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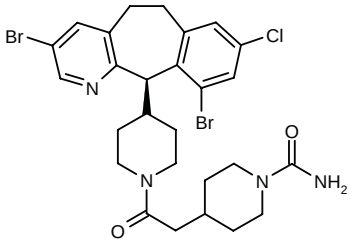
Lonafarnib

Prop INN; USAN

Farnesyltransferase Inhibitor
Solid Tumor Therapy
Leukemia Therapy

Sch-66336
Sarasar™

(+)-(*R*)-4-[2-[4-(3,10-Dibromo-8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)piperidin-1-yl]-2-oxoethyl]piperidine-1-carboxamide



$C_{27}H_{31}Br_2ClN_4O_2$

Mol wt: 638.8289

CAS: 193275-84-2

EN: 254680

Abstract

Ras proteins are involved in many crucial as well as housekeeping cellular processes such as growth, differentiation, apoptosis, cytoskeletal organization and membrane trafficking. Mutations of Ras proteins have been observed in as many as 30% of human cancers. Thus, interruption of Ras signaling has become a focus for the development of anticancer agents. One potentially effective approach that is currently being followed involves the prevention of the localization of Ras through inhibition of protein farnesyltransferase (FTase), the enzyme which catalyzes post-translational modification (*i.e.*, farnesylation) of Ras to enable localization of Ras proteins to the inner plasma membrane. Lonafarnib (Sch-66336) is a novel, orally active, heterocyclic peptidomimetic FTase inhibitor that competes with the enzyme for the CAAX portion of Ras. The agent has shown marked *in vitro* and *in vivo* antitumor activity and was chosen for further development. Lonafarnib has demonstrated efficacy and tolerability in numerous phase I and II trials as monotherapy or in combination with other chemotherapeutics, and is currently undergoing phase II/III development for the treatment of cancer.

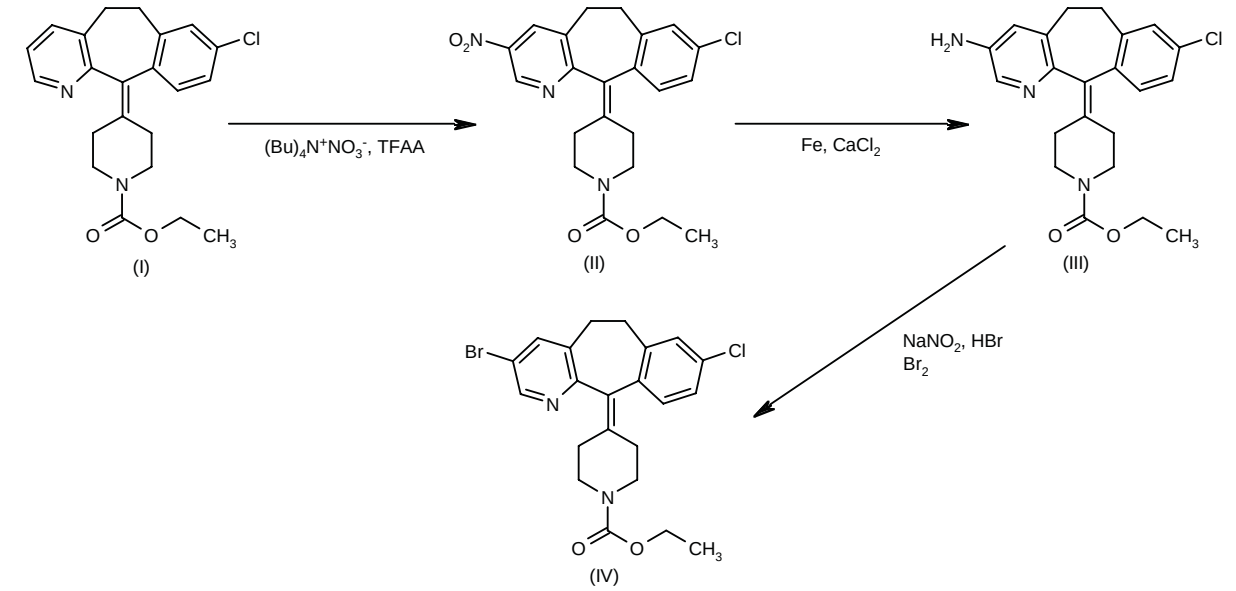
Synthesis

The nitration of loratadine (I) (1) by means of tetrabutylammonium nitrate and trifluoroacetic anhydride (TFAA) in dichloromethane gives the 3-nitro derivative (II), which is reduced with iron filings and $CaCl_2$ in refluxing ethanol/water to yield the 3-amino derivative (III). Treatment of compound (III) with $NaNO_2$, HBr and Br_2 provides 4-(3-bromo-8-chloro-5,6-dihydro-1*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidine-1-carboxylic acid ethyl ester (IV) (2). Scheme 1.

Introduction of a bromine atom at the 10-position of the benzocycloheptapyridine (IV) is achieved by the following sequence: Nitration of (IV) using $NaNO_3$ and H_2SO_4 affords a mixture of nitro compounds (V) and (VI), from which the major 9-nitro isomer (VI) is separated by silica gel chromatography. Reduction of the nitro group of (VI) with iron filings and $CaCl_2$ in refluxing aqueous ethanol gives the 9-amine derivative (VII), which is brominated at position 10 with Br_2 in AcOH. The brominated aniline (VIII) is then deaminated by diazotization, followed by reduction of the resulting diazonium salt with hypophosphorous acid to give the trihalogenated compound (IX), which by hydrolysis of the carbamate group in boiling concentrated HCl affords the piperidine derivative (X). Subsequent reduction of the C-11 double bond of compound (X) using DIBAL-H in refluxing toluene affords the corresponding racemic piperidine, which is submitted to enantiomeric separation by means of either HPLC on a ChiralPak AD column or chemical resolution using *N*-acetyl-L-phenylalanine as the resolving agent. The appropriate (+)-(*R*)-enantiomer (XI) is coupled with *N*-Boc-4-piperidineacetic acid (XII) in the presence of EDC and HOBt to yield the protected amide (XIII), which by hydrolysis of the Boc protecting group with trifluoroacetic acid results in the piperidine derivative (XIV) (3-5). Finally, this

L.A. Sorbera, J. Castañer. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of Intermediate (IV)



compound is treated with either trimethylsilyl isocyanate in dichloromethane (3-5) or urea in refluxing water (5). Scheme 2.

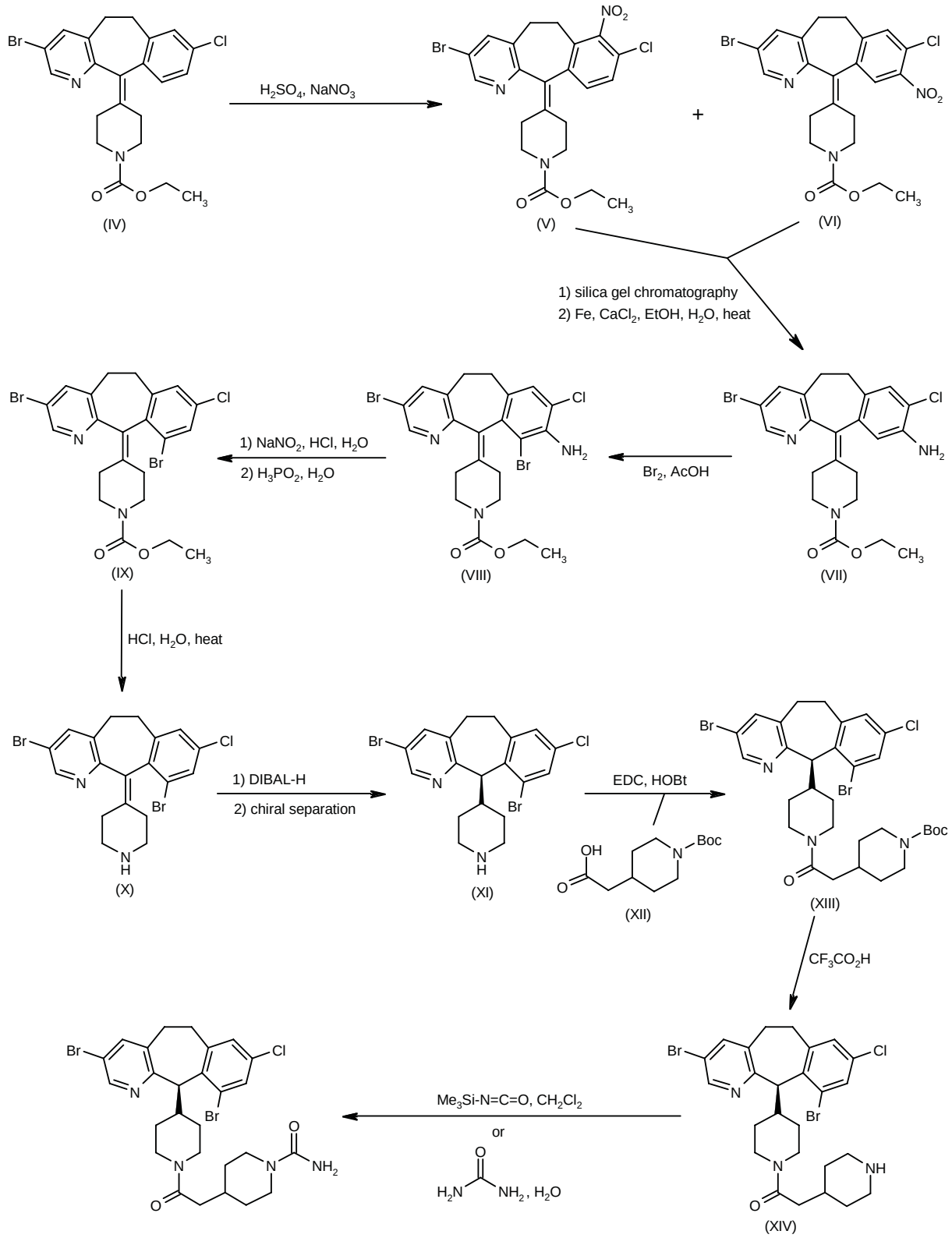
Alternatively, compound (X) can be resolved into its atropoisomers by digestion with Toyobo LIP-300 enzyme in the presence of trifluroethyl isobutyrate (XV) to give a mixture of the unreacted compound (–)-(XVI) and the acylated compound (+)-(XVII), which are separated by acid extraction. The undesired atropoisomer (–)-(XVI) is recovered by thermal razemization in diethyleneglycol dibutyl ether at 210 °C and new enzymatic separation. Acid hydrolysis of the separated amide (+)-(XVII) provides the desired atropoisomer (+)-(XVIII), which is finally reduced with DIBAL-H to the (+)-(*R*)-enantiomer (XI) (6, 7). Scheme 3.

Introduction

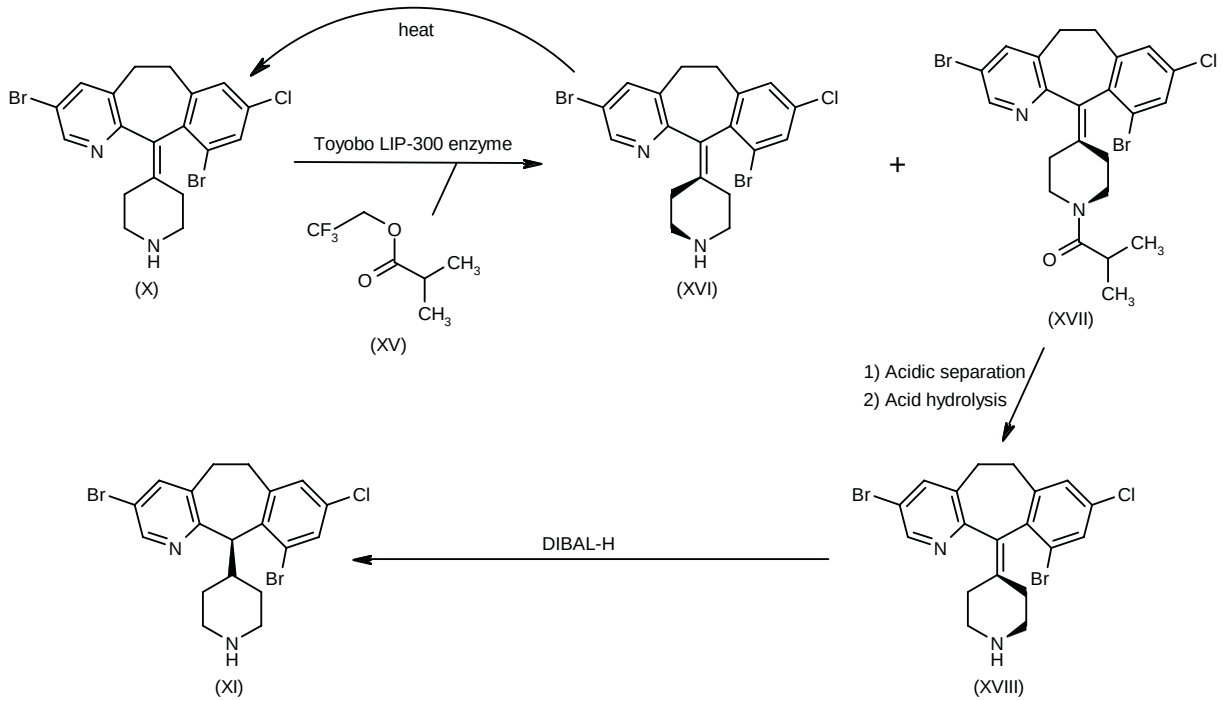
Genetic mutations are the underlying cause of tumorigenesis where they can cause the activation of oncogenes or the inactivation of tumor suppressor genes, leading to expression of a malignant phenotype. Ras oncogenes are known to be involved in signal transduction pathways regulating cell growth and differentiation in many human cancers, so that mutations of Ras protein are seen in about 30% of all cancers. There are 3 *ras* proto-oncogenes (H-*ras*, K-*ras* and N-*ras*) which encode 4 related and highly conserved 21-kDa Ras proteins (H-Ras, N-Ras, K-Ras4A and K-Ras4B). Ras proteins are members of the superfamily of GTPases comprised of proteins regulating protein synthesis and signal transduction involved in growth, differentiation, apoptosis, cytoskeletal organization and membrane trafficking. They are localized on the inner surface of the plasma membrane, where they act as molecular switches that cycle between an inactive GDP-bound form and an active GTP-bound state. In its active state, Ras mediates proliferative signals mainly upstream of receptor tyrosine kinases to a downstream cascade of protein kinases and their associated downstream pathways, including the mitogen-activated protein kinase (MAPK) cascade via Raf1, cell morphology via Rac/Rho, cell survival via phosphatidylinositol 3'-kinase (PI3-kinase) and stress response via mitogen-activated protein/ERK kinase kinase (MEKK) (8-12).

Localization of the Ras protein to the inner surface of the plasma membrane is required for signaling. Ras proteins are synthesized as inactive cytosolic precursors, after which they undergo a series of post-translational modifications at the C-terminus which yield the mature Ras protein that can be localized to the plasma membrane. The first post-translational modification is farnesylation, a prenylation reaction where a 15-carbon farnesyl isoprenoid moiety is added to Ras. This process is catalyzed by the zinc metalloenzyme protein farnesyltransferase (FTase), which transfers a farnesyl moiety from farnesyl pyrophosphate to the cysteine residue of CAAX-containing proteins (C = cysteine; A = aliphatic amino acid [leucine, isoleucine or valine]; X = methionine, serine, leucine or glutamine; Fig. 1). So far, studies have identified over 300 candidate peptides that contain CAAX and can undergo farnesylation, suggesting that a significant

Scheme 2: Synthesis of Lonafarnib



Scheme 3: Synthesis of Intermediate (XI)



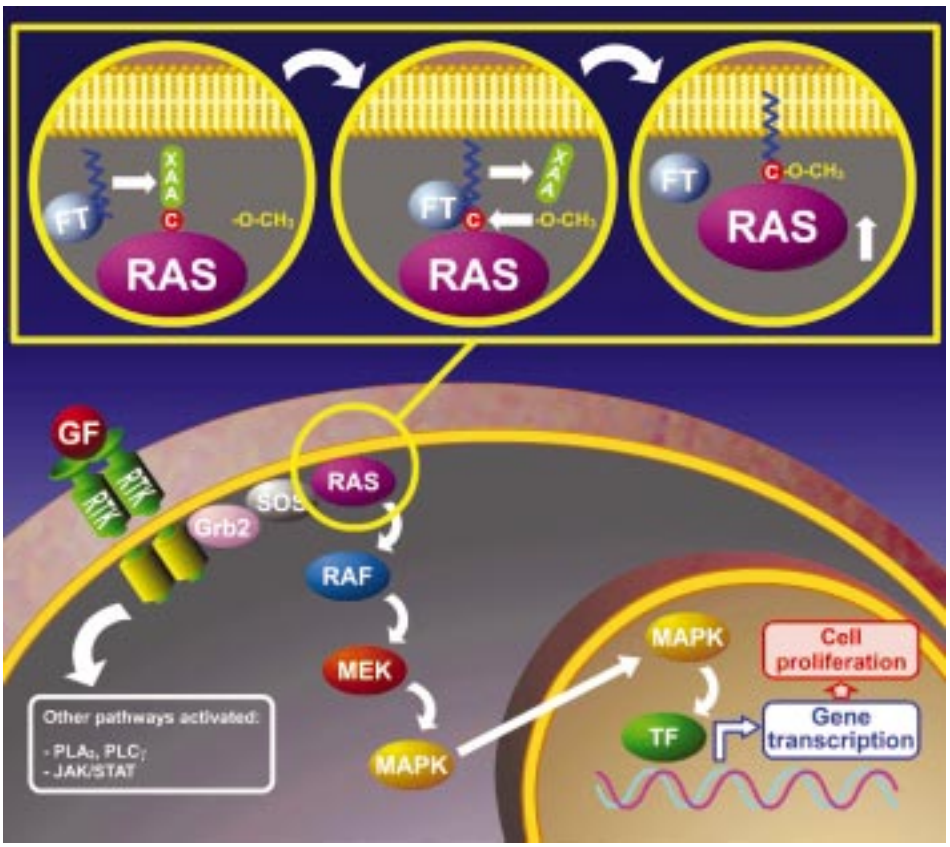


Fig. 1.

number of these proteins may be oncogenic and involved in mitogenic signaling (13, 14).

As mentioned above, a number of human tumors harbor Ras mutations which maintain Ras in a locked, activated state. K-*ras* is the most common, found with relative frequencies of 90, 55, 50, 35 and 23% in pancreatic, thyroid, colon, lung and ovarian carcinomas, respectively. N-*ras* mutations are less common, found with relative frequencies of 55, 43, 35, 30 and 20% in thyroid carcinoma, seminoma, lung cancer, myelodysplastic syndrome (MDS)/ acute myeloid leukemia (AML) and malignant melanoma, respectively, although they are more common than rare H-*ras* mutations (9, 10, 15-19). Because a large number of human cancers harbor mutated Ras, interruption of Ras signaling has become a focus for the development of anticancer agents. Approaches being developed include preventing membrane localization of Ras (*e.g.*, inhibition of FTase), inhibition of Ras protein expression through ribozyme, antisense nucleotides and inhibition of Ras downstream effectors. Small-molecule FTase inhibitors in particular are a novel class of antineoplastic agents of which at least 7 are currently under clinical development. Two of these agents, R-115777 (20) and lonafarnib (Sch-66336), are orally active heterocyclic peptidomimetics that compete with FTase for the CAAX portion of Ras. Lonafarnib in particular has exhibited marked antitumor activity *in vitro* and *in vivo* and has reached phase II/III development (3, 21).

Pharmacological Actions

Results from *in vitro* enzyme assays showed that lonafarnib potently and selectively inhibited the transfer of [^{3}H]-farnesyl pyrophosphate to activated H-Ras (IC_{50} = 1.9 nM); on the other hand, at concentrations up to 50 μM it was inactive in inhibiting protein geranylgeranyltransferase (GGPT)-mediated prenyl transfer. Lonafarnib also markedly inhibited farnesylation of H-Ras proteins in COS-7 cells transiently expressing H-ras[Val^{12}]-CVLS (IC_{50} = 10 nM) and inhibited anchorage-independent growth of tumor cell lines with and without H-*ras* and K-*ras* mutations. The IC_{50} values obtained from these colony-forming soft agar assays were 0.07, 0.50, 0.07, 0.25, 0.05 and 0.05 μM against H-*ras* NIH, K-*ras* NIH, colon HCT 116 bearing K-*ras*, pancreatic MIA PaCa-2 bearing K-*ras*, breast MCF7 and lung NCI-H146 cell lines, respectively (3, 21).

Lonafarnib was shown to have a good *in vitro* safety profile. Results from bacterial mutagenicity, human peripheral blood lymphocyte chromosome aberration and mouse bone marrow micronucleus assays indicated that the agent has no genotoxic potential and thus has an improved safety profile over other genotoxic anticancer agents (22).

Further studies examined the ability of lonafarnib to inhibit human tumor colony-forming units *in vitro*, with results indicating potent, broad-spectrum activity against ovarian, breast and non-small cell lung (NSCLC) cancers. The study using soft agar cloning assays and 70 primary tumor specimens from patients showed that 14-day exposure to lonafarnib (0.1-2.5 μM) resulted in concentration-related inhibition of tumor colony-forming units. At the highest concentration, lonafarnib was active against 3 of 6 (50%) breast tumors, 6 of 15 (40%) ovarian tumors and 5 of 13 (38%) NSCLC. Of the 69 specimens, lonafarnib showed activity against 27, 38, 33 and 27% of those samples resistant to doxorubicin, cisplatin, paclitaxel and etoposide, respectively (23).

In vitro studies using human head and neck squamous cell carcinoma (HNSCC) lines demonstrated the efficacy of lonafarnib in inhibiting Ras activity. In sulforhodamine B assays, the growth of 6 cell lines was time- and concentration-dependently inhibited with treatment (> 50% inhibition at 1 μM for 4 days). Anchorage-independent growth of all 6 cell lines was also inhibited in soft agar assays after 14 days of treatment with the agent. Reductions in cell number after treatment were found to be due to apoptosis. Experiments using the UMSCC38 cell line demonstrated that lonafarnib (1.5 μM) effectively inhibited Ras activity, since decreased phosphorylated Raf expression was observed at 5, 15 and 30 min and 1 h after treatment. Phosphorylated Akt expression was also reduced in a pattern similar to that observed for phosphorylated Raf, indicating an alteration of the PI3-kinase/Akt pathway activity. Similar effects were observed in 4 other HNSCC cell lines. A reduction in phosphorylated Bad, a proapoptotic protein which is phosphorylated and inactivated by Akt, was also observed in studies using SqCC/Y1 cells; reductions in expression of Bcl-xL and Bcl-2, but not Bax, cyclin D1 or cyclin B1, were also observed with treatment (24, 25).

An *in vitro* study using Ras-transformed Rat2 fibroblasts examining the mechanism of action of lonafarnib-induced apoptosis reported that treatment of H-Ras-CVLS-transformed fibroblasts with MEK1/2 (MAPK/ERK kinase) inhibitors (*e.g.*, PD-098059) significantly enhanced lonafarnib-induced apoptosis (approximately 60% vs. 30%). Combination treatment also resulted in markedly increased caspase 3 activity and a more complete and sustained inhibition of MAPK pathway activity than either agent alone; lonafarnib alone or in combination with the MEK1/2 inhibitor had no effect on Rat2 cells transformed with a geranylgeranylated form of H-Ras (H-Ras-CVLL). Interestingly, lonafarnib had no effect on K-Ras-transformed cells, although these cells underwent apoptosis in response to exposure to the MEK1/2 inhibitor (26).

The broad-spectrum antitumor efficacy of lonafarnib has been demonstrated *in vitro* in studies using several human cancer cell lines and *in vivo* in murine human tumor xenograft models. Antiproliferative activity (IC_{50} = 7.1-32.3 μM) was observed against 5 established human glioblastoma multiforme cell lines; cell lines expressing a large amount of H-Ras (*e.g.*, U-87 MG) and lesser amounts of K-Ras and N-Ras were more sensitive to the agent (27). Moreover, lonafarnib (2.5, 10 or 40 mg/kg q.i.d. p.o.) was effective in inhibiting tumor growth in nude

mice bearing human tumor xenografts including lung (A549, HTB-177), pancreatic (AsPC-1, HPAF-II, Hs 700T, MIA PaCa-2), colon (HCT 116, DLD-1) and prostate (DU 145) carcinomas. Significant inhibition of tumor volume was observed with all doses (*e.g.*, 67-86% inhibition at 40 mg/kg) (28).

Lonafarnib was also effective against chronic myeloid leukemia (CML) LLC-MK2 and LLC-MK1 Philadelphia chromosome (Ph)-positive cell lines, with increased apoptotic effects observed with increased exposure (96 h vs. 48 h) (29). Lonafarnib was also effective *in vivo* in reverting signs of leukemia and significantly increasing survival rates in a transgenic murine model of Bcr/Abl-positive lymphoblastic leukemia. Treatment (40 mg/kg p.o. b.i.d.) was well tolerated and no adverse events were noted. While all untreated control animals died within 103 days, 80% of the animals treated with lonafarnib survived until discontinuation of treatment at a median of 200 days. These animals showed no signs of leukemia or lymphoma (30).

Lonafarnib was shown to effectively prevent membrane association of H-Ras but not K-Ras or N-Ras in several human tumor cell lines in a study using cell fractionation and Western blot analysis (31). However, as mentioned above, the agent prevented anchorage-independent growth of cell lines harboring H-*ras*, K-*ras* and N-*ras* mutations in soft agar, and in an MTT assay it exhibited time- and concentration-dependent activity against NSCLC cell lines bearing 2 different K-*ras* mutations (A549: K-ras mutation GGT>AGT, codon 12; LX-1: GGT>GTT, codon 12) and against Calu-6 cells bearing wild-type K-*ras* (32). These observations suggest that lonafarnib may also block the farnesylation of other proteins in addition to Ras. A study has demonstrated that lonafarnib-sensitive human tumor cell lines (*e.g.*, lung NCI-H460, colon HCT 116, pancreatic MIA PaCa-2, breast MCF7) accumulated in the G_2/M phase following exposure to the agent (1 μM). However, those cell types with activated H-Ras (*e.g.*, H-*ras*-transformed NIH/3T3, bladder T24) accumulated in the G_1 phase, with the population of cells in the G_2/M phase unaffected. Genotypic analysis of the sensitive human tumor cell lines revealed that cells with wild-type *p53* were particularly sensitive to lonafarnib, and after exposure to the agent for 24 h, $p21^{Cip1}$, the downstream target of *p53*, was induced. It was concluded that *p53* status influences the sensitivity of cells to lonafarnib (33).

Treatment of the leukogenic murine cell line Bcr/Abl-BaF3A also resulted in accumulation of cells in the G_2/M phase, indicating an action of the agent on other proteins. It has been suggested that centromere-associated proteins CENP-E and CENP-F, which are involved in mitosis and regulate the G_2/M checkpoint, are critical farnesylated targets of lonafarnib (34, 35). Other farnesylated proteins such as the heat shock protein HDJ2 and the nuclear lamin prelamin A have also been implicated as farnesylated targets for lonafarnib. Experiments using biopsies or surgical tumor specimens from patients with head and neck cancer, melanoma or metastatic colorectal carcinoma who had been treated for 1-2 weeks with oral lonafarnib showed an accumulation of prelamin A and unfarnesylated HDJ2 (36). G_2/M arrest and accumulation of HDJ2 protein were also observed in B16 and COLO 853 human melanoma cells treated with the agent (37).

Further studies have also implicated other possible direct or indirect targets for the anticancer activity of lonafarnib. Treatment *in vitro* of melanoma LOX, breast carcinoma MDA-MB-231, NSCLC carcinoma NCI-H460, H-*ras*-transformed fibroblasts and *wap-ras* transgenic tumors resulted in concentration-dependent downregulation of expression of the angiogenic factors VEGF (vascular endothelial growth factor), IL-8 and angiogenin. Thus, inhibition of angiogenesis may also contribute to the potent antineoplastic effects of the agent (38). In addition, treatment of the MS-1 chronic myelomonocytic leukemia (CMML) cell line with lonafarnib (10 nM-1 μM) concentration-dependently upregulated LFA-1 integrin expression and induced inside-out activation of β_1 (to promote heterotypic adhesion) and β_2 integrin (to promote heterotypic adhesion). Lonafarnib had no stimulatory effects on antiinflammatory cytokines such as TNF-α, IL-1β, IL-8, SDF-1α or VEGF in these cells (39).

Results from an *in vitro* study using NIH-G185 cells which overexpress the gene product of human MDR1 (human transporter P-glycoprotein [Pgp]) indicate that lonafarnib should have synergistic activity when combined with Pgp substrate/inhibitors such as paclitaxel, tamoxifen and vinblastine by significantly enhancing their inhibitory effects on Pgp. In this study, lonafarnib was shown to directly interact with the substrate binding site of Pgp. Lonafarnib significantly inhibited daunorubicin transport (IC_{50} ~ 3 μM) and reduced Pgp-mediated ATP hydrolysis by more than 70% (K_m = 3 μM) (40).

The possible synergistic effects of lonafarnib with other chemotherapeutic agents were confirmed in several *in vitro* and *in vivo* studies. Lonafarnib enhanced the antitumor activity of paclitaxel in 10 of 11 (except breast adenocarcinoma MDA-MB-231) human breast, colon, lung, ovary, prostate and pancreatic tumor cell lines examined and was synergistic with docetaxel in 4 of 5 (except MDA-MB-231) cell lines tested. Synergistic activity was also observed *in vivo* with combination treatment including lonafarnib (20 mg/kg p.o. for 14 days) and paclitaxel (5 mg/kg i.p. once daily for 4 days) against human lung tumor NCI-H460 xenografts in nude mice. Moreover, lonafarnib (2.5, 10 or 40 mg/kg q.i.d. for 4 weeks) not only dose-dependently inhibited tumor growth in male *wap-ras*/F transgenic mice that spontaneously develop paclitaxel-resistant mammary tumors, but treatment with the agent also sensitized the tumors to paclitaxel. Tumor regression was observed with lonafarnib and was associated with increased apoptosis and a reduction in DNA synthesis (28, 41).

The antiproliferative effects *in vitro* of a combination of lonafarnib and cisplatin were additive or synergistic in A549 NSCLC and T98G human glioblastoma cells, with enhanced apoptosis observed, but they were less than

additive in breast MCF7, colon HCT 116 and pancreatic adenocarcinoma BxPC-3 cells. The synergistic effects of lonafarnib were drug sequence-dependent since treatment of A549 cells with cisplatin followed by lonafarnib resulted in antagonistic activity (42). Additive antiproliferative effects were observed in human lung cancer cell lines A549 and NCI-H460 treated with a combination of lonafarnib and cisplatin or carboplatin. Significantly enhanced antitumor efficacy was also observed *in vivo* in nude mice bearing human tumor xenografts and treated with lonafarnib + cisplatin (A549 xenograft model) or lonafarnib + temozolomide (human melanoma LOX xenograft model) (43).

Combination treatment of several human tumor cell lines *in vitro* with lonafarnib and Sch-58500, a replication-deficient recombinant *p53*, resulted in synergistic or additive cytotoxicity. Further synergy was not observed when paclitaxel was added to this combination, although additive effects were noted (44). Additive or synergistic effects were also seen when lonafarnib was combined with imatinib or cytosine arabinoside in a proliferation assay using imatinib-resistant Bcr/Abl-positive cells, indicating a possible efficacy for the agent in treating patients with imatinib-resistant Ph-positive leukemias. Moreover, lonafarnib potently sensitized imatinib-resistant cells to imatinib-induced apoptosis. In contrast, antagonistic effects were seen in these cells when lonafarnib was combined with daunorubicin or etoposide (45-48).

Synergistic activity in NSCLC cells was also observed when lonafarnib was combined with an adenovirus expressing insulin-like growth factor-binding protein 3 (IGFBP-3; Ad5CMV-BP3), which targets Ras and Akt (49).

Pharmacokinetics

Two HPLC methods were developed to determine levels of lonafarnib in plasma and its chiral inversion. The achiral method was found to be linear over a concentration range of 0.1-20 μg/ml in cynomolgus monkey plasma. The chiral method was linear in rat and cynomolgus monkey plasma over a concentration range of 0.25-10 μg/ml for both enantiomers and showed that the agent was not subjected to chiral inversion in these animals (50).

The pharmacokinetics of lonafarnib were determined in athymic nude mice, rats and cynomolgus monkeys. A serum C_{max} of 8.8 μM and an $AUC_{(0\text{-}24\ h)}$ value of 24.1 μg·h/ml were obtained in mice following oral administration of the agent (25 mg/kg); oral bioavailability was 76%. The half-life following i.v. administration was 1.4 h. Plasma C_{max} values in rats were 3, 10 and 30 μM following oral administration of 10, 30 and 100 mg/kg, respectively. In monkeys, C_{max} values were 1.8-2.5 μM following oral or i.v. administration of 10 mg/kg and the $AUC_{(0\text{-}48\ h)}$ value after oral administration was 14.7 μg·h/ml; the oral bioavailability was about 50% (3, 51).

The pharmacokinetics of lonafarnib (300 and 400 mg p.o. once daily in 28-day cycles) were determined in a phase I trial conducted in 12 patients with advanced solid tumors; pharmacokinetic analysis was performed on days 1 and 15. Grade 3 diarrhea was reported in 1 patient at 400 mg. However, 3 of 6 patients discontinued early at this dose level due to grade 1-3 diarrhea, uremia, creatinine elevation, asthenia, vomiting and/or weight loss. Similar toxicities were observed with 300 mg, although they were all grade 1-2; 300 mg once daily was the recommended dose for phase II studies. C_{max} and AUC values were dose-related and were increased on day 15 as compared to day 1, indicating drug accumulation. Steady state was achieved by day 14. A large volume of distribution was observed at steady state, suggesting marked distribution outside the plasma compartment. The plasma half-life appeared to increase with dose and was 5-9 h (52).

Similar pharmacokinetic results were obtained with a twice-daily dosing schedule. The pharmacokinetics of lonafarnib (25, 50, 100, 200, 300 and 400 mg p.o. b.i.d. in 28-day cycles) were examined in a dose-escalation phase I trial conducted in 24 patients with advanced solid tumors; pharmacokinetic analysis was performed on days 1 and 15. The dose-limiting toxicities (DLTs) were grade 4 vomiting, grade 4 neutropenia and thrombocytopenia and a combination of grade 3 anorexia and diarrhea with reversible grade 3 plasma creatinine elevation at 400 mg b.i.d., and grade 4 neutropenia, grade 3 neurocortical toxicity and a combination of grade 3 fatigue with grade 2 nausea and diarrhea at 300 mg b.i.d. The recommended dose for phase II studies was 200 mg b.i.d. Steady-state plasma concentrations of the agent were achieved at days 7-14. The volume of distribution was large at steady state, suggesting that there was extensive distribution of the agent outside the plasma compartment. A greater-than-dose-proportional increase in drug exposure and plasma C_{max} values was observed, so that higher values were obtained on day 15 as compared to day 1; these results suggest drug accumulation. Plasma half-life values appeared to increase with dose and ranged from 4 to 11 h (53).

The effects of food on the pharmacokinetic profile of lonafarnib were examined in 2 randomized, crossover phase I studies: a single-dose (100 mg after an overnight fast or a high-fat breakfast) study in 12 subjects and a multiple-dose (200 mg b.i.d. for a 28-day cycle under fasted conditions and a 28-day cycle under fed conditions separated by a 2-week washout period) in 12 patients with advanced cancer. The agent was safe and generally well tolerated in both studies. Results from the single-dose study showed that food intake reduced the rate (about 50%) and extent of absorption (about 23%) (C_{max} = 323 and 154 ng/ml, AUC = 2077 and 1556 ng·h/ml, t_{max} = 3 and 8 h, respectively, for fasted and fed states). However, in the multiple-dose study, no significant difference in the pharmacokinetics on day 15 were observed between fasted and fed states (C_{max} = 2.77 and 2.27 ng/ml, AUC = 24.1 and 21.6 ng·h/ml, t_{max} = 4 and 4 h, respectively, for fasted and fed states) (54, 55).

A phase I/II study conducted in patients with solid tumors (n=3-8/dose) showed that a single dose of paclitaxel (135 or 175 mg/m^2 by single 3-h i.v. infusion on a 3-week cycle) did not influence the pharmacokinetics of multiple-dose lonafarnib (100, 125 or 150 mg p.o. b.i.d. in 3-week cycles). Similarly, multiple-dose lonafarnib did not alter the pharmacokinetics of paclitaxel (56).

Results from an open-label phase I study conducted in patients (n=3-7/dose) indicated that multiple lonafarnib doses (100, 150 or 200 mg p.o. b.i.d. for 15 days) did not affect the pharmacokinetics of gemcitabine (600, 750 or 1000 mg/m^2 i.v. once a week) (57).

Clinical Studies

Phase I studies

A phase I dose-escalation trial in 20 patients with solid tumors determined the maximum tolerated dose (MTD) and efficacy of lonafarnib (25, 50, 100, 200, 300, 350 and 400 mg p.o. b.i.d. for 7 days on a 21-day cycle). Patients received 92 courses. At the highest dose, moderate, reversible renal insufficiency secondary to dehydration from gastrointestinal toxicity was observed. The DLTs at this dose level were gastrointestinal toxicities (nausea, vomiting and diarrhea) and fatigue. A patient with previously treated metastatic NSCLC who was on the study for 14 months had a partial response. Analysis of buccal mucosa cells of treated patients showed inhibition of prelamin A farnesylation, indicating successful inhibition of FTase (58).

A randomized phase IB trial in 22 previously untreated patients with squamous cell carcinoma of the head and neck who were scheduled for surgical resection examined the efficacy of induction therapy with lonafarnib (100, 200 or 300 mg p.o. b.i.d. for 8-14 days before surgery). Seventeen patients were treated with the agent and 5 were randomized to no therapy before surgery. Only moderate toxicity was observed, which included grade 1 nausea (n=4), grade 1/2 diarrhea (n=5) with grade 3 dehydration (n=1), grade 1/2 anemia (n=9) and grade 1 thrombocytopenia (n=7). Of the 17 treated patients, 3 patients with skin, oral cavity and oropharynx as primary sites (2 of whom received the highest dose) had partial remission before surgery. Resected surgical specimens from all treated patients showed inhibition of DNA-J (11-50% increases in unfarnesylated DNA-J) and prelamin A (59).

The efficacy of lonafarnib (200 mg p.o. b.i.d.) was examined in a pilot study involving 12 previously treated patients with chronic- or accelerated-phase CML resistant or refractory to imatinib. Because 2 patients experienced a rapid increase in white blood cells (WBCs) at 7-10 days after the initiation of therapy, transient use of hydroxyurea was allowed. Median duration of therapy was 10 weeks. Two patients continued on therapy after 13 and 17 weeks, respectively. Diarrhea was the most common adverse event seen in 10 patients, with grade 3 or greater reported in 4 patients requiring dose adjustments. One patient developed grade 3 nausea. One patient with accelerated-phase CML and who received hydroxyurea for 2 weeks had hematological remission and resolution of splenomegaly lasting for 3 months. Another patient with chronic-phase CML who did not receive hydroxyurea also had a response and continues to receive treatment after 4 months. An alteration in differential count was noted so that increases in myelocytes and promelocytes, and sometimes WBCs, were observed in 6 patients, possibly indicative of an effect on differentiation (60).

A phase I trial examined the tolerability and efficacy of lonafarnib (200 or 300 mg p.o. b.i.d.) in 19 patients with hematological malignancies including acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL), CML-BC (blast crisis), CMML and myelodysplastic syndrome (MDS). A DLT of grade 3 diarrhea and hypokalemia unresponsive to diarrhea therapy was seen at the higher dose. Other adverse events noted at both dose levels included grade 2 diarrhea, nausea, fatigue and weakness. The lower dose was concluded to be well tolerated. During the first cycle, 2 deaths occurred due to disease progression. Of 17 evaluable patients, 3 of 5 with CMML, 2 of 4 with Ph-positive CML-BC or ALL, and 1 of 7 with AML had clinical responses (*e.g.*, erythroid, neutrophil, platelet responses and/or a > 50% decrease in blast or monocyte count). Inhibition of DNA-J farnesylation was observed at both dose levels (61).

A phase I study in 24 patients with solid tumors examined the safety and efficacy of combination therapy with lonafarnib (100, 125 or 150 mg p.o. b.i.d.) and paclitaxel (135 or 175 mg/m^2). No grade 3 or higher toxicities were observed in the 3 patients receiving 100 mg lonafarnib + 135 mg/m^2 paclitaxel. However, 2 of 8 patients receiving 100 mg lonafarnib + 175 mg/m^2 paclitaxel had grade 3 dehydration and grade 3 hyperbilirubinemia and 1 of 3 patients given 150 mg lonafarnib + 175 mg/m^2 paclitaxel had grade 4 neutropenia with fever. Of the 18 evaluable patients, partial responses were obtained in 3 of 6 previously untreated patients (2 stage IV NSCLC and 1 metastatic salivary gland tumor) and 3 of 6 extensively pretreated patients with metastatic NSCLC. The maximum tolerated dose (MTD) was determined to be 100 mg lonafarnib + 175 mg/m^2 paclitaxel (62).

Another phase I trial in 27 patients with advanced malignancies (colon, NSCLC, renal, melanoma, pancreatic, adrenal, prostate, sarcoma, hepatoma, biliary tract and unknown) examined the tolerability of lonafarnib (100, 125, 150 or 200 mg p.o. b.i.d. on days 3-28 of cycle 1 and days 1-28 for subsequent cycles) combined with paclitaxel (40, 60 or 80 mg/m^2/week on day 1 of each week). A total of 87 cycles were administered. A DLT of myelosuppression was seen in 2 of 3 patients given 150 mg lonafarnib + 80 mg/m^2 paclitaxel. Other toxicities reported included nausea, vomiting, diarrhea, fatigue/weakness and taste alterations. The MTD was determined to be 125 mg lonafarnib + 80 mg/m^2 paclitaxel (63).

The tolerability of combination treatment including lonafarnib (75, 100, 125 or 100 mg b.i.d. on days 6-21 of

cycle 1 and days 1-21 for subsequent cycles), paclitaxel (125, 150, 175 or 200 mg/m^2 as 3-h i.v. infusion on day 1) and carboplatin (AUC = 5 as 30-min i.v. infusion following paclitaxel) was examined in a phase I trial conducted in 27 patients with solid tumors. Twenty-six patients have completed a total of 86 cycles (1 patient withdrew prior to treatment). Treatment was well tolerated. DLTs of neutropenia and diarrhea were seen in 2 patients receiving 75 mg lonafarnib + 125 mg/m^2 paclitaxel + carboplatin, and diarrhea was reported in 1 patient administered 125 mg lonafarnib + 175 mg/m^2 paclitaxel + carboplatin. Other toxicities observed were fatigue, nausea/vomiting, diarrhea, alopecia, neuropathy and dry mouth. One patient discontinued carboplatin in cycle 13 for hypersensitivity reactions. Patient enrollment continues (64).

The tolerability of lonafarnib (50, 100, 125, 150 or 200 mg p.o. b.i.d. continuously starting on day 1) combined with docetaxel (60 or 75 mg/m^2 as 1-h i.v. infusion every 3 weeks starting on day 8) was studied in a phase I trial in 13 patients with solid malignancies (NSCLC, esophageal, head and neck, mesothelioma, SCLC and thyroid) and no more than 2 prior chemotherapy regimens. Treatment was well tolerated. A DLT of diarrhea/fatigue was seen in 1 of 6 patients administered 125 mg lonafarnib + 75 mg/m^2 docetaxel. Other adverse events reported included nausea, vomiting, diarrhea, neutropenia, thrombocytopenia and fatigue. Patient enrollment continues (65).

A phase I dose-escalation trial conducted in 25 patients with advanced malignancies reported the tolerability and efficacy of lonafarnib (100, 150 or 200 mg p.o. b.i.d. continuously) combined with gemcitabine (600, 750 or 1000 mg/m^2 on days 1, 8 and 15 every 28 days). The median duration of treatment was 6 months. Dose-limiting toxicities seen included nausea, vomiting, diarrhea and moderate myelosuppression, which were controlled with dose changes. Two partial responses were obtained in patients with pancreatic malignancies. Two patients with pancreatic malignancies and mesothelioma achieved minor responses. Long-term disease stability of greater than 6 months was reported for 11 patients. Examination of buccal smears from patients showed accumulation of prelamin A (66).

A phase I trial in 14 patients with advanced cancers examined the tolerability and efficacy of combination treatment including lonafarnib (75-125 mg p.o. b.i.d.), cisplatin (75-100 mg/m^2) and increasing doses of gemcitabine (750-1000 mg/m^2 weekly x 3) in 28-day cycles. A total of 27 courses have been administered. Non-hematological toxicities reported were nausea/vomiting (grade 1/2 in 52% of the courses; grade 3 in 37%), grade 1/2 fatigue (in 41%), grade 1 transaminase elevations (in 41%), grade 1 tinnitus (in 26%) and grade 1/2 diarrhea (in 15%). Dose-limiting toxicities of grade 2 thrombocytopenia and neutropenic fever were seen in 2 patients receiving 75 mg lonafarnib + 75 mg/m^2 cisplatin + 1000 mg/m^2 gemcitabine and in 2 patients receiving 100 mg lonafarnib + 100 mg/m^2 cisplatin + 1000 mg/m^2 gemcitabine. Lonafarnib was discontinued for nausea/vomiting in 1 patient who had received 100 mg lonafarnib + 75 mg/m^2 cisplatin + 1000 mg/m^2 gemcitabine and 2 other patients withdrew due to persistent nausea/vomiting or fatigue. Analysis of peripheral blood mononuclear cells revealed a reduction in FTase (37.5 ± 16.7%) activity in 7 of 8 patients receiving a regimen including 75 mg lonafarnib. Accrual continues (67).

Phase I/II studies

The efficacy and tolerability of lonafarnib (200 mg p.o. b.i.d. for 3 courses of 4 weeks separated by 1-4 weeks off treatment) as a treatment for MDS or secondary AML were examined in a phase I/II trial conducted in 16 patients. The major adverse events reported were gastrointestinal toxicities (diarrhea, nausea and anorexia) and myelosuppression. Other adverse events included infections, fatigue, elevations in liver enzymes, arrhythmia and skin rash. One patient was discontinued due to atrial fibrillation and another died from infection. Dose reductions were required in all but 1 patient treated with more than 1 course. Of the 12 patients evaluable for response, 2 partial responses were observed in patients who did not present Ras mutations. One of these patients, who had secondary AML with complex chromosome abnormalities, had reductions in blasts of 8% after the first course and remained stable after the second course. However, the patient discontinued due to gastrointestinal toxicity. The other patient had RAEB (with 5q- and 20 q-) and experienced reductions in blasts of 3% with normal karyotype and FISH (fluorescence *in situ* hybridization) screening after 1 course. However, excess blasts and chromosomal abnormalities returned despite continued treatment (68).

A phase I/II trial examined the efficacy and tolerability of lonafarnib (100 mg p.o. b.i.d.) in combination with paclitaxel (175 mg/m^2 i.v. over 3 h) in patients with NSCLC who failed previous therapy with paclitaxel (76%), docetaxel (39%), or both (15%). A median of 5 treatment cycles were administered. Combination treatment was well tolerated. Only minimal toxicity was observed, which included grade 3 fatigue in 3 patients, diarrhea, dyspnea and weakness in 2 patients and neutropenia in 1 patient. Two patients suffered grade 4 respiratory insufficiency and 1 patient each had grade 4 fatigue or neutropenic fever, respectively. Of the 34 evaluable patients, 5 and 15 patients had partial responses and stable disease, respectively (69).

Phase II studies

A phase II trial examining the efficacy and tolerability of lonafarnib monotherapy (starting dose of 200 mg p.o. b.i.d. as continuous daily dosing) in 21 patients with metastatic colorectal cancer who were refractory to first- and second-line therapy (5-fluorouracil and irinotecan) did not recommend future development of the agent for

this indication. The major adverse events reported were grade 1 (42%), 2 (42%) and 3 (14%) fatigue, grade 1 (23%) and 3 (42%) diarrhea and grade 2 (16%) nausea. Grade 2 or 3 elevations in serum creatinine, probably due to diarrhea-induced dehydration, were seen in 19% of the patients. No significant hematological toxicities were observed, although 19% of the patients developed grade 1 thrombocytopenia and 28% had grade 2 or 3 anemia. No objective responses were obtained, although 3 patients did have stable disease lasting for months (70).

A multicenter, randomized phase II trial in 15 patients with unresectable or metastatic transitional cell carcinoma of the urothelial tract who failed prior chemotherapy examined the efficacy and tolerability of lonafarnib (200 mg p.o. b.i.d. daily repeated every 28 days with allowance for dose escalation). Fourteen patients were evaluable, with 7, 3 and 4 receiving less than 4 weeks, 4-7 weeks and 8 weeks of therapy, respectively. Grade 3 and 4 toxicities related to treatment were fatigue, anorexia, nausea/vomiting, confusion, dehydration and dyspnea. Significant hematological toxicities were less common and included grade 3 and 4 anemia, neutropenia and thrombocytopenia. Four patients were discontinued and 3 required hospitalization for toxicities. Of the 7 patients evaluable for response, 1 had stable disease while 6 had disease progression. The trial will be stopped if more than 9 patients of the total 15 patients have progressive disease at 8 weeks (71).

An ongoing, open-label phase II trial in 54 adult patients with CML-BC, CMML, ALL, advanced MDS or relapsed, refractory or poor-risk AML examined the tolerability and efficacy of lonafarnib (200 mg p.o. b.i.d. continuously). Treatment was well tolerated. Ten patients have developed hematological toxicities. One of the 19 patients with AML had a pathological response (decrease in marrow blast count from 64% to 32%). Of the 15 patients with MDS, 1 patient had minor erythroid improvement and major platelet improvement, 1 patient had minor erythroid improvement and 1 patient had minor platelet improvement. Normalization of monocyte counts was seen in 6 of the 12 patients with CMML (72).

The safety and efficacy of lonafarnib (200 mg p.o. b.i.d.) were compared to gemcitabine (1000 mg/m^2 weekly for 7 weeks followed by 1 week of rest) in a randomized phase II trial in 63 patients with metastatic adenocarcinoma of the pancreas. A similar incidence of nausea, vomiting and diarrhea was seen in both treatment groups, although cases were more severe with gemcitabine. Moreover, fewer cases of thrombocytopenia and neutropenia were seen in the group receiving lonafarnib (0% vs. 17% and 3% vs. 17%, respectively). The 3-month progression-free survival rate for patients administered lonafarnib was 23% as compared to 31% for gemcitabine. Partial responses and stable disease were seen in 2 and 6 patients receiving lonafarnib, respectively, while 1 and 11 patients receiving gemcitabine had a partial response and stable disease, respectively. Due to the favorable results obtained in this study, future evaluation of lonafarnib in combination with gemcitabine will be performed in patients with advanced pancreatic cancer (73).

Source

Schering-Plough Corp. (US).

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