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Deforolimus

USAN

mTOR Inhibitor
Oncolytic

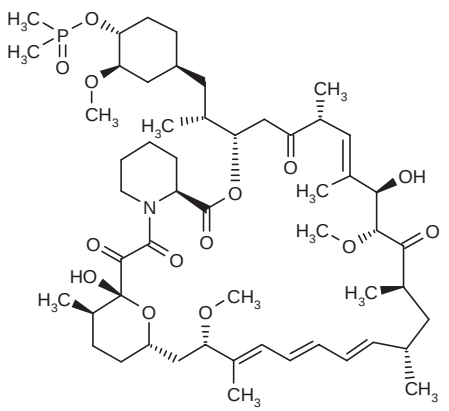
AP-23573

(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*,23*S*,24*E*,26*E*,28*E*,30*S*,32*S*,35*R*)-12-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-[(Dimethylphosphinoyl)oxy]-3-methoxycyclohexyl]-1-methylethyl]-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.0^{4,9}]hexatriaconta-16,24,26,28-tetraene- 2,3,10,14,20-pentone

Dimethylphosphinic acid (1*R*,2*R*,4*S*)-4-[2(*R*)-[(3*S*,6*R*,9*R*,10*R*,12*R*,14*S*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,27-dihydroxy-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-1,5,11,28,29-pentaoxo-3,4,5,6,9,10,11,12,13,14,21,22,23,24,25,26,27,28,29,31,32,33,34,34a-tetracosahydro-1*H*-23,27-epoxypyrido[2,1-*c*][1,4]oxazacyclohentriacontin-3-yl]propyl]-2-methoxycyclohexyl ester

Dimethylphosphinic acid rapamycin-40-*O*-yl ester

InChI=1/C53H84NO14P/c1-32-18-14-13-15-19-33(2)44(63-8)30-40-23-21-38(7)53(61,67-40)50(58)51(59)54-25-17-16-20-41(54)52(60)66-45(35(4)28-39-22-24-43(46(29-39)64-9)68-69(11,12)62)31-42(55)34(3)27-37(6)48(57)49(65-10)47(56)36(5)26-32/h13-15,18-19,27,32,34-36,38-41,43-46,48-49,57,61H,16-17,20-26,28-31H2,1-12H3/b15-13+,18-14+,33-19+,37-27+/t32-,34-,35-,36-,38-,39+,40+,41+,43-,44+,45+,46-,48-,49+,53-/m1/s1



Abstract

The phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B, PKB) signal transduction cascade is crucial for the growth and proliferation of cancer cells and its components represent attractive targets for the design of anticancer agents. One viable target within the PI3K/Akt signaling pathway is mammalian target of rapamycin (mTOR), a key serine/threonine kinase acting downstream of PI3K activation to intracellularly mediate prosurvival activity. Inhibition of mTOR by the macrolide antibiotic rapamycin (sirolimus) results in potent antiproliferative, proapoptotic and antiangiogenic activity. Deforolimus (AP-23573) is a novel semi-synthetic phosphorus-containing C43-modified nonprodrug rapamycin analogue that exerts marked antiproliferative activity against several *PTEN*-deficient tumor cell lines and displays proapoptotic and antiangiogenic effects in a variety of human tumor xenograft models. Moreover, deforolimus exhibits potent clinical activity and is presently undergoing phase II development for the treatment of cancer.

$C_{53}H_{84}NO_{14}P$
Mol wt: 990.2062

CAS: 572924-54-0
EN: 347892

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Synthesis

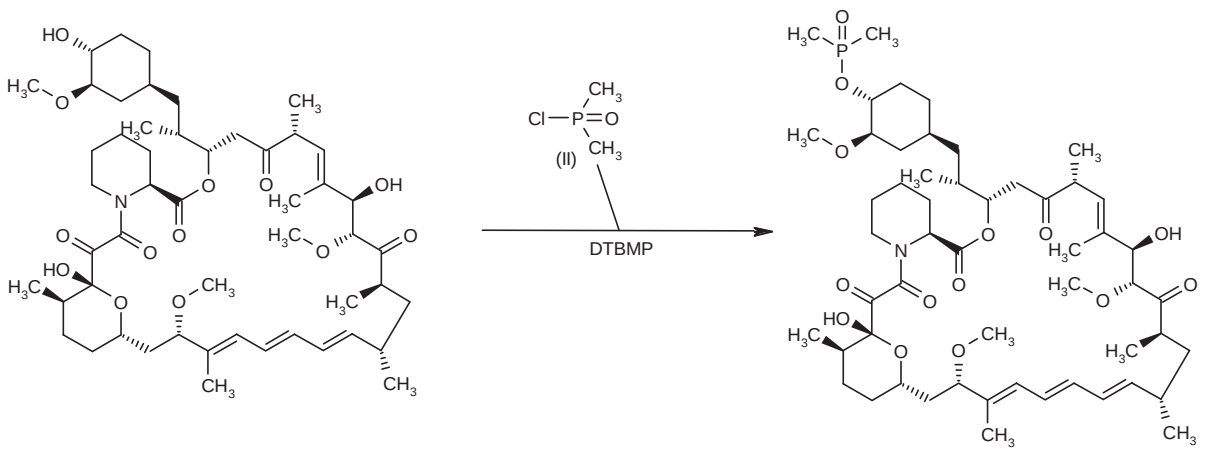
AP-23573 can be prepared by condensation of rapamycin (I) with dimethylphosphinyl chloride (II) by means of DTBMP in dichloromethane (1, 2). Scheme 1.

Background

The growth and proliferation of cancer cells are mediated by signal transduction pathways triggered by the conditional or constitutive activation of receptor tyrosine kinases (RTKs). RTKs in turn activate cytoplasmic kinases, many of which are serine/threonine kinases which ultimately lead to signaling promoting cancer development. Three signaling pathways, the phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B, PKB) and protein kinase C (PKC) family and the mitogen-activated protein kinase (MAPK)/Ras cascades, have been recognized as crucial for the development of cancer and are therefore attractive targets for the design of anticancer agents (3-12).

One promising anticancer target is mammalian target of rapamycin (mTOR, also known as FRAP, FKBP12, RAFT1 and RAP1), a key serine/threonine kinase acting downstream of PI3K activation. Activation of mTOR results in prosurvival signaling and consequent progression of the cell cycle from the G1 to the S phase. mTOR was discovered following the observation that rapamycin (sirolimus), a macrolide antibiotic discovered in 1975 to be a potent antifungal agent and later approved in 1990 for use as an immunosuppressive agent, exhibited potent antiproliferative and proapoptotic activity and specifically inhibited mTOR. mTOR has been highly conserved from yeast to mammals, mediating cell growth and proliferation at the translational level. mTOR consists of a FAT and FATC domains, a catalytic kinase domain, an FKBP12-rapamycin binding domain (FRB), a repressor domain near the *C*-terminus and up to 20 tandemly repeated HEAT motifs at the amino terminus, including huntingtin, eukaryotic translation elongation factor 3 (eEF3), the A subunit of protein phosphatase 2A (PP2A) and TOR. mTOR kinase is co-localized in the cytoplasm with three other peptides. These include: regulatory-associated protein of mTOR (Raptor), a scaffold protein which presents substrates to the mTOR kinase domain for optimal phosphorylation of downstream targets and inhibits mTOR kinase activity under nutrient-depleted condition; GβL, which increases the activity of mTOR through binding to the catalytic kinase domain of mTOR and stabilizing the Raptor-mTOR interaction; and mLST8, the function of which is unknown. Activation of mTOR kinase ultimately leads to enhancement of TOP (5'-terminal oligopyrimidine tract; *e.g.*, IGF-II)- and cap (*e.g.*, cyclin D1, Myc, HIF-1)-dependent translation of proteins involved in cellular catabolism, anabolism and apoptosis (Fig. 1). Growth factors or cytokines (*i.e.*, mitogenic stimuli) binding to membrane RTKs trigger the PI3K/Akt signal transduction cascade. After cytokine binding, the receptor dimerizes and becomes phosphorylated, inducing activation of PI3K, which phosphorylates phosphatidylinositol (4,5)-bisphosphate (PIP_2) to give phosphatidylinositol (3,4,5)-triphosphate (PIP_3). PIP_3 activates Akt, which subsequently activates mTOR. The downstream targets of mTOR are ribosomal p70S6 kinase (p70S6K) and eukaryotic translation initiation factor 4E (eIF4E)-binding protein (4E-BP1). mTOR-mediated activation of 4E-BP1 causes its dissociation from the RNA cap-binding protein eIF4E and formation of the eIF4F complex composed of eIF4E, the scaffold protein eIF4G and the RNA helicase eIF4A. This eIF4F complex enhances cap-dependent protein translation. Thus, inhibition of mTOR would block p70S6K and 4E-BP1 signaling and prevent translation of RNAs required for cell cycle progression from the G1 to the S phase. Inhibition also induces a proapoptotic effect and results in a deficiency in active cyclin-dependent kinase 4 (CDK4)/cyclin D1, which contributes to G1 arrest. Moreover, studies have shown that blockade of mTOR

Scheme 1: Synthesis of AP-23573



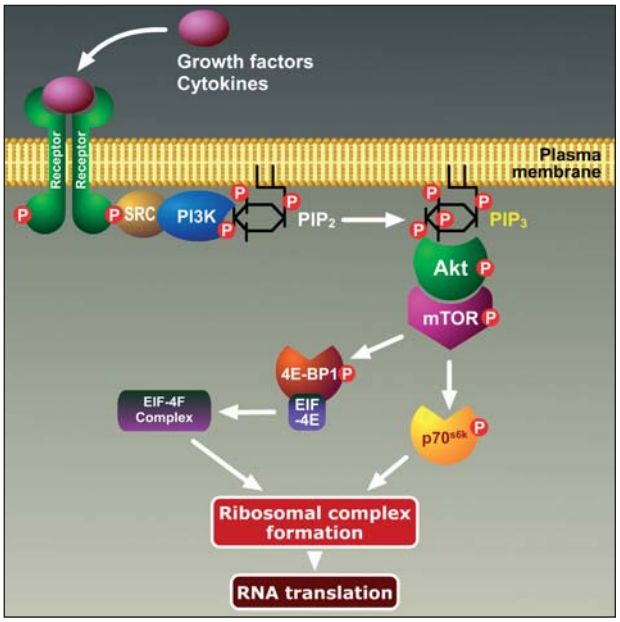


Fig. 1. Growth factors or cytokines binding to membrane receptor tyrosine kinases (RTKs) trigger the phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B, PKB) signal transduction cascade. After cytokine binding, the receptor dimerizes and becomes phosphorylated, inducing activation of PI3K, which phosphorylates phosphatidylinositol (4,5)-bisphosphate (PIP_2) to give phosphatidylinositol (3,4,5)-triphosphate (PIP_3). PIP_3 activates Akt, which subsequently activates mTOR. The downstream targets of mTOR are ribosomal p70S6 kinase (p70S6K) and eukaryotic translation initiation factor 4E (eIF4E)-binding protein (4E-BP1). mTOR-mediated activation of 4E-BP1 causes its dissociation from the RNA cap-binding protein eIF4E and formation of the eIF4F complex composed of eIF4E, the scaffold protein eIF4G and the RNA helicase eIF4A. This eIF4F complex enhances cap-dependent protein translation. Inhibition of mTOR would block p70S6K and 4E-BP1 signaling and prevent translation of RNAs required for cell cycle progression from the G1 to the S phase.

produces antiangiogenic effects due to reductions in vascular endothelial growth factor (VEGF) and the response of endothelial cells to VEGF stimulation (6, 13-16).

Further supporting targeting mTOR as a potential strategy for the treatment of cancer is the observation that several different tumor types exhibit aberrant activated mTOR signaling, resulting in uncontrolled proliferation of mutant clones. Mutations upstream and downstream of mTOR have been detected in cancer cells, such as amplification of a catalytic subunit of PI3K, loss of *PTEN* (phosphatase and tensin homologue deleted on chromosome 10; a tumor suppressor gene downregulating PI3K or Akt kinase expression) and overexpression or amplification of eIF4E or S6K1. The search for inhibitors of mTOR has become the focus of many researchers in an effort to design more effective and specific anticancer agents. Those mTOR inhibitors currently under active development for the treatment of cancer are shown in Table I (17-29).

One promising mTOR inhibitor is deforolimus (AP-23573), which was identified from a series of semisynthetic phosphorus-containing C43-modified nonprodrug rapamycin analogues. It is stable in organic solvents, aqueous solutions at different pH values, plasma and whole blood, and possesses undiminished affinity for FKBP. The agent was shown to exert marked antiproliferative activity against several *PTEN*-deficient tumor cell lines and was effective in a variety of human tumor xenograft models. Deforolimus was therefore selected for further development as an oncolytic agent (1, 14, 30).

Preclinical Pharmacology

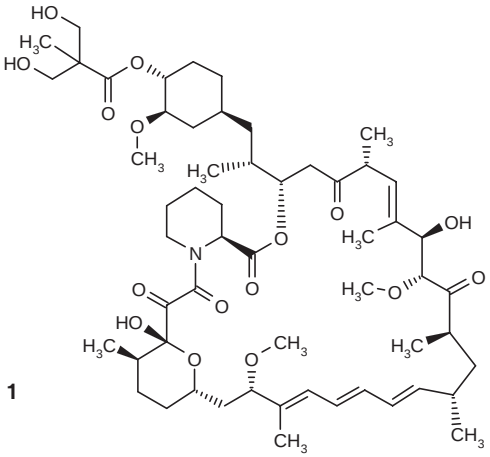
Deforolimus potently inhibited the growth of several human tumor cell lines *in vitro*, including *PTEN*-deficient glioblastoma (U-87 MG) and prostate (DU 145), breast, pancreatic, lung and colon cancers, with activity similar to that of rapamycin (IC_{50} about 0.1 nM). The agent also inhibited growth factor-induced proliferation of primary human umbilical vein endothelial cells (HUVEC) *in vitro*. Investigation of the mechanism of action of deforolimus using HT-1080 human fibrosarcoma cells showed that the concentration-dependent accumulation of cells in the G0/G1 phase of the cell cycle and the significant decrease in cell size (15-20% at 1-10 nM) observed were indicative of cytostatic effects comparable to those observed under conditions of nutrient or growth factor starvation due to mTOR inhibition. Further experiments revealed an antiangiogenic effect. Treatment of HT-1080 cells engineered to constitutively secrete human growth hormone (hGH) with deforolimus (10 nM) decreased VEGF secretion by more than 50% and the agent inhibited VEGF-induced proliferation of HUVEC with an IC_{50} value of 0.1 nM (1, 30).

Deforolimus markedly decreased survival and induced apoptosis in human malignant glioblastoma cell lines (U251, D54, U-87 MG, wild-type epidermal growth factor receptor [EGFR]- and mutant vIII EGFR-overexpressing U-87 MG cells) regardless of EGFR phenotype. Survival of glioblastoma cell lines was decreased by 40% following 72-h exposure to 1 nM deforolimus and apoptosis was induced in serum and under serum-free conditions (31).

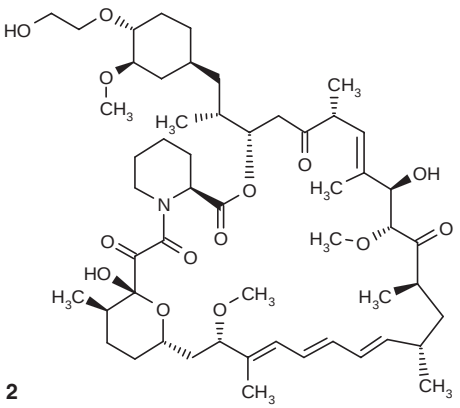
When combined simultaneously *in vitro* with other cytotoxic agents (camptothecin, 5-fluorouracil [5-FU], docetaxel, doxorubicin, cisplatin and topotecan) against human breast (MCF7), ovarian (SK-OV-3) and endometrial (AN3 CA) tumor cell lines, deforolimus exhibited additive and, at higher concentrations, possibly synergistic antiproliferative activity. Additive activity was also observed when deforolimus was combined with the tumor-targeting antibodies trastuzumab and imatinib and an EGFR inhibitor in breast BT-474, breast SK-BR-3 and *BCR-ABL*-positive K-562 chronic myelogenous leukemia (CML) cell lines, respectively. Synergistic activity was detected in experiments combining deforolimus concomitantly with the MEK inhibitor CI-1040 against four non-small cell lung cancer (NSCLC) cell lines, including *K-RAS* mutant and *TP53* wild-type A549 cells; enhanced caspase-mediated or autophagic cell death was observed

Table I: mTOR inhibitors under active development for the treatment of cancer (from Prous Science Integrity®).

Drug	Source	Phase
1. Temsirolimus	Wyeth Pharmaceuticals	L-2007
2. Everolimus	Novartis	III
3. Deforolimus	Ariad Pharmaceuticals	II
4. Sirolimus	Wyeth Pharmaceuticals	II
5. BEZ-235	Novartis	I
6. XL-765*	Exelixis	I
7. ABI-009*	Abraxis BioScience	IND Filed
8. TOP-216*	TopoTarget	Preclinical



1



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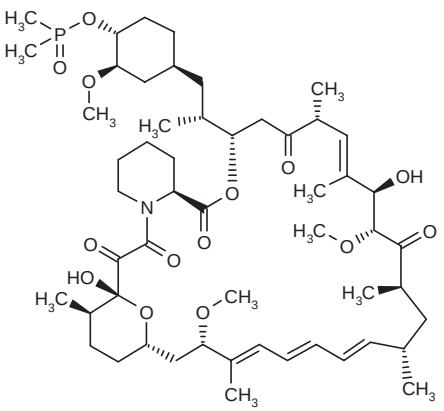

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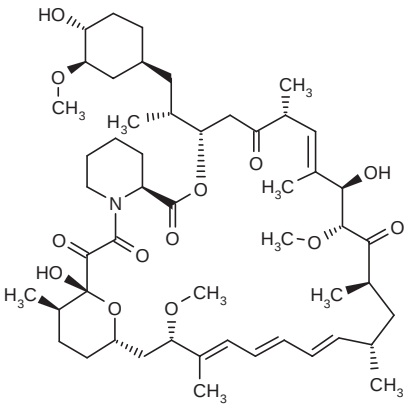

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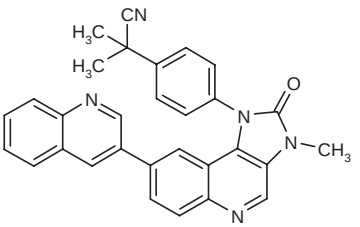

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*Structure not available.

as compared to either agent alone. Additive anticancer activity was noted when cells were exposed to higher concentrations of the two agents and antagonism was observed at low concentrations (32, 33).

The anticancer activity of deforolimus was demonstrated in several murine tumor xenograft models, including human U-87 MG glioblastoma, PC-3 prostate, MCF7 breast, PANC-1 pancreas, A549 lung and HCT 116 colon tumor models. The agent was effective in inhibiting growth when administered i.p. and p.o. at doses ranging from 0.1 to 10 mg/kg for 5 consecutive days followed by a 9-day rest period, or when given on a weekly dosing schedule. When administered early after tumor inoculation, partial regression of up to 90% was observed, which

persisted for up to 2 weeks. Moreover, substantial decreases in growth rate were seen when deforolimus was administered at a later stage of tumor development in all xenograft models. Examination of tumors from treated animals revealed inhibition of mTOR signaling, since levels of phosphorylated ribosomal S6 protein were completely abolished after a single dose of 1 mg/kg; inhibition of S6 persisted for 2-3 days. Results using the U-87 MG xenograft model showed that treatment with deforolimus (0.3 mg/kg/day i.p. for 5 days) significantly decreased tumor growth and size compared to untreated controls. Effects of the agent were sustained, with a 46% reduction in mean tumor volume at about 2 weeks postdosing as compared to the 150% increase observed in untreated animals. Deforolimus was also effective in this model when administered orally at a dose of 1 mg/kg/day for 5 days (1, 34, 35).

Pharmacokinetics and Metabolism

The pharmacokinetics of deforolimus were evaluated in two dose-escalating trials in which it was administered as a 30-min i.v. infusion once weekly (6.25-100 mg on 4-week cycles) or once daily (3-28 mg every 2 weeks on 4-week cycles) to patients with refractory or advanced solid malignancies. The pharmacokinetics of the agent were best described using a 3-compartment model, with estimated AUC values increasing in a less than dose-proportional manner on both dosing schedules. AUC was inversely related to body surface area (BSA) and proportional to red blood cell (RBC) concentrations. The nonlinear pharmacokinetics are consistent with saturation of the RBC compartment. The mean steady-state volume of distribution (V_{ss}) increased with dose and was significantly altered by BSA and RBC. The mean half-life ranged from 45 to 75 h and was independent of dose. Safety, tolerability and efficacy data from these trials are discussed below (36-38).

Clinical Studies

A phase I trial conducted in patients with advanced or refractory malignancies examined the safety, tolerability, maximum tolerated dose (MTD) and efficacy of i.v. deforolimus (6.25-100 mg by 30-min infusion weekly on a 4-week cycle). Seventeen patients received 34 cycles. Dose-limiting toxicity (DLT) of reversible grade 3 oral mucositis was observed in 2 patients at the 100-mg dose, which was concluded to exceed the MTD. Other frequent reversible grade 1-2 adverse events seen during the first cycle were anorexia, diarrhea, fatigue, rash, mucositis, thrombocytopenia and anemia. Analysis of peripheral blood mononuclear cells (PBMCs) collected from patient whole blood showed significant reductions in phosphorylated 4E-BP1, which were sustained until the next weekly dose. Of the 12 patients evaluable for efficacy, 1 patient with metastatic cholangiocarcinoma and another with medullary thyroid cancer had stable disease for at least 4 months (37).

The safety, tolerability, MTD and efficacy of i.v. deforolimus (3-28 mg by 30-min infusion for 5 days every 2 weeks on a 4-week cycle) were determined in another phase I trial in 32 patients with refractory or advanced malignancies. The MTD was determined to be 18.75 mg, since 2 patients developed DLT of grade 3 oral mucositis at a dose of 28 mg. Other adverse events observed during the first cycle were reversible grade 1 or 2 mucositis, fatigue, nausea, rash, anemia and neutropenia, and reversible grade 1 diarrhea, hyperlipidemia and thrombocytopenia. Phosphorylated 4E-BP1 levels in PBMCs were rapidly decreased by more than 80% within 1 h of dosing during the first cycle. Analysis of skin biopsies from 23 patients taken on days 1, 3 and 15 predosing and 4 h postinfusion indicated that phosphorylated S6 levels (Ser235/236) were inhibited by 50% or more at 1 or more time points postinfusion; an average reduction of 27-33% in phosphorylated S6 staining index was observed at 4 h and 3 days, and inhibition was sustained for at least 10 days postdosing in 8 samples analyzed. In contrast, p70S6K staining was constant during all time points. Inhibition of S6 phosphorylation in the skin did not correlate with dose or response. Of the 16 evaluable patients, minor responses were observed in a patient with renal cell cancer receiving 6.25 mg for 9 months (28% decrease in overall tumor burden) and another patient with an imatinib-refractory gastrointestinal stromal tumor (GIST) receiving 12.5 mg for more than 4 months. Stable disease for more than 4 months was observed in a patient with metastatic uterine sarcoma at 3 mg and 9 other patients had stable disease for 2-> 6 months (38, 39).

Archival tumor samples from at least 54 patients with advanced malignancies who participated in the above-described phase I trials (36, 37) were examined for *PTEN* expression. Results from analysis of 22 patient samples have been reported and showed that 91% of the tumor samples expressed near normal *PTEN* levels. Levels of *PTEN* expression did not correlate with antitumor responses to deforolimus. For example, *PTEN* expression was absent from a tumor sample from a patient with mixed mullerian uterine sarcoma who received 3 mg on the daily x 5 days schedule and had a partial response for 20+ months. In contrast only a modest reduction in tumor *PTEN* expression was noted in a patient with clear cell renal carcinoma treated with 18.75 mg on the daily x 5 days dose schedule and having stable disease for 10 months (40).

A phase I dose-escalating trial in 24 patients with refractory or advanced malignancies determined the safety, tolerability, MTD and antitumor efficacy of orally administered deforolimus (10-30 mg/day once daily for 4 days every week, 21 days or 28 days of a 28-day cycle). DLTs reported in cohorts receiving a dose of 20 mg/day for 21 or 28 days included severe mucositis and fatigue. Other adverse events seen during cycle 1 in at least 2 patients were reversible, mild to moderate mucositis, fatigue, rash, diarrhea, anorexia and nausea. Analysis of PBMCs revealed that levels of phosphorylated 4E-BP1 were decreased by more than 80% by day 1 during the

first cycle; these reductions were sustained, indicating potent and durable mTOR inhibition. Of the 3 patients evaluable for antitumor efficacy following completion of 2 treatment cycles, 1 patient with metastatic breast carcinoma receiving 20 mg/day once daily for 4 days/week experienced a 22% decrease in overall tumor burden, another patient with metastatic renal cell carcinoma receiving 20 mg/day for 21 days had a 21% decrease in tumor burden, and a patient with soft tissue sarcoma receiving 20 mg/day once daily for 4 days/week had stable disease (41).

Another phase I trial examined the MTD, safety and efficacy of deforolimus (starting dose of 12.5 mg by 30-min i.v. infusion daily x 4 days prior to tumor resection followed by daily x 5 days every 2 weeks on a 4-week cycle after surgical recovery) in patients with recurrent malignant gliomas. Patients were stratified according to concurrent use of enzyme-inducing anticonvulsants (EIACs). Results were reported from 9 patients in the non-EIAC arm (all received prior radiotherapy), of whom 5 received 12.5 mg and 2 received 15 mg. Of these patients, discontinuations included 5 due to progressive disease, 2 due to serious adverse events unrelated to the study drug, 1 at the investigator's discretion and 1 in the 12.5-mg cohort due to declining performance status after 2 cycles. No DLTs or serious adverse events were reported. Drug-related adverse events seen presurgery and during cycle 1 were hypertriglyceridemia/hyperlipidemia (n=3), thrombocytopenia (n=2), hypercholesterolemia (n=2), hyperglycemia (n=2), diarrhea (n=2) and mucositis (n=1). Analysis of PBMCs from 2 patients revealed rapid reductions in phosphorylated 4E-BP1 within hours postdosing, which were sustained for at least 6 days. In addition, marked decreases (50-100%) in phosphorylated S6 were observed in 6 of 8 surgically obtained tumor samples following deforolimus treatment (42).

The safety, tolerability and efficacy were further evaluated in a phase Ib study in 15 patients with solid tumors given deforolimus at doses of 25, 37.5 and 50 mg i.v. on days 1, 8 and 15 of a 28-day cycle, combined with capecitabine (1650 and 1850 mg/m^2/day). No DLTs were reported. The majority of adverse events related to treatment were mild to moderate (grade 1 or 2), with mucositis/stomatitis being the most common. A partial response was observed in a patient with endometrial cancer and stable disease for more than 4 months was seen in 3 patients with renal, uterine and head and neck cancers (43).

A modified, sequential (3+3) dose-finding study in 29 patients with solid tumors (sarcoma, pancreas, head and neck, melanoma, thymoma) determined the MTD, safety, tolerability and efficacy of i.v. deforolimus combined with i.v. paclitaxel (starting at 12.5 mg deforolimus + 80 mg/m^2 paclitaxel on days 1, 8 and 15 of a 28-day cycle). At the starting dose levels, grade 3 thrombocytopenia, grade 2 mouth sores and missed doses due to grade 2 neutropenia were reported. Other adverse events included mild and reversible mouth sores and fatigue. Examination of PBMCs revealed no interference of paclitaxel in deforolimus-induced inhibition of mTOR. Dose levels of 12.5 mg deforolimus + 80 mg/m^2 paclitaxel and 37.5 mg deforolimus + 60 mg/m^2 paclitaxel were well tolerated and concluded to be the MTDs. Antitumor activity was observed at several deforolimus/paclitaxel dose levels, including 25 mg/60 mg/m^2, 12.5 mg/80 mg/m^2 and 25 mg/80 mg/m^2. Five patients have remained on the study for more than 4 cycles; 1 with head and neck cancer and another with pancreatic cancer had partial responses (44).

An open-label phase II trial in patients with taxane-resistant androgen-independent prostate cancer examined the safety and efficacy of deforolimus (50 mg by 30-min i.v. infusion once weekly on 4-week cycles). Of the 38 patients enrolled (median prior taxane treatment = 5.8 months), 26 received more than 4 cycles of treatment and 11 continued on treatment at the time of reporting. Treatment-related adverse events were generally mild to moderate, mouth sores, fatigue, nausea, diarrhea and thrombocytopenia being the most common (> 20% of patients). Of 16 patients with measurable disease and evaluable for response, 1 partial response, 13 disease stabilizations and 2 cases of disease progression were observed. Of the 34 patients evaluable for prostate-specific antigen (PSA) response, 12 had stable disease and 22 had progressive disease. From the 18 patients evaluated after 4 cycles using the FACT-Prostate questionnaire, 14 reported improvement or stabilization of pain compared to baseline (45).

In an open-label phase II study conducted in 45 patients with advanced endometrial cancer, the safety and efficacy of deforolimus (12.5 mg once daily by 30-min infusion x 5 days every 2 weeks on 28-day cycles) were demonstrated. According to analysis of data from the 27 patients evaluable for response, the agent was well tolerated. The most common adverse events were fatigue (33%), anemia (33%), mouth sores (30%) and nausea/vomiting (30%). In addition, 16 grade 3/4 adverse events were reported, including 2 cases of hyperglycemia and 14 other adverse events similar to those reported in other deforolimus trials. Twenty-seven patients were evaluable for response, 14 of whom discontinued before 4 cycles due to progressive disease, 1 due to consent withdrawal and 3 due to adverse events unrelated to treatment. Of the 27 patients evaluable for response, 9 had a clinical benefit response (CBR; includes complete or partial responses or prolonged [16 weeks] stable disease), including 2 partial responses in patients with adenocarcinomas (46).

The safety and efficacy of deforolimus (12.5 mg i.v. daily x 5 days every 2 weeks) were also assessed in a phase II trial in patients with advanced sarcoma (bone sarcomas, leiomyosarcoma, liposarcoma and others). A total of 64 courses were administered, with 25, 19, 11 and 9 patients receiving 1, 2, 3 and 4 courses, respectively. The majority of adverse events related to treatment were mild to moderate in severity and the most common included mucositis (n=16), anemia (n=10), thrombocytopenia (n=8) and maculopapular rash (n=5). One patient with advanced abdominal liposarcoma died after 1 course of treatment. Of the 23 patients who had tumor-

associated uptake of [^{18}F]-2-fluoro-2-deoxy-D-glucose (FDG) on PET imaging at baseline, 9 and 25 patients exhibited a reduction in overall uptake of 25% or more and < 25%, respectively. Thirteen patients had symptomatic improvements in pain, cough and dyspnea. Response assessment in this trial was ongoing at the time of reporting. Of the 36 patients evaluable for response, half were considered responders (*i.e.*, objective response or prolonged stable disease for 16 weeks or more). Analysis of plasma VEGF levels from these patients revealed a > 50% reduction in 5 of 14 (36%) evaluable responders and 1 of 16 evaluable nonresponders (median decrease in VEGF levels of 35%). Tumor sample *PTEN*, phosphorylated S6 and p27Kip1 levels were examined in 20 patients, 8 of whom were responders. Three of these responders displayed low *PTEN* levels; phosphorylated S6 and p27Kip1 levels did not correlate with clinical response (47-49).

Another phase II trial examined the efficacy and safety of deforolimus (12.5 mg i.v. daily x 5 days every 2 weeks) in 212 patients with advanced soft tissue or bone sarcomas. Treatment was well tolerated. The most frequent adverse events were mucositis, rash, hyperlipidemia, fatigue and thrombocytopenia. The median overall CBR rate was 29%, which included 5 partial responses in 3 patients with osteosarcoma, 1 patient with spindle cell sarcoma and 1 patient with malignant fibrous histiocytoma of the bone (MFH). Of these responding patients, the overall survival was 67.6 weeks compared to 40.1 weeks for the entire study population (50, 51).

Preliminary results were reported from a phase II trial in 12 patients with relapsed or refractory hematological malignances (6 acute myelogenous leukemia [AML], 3 agnogenic myeloid metaplasia [AMM], 2 acute lymphocytic leukemia [ALL] and 1 myelodysplasia [MDS]) investigating the safety and efficacy of deforolimus (12.5 mg i.v. daily x 5 days every 2 weeks). Serious treatment-related adverse events reported were 1 case each of hypertriglyceridemia, neutropenic sepsis and mucositis. Of the 11 patients evaluable for response after cycle 1, 3 had stable disease and 4 had disease progression (52).

Preliminary data on the efficacy and safety of deforolimus (12.5 mg i.v. daily x 5 days every 2 weeks) were reported from another phase II trial conducted in 51 patients with relapsed or refractory malignancies. Patients were stratified into 5 disease cohorts: AML/MDS, ALL, AMM, chronic lymphocytic leukemia (CLL) and T-cell lymphoma/mantle cell lymphoma (MCL). The agent was generally well tolerated. Serious, possibly treatment-related adverse events included diarrhea, mucositis, hypertriglyceridemia, neutropenic sepsis, dyspnea, syncope, pleural effusion and pneumonia. The most common adverse events were mild to moderate nausea, mucositis, hyponatremia, pruritus, rash and hypokalemia. Of the 46 patients evaluable for response, anticancer activity was observed in all disease cohorts, with a total of 41% of the patients exhibiting a minimum of stable disease. Best or most recent response data included: 2 partial responses, 3 stable diseases and 2 disease progressions in the AMM cohort (n=7); 2 hematological improvements, 6 stable diseases and 18 progressive diseases in the AML/MDS cohort (n=26); 4 stable diseases and 3 disease progressions in the CLL cohort (n=9); 2 stable diseases and 2 disease progressions in the T-cell lymphoma/MCL cohort (n=2/5); and 2 disease progressions in the ALL cohort (n=2) (53).

A phase III trial in patients with metastatic sarcoma is scheduled to begin soon (54).

Drug Interactions

A phase Ib study in 15 patients with solid tumors examined the pharmacokinetics of deforolimus (25, 37.5, 50 and 75 mg i.v. on days 1, 8 and 15 of a 28-day cycle) combined with capecitabine (1650 and 1850 mg/m^2/day p.o. on days 1-14) or 5-FU. Deforolimus did not significantly affect the pharmacokinetics of either chemotherapeutic. However, exposure to the catabolite 5-fluoro-5,6-dihydrouracil tended to decrease in the presence of deforolimus and a gradual 60% reduction in dihydropyridine dehydrogenase was observed as compared to activity in the absence of deforolimus (43).

Sources

Ariad Pharmaceuticals, Inc. (US); licensed to Merck & Co. for co-development and co-marketing.

References

1. Metcalf, C.A. III, Bohacek, R., Rozamus, L.W. et al. *Structure-based design of AP23573, a phosphorus-containing analog of rapamycin for anti-tumor therapy*. Proc Am Assoc Cancer Res (AACR) 2004, 45: Abst 2476.

2. Berstein, D.L., Wang, Y., Rozamus, L.W., Metcalf, C.A. III (Ariad Gene Therapeutics, Inc.). *Phosphorus-containing compounds & uses thereof*. EP 1478648, JP 2005516065, WO 2003064383.

3. Larue, L., Bellacosa, A. *Epithelial-mesenchymal transition in development and cancer: Role of phosphatidylinositol 3'kinase/AKT pathways*. Oncogene 2005, 24(50): 7443-54.

4. Steinberg, S.F. *Distinctive activation mechanisms and functions for protein kinase Cdelta*. Biochem J 2004, 384(Pt. 3): 449-59.

5. Fang, J.Y., Richardson, B.C. *The MAPK signaling pathways and colorectal cancer*. Lancet Oncol 2005, 6(5): 322-7.

6. Bjornsti, M.-A., Houghton, P.J. *The TOR pathway: A target for cancer therapy*. Nat Rev Cancer 2004, 4(5): 335-48.

7. Faivre, S., Kroemer, G., Raymond, E. *Current development of mTOR inhibitors as anticancer agents*. Nat Rev Drug Discov 2006, 5(8): 671-88.

8. Prous Science Disease Briefings: *Breast Cancer* (online publication). Updated 2007.

9. Prous Science Disease Briefings: *Leukemia* (online publication). Updated 2007.

10. Prous Science Disease Briefings: *Prostate Cancer* (online publication). Updated 2007.

11. Prous Science Disease Briefings: *Head & Neck Cancer* (online publication). Updated 2007.

12. Prous Science Disease Briefings: *Lung Cancer* (online publication). Updated 2007.

13. Vezina, C., Kudelski, A., Sehgal, S.N. *Rapamycin (AY-22,989) a new antifungal antibiotic. Taxonomy of the producing streptomycete and isolation of the active principle*. J Antibiot (Tokyo) 1975, 28(10): 721-6.

14. Smolewski, P. *Recent developments in targeting the mammalian target of rapamycin (mTOR) kinase pathway*. Anti-Cancer Drugs 2006, 17(5): 487-94.

15. Kim, D.H., Sarbassov, D.D., Ali, S.M. et al. *mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery*. Cell 2002, 110(2): 163-75.

16. Kim, D.H., Sarbassov, D.D., Ali, S.M. et al. *GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR*. Mol Cell 2003, 11(4): 895-904.

17. Raftopoulou, M., Etienne-Manneville, S., Self, A., Nicholls, S., Hall, A. *Regulation of cell migration by the tumor suppressor PTEN*. Science 2004, 303(5661): 1179-81.

18. De Benedetti, A., Graff, J.R. *eIF4E expression and its role in malignancies and metastases*. Oncogene 2004, 23(18): 3189-99.

19. Mita, M.M., Mita, A., Rowinsky, E.K. *Mammalian target of rapamycin: A new molecular target for breast cancer*. Clin Breast Cancer 2003, 4(2): 126-37.

20. Zhou, X., Tan, M., Hawthorne, V.S. et al. *Activation of the Akt/mammalian target of rapamycin/4E-BP1 pathway by ErbB2 overexpression predicts tumor progression in breast cancers*. Clin Cancer Res 2004, 10(20): 6779-88.

21. David, O., Jett, J., LeBeau, H., Dy, G., Hughes, J., Friedman, M., Brody, A.R. *Phospho-Akt overexpression in non-small cell lung cancer confers significant stage-independent survival disadvantage*. Clin Cancer Res 2004, 10(20): 6865-71.

22. Roy, H.K., Olusola, B.F., Clemens, D.L., Karolski, W.J., Ratashak, A., Lynch, H.T., Smyrk, T.C. *AKT proto-oncogene overexpression is an early event during sporadic colon carcinogenesis*. Carcinogenesis 2002, 23(1): 201-5.

23. Witzig, T.E., Kaufmann, S.H. *Inhibition of the phosphatidylinositol 3-kinase/mammalian target of rapamycin pathway in hematologic malignancies*. Curr Treat Options Oncol 2006, 7(4): 285-94.

24. Giles, F.J., Albitar, M. *Mammalian target of rapamycin as a therapeutic target in leukemia*. Curr Mol Med 2005, 5(7): 653-61.

25. Smith, S.M., van Besien, K. *mTOR inhibition in lymphoma: A rational and promising strategy*. Lett Drug Des Discov 2007, 4(3): 224-31.

26. Reddy, G.K., Mughal, T.I., Rini, B.I. *Current data with mammalian target of rapamycin inhibitors in advanced-stage renal cell carcinoma*. Clin Genitourin Cancer 2006, 5(2): 110-3.

27. von Mehren, M. *New therapeutics for soft-tissue sarcomas in adults*. Oncology 2007, 21(1): 123-6.

28. Pantuck, A.J., Thomas, G., Belldegrun, A.S., Figlin, R.A. *Mammalian target of rapamycin inhibitors in renal cell carcinoma: Current status and future applications*. Semin Oncol 2006, 33(5): 607-13.

29. Sathormsumetee, S., Rich, J.N. *New approaches to primary brain tumor treatment*. Anti-Cancer Drugs 2006, 17(9): 1003-16.

30. Pollock, R., Rivera, V.M., Tang, H., Berk, L., Gaschet, J., Metcalf, C.A.III, Clackson, T. *Cell shrinkage, cell cycle arrest and anti-angiogenesis underlie the anti-tumor activity of the mTOR inhibitor APA23573*. 15th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther (Nov 17-21, Boston) 2003, Abst B160.

31. Nelms, J.L., Goldlust, S.A., Kanner, A., Vogelbaum, M.A. *A novel mTOR inhibitor (AP23573) produces cell loss and apoptosis in glioblastoma multiforme independent of EGFR status*. Neuro-Oncology 2003, 5(4): Abst ET-29.

32. Rivera, V.M., Tang, H., Metcalf, C.A. III et al. *Anti-proliferative activity of the mTOR inhibitor AP23573 in combination with cytotoxic and targeted agents*. Proc Am Assoc Cancer Res (AACR) 2004, 45: Abst 3887.

33. Legrier, M.-E., D'Silva, S., Yan, H.-G., Horwitz, S.B., McDaid, H.M. *Chemosensitization by dual pharmacological inhibition of MEK and mTOR signaling pathways in human non-small cell lung cancer (NSCLC)*. Proc Am Assoc Cancer Res (AACR) 2006, 47: Abst 4867.

34. Clackson, T., Metcalf, C.A., Rivera, V.M. et al. *Broad antitumor activity of AP23573, an mTOR inhibitor in clinical development*. Proc Am Soc Clin Oncol (ASCO) 2003, 22: Abst 882.

35. Clackson, T., Metcalf, C.A. III, Rozamus, L.W. et al. *Regression of tumor xenografts in mice after oral administration of AP23573, a novel mTOR inhibitor that induces tumor starvation*. Proc Am Assoc Cancer Res (AACR) 2002, 43(Suppl.): Abst LB-95.

36. Desai, A.A., Mita, M., Fetterly, G.J. et al. *Development of a pharmacokinetic (PK) model and assessment of patient (pt) covariate effects on dose-dependent PK following different dosing schedules in two phase I trials of AP23573 (AP), a mTOR inhibitor*. 41st Annu Meet Am Soc Clin Oncol (ASCO) (May 13-17, Orlando) 2005, Abst 3043.

37. Desai, A.A., Janisch, L., Berk, L. et al. *A phase I trial of weekly AP23573, a novel mTOR inhibitor, in patients with advanced or refractory malignancies: A pharmacokinetic (PK) and pharmacodynamic (PD) analysis*. Eur J Cancer - Suppl [16th EORTC-NCI-AACR Symp Mol Targets Cancer Ther (Sept 28-Oct 1, Geneva) 2004] 2004, 2(8): Abst 390.

38. Mita, M., Rowinsky, E., Mita, A. et al. *AP23573, an mTOR inhibitor, administered IV daily x 5 every other week in patients with refractory or advanced malignancies – A phase I, pharmacokinetic (PK), and pharmacodynamic (PD) study*. Eur J Cancer - Suppl [16th EORTC-NCI-AACR Symp Mol Targets Cancer Ther (Sept 28-Oct 1, Geneva) 2004] 2004, 2(8): Abst 409.

39. Rivera, V.M., Kriesberg, J.I., Mita, M.M. et al. *Pharmacodynamic study of skin biopsy specimens in patients (pts) with refractory or advanced malignancies following administration of AP23573, an mTOR inhibitor*. 41st Annu Meet Am Soc Clin Oncol (ASCO) (May 13-17, Orlando) 2005, Abst 3033.

40. Janisch, L.A., Desai, A.A., Mita, M. et al. *PTEN expression in archival tumor samples in patients (pts) with advanced malignancies in two phase I studies of AP23573 (AP), an mTOR inhibitor*. 41st Annu Meet Am Soc Clin Oncol (ASCO) (May 13-17, Orlando) 2005, Abst 9661.

41. Tolcher, A.W., Poplin, E., Britten, C.D. et al. *A phase I dose escalation trial of oral AP23573, a novel mTOR inhibitor, in patients with refractory or advanced malignancies*. 17th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther (Nov 14-18, Philadelphia) 2005, Abst C93.

42. Reardon, D.A., Wen, P.Y., Lyons, P. et al. *A phase I trial of AP23573, a novel mTOR inhibitor, in patients (Pts) with recurrent malignant glioma*. 17th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther (Nov 14-18, Philadelphia) 2005, Abst A195.

43. Perotti, A., Maur, M., Viganò, L. et al. *Phase Ib pharmacokinetic (PK) and pharmacodynamic (PD) study to define the optimal dose for combining the mTOR inhibitor AP23573 with capecitabine (CAPE)*. 42nd Annu Meet Am Soc Clin Oncol (ASCO) (June 3-6, Atlanta) 2006, Abst 3065.

44. Cresta, S., Tosi, D., Sessa, C. et al. *Phase 1b study defining the optimal dosing combinations of the mTOR inhibitor AP23573 and paclitaxel (PTX)*. J Clin Oncol [43rd Annu Meet Am Soc Clin Oncol (ASCO) (June 1-5, Chicago) 2007] 2007, 25(18, Suppl.): Abst 3509.

45. Gross, M.E., Amato, R.J., Mushtaq, M. et al. *A phase 2 trial of AP23573, an mTOR inhibitor, in patients (pts) with taxane-resistant androgen-independent prostate cancer (AIPC)*. Eur J Cancer - Suppl [18th EORTC-NCI-AACR Symp Mol Targets Cancer Ther (Nov 7-10, Prague) 2006] 2006, 4(12): Abst 362.

46. Colombo, N., McMeekin, S., Schwartz, P. et al. *A phase II trial of an mTOR inhibitor AP23573 as a single agent in advanced endometrial cancer*. J Clin Oncol [43rd Annu Meet Am Soc Clin Oncol (ASCO) (June 1-5, Chicago) 2007] 2007, 25(18, Suppl.): Abst 5516.

47. Chawla, S.P., Sankhala, K.K., Chua, V. et al. *A phase II study of AP23573 (an mTOR inhibitor) in patients (pts) with advanced sarcomas*. 41st Annu Meet Am Soc Clin Oncol (ASCO) (May 13-17, Orlando) 2005, Abst 9068.

48. Rivera, V.M., Berk, L., Chawla, S.P. et al. *Analysis of potential biomarkers of AP23573 activity in a phase II trial in sarcoma patients*. 17th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther (Nov 14-18, Philadelphia) 2005, Abst B181.

49. Sankhala, K.K., Chawla, S.P., Iagaru, A. et al. *Early response evaluation of therapy with AP23573 (an mTOR inhibitor) in sarcoma using [18F]2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) scan*. 41st Annu Meet Am Soc Clin Oncol (ASCO) (May 13-17, Orlando) 2005, Abst 9028.

50. Chawla, S.P, Tolcher, A.W., Staddon, A.P. et al. *Updated results of a phase II trial of AP23573, a novel mTOR inhibitor, in patients (pts) with advanced soft tissue or bone sarcomas*. J Clin Oncol [42nd Annu Meet Am Soc Clin Oncol (ASCO) (June 3-6, Atlanta) 2006] 2006, 24(18, Suppl.): Abst 9505.

51. Chawla, S.P., Tolcher, A.W., Staddon, A.P. et al. *Survival results with AP23573, a novel mTOR inhibitor, in patients (pts) with advanced soft tissue or bone sarcomas: Update of phase II trial*. J Clin Oncol [43rd Annu Meet Am Soc Clin Oncol (ASCO) (June 1-5, Chicago) 2007] 2007, 25(18, Suppl.): Abst 10076.

52. Feldman, E., Giles, F., Roboz, G. et al. *A phase 2 clinical trial of AP23573, an mTOR inhibitor, in patients with relapsed or refractory hematologic malignancies*. 41st Annu Meet Am Soc Clin Oncol (ASCO) (May 13-17, Orlando) 2005, Abst 6631.

53. Rizzieri, D.A., Feldman, E., Moore, J.O. et al. *A phase 2 clinical trial of AP23573, an mTOR inhibitor, in patients with relapsed or refractory hematological malignancies*. Blood [47th Annu Meet Am Soc Hematol (Dec 10-13, Atlanta) 2005] 2005, 106(11): Abst 2980.

54. *Ariad and FDA agree on SPA for phase III trial of deforolimus for metastatic sarcomas*. DailyDrugNews.com, September 13, 2007.