro by leflunomide, a new immunosupressant, is antagonized by uridine.* Immunol Lett 1995, 47: 171-4.

9. Cao, W.W., Kao, P.N., Aoki, Y., Xu, J.C., Shorthouse, R.A., Morris, R.E. *A novel mechanism of action of the immunomodulatory drug, leflunomide: Augmentation of the immunosuppressive cytokine, TGF-β_1, and suppression of the immunostimulatory cytokine, IL-2.* Transplant Proc 1996, 28: 3079-80.

10. Cherwinski, H.M., Cohn, R.G., Cheung, P., Webster, D.J., Xu, Y.-Z., Caulfield, J.P., Young, J.M., Nakano, G., Ransom, J.T. *The immunosuppressant leflunomide inhibits lymphocyte proliferation by inhibiting pyrimidine biosynthesis.* J Pharmacol Exp Ther 1995, 275: 1043-9.

11. Silva, H.S. Jr., Cao, W., Shorthouse, R., Löefller, M., Morris, R.E. *Evidence that in vitro and in vivo anti-proliferative effects of leflunomide (LFM) are mediated by inhibition of de novo pyrimidine biosynthesis: In vivo inhibition of dihydroorotate dehydrogenase (DHOdehase).* J Heart Lung Transplant 1997, 16(1): Abst 252.

12. Silva, H.T. Jr., Slauson, S.D., Shorthouse, R., Cao, W., Löffler, M., Morris, R.E. *Elucidation of the molecular mechanism by which leflunomide (LFM) prolongs graft survival in vivo: Inhibition of dihydroorotate dehydrogenase (DHODH) activity and pyrimidine biosynthesis.* 16th Annu Meet Sci Sess Bus Meet Amer Soc Transplant Physicians (May 10-14, Chicago) 1997, Abst 416.

13. Xu, X., Williams, J.W., Gong, H., Finnegan, A., Chong, A.S.-F. *Two activities of the immunosuppressive metabolite of leflunomide, A771726.* Biochem Pharmacol 1996, 52: 527-34.

14. Lucien, J., Dias, V.C., LeGatt, D.F., Yatscoff, R.W. *Blood distribution and single-dose pharmacokinetics of leflunomide.* Ther Drug Monit 1995, 17: 454-9.

15. Silva, H.T., Shorthouse, R., Morris, R.E. *Single- and multiple-dose pharmacokinetics and pharmacodynamics of leflunomide's active metabolite A771726 in normal Lewis rats.* Transplant Proc 1996, 28: 3092-4.

16. Furst, D.E. *Cyclosporin, leflunomide and nitrogen mustard.* Bailliere's Clin Rheumatol 1995, 9: 711-29.

17. Hsieh, G.C., Andrews, J.M., Fey, T.A., Krause, R.A., Bretheim, P.T., Coghlan, M.J., Mollison, K.W., Luly, J.R. *Pharmacokinetics in rats of the immunosuppressive and antirheumatic drug leflunomide measured by high-performance liquid chromatography (HPLC).* FASEB J 1995, 9(4, Part 2): Abst 5495.

18. Dias, V.C., Lucien, J., LeGatt, D.F., Yatscoff, R.W. *Measurement of the active leflunomide metabolite (A771726) by reverse-phase high-performance liquid chromatography.* Ther Drug Monit 1995, 17: 84-8.

19. Bertolini, G., Aquino, M., Biffi, M., D'Atri, G., Di Pierro, F., Ferrario, F., Mascagni, P., Somenzi, F., Zaliani, A., Leoni, F. *A new rational hypothesis for the pharmacophore of the active metabolite of leflunomide, a potent immunosuppressive drug.* J Med Chem 1997, 40: 2011-6.

20. Bartlett, R.R., Brendel, S., Zielinski, T., Schorlemmer, H.-U. *Leflunomide, an immunorestoring drug for the therapy of autoimmune disorders, especially rheumatoid arthritis.* Transplant Proc 1996, 28: 3074-8.

21. Glant, T.T., Mikecz, K., Brennan, F., Negroiu, G., Bartlett, R.R. *Suppression of autoimmune responses and inflammatory events by leflunomide in an animal model for rheumatoid arthritis.* Agents Actions 1994, 41: C267-70.

22. Glant, T.T., Mikecz, K., Bartlett, R.R., Deák, F., Thonar, E.J.-M.A., Williams, J.M., Mattar, T., Kuettner, K.E., Schleyerbach, R. *Immunomodulation of proteoglycan-induced progressive polyarthritis by leflunomide.* Immunopharmacology 1992, 23: 105-16.

23. Thoss, K., Henzgen, S., Petrow, P.K., Katenkamp, D., Bräuer, R. *Immunomodulation of rat antigen-induced arthritis by leflunomide alone and in combination with cyclosporin A.* Inflamm Res 1996, 45: 103-7.

24. Silva, H.T. Jr., Morris, R.E. *Leflunomide and malononitrilamides.* Amer J Med Sci 1997, 313: 289-301.

25. Mladenovic, V., Domljan, Z., Rozman, B. et al. *Safety and effectiveness of leflunomide in the treatment of patients with active rheumatoid arthritis. Results of a randomized, placebo-controlled, phase II study.* Arthritis Rheum 1995, 38: 1595-603.

26. Popovic, M., Stefanovic, D., Pejnovic, N., Popovic, R., Dimitrijevic, M. *Comparative study of clinical efficacy of four DMARDs, leflunomide, methotrexate, cyclosporine A and levamisole in patients with RA.* 3rd Int Conf New Trends Clin Exp Immunosuppr (Feb 12-15, Geneva) 1998, 147.

27. Plosker, G.L., Wagstaff, A.J. *Leflunomide.* Clin Immunother 1996, 6: 300-5.

28. Dimitrijevic, M., Stupar, N., Pilipovic, N. *Leflunomide in the treatment of patients with severe rheumatoid arthritis: Influence on immunological parameters.* Clin Exp Rheumatol 1995, 13(Suppl. 12): Abst 15-B-P.

29. *FDA approves, HMR begins shipping new treatment for adult rheumatoid arthritis.* Prous Science Daily Essentials September 14, 1998.

30. *HMR files for European, American approval of arthritis drug.* Prous Science Daily Essentials April 7, 1998.

31. *Leflunomide preclinical results presented at transplant congress.* Prous Science Daily Essentials June 2, 1997.

32. Trajkovic, V., Stosic-Grujicic, S., Popadic, D., Pantelic, D., Mostarica Stojkovic, M., Bartlett, R.R. *Leflunomide inhibits nitric oxide production in LPS- or IFN-γ-treated rat macrophages.* 3rd Int Conf New Trends Clin Exp Immunosuppr (Feb 12-15, Geneva) 1998, 23.

33. Foster, P.F., Xiao, F., Kociss, K., Chong, A.-S., Sankary, H.N., McChesney, L., Cohn, S., Williams, J.W. *Leflunomide immunosuppression in rat small intestinal transplantation.* Transplant Proc 1994, 26: 1599-600.

34. Silva, H.T. Jr., Sherwood, S., Huang, X., Cheung, A., Shorthouse, R., Morris, R.E. *Prevention of acute rat heart allograft rejection by leflunomide (LFM): Study of relationships among drug dose, treatment schedule, pharmacokinetics (PK), pharmacodynamics (PD) and grade of rejection.* 16th Annu Meet Sci Sess Bus Meet Amer Soc Transplant Physicians (May 10-14, Chicago) 1997, Abst 411.

35. Küchle, C.C.A., Thoenes, G.H., Langer, K.H., Schorlemmer, H.U., Bartlett, R.R., Schleyerbach, R. *Prevention of kidney and skin graft rejection in rats by leflunomide, a new immunomodulating agent.* Transplant Proc 1991, 23: 1083-6.

36. Bartlett, R.R., Dimitrijevic, M., Mattar, T. et al. *Leflunomide (HWA 486), a novel immunomodulating compound for the treatment of autoimmune disorders and reactions leading to transplantation rejection*. Agents Actions 1991, 32: 10-21.

37. Schorlemmer, H.U., Seiler, F.R., Bartlett, R.R. *Prolongation of allogeneic transplanted skin grafts and induction of tolerance by leflunomide, a new immunosuppressive isoxazol derivative.* Transplant Proc 1993, 25: 763-7.

38. D'Silva, M., Candinas, D., Achilleos, O. et al. *The immunomodulatory effect of leflunomide in rat cardiac allotransplantation.* Transplantation 1995, 60: 430-7.

39. Williams, J.W., Xiao, F., Foster, P.F., Chong, A., Sharma, S., Bartlett, R., Sankary, H.N. *Immunosuppressive effects of leflunomide in a cardiac allograft model.* Transplant Proc 1993, 25: 745-6.

40. Xiao, F., Chong, A., Foster, P., Sankary, H., McChesney, L., Williams, J.M., Frieders, D., Williams, J.W. *Effect of leflunomide in control of acute rejection in hamster-to-rat cardiac xenografts.* Transplant Proc 1994, 26: 1263-5.

41. Lin, Y., Sobis, H., Vandeputte, M., Waer, M. *Induction therapy of leflunomide and cyclosporine allows for long-term xenograft survival under cyclosporine alone.* Transplant Proc 1994, 26: 3052.

42. Ulrichs, K., Kaitschick, J., Bartlett, R., Müller-Ruchholtz, W. *Suppression of natural xenophile antibodies with the novel immunomodulating drug leflunomide.* Transplant Proc 1992, 24: 718-9.

43. Guo, Z., Chong, A.S.F., Shen, J., Foster, P., Sankary, H.N., McChesney, L., Mital, D., Jensik, J.C., Gebel, H., Williams, J.W. *In vivo effects of leflunomide on normal pancreatic islet and syngeneic islet graft function.* Transplantation 1997, 63: 716-21.

44. Mital, D., Guo, Z., Tian, Y. et al. *Leflunomide-based immunosuppression for porcine islet xenotransplantation.* Transplant Proc 1998, 30: 515.

45. Coupland, S.E., Klebe, S., Karow, A.-C., Krause, L., Kruse, H., Bartlett, R.R., Hoffman, F. *Leflunomide therapy following penetrating keratoplasty in the rat.* Graefe's Arch Clin Exp Ophthalmol 1994, 232: 622-7.

46. Niederkorn, J.Y., Lang, L.S., Ross, J., Mellon, J., Robertson, S.M. *Promotion of corneal allograft survival with leflunomide.* Invest Ophthalmol Visual Sci 1994, 35: 3783-5.

47. McChesney, L.P., Xiao, F., Sankary, H.N., Foster, P.F., Sharma, S., Haklin, M., Williams, J.W. *An evaluation of leflunomide in the canine renal transplantation model.* Transplantation 1994, 57: 1717-22.

48. Williams, J.W., Xiao, F., Foster, P., Clardy, C., McChesney, L., Sankary, H., Chong, A.S.-F. *Leflunomide in experimental transplantation. Control of rejection and alloantibody production, reversal of acute rejection, and interaction with cyclosporine.* Transplantation 1994, 57: 1223-31.

49. Lang, R., Wagner, H., Heeg, K. *Differential effects of the immunosuppressive agents cyclosporine and leflunomide in vivo.* Transplantation 1995, 59: 382-9.

50. Rastellini, C., Cicalese, L., Leach, R., Braun, M., Fung, J.J., Starzl, T.E., Rao, A.S. *Combined use of tacrolimus (FK506) and leflunomide (LEF) results in prolongation of pancreatic islet allograft survival.* Transplantation 1998, 65(8, Suppl.): Abst 513.

51. Candinas, D., Achilleos, O., Lee, S.J., Dalgic, A., Mayer, A.D., Buckels, J.A.C., McMaster, P., D'Silva, M. *Efficacy of leflunomide in combination with current immunosuppressive agents in rat cardiac allotransplantation.* Transplant Proc 1994, 26: 3251-2.

52. D'Silva, M., Antiniou, E., DeRoover, A., Nishimura, Y., Howie, A., McMaster, P. *Immunosuppressive effect of brequinar on rat cardiac allograft survival in combination with leflunomide or FK 506.* Transplant Proc 28: 950-1.

53. Chong, A.S.-F., Rezai, K., Gebel, H., Finnegan, A., Foster, P., Xu, X., Williams, J.W. *Effects of leflunomide and other immunosuppressive agents on T cell proliferation in vitro.* Transplantation 1996, 61: 140-5.

54. Weinberg, L., Karlsson-Parra, A., Sundberg, B, Rafael, E., Liu, J., Zhu, S., Groth, C.G., Korsgren, O. *Efficacy of immunosuppressive drugs in islet xenotransplantation.* Transplantation 1997, 63: 1234-42.

55. Kurtz, E.S., Bailey, S.C., Arshad, F., Lee, A.A., Przekop, P.A. *Leflunomide: An active antiinflammatory and antiproliferative agent in models of dermatologic disease.* Inflamm Res 1995, 44(Suppl. 2): S187-8.

56. Johnston, G.A., Rees, J.L., Reynolds, N.J. *Leflunomide therapy in severe, unstable psoriasis.* Australas J Dermatol 1997, 38(Suppl. 2): Abst 2062.

57. Solbach, W., Asmuss, P.A., Zimmermann, S., Humborg, C., Röllinghoff, M. *Protective effect of leflunomide on the natural course of Leishmania major-induced disease in genetically susceptible BALB/c mice.* Int J Immunopharmacol 1995, 17: 481-8.

58. Ogawa, T., Inazu, M., Gotoh, K., Hayashi, S. *Effects of leflunomide on glomerulonephritis induced by antibasement membrane antibody in rats.* Agent Actions 1990, 31: 321-8.

59. Ogawa, T., Inazu, M., Gotoh, K., Inoue, T., Hayashi, S. *Therapeutic effects of leflunomide, a new antirheumatic drug, on glomerulonephritis induced by the antibasement membrane antibody in rats.* Clin Immunol Immunopathol 1991, 61: 103-18.

60. Schorlemmer, H.U., Bartlett, R.R. *Therapeutic activity of leflunomide in acute and chronic relapsing experimental allergic encephalomyelitis.* Agents Actions 1994, 41: C271-3.

61. Stosic-Grujicic, S., Dimitrijevic, M., Bartlett, R.R. *Prevention of experimental autoimmune diabetes in mice by treatment with leflunomide.* 3rd Int Conf New Trends Clin Exp Immunosuppr (Feb 12-15, Geneva) 1998, 47.

62. Gregory, C.R., Stewart, A., Sturges, B., Cannon, A., Ortega, T., Morris, R.E. *Leflunomide effectively treats naturally-occurring immune mediated and inflammatory diseases of dogs that are unresponsive to conventional therapies.* 3rd Int Conf New Trends Clin Exp Immunosuppr (Feb 12-15, Geneva) 1998, 48.

63. Hirth, K.P., Schwartz, D.P., Mann, E. et al. (Univ. California). *Treatment of platelet derived growth factor related disorders such as cancers.* US 5700822.

64. Schwart, D.P., Shawver, L.K. (Sugen, Inc.). *Formulations for lipophilic cpds.* US 5610173.

65. Lipson, K.E., Wang, X., Chen, H. *SU101, and not its major metabolite, inhibits PDGF-dependent receptor tyrosine phosphorylation, cell cycle progression, and human tumor cell growth.* 10th NCI-EORTC Symp New Drugs Cancer Ther (June 16-19, Amsterdam) 1998, Abst 423.

66. Lipson, K.E., Wang, X., Chen, H., Chu, L., Nematalla, A., Tang, C., Strawn, L.M., Shawver, L.K., Hirth, K.P., McMahon, G. *SU101, and not its major metabolite, inhibits PDGF-dependent receptor tyrosine phosphorylation, cell cycle progression, and human tumor cell growth.* Proc Amer Assoc Cancer Res 1998, 39: Abst 3797.

67. Crop, G.F., Hannah, A.L., Malkin, M., Eckhardt, S.G., Marshall, J., Lombardi, C.C., Rosen, L., Lopez, A.M., Sweeney, K. *Pharmacokinetics of SU101, a PDGF-R signal transduction inhibitor, and metabolite, SU0020.* 10th NCI-EORTC Symp New Drugs Cancer Ther (June 16-19, Amsterdam) 1998, Abst 422.

68. Hannah, A., Malkin, M., Rosen, L., Lopez, A.M., Marshall, J., Lombardi, C.C., Eckhardt, S.G. *An overview of phase I/II studies of SU101, a tyrosine kinase inhibitor, in patients with recurrent malignant glioma and advanced solid tumors.* 10th NCI-EORTC Symp New Drugs Cancer Ther (June 16-19, Amsterdam) 1998, Abst 052.

69. Ko, Y.-J., Balk, S., Hannah, A., Keith, K., Huang, M.-Y., Bubley, G. *SU101, a platelet derived growth factor receptor antagonist in hormone refractory prostate carcinoma.* 10th NCI-EORTC Symp New Drugs Cancer Ther (June 16-19, Amsterdam) 1998, Abst 421.

70. Malkin, M.G., Rosen, L., Lopez, A.M., Mulay, M., Cloughesy, T., Hannah, A.L. *Phase 2 study of SU101, a PGDF-R signal transduction inhibitor, in recurrent malignant glioma.* Proc Amer Soc Clin Oncol 1998, 17: Abst 1504.

71. *SU-101 enters phase II testing in ovarian cancer patients.* Prous Science Daily Essentials 1998, July 16.

Additional References

Bartlett, R.R., Schleyerbach, R., Kämmerer, F.-J. (Hoechst AG). *Medicines against chronic graft-vs-host disease as well as against autoimmune diseases, especially systemic lupus erythematosus.* DE 3534440, EP 217206, JP 87072614, JP 93208908, US 4965276, US 5268382, US5459163.

Bartlett, R.R. (Hoechst AG). *Medicament for the treatment of rejection reactions during organ transplantations. EP 529500, JP 93208909.*

Robertson, S.M., Lang, L.S. (Alcon Labs., Inc.). *5-Methyl-isoxazole-4-carboxylic acid anilides and 2-hydroxyethylidene-cyano acetic acid anilides for the treatment of ocular diseases.* EP 413329.

Kurtz, E.S., Welthmann, K.U., Bartlett, R.R. (Hoechst AG). *Pharmaceutical for the treatment of skin disorders.* EP 617959, US 5504084,

Bartlett, R.R., Kämmerer, F.-J. (Hoechst AG). *Isoxazole-4-carboxamides and hydroxyalkylidene-cyanoacetamides, drugs containing these cpds. and use of such drugs.* WO 9117748.

Bartlett, R.R., Morris, R.E. (Hoechst AG). *Method of treating hyperproliferative vascular disease.* EP 665013.

Silva, H.T., Slauson, S.D., Shorthouse, R., Löffler, M., Morris, R.E. *Molecular mechanism of immunosuppression by the malononitrilamides (MNA) in vivo: Inhibition of dihydroorotate dehydrogenase (DHODH).* 17th World Cong Transplant Soc (July 12-17, Montreal) 1998, Abst 1657.

Mirmohammadsadegh, A., Homey, B., Abts, H.F., Kohrer, K., Ruzicka, T., Michel, G. *Differential modulation of pro- and antiinflammatory cytokine receptors by N-(4-trifluoromethyphenyl)-2-cyano-3-hydroxy-crotonic acid amide (A771726), the physiologically active metabolite of the novel immunomodulator leflunomide.* Biochem Pharmacol 1998, 55: 1523-9.

Fox, R.I. *Mechanism of action of leflunomide in rheumatoid arthritis.* J Rheumatol 1998, 25(Suppl. 53): 20-6.

Brazelton, T.R., Shorthouse, R., Huang, X., Morris, R.E. *Efficacy of immunosuppressive agents corresponds to their ability to maintain donor tissue phenotype in allografted tracheas.* J Heart Lung Transplant 1997, 16(1): Abst 125.

Elder, R.T., Xu, X.L., Williams, J.W., Gong, H.H., Finnegan, A., Chong, A.S.F. *The immunosuppressive metabolite of leflunomide, A771726, affects murine T cells through two biochemical mechanisms.* J Immunol 1997, 159: 22-7.

Curnock, A.P., Robson, P.A., Yea, C.M., Moss, D., Gadher, S., Thomson, T.A., Westwood, R., Ruuth, E., Williamson, R.A. *Potencies of leflunomide and HR325 as inhibitors of prostaglandin endoperoxide H synthase-1 and -2: Comparison with nonsteroidal anti-inflammatory drugs.* J Pharmacol Exp Ther 1997, 282: 339-47.

Östraat, Ö., Qi, Z.-Q., Tufveson, G., Hedlund, G., Ekberg, H. *The effects of leflunomide and cyclosporin A on rejection of cardiac allografts in the rat.* Scand J Immunol 1998, 47: 236-42.

Hancock, W.W., Miyatake, T., Koyamada, N., Kut, J.P., Soares, M., Russell, M.E., Bach, F.H., Sayegh, M.H. *Effects of leflunomide and deoxyspergualin in the guinea pig-rat cardiac model of delayed xenograft rejection.* Transplantation 1997, 64: 696-704.

Yeh, L.-S., Gregory, C.R., Griffey, S.M., Lecouter, R.A., Hou, S.-M., Morris, R.E. *Combination leflunomide and cyclosporine prevents rejection of functional whole limb allografts in the rat.* Transplantation 1997, 64: 919-22.

Silva, H.T., Slauson, S., Sherwood, S., Bryce, T., Wade, A., Wang, X., Cheung, A., Wang, Y., Shourthouse, R., Billingham, M., Morris, R.E. *Monitoring of pharmacodynamics (PD), but not pharmacokinetics (PK), differentiates the relative immunosuppressive potencies of leflunomide and its malononitrilamide (MNA) analogs.* Transplantation 1998, 65(8, Suppl.): Abst 160.

Waldman, W.J., Knight, D.A., Lurain, N., Miller, D.M., Sedmak, D.D., Williams, J.W., Chong, A.S.-F. *Novel mechanism of cytomegalovirus inhibition by leflunomide: Equivalent activity against ganciclovir/foscarnet-sensitive and resistant isolates.* Transplantation 1998, 65(8, Suppl.): Abst 444.

Hausen, B., Boeke, K., Berry, G.J., Christians, U., Gummert, J., Benet, L.Z., Morris, R.E. *Coadministration of microemulsion cyclosporine and the novel leflunomide malononitrilamide analog, HR1279, more effectively suppresses rat lung allograft rejection than microemulsion cyclosporine plus leflunomide.* Transplantation 1998, 65(8, Suppl.): Abst 522.

Slauson, S., Silva, H., Sherwood, S., Morris, R.E. *Flow cytometric (FCM) analysis of the molecular mechanisms of immunosuppressive action of the active metabolite of leflunomide and its malononitrilamide (MNA) analogs in a novel whole blood assay.* Transplantation 1998, 65(8, Suppl.): Abst 526.

Guo, Z., Chong, A.S.F., Tian, Y. et al. *Effect of leflunomide and cyclosporine on concordant xenogeneic islet transplantation in streptozotocin-induced and autoimmune diabetic mice.* Transplant Proc 1997, 29: 2155.

Wennberg, L., Groth, C.G., Tibell, A. et al. *Triple drug treatment with cyclosporine, leflunomide and mycophenolate mofetil prevents rejection of pig islets transplanted into rats and primates.* Transplant Proc 1997, 29: 2498.

Guo, Z., Mital, D., Shen, J., Chong, A.S.F., Tian, Y., Foster, P., Sankary, H., McChesney, L., Jensik, S.C., Williams, J.W. *Differential effect of leflunomide on concordant xenogeneic islet graft rejection and recurrence of autoimmune diabetes.* Transplant Proc 1998, 30: 463-4.

Uhlig, T., Eber, E., McMenamin, C., Cooper, D., Wildhaber, J.H., Sly, P.D. *Long term treatment with leflunomide inhibits allergic sensitisation, airway inflammation and lymphocyte proliferation in a model of asthma.* Annu Cong Eur Respir Soc (Sept 20-24, Berlin) 1997, Abst P2828.

Eber, E., Uhlig, T., McMenamin, C., Sly, P.D. *Leflunomide prevents sensitisation of mast cells in vivo, but topical administration does not protect against allergen-induced early airway response.* Annu Cong Eur Respir Soc (Sept 20-24, Berlin) 1997, Abst P3022.

Xu, X., Blinder, L., Gong, H., Shen, J., Finnegan, A., Williams, J.W., Chong, A.S.-F. *In vivo mechanism by which leflunomide controls lymphoproliferative and autoimmune disease in MRL/mpJ-lpr/lpr mice.* 16th Annu Meet Sci Sess Bus Meet Amer Soc Transplant Physicians (May 10-14, Chicago) 1997, Abst 405.

Chong, A.S.-F., Yin, D.-P., Blinder, L., Ma, L., Shen, J., Williams, J.W. *Modification of humoral responses in Lewis rats to hamster heart xenografts by the combination of leflunomide and cyclosporine (CsA).* 16th Annu Meet Sci Sess Bus Meet Amer Soc Transplant Physicians (May 10-14, Chicago) 1997, Abst 606.

Dimitrijevic, M., Milenkovic, M., Misolavljevic, P., Colic, M. *Effects of leflunomide on experimental autoimmune myocarditis with special reference to the dendritic cells.* 3rd Int Conf New Trends Clin Exp Immunosuppr (Feb 12-15, Geneva) 1998, 45.