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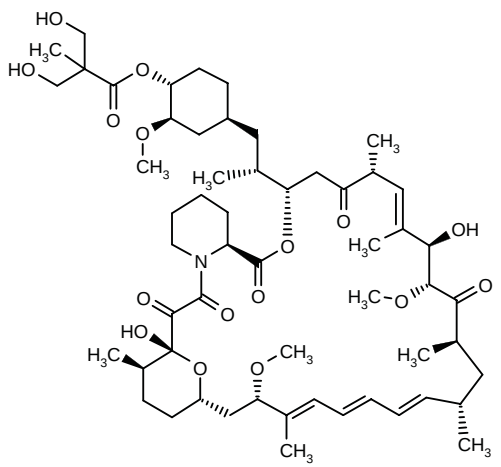
CCC: 0377-8282/2002

CCI-779

Oncolytic
mTOR Inhibitor

NSC-683864

[1*R*,9*S*,12*S*[1'*R*(1''*R*,3''*R*,4''*R*)],15*R*,18*R*,19*R*,21*R*,23*S*,30*S*,32*S*,35*R*]-1,18-Dihydroxy-12-[2-[4-[3-hydroxy-2-(hydroxymethyl)-2-methylpropionyloxy]-3-methoxycyclohexyl]-1-methylethyl]-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.0(4,9)]hexatriaconta-16(*E*),24(*E*),26(*E*),28(*E*)-tetraene-2,3,10,14,20-pentaone



$C_{56}H_{87}NO1_6$ Mol wt: 1030.3100

CAS: 162635-04-3
CAS: 343261-52-9

EN: 218793

Synthesis

CCI-779 can be prepared by two related ways:

a) 2,2-Bis(hydroxymethyl)propionic acid isopropylidene ketal (I) is activated by treatment with 2,4,6-trichlorobenzoyl chloride (II) and triethylamine in anhydrous THF at 0 °C. The resulting activated acid is then coupled with rapamycin (III) by means of DMAP in benzene to provide the protected rapamycin derivative (IV). Finally, CCI-779 is obtained by hydrolysis of the ketal group with HCl in THF (1). Scheme 1.

b) The reaction of rapamycin (III) with chlorotrimethylsilane and imidazole in AcOEt gives 31,42-bis-*O*-(trimethylsilyl)rapamycin, which, without isolation, is partially deprotected with sulfuric acid to provide 31-*O*-(trimethylsilyl)rapamycin (V). Coupling ether (V) with either 2,2,5-trimethyl-1,3-dioxane-5-carboxylic acid chloride (VI) by means of DMAP in CH_2Cl_2/DMF or with carboxylic acid (I) pretreated with 2,4,6-trichlorobenzoyl chloride (II) and DIEA by means of DMAP in CH_2Cl_2 yields the protected rapamycin 42-ester (VII). Removing the trimethylsilyl group of (VII) by treatment with 0.5N sulfuric acid in acetone affords ester (VIII), which is finally converted into CCI-779 by hydrolysis of the ketal group by means of 2N sulfuric acid in THF (2). Scheme 2.

Introduction

Cancer cells exhibit a high frequency of mutations which cause complex alterations in cell cycle regulation and growth signal transduction and result in enhancement of proliferation. Thus, the highly regulated processes involved in the transduction of extracellular and autocrine proliferative stimuli are potential targets for development of therapies against cancer. In this regard, several novel anticancer agents have been designed which alter specific elements of aberrant signal transduction and cell cycle regulation. These include inhibitors of receptor tyrosine kinases, oncogenes, proteins involved in signal transduction (*e.g.*, Ras, Raf) and cyclin-dependent kinases (3, 4).

Rapamycin (sirolimus; Rapamune®), a macrolide fungicide isolated from *Streptomyces hygroscopicus*, is one such agent that displays potent antimicrobial and immunosuppressant effects as well as antitumor properties (5-7). Rapamycin's potent antiproliferative actions are due to its ability to modulate key signal transduction pathways that link mitogenic stimuli to the synthesis of proteins necessary for the cell cycle to progress from the G_1 to S phase (8). Specifically, rapamycin binds intracellularly to the immunophilin family of FK506 binding

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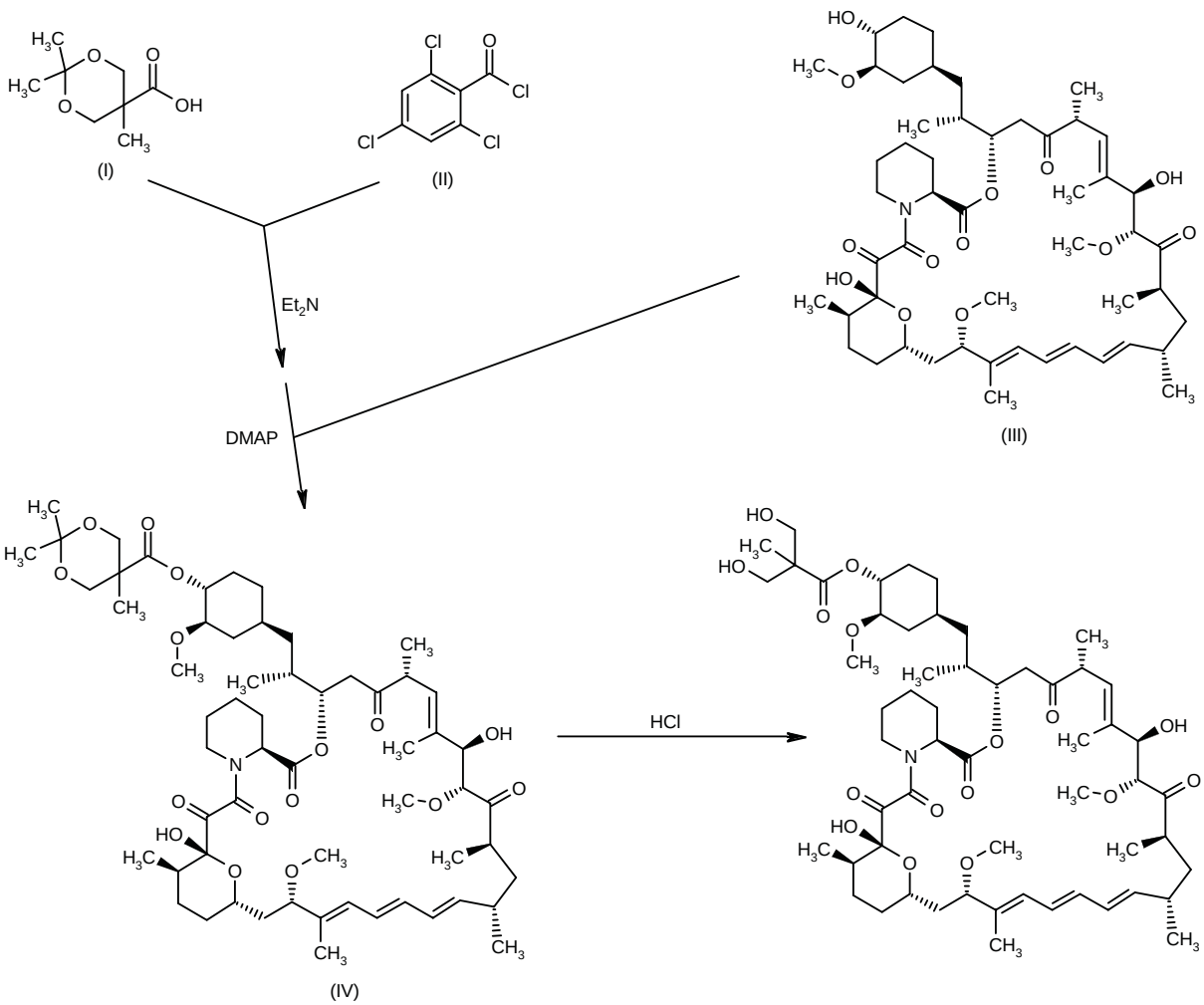
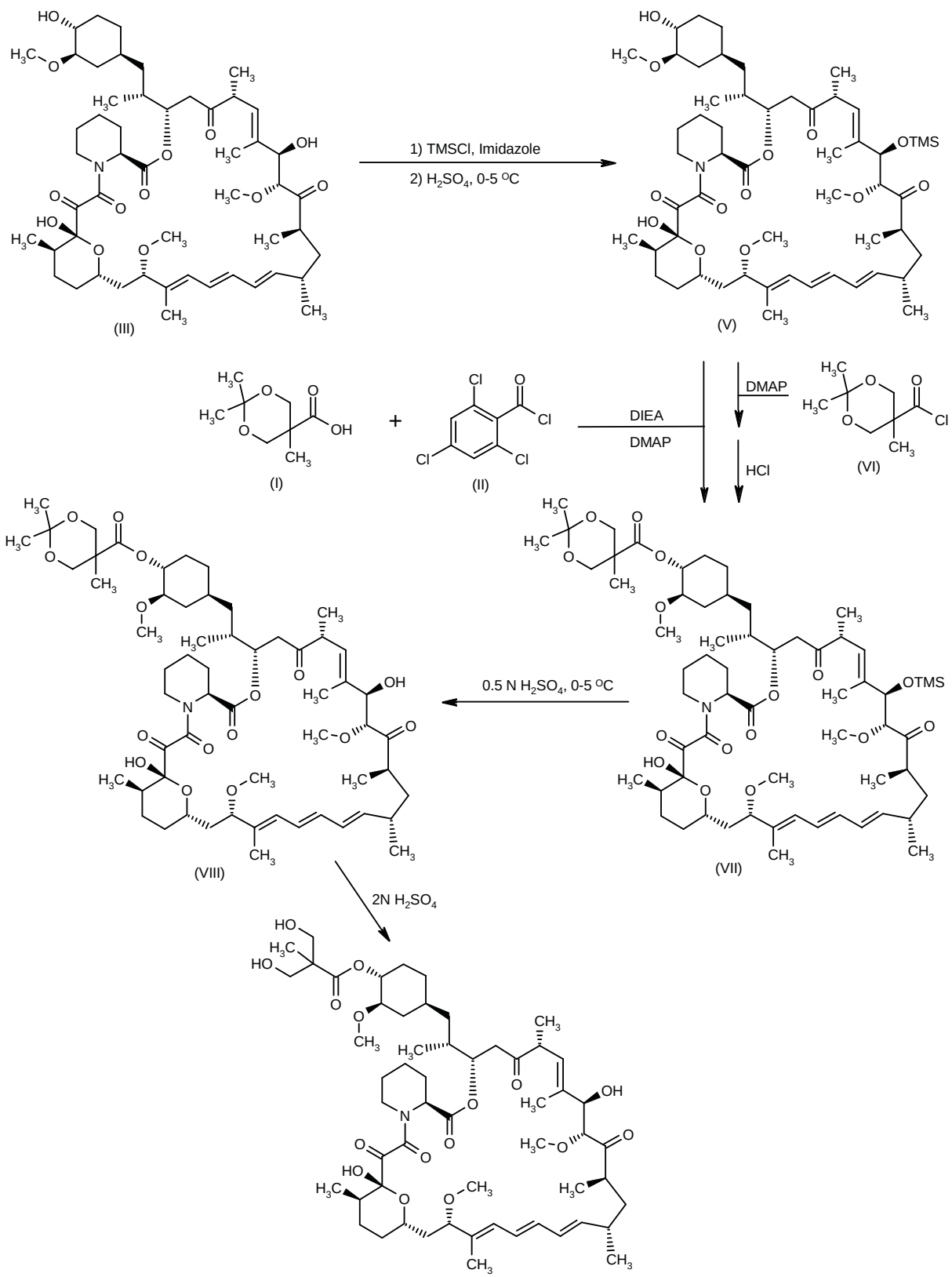
Scheme 1: Synthesis of CCI-779



proteins (FKBPs) inhibiting their prolyl isomerase activity; evidence suggests that FKBP12 is the most sensitive target for rapamycin. In turn, the rapamycin-FKBP12 complex inhibits the activity of a recently identified 290-kDa member of the family of phosphoinositide 3-kinase related kinases (PI3Ks) known as mammalian target of rapamycin (mTOR; also known as FRAP, RAFT1 and RAP1) (8-10). PI3Ks regulate cell cycle progression, cell cycle checkpoints that mediate cellular responses to DNA damage, DNA repair and DNA recombination (11). Activation of mTOR results in transduction of signals initiating synthesis of ribosomal proteins, translation of specific mRNA transcript subsets and generation of cyclin-dependent kinases promoting the progression of the cell cycle. The end result is T cell proliferation and activation, B cell proliferation, activation and antibody production in addition to proliferation of nonimmune cells such as fibroblasts, endothelial cells, hepatocytes and smooth muscle cells (8-12).

There are 2 known main functions of mTOR following its phosphorylation-induced activation which include the modulation of 2 distinct downstream pathways that control translation of specific subsets of mRNA: eukaryotic initiation factor 4E (eIF-4E) binding protein-1 (4E-BP1; also known as phosphorylated heat and acid-stable protein-1 [PHAS-1]) and the 40S ribosomal protein S6 ($p70^{S6}$) kinase. Treatment with rapamycin results in dephosphorylation of 4E-BP1/PHAS-1, an increase in eIF-4E binding and a decrease in translation of mRNAs for cell cycle progression from the G_1 to S phase. Furthermore, rapamycin treatment dephosphorylates $p70^{S6}$ kinase thus inactivating it. This results in suppression of translation of mRNAs encoding for ribosomal proteins, elongation factors and insulin growth

Scheme 2: Synthesis of CCI-779



factor-II (8, 13-16). Other effects of rapamycin include an interference with the balance of cyclin/cyclin-dependent-kinase/cyclin-dependent kinase inhibitors in the early phases of the cell cycle (17-19) which together with rapamycin's ability to inhibit translation of crucial subsets of mRNAs involved in the cell cycle progression from G_1 to the S phase can suppress the growth of cancer cells.

Although rapamycin has shown excellent preclinical anticancer activity, its clinical development has been hampered due to the poor aqueous solubility and chemical stability of the macrolide. The response has been development of soluble C-42 hydroxyester and amidino carbamate analogs. CCI-779, a rapamycin ester derived from 2,2-bis(hydroxymethyl)propionic acid, is one such analog that was selected for further development as an i.v. anticancer agent due to its promising pharmacological, toxicological and antitumor profiles (20).

Pharmacological Actions

CCI-779 potently inhibits T cell proliferation (IC_{50} = 0.8 nM) and binds to FKBP (IC_{50} = 8.2 ng/ml) (20).

The antitumor activity of CCI-779 has been demonstrated in studies using a number of tumor models *in vitro* and *in vivo*. *In vitro* studies using several types of human tumor cell lines and embryonic stem cells bearing deletions of the phosphatase PTEN tumor suppressor gene showed that CCI-779 treatment resulted in growth inhibition, accumulation of cells in the G_1 phase and apoptosis. PTEN is upstream in the mTOR pathway and inhibits PI3K-dependent activation of Akt (protein kinase B; Akt activates the mTOR pathway via phosphorylation) and deletion or inactivation of PTEN results in constitutive activation of Akt. Tumor cells were found to be either very sensitive (IC_{50} = ~ 1 nM) or resistant (IC_{50} > 1 μM) to the agent. The FKBP inhibitor ascomycin blocked the growth inhibitory effects of CCI-779, suggesting that the agent acts via FKBP binding. CCI-779 was extremely potent in inhibiting platelet-derived growth factor (PDGF)-stimulated growth of human glioblastoma cells (T98G; IC = about 1 pM) (21).

CCI-779 appears to be target specific and therefore it is possible that the molecular profiles of tumor cells would determine their sensitivity to the agent. Since the cytostatic effect of CCI-779 may be due to insufficient translation of proteins required for progression through the G_1 phase, it is possible that abnormalities in the regulators of the G_1 checkpoint predict sensitivity to CCI-779. A study further analyzing the sensitivity and resistance of tumor cell lines (*e.g.*, brain, prostate, breast, colon, uterine) both *in vitro* (human tumor cell lines, cells from PTEN knockout mice [$PTEN^{-/-}$]) and *in vivo* (*i.e.*, human tumors implanted in nude mice; $PTEN^{+/-}$ mice) revealed that those cells with a loss of PTEN and/or activated Akt displayed an increased sensitivity to CCI-779 and those cells resistant to the agent had low levels of activated Akt; cells with moderate Akt levels were either sensitive or resistant to CCI-779 (22-24).

The antigrowth effects of CCI-779 were also demonstrated *in vivo* in several studies using nude mouse xenograft models. Growth of staged human glioblastoma (U87MG) tumors was blocked by 5-day treatment with CCI-779 (minimum effective dose = 0.1-1 mg/kg). CCI-779 was cytostatic. However, while the immunosuppressive effects of CCI-779 were lost as early as 1 day postdosing, the antigrowth effects of the agent were sustained to 14 days postdosing. Similar results were obtained with pancreatic, breast and prostate tumor types (21).

The potential efficacy of CCI-779 *in vivo* was further demonstrated in a study that reported development of 2 assays involving determination of 4E-BP1 bound to eIF-4E and direct Western blot analysis of phosphorylation of the Thr^{70} residue of 4E-BP1 to be used in assessing mTOR activity in tumor specimens. Treatment of mice bearing rhabdomyosarcoma (Rh18) xenografts with CCI-779 at a daily dose of 8.7 mg/kg or more resulted in tumor growth inhibition. Similarly, mice bearing prostate (DU-145, PC-3), glioma (SF295) or ovarian (OVCAR5) carcinoma xenografts were also treated for 5 days with the agent (20 mg/kg/day). Tumor growth was suppressed with treatment in all cases except in mice bearing ovarian tumors, where no significant effect was observed (25).

CCI-779 also showed efficacy against a panel of breast cancer cell lines both *in vitro* and *in vivo*. Those cell lines that were sensitive to CCI-779 (IC_{50} = 50 nM or less) were either estradiol responsive (MCF-7, BT-474, T-47D) or lacked PTEN expression (MDA-MB-468, BT-549) and/or overexpressed Her-2/neu (SKBR-3, BT-474). The two resistant lines (IC_{50} = > 1 μM; MDA-MB-435, MDA-MB-231) were not responsive to estradiol, did not overexpress Her-2/neu and were wild-type for PTEN. CCI-779 treatment of sensitive cells caused a reduction in D-type cyclin and c-myc levels and an increase in $p27^{Kip1}$ levels. Sensitivity of cells to the agent correlated with activation of the Akt pathway and results from experiments using MCF-7 cells suggested that amplification of mTOR-regulated $p70^{S6}$ kinase may also confer CCI-779 sensitivity. CCI-779 was also effective *in vivo* where doses of 10, 20 or 40 mg/kg i.p. administered for 5 days inhibited growth of sensitive MDA-MB-468 but not resistant MDA-MB-435 xenografts (26).

The antitumor activity of CCI-779 was further exemplified *in vivo* in a study examining the effects of the agent alone and in combination with cisplatin on human brain tumors (DAOY and D283 human medulloblastoma cells) implanted s.c. on the flanks of athymic nude mice. Treatment with CCI-779 (20 mg/kg/day i.p. for 5 days for up to 4 weeks starting 3 weeks after tumor implantation) delayed DAOY tumor growth by 160 and 240% after 1 and 2 weeks of treatment, respectively. Treatment with a single high dose of CCI-779 (100 mg/kg i.p.) resulted in a 37% regression in DAOY tumor volume within 1 week. However, growth of the tumor resumed after 1 week and a subsequent high dose on day 12 did not cause a second growth regression. Combination treatment of DAOY tumors with CCI-779 (20 mg/kg i.p. 5 days/week

for 2 weeks) and cisplatin (5 mg/kg i.p. single dose) caused a 1.3 times greater inhibition of growth as compared to cisplatin alone. Interestingly, CCI-779 (20 mg/kg i.p. 5 days/week) was also effective against U251 human glioma xenografts, a cell line that is resistant to rapamycin *in vitro* (27).

Clinical Studies

An ongoing phase I study is evaluating the safety, pharmacokinetics and efficacy of escalating doses of CCI-779 (0.75-19.1 mg/m^2/day for 5 days every 2 weeks as a 30-min i.v. infusion) in patients with solid neoplasms. Of the 51 evaluable patients who have received 262 courses to date, 3 episodes of dose-limiting toxicity (DLT) were observed in the first cycle which included asymptomatic grade 3 hypocalcemia at a dose of 2.16 mg/m^2/day (1 patient), grade 3 transaminase elevations (1 patient) and grade 3 vomiting, grade 2 diarrhea and grade 2 asthenia (1 patient) at 19.1 mg/m^2/day. Three heavily pretreated patients receiving 19.1 mg/m^2/day developed grade 3 thrombocytopenia requiring dose reductions. It was concluded that this dose is not well tolerated in heavily pretreated patients and no further dose-escalations above 15 mg/m^2 were performed in these patients. Mild to moderate toxicities observed were neutropenia, rash, mucositis, asthenia, fever, hypertriglyceridemia and allergic phenomena. Peak plasma concentrations of the agent increased with dose in 17 patients treated with 0.75-3.12 mg/m^2/day; the median terminal $t_{1/2}$ was 15.2 h in these patients. A partial response was observed in 1 patient with non-small cell lung carcinoma and minor responses and/or prolonged stable disease of more than 4 months have been observed in patients with drug-refractory cancers such as soft-tissue sarcoma (3 patients) and cervical (1 patient), uterine (1 patients) and renal cell (3 patients) carcinomas. Dose escalation continues in minimally pretreated patients (28-30).

Promising results were obtained for CCI-779 (7.5, 15, 22.5, 34, 45, 60, 80, 110, 165 and 220 mg/m^2/week as a 30-min i.v. infusion) in another phase I dose escalation study utilizing a weekly dosing schedule and conducted in 18 patients with advanced solid tumors. The maximum tolerated dose (MTD) has not been achieved as of yet and only 1 DLT was observed. Grade 1-2, dose-independent skin toxicities such as dryness with mild itching (6 patients), eczema-like lesions (2 patients), subacute urticaria (2 patients) and aseptic folliculitis (11 patients) have been observed. All patients who received 8 doses or more had grade 1 nail changes (*i.e.*, thickness and dystrophia). Thrombocytopenia was seen in 9 patients of whom 2 patients treated with 34 and 45 mg/m^2/week experienced grade 3. Other toxicities reported were leukopenia (4 patients), anemia (7 patients), grade 1-2 (10 patients) and grade 3 (1 patient) mucositis/stomatitis, reactivation of perioral herpes lesions (5 patients) and asymptomatic increases in triglyceride (9 patients) and cholesterol levels (5 patients). In addition, 5 of 9 male patients receiving 4 or more doses of 15 mg/m^2/week or higher displayed a reversible reduction in testosterone associated with increased LH and FSH. No significant or prolonged immunosuppression was detected following subjection of blood samples to peripheral lymphocyte immunophenotype and mitogen proliferation assays. Results from 12 patients receiving doses up to 60 mg/m^2/week suggest that C_{max} increased linearly, AUC increased subproportionately and clearance and volume of distribution at steady state increased with dose; the mean $t_{1/2}$ was 20 h. Of the 16 patients evaluable for efficacy, 3 partial responses were seen (renal cell carcinoma with lung metastases; neuroendocrine tumor with hepatic metastases; and breast cancer with liver, lymph node and periorbital metastases) (31-34).

A phase I study conducted in a total of 28 patients with advanced solid tumors (colorectal, gastric, esophagus, head and neck and cholangio carcinoma and 4 others) reported the preliminary results from 24 patients on the efficacy and safety of escalated-dose CCI-779 in combination with 5-fluorouracil/leucovorin (5-FU/LV). All agents were administered once weekly for 6 weeks followed by a 1-week rest period. CCI-779 was started in week 2 and administered at doses of 15, 25, 45 and 75 mg/m^2 (30-min i.v. infusion) followed by LV (200 mg/m^2, 1-h i.v. infusion) and 5-FU (2600 mg/m^2, 24-h i.v. infusion). Since stomatitis was dose-limiting requiring discontinuation, dose reduction or medical intervention in 6/6 patients administered 75 mg/m^2, dose escalation was stopped at 45 mg/m^2. Of the 11 evaluable patients at the 45 mg/m^2 dose level, 2 toxic deaths due to gastrointestinal perforation were seen in addition to 15 cases of grade 3 toxicities in 9 patients and 4 cases of grade 4 toxicities in 4 patients. These included fatigue, dehydration, leukopenia and acute abdominal toxicities requiring discontinuation in 1 patient or dose reductions in the others. Other toxicities such as rash, folliculitis, pruritus, ulceration, nail changes, stomatitis and asthenia were also observed. To date, 1 complete response was observed in a patient with colorectal carcinoma receiving the 15 mg/m^2 dose in week 42. In addition, 10 stable diseases of a maximum duration of 12 months were seen. It was concluded that the clinical efficacy of CCI-779 was dose-independent possibly due to concomitant 5-FU/LV administration. The safety profile obtained suggests an overlap of CCI-779 and 5-FU/LV toxicities. Furthermore, the high frequency and severity of toxicities observed even at 45 mg/m^2 indicate a narrow therapeutic window for CCI-779 in combination with 5-FU/LV. Further studies are required to determine appropriate regimens with this combination treat- ment (35).

CCI-779 has completed phase II trials in renal cell cancer with phase III trials planned. Phase II trials with CCI-779 in breast cancer continue (36).

Manufacturer

Wyeth-Ayerst Pharmaceuticals, Inc. (US).

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