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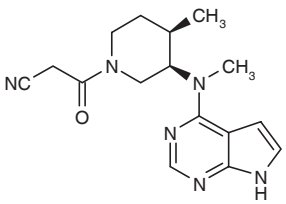
CP-690550

JAK3 Inhibitor
Immunosuppressant
Treatment of Rheumatoid Arthritis
Treatment of Transplant Rejection

(3*R*,4*R*)-3-[4-Methyl-3-[*N*-methyl-*N*-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino]piperidin-1-yl]-3-oxopropionitrile

InChI=1/C16H20N6O/c1-11-5-8-22(14(23)3-6-17)9-13(11)21(2)16-12-4-7-18-15(12)19-10-20-16/h4,7,10-11,13



$C_{16}H_{20}N_6O$
Mol wt: 312.3698

CAS: 477600-75-2

EN: 306518

Abstract

Standard immunosuppressive agents used in the clinic for the prevention of organ transplant rejection and autoimmune diseases, although generally effective, target ubiquitously expressed molecules and are associated with significant adverse events. Identification of potential targets selectively expressed by immune cells has become a research priority in order to develop immunosuppressants devoid of the toxicities observed with current therapies. In this regard, targeting of Janus kinase 3 (JAK3) represents a potentially effective immunosuppressive strategy since expression of this signaling molecule is relatively restricted to immune cells and it is only used by cytokine receptors containing the γ-chain (γc). CP-690550 is a novel JAK3 inhibitor that has exhibited potent immunosuppressive effects in preclinical transplantation and arthritis models and has been shown to be clinically safe and effective in preventing transplant rejection and improving symptoms of rheumatoid arthritis and psoriasis. CP-690550 continues to undergo phase II development as an immunosuppressive agent.

Synthesis

Racemic CP-690550 can be obtained by the following method. Reductive amination of 1-benzyl-4-methylpiperidin-3-one (I) with methylamine and sodium triacetoxyborohydride in THF gives *N*-methyl-1-benzyl-4-methylpiperidin-3-amine (II), which is condensed with 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (III) by means of triethylamine in a sealed tube at 100 °C to yield the tertiary amine (IV). After deprotection of (IV) by catalytic hydrogenolysis over $Pd(OH)_2/C$, the debenzylated piperidine (V) is acylated with cyanoacetyl chloride (VI) and pyridine in CH_2Cl_2 to provide the target compound (1). Scheme 1.

An enantioselective synthesis of the (3*R*,4*R*)-isomer has also been reported. Quaternization of 3-(methoxycarbonylamino)-4-methylpyridine (VII) with benzyl chloride in toluene at 80 °C, followed by reduction of the resulting pyridinium salt (VIII) with $NaBH_4$ in ethanol, yields the tetrahydropyridine (IX). Enantioselective hydrogenation of (IX) in the presence of chiral rhodium catalyst in ethanol, and subsequent purification of the obtained piperidine by crystallization as the corresponding di-*p*-toluoyltartrate salt, provides the pure (3*R*,4*R*)-enantiomer (X). This is then condensed with 2,4-dichloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (XI) by means of K_2CO_3 in boiling water to give the tertiary amine (XII), which is subjected to simultaneous debenzylation and dechlorination by means of H_2 and $Pd(OH)_2/C$ to yield the deprotected piperidine (XIII) (2). This compound is finally condensed with cyanoacetyl chloride (VI) (prepared from cyanoacetic acid [XIV] and oxalyl chloride) by means of triethylamine in dichloromethane to obtain the chiral CP-690550 (2, 3). Scheme 2.

Background

Numerous therapeutic options are available to achieve immunosuppression in organ transplantation and autoimmune diseases such as rheumatoid arthritis, psoriasis, psoriatic arthritis, multiple sclerosis (MS), inflammatory bowel disease (IBD) and systemic lupus erythematosus (SLE). Some standard immunosuppressive agents used in the clinic include steroids, ciclosporin, tacrolimus, mycophenolate motefil and sirolimus. Although generally effective, these agents target ubiquitously expressed molecules and are thus associated with significant adverse

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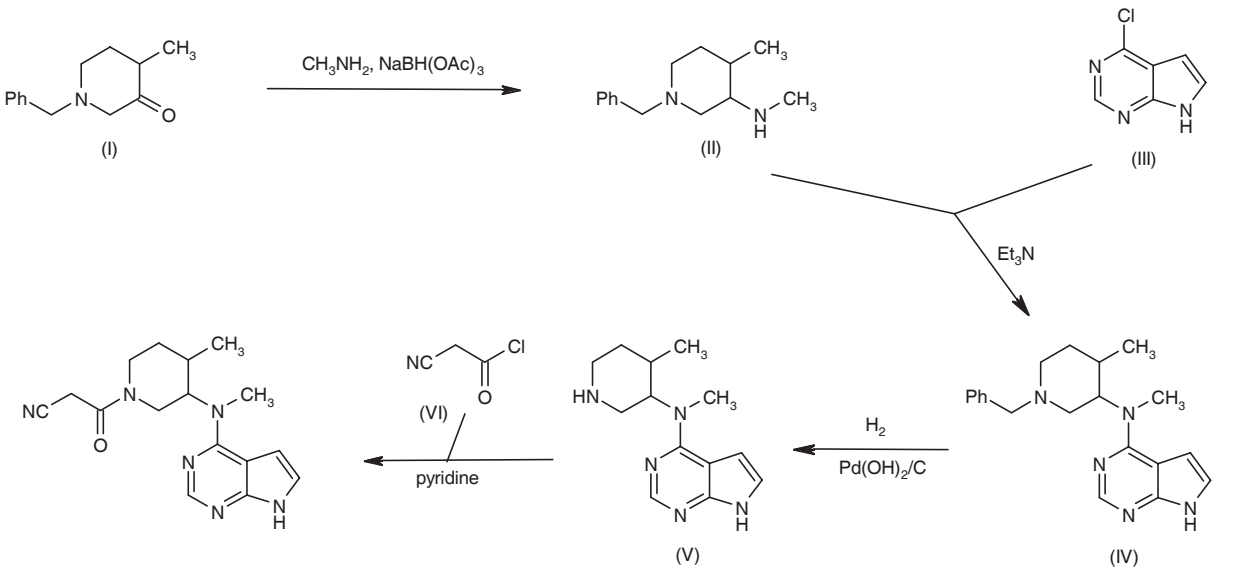
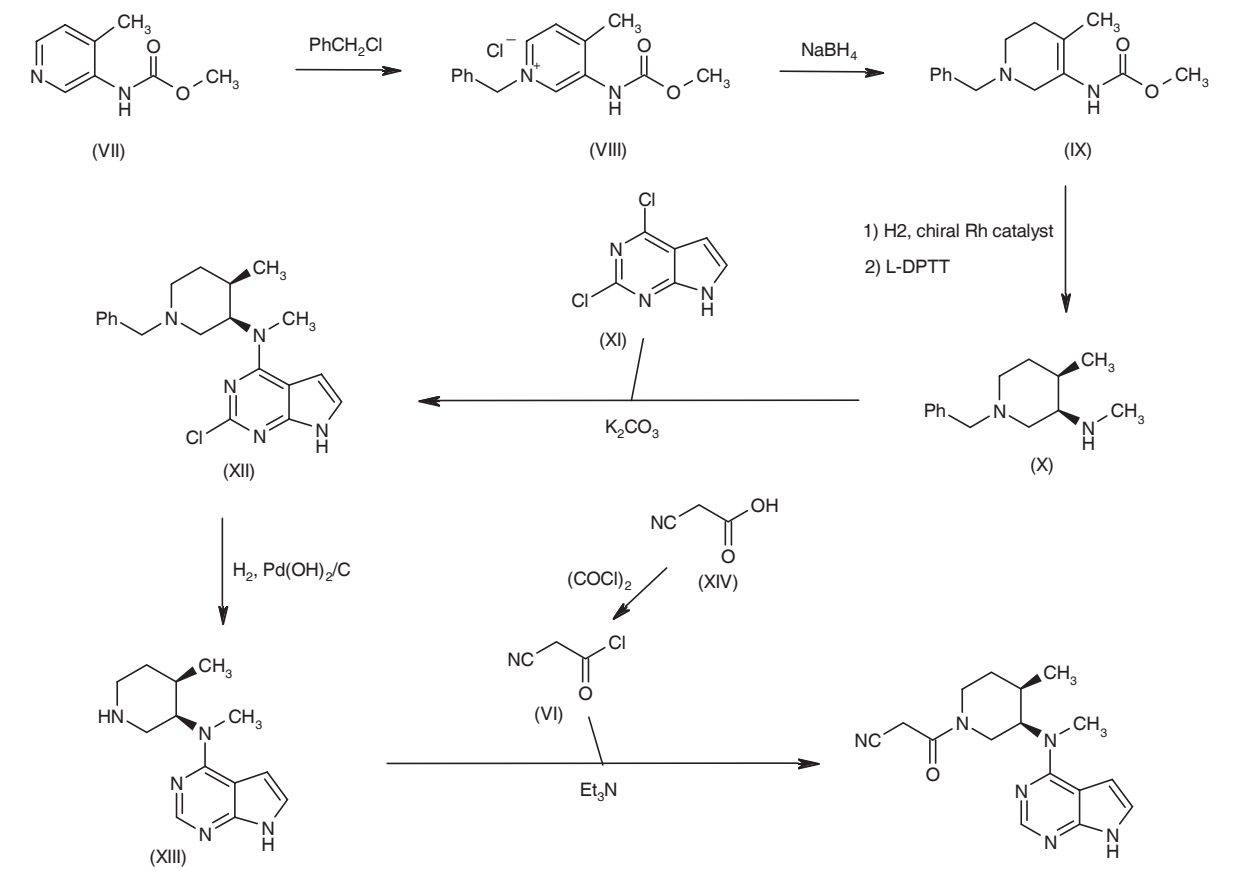
Scheme 1: Synthesis of Racemic CP-690550



Scheme 2: Synthesis of CP-690550



events, such as nephrotoxicity, neurotoxicity, new-onset post-transplant diabetes, hyperlipidemia and hypertension. In theory, molecular targeting should therefore be restricted to components expressed solely by immune cells, thus ensuring immunosuppressive effects without the toxicities observed with current therapies. Researchers have focused their efforts on identifying potential targets selectively expressed by immune cells in an attempt to develop novel agents to achieve immunosuppression without associated toxicity (4-6).

Several cytokines are critical for immune cell development and homeostasis. In particular, interleukins IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, which bind to receptors sharing the common cytokine receptor γ-chain (γc), have emerged as crucial entities regulating immune function. Severe combined immunodeficiency (SCID) is a condition characterized by developmental and functional immune cell deficiencies resulting in a T^-B^+natural killer $(NK)^-$ phenotype, and many, but not all, patients exhibit mutations in the gene encoding this common cytokine γc. Following cytokine binding to cell-surface receptors, signal transduction to the nuclei is achieved via activation of the large cytoplasmic tyrosine kinases, the Janus kinases (JAKs; Fig. 1). Activated JAKs consequently phosphorylate the cytokine receptor, creating a site for docking of STATs (signal transducers and activators of transcription). STATs in turn are phosphorylated by JAKs, and dimerization occurs followed by transport to the nucleus, DNA binding and, ultimately, transcriptional modulation. The JAK family includes JAK1, JAK2, JAK3 and TYK2. In contrast to JAK1, JAK2 and TYK2, which are used by a variety of cytokine receptors and are ubiquitously expressed,

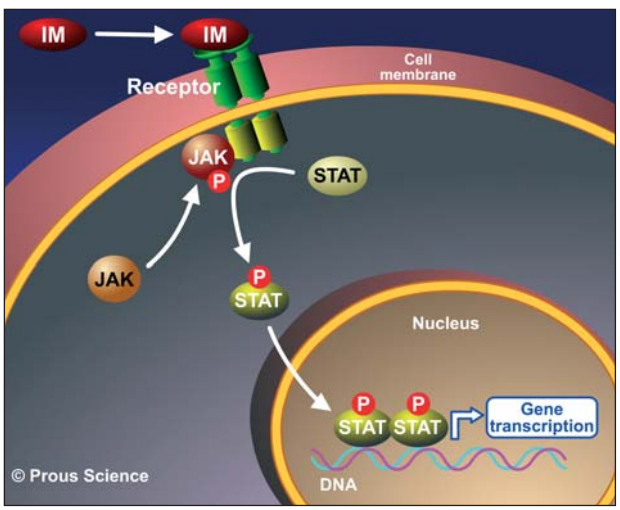


Fig.1. Following cytokine (*i.e.*, immunomodulator; IM) binding and receptor dimerization, Janus kinase (JAK) is recruited to the receptor dimer and becomes phosphorylated. Activated JAK serves as the docking site for the recruitment of the monomeric latent cytosolic STATs (signal transducers and activators of transcription), which are phosphorylated, dissociate from the receptor and dimerize. The STAT dimer translocates into the nucleus, where it binds to DNA and directly induces the expression of genes.

JAK3 expression is relatively restricted and is only used by cytokine receptors containing γc. High levels of JAK3 are found in NK cells and thymocytes and it is inducible in T-cells, B-cells and myeloid cells; it is not expressed in resting T-cells. Interestingly, studies have shown that patients carrying mutations in the gene encoding JAK3 present the $T^-B^+NK^-$ SCID phenotype. Thus, JAK3 was recognized as a potentially effective and selective target for the development of novel immunosuppressive agents (4-12).

Screening of thousands of primary compounds for *in vitro* inhibition of JAK3 led to the identification of a lead compound (CP-352664). This compound was extensively modified to improve kinase-inhibitory activity, yielding the novel and selective immunosuppressive agent CP-690550. The agent potently and selectively inhibited JAK3 and blocked cytokine signaling and cytokine-induced gene expression. The lack of marked activity against other JAKs suggests that CP-690550 may be devoid of the adverse events typically associated with immunosuppression. CP-690550 was chosen for further development as an immunosuppressive agent to prevent organ transplant rejection and treat various autoimmune diseases (13).

Preclinical Pharmacology

Results from *in vitro* assays using enzymes purified from insect cells or *Escherichia coli* showed that CP-690550 potently inhibited JAK3 (IC_{50} = 1 nM). The agent was 20- and 100-fold less active in inhibiting JAK2 and JAK1, respectively, and was even less active against 30 other kinases (IC_{50} > 3000 nM for Lck and rho-associated protein kinase 2 [ROCK2]; IC_{50} > 10,000 nM for cyclin-dependent kinase 5 [CDK5], epidermal growth factor receptor [EGFR], mitogen-activated protein kinase 2/extracellular signal-regulated kinase 2 [MAPK2/ERK2], 5'-AMP-activated protein kinase [AMPK], phosphatidylinositol 3-kinase [PI3K], among others) (13).

CP-690550 potently inhibited IL-2-induced proliferation of human T-cells, which occurs via JAK3 signaling. The agent was 30-fold more effective in this assay as compared to its ability to inhibit JAK2-mediated granulocyte-macrophage colony-stimulating (GM-CSF)-induced proliferation of the human myelomonocytic cell line HUO3 (IC_{50} = 11 nM vs. 324 nM). CP-690550 displayed little activity against T-cell signaling and serum-induced proliferation. However, in contrast, potent inhibitory activity was observed in the mixed lymphocyte reaction (MLR) using murine (IC_{50} = 91 ± 66 nM), monkey or human cells, and experiments using an IL-2-stimulated NK-like cell line (YT cells) revealed that CP-690550 inhibited IL-2-induced phosphorylation of JAK3 and STAT5, one of the major substrates for JAK3 (13, 14).

A preclinical study using C57BL/6 mice reported time- and dose-dependent reductions in lymphocyte subsets following chronic treatment with CP-680550 (1.5-15 mg/kg/day via osmotic pump), which is indicative of elimination of γc signaling. After 21 days of CP-690550 dosing,

mice exhibited a 96% decrease in splenic NK1.1$^+$TCRβ^- cells. In addition, the immunosuppressive efficacy of the agent was demonstrated in the murine delayed-type hypersensitivity model (*i.e.*, sheep red blood cell-induced footpad swelling). In this model, mice administered the agent s.c. once daily during sensitization and challenge had a maximum inhibition of swelling of approximately 80% at a dose of 30 mg/kg (14).

Chronic CP-690550 dosing in cynomolgus monkeys (1, 10 or 30 mg/kg/day p.o. for 3 weeks or 14 days) resulted in dose- and time-dependent and selective decreases in circulating NK (about 80%) and CD8$^+$ T-cells (43%). Further experiments analyzing whole blood from cynomolgus monkeys revealed that CP-690550 treatment inhibited IL-15-induced CD69 expression in NK cells (IC_{50} = 48.0 ± 8.4 nM) and CD8$^+$ T-cells (IC_{50} = 16.2 ± 1.5 nM) (15).

The immunosuppressive effects of CP-690550 were demonstrated in several *in vivo* transplantation models. Treatment of C57/BL6 mice bearing DBA2 mouse heart transplants with the agent (81 ± 71 and 136 ± 45 ng/ml via osmotic pump for 28 days) extended the median survival time (MST) of transplants from 12 days in all control animals to more than 60 days (EC_{50} to maintain graft for more than 28 days about 60 ng/ml). Examination of RNA prepared from peripheral blood of transplant recipients and transplanted hearts revealed that treatment with CP-690550 most markedly inhibited the expression of granzyme B, Fas ligand (FasL), RANTES (regulated on activation, normal T expressed and secreted), MIG (monokine induced by interferon gamma) and IP-10. The genes encoding these chemokines are induced by interferon gamma (IFN-γ), which is the product of an IL-2-induced gene (13).

CP-690550 monotherapy (10, 15 and 30 mg/kg/day via osmotic minipumps for 28 days) dose-dependently prolonged cardiac allograft (neonatal BALB/c donors implanted into the ear of C3H/HEN mice) from an MST of 11 days in controls to 13, 17 and 18 days, respectively; similar effects were observed with i.p. ciclosporin. Lower doses of CP-690550 (0.5, 1.5 and 5 mg/kg/day) resulted in little or only modest effects on graft survival. However, when these low doses were combined with a suboptimal dose of ciclosporin (10 mg/kg/day), the efficacy in prolonging MST of grafts was enhanced (14).

The immunosuppressive efficacy of CP-690550 was demonstrated in a heterotopic cynomolgus monkey model of renal transplantation. Treatment with the agent (p.o. b.i.d. at doses to achieve 12-h trough blood levels of 1-147 ng/ml) significantly prolonged kidney allograft survival from 7 ± 1 days in controls to 53 ± 7 days. Histological examination of renal grafts 90 days after transplantation revealed that treatment also significantly delayed graft rejection (46 ± 7 days from transplantation *vs.* 7 ± 1 days in controls); NK cells and CD4$^+$ and CD8$^+$ T-cells were also significantly decreased in treated animals. Toxicities developed in animals with high exposure to the agent and included persistent anemia and polyoma virus-like nephritis and increases in urinary calcium (16, 17).

Further experiments using a nonhuman primate (cynomolgus monkey) model of kidney transplantation revealed that administration of the agent (p.o. b.i.d. starting at the time of transplantation and continuing for up to 90 days) at doses to achieve low (50-100 ng/ml) and high (200-400 ng/ml) 12-h trough levels significantly prolonged graft survival as compared to controls (MST = 62 ± 6 and 83 ± 6 days, respectively, *vs.* 6 ± 1 days). CP-690500 also significantly enhanced the prolonging effects on graft survival of mycophenolate mofetil (MMF) in this same model (MST = 23 ± 1 days with MMF alone *vs.* 59.5 ± 9.8 days with combination treatment) (13, 18).

CP-690500 (via osmotic pump for up to 56 days; mean drug exposure = 110 ± 38 ng/ml) was shown to prevent allograft vasculopathy in a study using a rat model of aorta transplantation (AxC Irish rat aortas heterotopically transplanted into Lewis rats). A significant reduction in intimal hyperplasia (51%), luminal obliteration (2.61 ± 0.54%), donor IgG production and SOCS-3 (suppressor of cytokine signaling-3) gene expression were observed in treated animals at day 56; treatment had no effect on RANTES, IP-10 and TGF-β1 (transforming growth factor-β1) expression (19).

CP-690550 treatment for up to 28 days significantly prevented obliterative bronchiolitis in a rat tracheal transplant model. Treatment significantly reduced airways obliteration (2.5% *vs.* 90%) and loss of epithelial coverage (0% *vs.* 100%) at day 28 and inhibited lymphocyte and macrophage infiltration into grafts. The effects observed with CP-690550 were similar to those of sirolimus. In addition, treatment was associated with induction of TGF-β3 gene expression on days 7, 14 and 28 post-transplantation; TGF-β1, PDGFRα (platelet-derived growth factor α) and PDGFRβ expression were unaffected by treatment (20, 21).

The preclinical immunosuppressive effects of CP-690550 were also demonstrated in murine collagen-induced and rat adjuvant-induced arthritis models. Treatment with the agent (up to 15 mg/kg/day for 14 and 28 days in mice and rats, respectively) resulted in dose-dependent reductions in rheumatoid arthritis in both models (ED_{50} about 1.5 mg/kg/day). Significant improvements were observed in clinical scores, inflammation and joint damage (22).

Pharmacokinetics and Metabolism

A novel dual-pump liquid chromatography/liquid chromatography/mass spectrometry (LC/LC/MS) method was described to quantitatively analyze CP-690550 levels in whole blood from transplant recipients. Linearity was observed over the range 2.5 to 750 ng/ml, with a lower limit of quantification of 2.5 mg/ml. The intra-assay and interday accuracy rates ranged from 103% to 105.4% and 98.1% to 103.8%, respectively, and the intra-assay and interday precision ranged from 2.7% to 4.3% and 8.7% to 11.1%, respectively. The assay was validated using cynomolgus monkey blood (23).

The multiple-dose pharmacokinetics of CP-690550 (5, 15 and 30 mg b.i.d.) were examined in a 29-day, randomized, double-blind, placebo-controlled, dose-escalating trial conducted in 28 stable renal allograft recipients. Only patients receiving the two lower doses were on concomitant stable calcineurin inhibitors and MMF, and steroid doses were unchanged throughout the treatment period. The AUC_{τ} values for the three doses were 273, 1090 and 1420 ng.h/ml, respectively. Moderate increases in CP-690550 exposure were detected when the agent was given concomitantly with calcineurin inhibitors, suggesting a possible interaction between the two therapies. CP-690550 did not appear to significantly interact with MMF since the slight increases observed in mycophenolic acid levels were not significantly different from placebo. Safety and tolerability data from this trial are discussed below (24).

Safety

A total of 27 patients completed the above-mentioned study and were followed for an additional 28 days after the treatment period. The majority of adverse events reported were mild to moderate in severity and were manageable with appropriate treatment. The most common adverse events were infections (12/22 *vs.* 3/6 on placebo) and gastrointestinal disorders (7/22 *vs.* 2/6 on placebo). No significant changes in blood pressure, heart rate, Q-T_c interval, serum glucose, hepatic or renal function were associated with treatment as compared to placebo. A reversible 10-13% reduction from baseline in nadir mean hemoglobin was observed at 14 days after the last CP-6690550 dose in the 15- and 30-mg cohorts. No changes in neutrophil, platelet or monocyte counts were detected with treatment. Treatment tended to increase blood $CD19^+$ cell counts and decrease $CD56^+$ cell counts, with no effects on $CD3^+$, $CD4^+$ or $CD8^+$ cell counts (25).

Clinical Studies

The safety and efficacy of CP-690550 (15 or 30 mg b.i.d. for 6 months) were examined and compared to tacrolimus in a multicenter, randomized, parallel-group, open-label phase IIa trial conducted in 61 *de novo* kidney transplant recipients who also received an IL-2 receptor antagonist for induction, MMF (2 g/day) and steroids; no patient received concomitant calcineurin inhibitors. MMF was discontinued and steroids were reduced in the higher dose CP-690550 cohort due to a high incidence of polyoma virus-associated nephropathy indicative of excess immunosuppression (4 patients *vs.* 0 in the other groups). No deaths, graft loss or malignancies were observed 6 months post-transplant. Discontinuations included 2, 3 and 7 patients from the tacrolimus and CP-690550 15- and 30-mg groups, respectively. The most common adverse events seen in the tacrolimus and CP-690550 15- and 30-mg cohorts were gastrointestinal disorders in 12, 11 and 13 patients, respectively, and general disorders in 11, 11 and 10 patients, respectively. When compared to tacrolimus and 15 mg CP-690550, significantly more patients in the 30-mg CP-690550 dose group developed infections (12 *vs.* 5 and 8 patients, respectively) and cytomegalovirus (CMV) disease (4 *vs.* 0 and 2 patients, respectively). No significant differences in white blood cell counts and hemoglobin were noted between treatment groups at 6 months. However, a 15-27% increase in serum lipids, including total, LDL cholesterol, HDL cholesterol and triglycerides, was observed in the CP-690550 dose groups as compared to the tacrolimus group. Biopsy-proven acute rejection by 6 months post-transplant was seen in 1, 1 and 4 patients, respectively, on tacrolimus and 15 and 30 mg CP-690550. The least squares mean glomerular filtration rate (GFR) at month 6 was 77.4 ± 12.6, 76.9 ± 10.0 and 72.8 ± 14.7 ml/min, respectively (26).

The safety, tolerability and efficacy of CP-690550 (5, 15 or 30 mg b.i.d. for 6 weeks) were examined in another randomized, double-blind, placebo-controlled phase IIa trial conducted in 264 subjects with moderate to severe active rheumatoid arthritis (at least 9 painful or tender joints, 6 swollen joints and evidence of systemic inflammation) who were intolerant of or unresponsive to methotrexate or tumor necrosis factor (TNF) inhibitors and had discontinued disease-modifying antirheumatic drugs (DMARDs) or biological therapies; stable background nonsteroidal antiinflammatory drugs (NSAIDs), coxibs, low-dose glucocorticoids and analgesics were allowed. The most common adverse events were headache and nausea, and the incidence appeared to increase in a dose-dependent manner. Decreases in neutrophil counts to 1000 cells/mm^3 or less developed in 1 and 2 subjects in the 5- and 30-mg cohorts, respectively. Infection rates were 30.4% on 15 and 30 mg of CP-690550 and 26.2% on placebo. Patients receiving CP-690550 also exhibited dose-dependent increases in LDL and HDL cholesterol and a reversible increase in mean serum creatinine (0.04-0.06 mg/dl). All doses of CP-690550 were effective in improving arthritis symptoms, functional disability and health status starting at week 1, as measured using the American College of Rheumatology (ACR) response criteria, patient's assessment of pain, the Health Assessment Questionnaire-Disability Index (HAQ-DI) and the SF-36v2™ Health Survey. ACR20, ACR50 and ACR70 responses at week 6 were achieved in 70-81%, 33-54% and 13-28%, respectively, of the patients receiving CP-690550 as compared to only 29%, 6% and 3%, respectively, of those on placebo. Moreover, significant improvements from baseline were noted in pain as compared to placebo for the 15- and 30-mg groups at all weeks and in the 5-mg group at weeks 4 and 6. An improved functional status was associated with treatment at all weeks, as reflected by significant dose-related decreases in HAQ-DI scores. Clinically significant reductions in HAQ-DI (0.3 units or more) at week 6 were seen in 57%, 75% and 76% of the patients in the respective CP-690550 dose groups as compared to 36% on placebo. SF-36v2™ scores significantly improved for all dose groups starting at 1 week and

continuing over the 6-week treatment period. Moreover, significant differences were also detected for the 15- and 30-mg CP-690550 dose groups compared to placebo in remission rates at week 6, as measured using the Disease Activity Score (DAS; 30.4% and 25%, respectively, *vs.* 2.1%), Simplified Disease Activity Index (SDAI; 7.4% and 7.6%, respectively, *vs.* 0%) and Clinical Disease Activity Index (CDAI; 12.1% and 12.5%, respectively, *vs.* 0%) (27-29).

A multiple-dose, randomized, double-blind, placebo-controlled phase I trial examined the safety, tolerability and efficacy of CP-690550 (5, 10, 20, 30 or 50 mg b.i.d. or 60 mg once daily for 14 days) as a treatment for psoriatic lesions. A total of 59 otherwise healthy patients with psoriatic lesions were enrolled, 58 of whom completed the study; the study included a 28-day follow-up period for safety assessment. CP-690550 was well tolerated. The most common adverse events were mild headache and nausea. A slight decrease in neutrophil counts occurred in the 50-mg b.i.d. group, although this effect was reversed during follow-up. Marked alterations in lymphocyte subsets were observed in the 50-mg b.i.d dose group, which included changes in $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+CD56^+$ and $CD19^+$ cells; these effects were not seen in the 5-mg group and were variable in the other cohorts. With the exception of the lowest dose group, all doses of the agent resulted in significant improvement in a modified Psoriasis Area and Severity Index (mPSAI) at day 14 as compared to placebo; reductions in these scores were dose-related. Physician Global Assessment (PGA) of overall disease status significantly improved on day 14 in the 20-, 30- and 50-mg b.i.d. dose groups as compared to placebo. A complete reversal of keratin 16 expression, indicating reversal of hyperplasia and other disease pathologies, was observed in 3 of 4 skin biopsies from the 30-mg b.i.d. group, but not in the 5- or 10-mg CP-690550 dose groups or on placebo (30).

CP-690550 continues to undergo phase II development for the prevention of acute rejection in kidney transplant recipients and for the treatment of rheumatoid arthritis (31-35).

Source

Pfizer, Inc. (US).

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