Aminoflavone Toxicology Summary

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Range-finding toxicology studies with aminoflavone (NSC-686288) and aminoflavone prodrugs (bromide salt, NSC-702295 and methane sulfonate salt, NSC-710464) were conducted in mice, dogs and non-human primates. Bolus i.v. doses of aminoflavone (NSC-686288) produced pulmonary toxicity in dogs but were non-toxic in mice. In vitro efficacy data predicted that a plasma concentration of ~1 μM would be therapeutically active, so the toxicity of NSC-702295 and NSC-710464 was evaluated in non-human primates and dogs, respectively following a 3 hour infusion. In non-human primates, doses of 180 mg/m² of the trihydrobromide aminoflavone prodrug given as a 3-hour infusion once a week for 3 weeks (total dose: 540 mg/m²) were well tolerated with no clinical signs of toxicity or changes in clinical pathology parameters. Plasma aminoflavone concentrations at the end of the 3-hour infusion ranged from 1.2-1.5 μM. No histopathological lesions were present in the lungs. In a similar study using dogs (1), aminoflavone prodrug AFP-464 (methane sulfonate salt, NSC-710464) was administered as a 3-hour infusion once a week for 3 weeks at a dose of 228 mg/m² (total dose: 684 mg/m²). Drug-related emesis occurred on dosing days, but there were no other clinical signs of toxicity or changes in clinical pathology parameters. Plasma aminoflavone levels during the 3-hour infusion ranged from 0.96-0.99 μM. Drug-related histopathological lesions in the lungs included inflammation, fibrosis and cellular hyperplasia. No lesions were present in heart, liver, kidney, spleen or brain.

Two groups of mice were treated with AFP-464 with intravenous doses of 75 and 150 mg/m^2/dose given i.v. hourly for a total of 3 doses (total dose: 225 and 450 mg/m^2), while a third group was administered 600 mg/m^2/dose i.p. hourly for a total of 3 doses (total dose: 1800 mg/m^2). Drug was administered in 10% DMSO in saline. Doses of 150 mg/m^2/dose were lethal after the first or second injection. In the 75 mg/m^2/dose group, 10% of the mice died after the third dose was administered. In the 600 mg/m^2/dose (i.p.) group, 2/10 male and 10/10 female mice died 2-3 days after dosing. Drug-related body weight loss and leukopenia (lymphopenia) occurred in the 200 mg/kg/dose (i.p.) group. In mice, drug-related histopathological lesions were present in the intestines, bone marrow, lymph tissue/nodes and testes.

In a GLP toxicology study (2), dogs were administered either 114 mg/m² or 228 mg/m² of aminoflavone prodrug AFP-464 as a 3-hour infusion, once a week for 3 weeks (total dose: 342 mg/m² and 684 mg/m², respectively). Parameters examined included clinical observations, body temperature, body weight, diffusion lung capacity of carbon monoxide (DL_{CO}), thoracic auscultation with respiratory rate, bronchial alveolar lavage (BAL) and serum sampling for cytokine analysis, hematology, clinical chemistry, gross necropsy examination and microscopic examination of the lungs. Drug-related emesis and swelling at the infusion site(s) occurred primarily after the second and third administration of AFP-464. Lethargy was noted in the 228 mg/m^2 group and was

attributed to AFP-464 administration. Dose-related decreases in body weights were noted in animals administered 114 and 228 mg/m^2 from Day 8 to 23. Increases in respiratory rates with associated breath sounds were noted in dogs receiving 228 mg/m^2. A 24% decline from baseline in diffusion lung capacity of carbon monoxide was noted in animals receiving 114 mg/m^2 following the third dose. Dogs receiving 228 mg/m^2 of AFP-464 had a 30% decrease in diffusion lung capacity of carbon monoxide following the second dose and a 43% decrease in diffusion lung capacity of carbon monoxide following the third dose. A similar decline in the diffusion constant (K_{CO}) was also noted. At the end of recovery (Day 30), the DL_{CO} and K_{CO} values returned towards baseline values. There was no apparent change in total lung capacity (TLC_{STPD}) indicating no impairment of overall mechanical ventilation capacity. No significant changes were noted in serum or BAL cytokine analyses. Microscopically, the lungs of all treated dogs and three of the four vehicle 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. Administration of aminoflavone prodrug AFP-464 (NSC-710464D) to dogs by a three-hour continuous intravenous infusion at 114 or 228 mg/m^2 once a week for three consecutive weeks was determined to produce pulmonary effects which appeared to be reversible.

In conclusion, target plasma concentrations of ~1.0 μM given as a 3 hour infusion were well tolerated in non-human primates with no evidence of pulmonary toxicity, while dogs were more sensitive to the pulmonary toxic effects of AFP-464. In contrast, in mice, AFP-464 produced bone marrow and intestinal toxicity. Although these data would support recommending a starting dose of 38 mg/m^2 for Phase I clinical trials based on one-sixth of the highest nonlethal dose in dogs, 19 mg/m^2 was actually used. In addition, DL_{CO} measurements were incorporated into the clinical protocol to monitor possible changes in pulmonary function due to toxicity.

VI. REFERENCES

1. Brown, AP, Morrissey, RL, Rodvold, ka, Tolhurst, TA, Donohue SJ, Tomaszewski, JE and Levine, BS. (1999) Intravenous plasma elimination kinetics and toxicity of an aminoflavone in the dog. Proc. Amer. Assoc. Cancer Res 40:390

2. Grossi IM, Lynch M, Merrill JW, Covey JM, Tomaszewski JE and Peggins JO. Pulmonary function effects of a three-hour infusion of aminoflavone prodrug (NSC-694501) in dogs. [Abstract Number: 686] *The Toxicologist*, 73, March 2004.