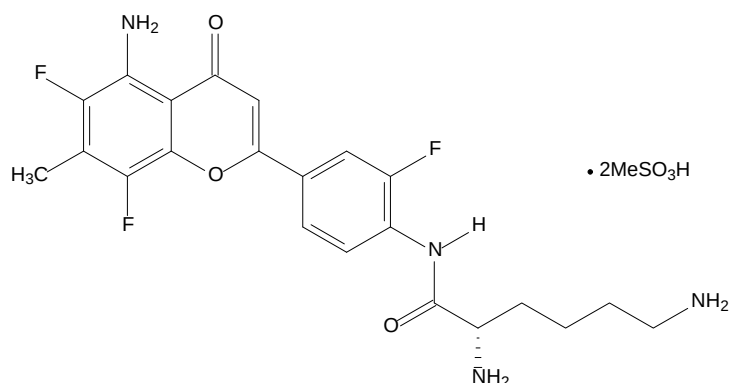


CTEP RAPID COMMUNICATION

SOLICITATION FOR LETTERS OF INTENT FOR PHASE 1 TRIALS

AMINOFLAVONE PRODRUG (AFP-464) NSC 710464



CTEP is soliciting proposals for phase 1 single agent trials of the aminoflavone prodrug (NSC 710464, AFP-464), a lysyl prodrug of aminoflavone (NSC 686288, AF). AF is a flavone analog that has differential activity in the 60-cell-line screen and marked antitumor effect in mice bearing human tumor xenografts. In *in vitro* studies, AF was cytotoxic in certain human renal and breast cancer cell lines, which correlated with metabolism of AF by CYP1A1 and CYP1B1. These metabolites covalently bind DNA and exert antiproliferative effects in AF sensitive tumor cell lines. In these cells, AF was also shown to induce CYP1A1 and CYP1B1 gene expression, via the aryl hydrocarbon receptor (AhR) transduction pathway, a mechanism by which AF may induce its own activation. Trial proposals should include a 3-hour infusion of AFP-464 weekly schedule. Although nonclinical studies suggest that modulatory activity is achieved at concentrations of approximately 1-2 μM , trial proposals should be designed to characterize the full toxicity profile up to the maximum tolerated dose (MTD). Based on *in vivo* toxicology studies, pulmonary toxicity may possibly be a significant and dose limiting toxicity. In a dog study, pulmonary toxicity has been observed with impaired diffusion lung capacity of carbon monoxide (DL_{CO}) that improved after stopping treatment. Thus, baseline and continued serial monitoring of DL_{CO} should be incorporated into the treatment monitoring plan. Patients with prior lung radiation therapy as well as symptomatic pulmonary disease should be excluded from participating in these studies.

Background

Flavones and flavonoids are a widely diverse class of compounds that produce biologic, pharmacologic, and toxicologic effects, including tumor inhibition (Akama *et al.*, 1996). Aminoflavone Prodrug (AFP-464) NSC 710464 is a lysine prodrug of the aminoflavone (AF) derivative NSC 686288, 4H-1-benzopyran-4-one, 5-amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-methyl. AF has a flavonoid ring system with unique substitutions, including two amino groups and three fluorine atoms. AFP-464 has been shown to rapidly convert to the parent

compound AF in plasma. *In vitro* studies have demonstrated that AFP-464 and AF are cytotoxic in renal and breast cancer cell lines. This activity was correlated to the production of metabolites of AF due to CYP1A1 and CYP1A2 metabolism (Kuffel, *et al.*, 2002, Loaiza-Pérez *et al.*, 2004a). Additionally, AF induces activation of the AhR transduction pathway in sensitive but not in resistant breast cancer cell lines (Loaiza-Pérez *et al.*, 2004b).

***In Vitro* Cytotoxic Assays**

In vitro, AFP-464 and AF have demonstrated antiproliferative activity in several tumor cell lines (Loaiza-Pérez *et al.*, 2004a, b). In the NCI 60-cell-line screen (a 48-hour exposure assay), AF was active against CaKi-1 and A498 renal, MCF-7 breast, and OVCAR-5 ovarian cancer cell lines. In this assay, AFP-464 showed similar activity comparable to its parent compound AF with a correlation coefficient of 0.79 at the GI₅₀ level. AF induced growth inhibition of several breast and lung cancer cell lines after brief exposures (0.75-6 hours) to <6 µM. Specifically, AF exhibited the highest growth inhibition activity in MCF-7, T-47D and NCI-H226 cell cultures after short-term exposures at 1-3 µM or >24 hours exposures at 0.7-0.9 µM. Similarly, AF showed prominent inhibitory effects in A498 and CaKi-1 cell cultures, but failed to be cytotoxic at prolonged exposure periods and/or high drug concentrations.

***In Vivo* Antitumor Activity**

In vivo antitumor activity of AF was demonstrated against two of three human renal carcinoma xenografts in mice, A-498 and CaKi-1, and against papillary renal cell carcinomas more often than clear cell carcinomas (Loaiza-Pérez *et al.*, 2004a). AF (40-120 mg/kg) was given through an intraperitoneal (IP) or intravenous (IV) route once daily for a total of 5 days with the first day of treatment being on day 13. Both of these models exhibited multiple tumor-free host animals. In contrast, AF was inactive in several other human xenograft models, including SW-620 (colon), IGROV-1 (ovarian), OVACR-5 (ovarian), and RXF-393 (renal cell), most of which were found to be sensitive to AF in the *in vitro* cancer screen. In addition, AF was found to have a modest activity against the MCF-7 breast cancer xenograft model after a brief IP and IV treatment regimens (Loaiza-Pérez *et al.*, 2004b). However, the efficacy of AF was found to be greater in this model after repeated treatments over eight doses (days 0-4 and 7-11) using the IP, IV or oral (PO) routes of administration. AFP-464 was also found to be active in CaKi-1 and MCF-7 xenografts models, though more toxic than its parent compound on a molar basis when administered IV. AFP-464 was active in 27% of MCF-7 tumor-bearing animals and 100% with 4/6 tumor-free animals in the CaKi-1 xenograft model.

Mechanism of Action

The mechanism by which aminoflavones exert their cytotoxic activity is unknown. The antiproliferative effect of other flavonoid analogs has been attributed to the inhibition of topoisomerases, tubulin polymerization, and/or protein kinase activities (Kuffel *et al.*, 2002). However, AF has been shown to be metabolized to reactive species that covalently bind DNA and other macromolecules by human and rat hepatic microsomes and by recombinant CYP1A1 and CYP1A2. In MCF-7 cells, AF induced CYP1A1 mRNA and CYP1A1/1A2 protein expression that converted AF to reactive metabolites that covalently bound to macromolecules, including DNA (Kuffel *et al.*, 2002; Loaiza-Pérez *et al.*, 2004b). Pretreatment with a CYP1A inducer (3-methylcholanthrene) augmented the amount of metabolite bound to macromolecules, while these

levels were decreased by coincubation with a CYP1A inhibitor (α -naphthoflavone). The covalent binding of AF metabolites to macromolecules induced DNA damage through a p53/p21 dependent mechanism. Subsequent studies showed that covalent binding was highest in tumor cell lines that were most sensitive to AF and much lower in resistant cell lines. Further analysis demonstrated that AF induced CYP1A1 and CYP1B1 mRNA expression in MCF-7 cells that correlated with the translocation of AhR to the nucleus after treatment with AF (Loaiza-Pérez *et al.*, 2004b). In addition, AF increased the activity of CYP1A1 in sensitive cells. In studies using renal cancer cell lines, similar findings showed that AF increased CYP1A1 mRNA expression in sensitive cell lines. Experimental data from renal cell isolates from 13 patients with renal cell carcinoma found that cell isolates that were sensitive to AF had increased binding of AF metabolites when compared to resistant isolates.

Pharmacokinetic Studies

Analysis of plasma samples after an IV injection of AFP-464 (5 mg/kg) to two dogs revealed that AFP-464 was rapidly converted to the parent compound (AF). The disposition of AF was biexponential with an initial phase half-life of 25 minutes and a terminal phase of 161 minutes. The total plasma clearance rate was 63 mL/min/kg, and the apparent volume of distribution was 15 L/kg. Additional pharmacokinetic parameters were obtained in mice and rats administered AF. Similar to the findings in dogs, the IV injection of the parent drug in mice showed a biexponential decline of plasma AF.

Toxicology

Toxicology studies of aminoflavone have been performed in mice, dogs, and monkeys. Two dogs that were administered 114 and 228 mg/m² AFP-464, respectively, as a 3-hour infusion once a week for 3 weeks, had lowered body weight and a decreased DL_{CO}; however, there were no apparent changes in total lung capacity and serum and bronchial alveolar lavage (BAL) cytokine analyses. Pulmonary arterial/thromboembolism was observed microscopically in one of four control dogs, in three of four treated dogs necropsied at day 16, and in one of four treated dogs necropsied at day 30. Lung histopathology of all treated dogs and three of the four control dogs had alterations characterized as multifocal chronic-active inflammation. The increased severity of pulmonary multifocal inflammation in the treated or previously-treated recovery dogs in comparison to vehicle dogs was interpreted to be due to AFP-464 administration. Other studies in mice administered three doses of 75 (IV), 150 (IV), or 600 (IP) mg/m²/dose AFP-464 showed that drug-related histopathologic lesions were present in the intestines, bone marrow, lymph tissue/nodes, and testes. AF also produced pulmonary toxicity in dogs and was non-toxic in mice after IV injections. In monkeys, administration of 180 mg/m² of the bromide salt of the aminoflavone prodrug (702295) given as a 3-hour infusion once a week for 3 weeks did not show any signs of toxicity and no histopathologic changes in the lungs were observed. The plasma concentration at the end of the 3-hour infusion was 1.2-1.5 μ M.

Request for Trials

CTEP is soliciting for phase 1 single agent trials of the aminoflavone prodrug, AFP-464, a flavone analog that shows antitumor activity possibly through the production of metabolites that covalently bind to DNA resulting in apoptosis or growth arrest of tumor cells. The proposed trial should include a weekly schedule of a 3-hour infusion of AFP-464 for 3 weeks, with an initial dose of 19

mg/m² IV based on one-sixth of the 114 mg/m² toxic nonlethal dose tested in dogs. Although nonclinical studies suggest that modulatory activity is achieved at concentrations of approximately 1-2 μ M, trial proposals should be designed to characterize the full toxicity profile up to the maximum tolerated dose (MTD). Based on *in vivo* toxicology studies, reversible pulmonary toxicity may possibly be a significant and dose limiting toxicity. Because impaired diffusion lung capacity of carbon monoxide (DL_{CO}) was the initial measurable pulmonary abnormality, baseline and continued serial monitoring of DL_{CO} should be incorporated into the treatment monitoring plan. Patients with prior lung radiation therapy as well as symptomatic pulmonary disease should be excluded from participating in these studies. A general phase I population should be evaluated, but proposals that enrich accrual of patients with papillary renal cell and hormone receptor positive breast cancer to determine an early signal of activity are encouraged.

Correlative studies should be included in the proposal. Evaluation of the pharmacokinetic profile of AFP-464 is considered a priority, but proposals for other studies will also be considered. Specifically, studies that evaluate the proposed mechanism of activation (activation of AhR pathway and induction of CYP1A1 and CYP1B1) and confirmation of mechanism of action (covalent binding of macromolecules) are of interest. Studies that will be helpful for predicting agent toxicity are also encouraged. Translational Research Initiative (TRI) funding may be available for some of these studies.

Evaluation criteria for proposals include scientific rationale, accrual potential, past performance in accrual to phase 1 studies, and adequacy of trial design, as well as the ability to perform relevant correlative laboratory studies. If an investigator intends to apply for TRI support for laboratory correlative studies, this should be noted in the LOI, and the LOI should include an estimated budget along with a very brief justification of cost (e.g., number of procedures or tests and cost per each). If the LOI is approved, CTEP will request that a formal budget for the TRI be sent with the protocol.

Questions regarding this solicitation may be addressed to Jennifer Low, M.D., Ph.D., Senior Investigator, Investigational Drug Branch, CTEP, NCI (phone: 301-496-1196; fax: 301-402-0428; e-mail: lowj@mail.nih.gov).

Letters of Intent should be e-mailed to the LOI Coordinator at the e-mail address below by midnight (EST) **April 12, 2005**. The most recent version of the LOI form available on the CTEP Website (<http://ctep.info.nih.gov>) must be used. **Electronic submission of LOIs is required.**

E-mail address: pio@ctep.nci.nih.gov

CTEP Website: <http://ctep.info.nih.gov/>

Questions: LOI Coordinator, phone (301) 496-1367

We ask that the approved site(s) plan to submit the protocol within 1 month of final approval of the LOI. We will provide information to facilitate rapid development of the protocol.

References

Akama, T., Y. Shida, T. Sugaya, *et al.* (1996). Novel 5-aminoflavone derivatives as specific antitumor agents in breast cancer. *J Med Chem* 39:3461-3469.

Kuffel, M.J., J.C. Schroeder, L.J. Probst, *et al.* (2002). Activation of the antitumor agent aminoflavone (NSC 686288) is mediated by induction of tumor cell cytochrome P450 1A1/1A2. *Mol Pharmacol.* 62:143-153.

Loaiza-Pérez, A.I., S. Kenney, J. Boswell, *et al.* (2004a). Sensitivity of renal cell carcinoma to aminoflavone: role of CYP1A1. *J Urology.* 171:1688-1697.

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