

PHASE I ACCELERATED DOSE-ESCALATING SAFETY AND PHARMACOKINETIC (PK) STUDY OF ARYL-HYDROCARBON RECEPTOR-MEDIATED AMINOFLAVONE PRODRUG, AFP464, IN ADVANCED SOLID TUMORS.

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ABSTRACT

Background: AFP464 is a dimethanesulfonate derivative of aminoflavone that has strong anti-tumor activity against a variety of solid tumors through the Aryl hydrocarbon receptor (AhR) mediated signal transduction. In-vitro and in-vivo studies have shown that tumors having AhR localized in the cytoplasm are very sensitive to AFP464 while those having AhR localized in the nucleus are not. **Methods:** Patients (pts) with advanced solid tumors refractory to conventional therapy received AFP464 as a 3-hr IV infusion on Days 1 and 8 every 21 days. Cohorts of one patient per dose level were enrolled until a Grade 2 adverse event was observed, after which cohorts of 3-6 pts were treated. Ten additional pts were treated at the MTD for pharmacodynamic evaluation. Inclusion criteria included age > 18 yrs, ECOG PS 0-2 and adequate organ functions. Tumor response was assessed by RECIST. Plasma was sampled for PK. **Results:** 30 heavily pretreated pts (median of 6 prior regimens, range 1-14) were enrolled, with a median age of 59 yrs. Seven dose levels from 14 to 103 mg/m² were evaluated. At 103 mg/m², 2/3 pts had DLT, one with a G3 decreased DLco and one with bilateral pulmonary infiltrates which resolved with steroid treatment. The MTD and recommended phase II dose was 74 mg/m² where 1/6 patient had asymptomatic G3 decreased DLco. Across all dose levels, regardless of drug causality, nausea/vomiting (42%) and asthenia (35%) are the most common adverse events, followed by pyrexia (27%), anemia (19%), dyspnea (19%), diarrhea (15%), cough (12%) and fatigue (12%). DLco decreases were observed in 11 (37%, 8 being G2 or 3) of patients across different dose levels. PKs of AF were dose proportional with C_{max} at the MTD dose being 30 times the I_{C₅₀ of sensitive breast cancer cell lines. 9/25 (36%) evaluable pts to date had stable disease of up to 6 cycles (median 4, range 2-6, breast, ovarian, renal, CRC, prostate, thyroid and mesothelioma). **Conclusions:** AFP464 administration is feasible with minimal bone marrow suppression. The high percentage of stable disease observed in a highly refractory patient population merits further phase II testing. Immunohistochemistry analysis of AhR localization from patients' tumor biopsies is ongoing and may be used to select pts who are most likely to benefit from treatment with AFP464 in future phase II-III studies.}

STUDY RATIONALE

Through in-vivo and in-vitro testing AFP464 has been shown to promote translocation of the arylhydrocarbon receptor (AhR) from the cytoplasm to the nucleus, resulting in induction of CYP1A1 transcription. This enzyme expression converts AF to metabolites that are covalently bound to DNA. This results in phosphorylation of p53 with induction of the p53 downstream target p21^{Waf1/Cip1} and apoptosis.

STUDY OBJECTIVES

- To determine the dose limiting toxicity (DLT) and maximum tolerated dose (MTD)
- To assess the safety, tolerability and pharmacokinetics
- To observe antitumor activity and explore correlative biomarkers and pharmacogenetics predictive of AFP464 efficacy

TREATMENT ADMINISTRATION

AFP464 is administered as a 3-hour infusion on Days 1 and 8 of a 21-Day cycle. The following seven dose levels were evaluated in this study: 14, 19, 27, 38, 53, 74, and 103 mg/m²/day.

STUDY METHODS

Patient Inclusion: Patients with advanced stage, treatment-refractory solid tumors for whom standard therapies are not available were considered for enrollment. Additional inclusion criteria included ≥ 18 years of age, life expectancy > 12 weeks, ECOG performance status ≤ 2, normal organ and marrow function, prior chemotherapy is allowed provided there is a 4-week washout and the patient must have full recovery from the effects of the chemotherapy agent. Patients must have adequate pulmonary function and DLco grade of 0 or 1 at baseline (within 4 days of first dose of study medication) as defined:

- Grade 0** – DLco is within the normal range: greater than the institutional lower limit of normal (LLN) or less than 3 units (mL/min/mmHg) below the LLN
- Grade 1** – DLco is 3 to 5 units (mL/min/mmHg) below the LLN
- Grade 2** – DLco is greater than 5 units (mL/min/mmHg) below LLN

STUDY METHODS (CONCLUDED)

Patient Exclusion: pregnant females, symptomatic pulmonary disease, brain metastases, receipt of other investigational agents, history of allergy to compounds with similar structure or biologic composition, uncontrolled intercurrent illness, and patients known to be HIV positive.

Dose Limiting Toxicity: Drug related toxicities that occur during the 21-day period of Cycle 1 will be considered when determining the DLT. DLT is defined as one or more of the following:

- ≥ Grade 3 non-hematological toxicity (excluding untreated nausea or vomiting, or alopecia)
- ≥ Grade 3 nausea, vomiting or diarrhea uncontrolled by aggressive treatment
- Grade 4 granulocytopenia lasting ≥ 5 days without hematopoietic growth factor supplement; or febrile/neutropenia
- Inability to receive subsequently planned weekly dose due to toxicity
- Inability to begin the next cycle of treatment (at full dose) within two weeks of the scheduled dose due to unresolved toxicity.
- Thrombocytopenia (platelet count < 25,000 cells/mm³)
- Certain Grade 2 toxicities, which, in the judgment of the investigator and sponsor are of clinical significance.
- Grade 3 DLco or Grade 2 DLco persisting for more than 14 days

DLco Grading Post Baseline

DLco assessments are scheduled before the initiation of every cycle and before D8 of cycle 1. Additionally, if a patient presents at any time with pulmonary symptoms (dyspnea, cough) a DLco assessment should be completed to ensure stable lung functioning before continued dosing. A patient's dose should be held if their DLco is grade 2 or greater. Patient's who have had their dose held due to G2 DLco should be monitored weekly until their DLco returns to G1 or G0. Patients who recover within 2 weeks may, at the discretion of the investigator, be dosed at one dose level lower. These patients should have their DLco monitored prior to every future dose.

- Grade 0** – A less than 3 units (mL/min/mmHg) decrease in DLco from baseline
- Grade 1** – A 3 to 5 unit (mL/min/mmHg) decrease in DLco from baseline
- Grade 2** – A >5 to 8 unit (mL/min/mmHg) decrease in DLco from baseline
- Grade 3** – A >8 unit (mL/min/mmHg) asymptomatic decrease in DLco from baseline or >5 unit decrease along with the presence of G2 pulmonary symptoms (e.g. dyspnea or hypoxia)
- Grade 4** – Definition of G3 along with pulmonary symptoms meeting the definition of a serious adverse event

Dose Escalation Dose Escalation began with single patient cohorts and expanded to 3 patient cohorts when Grade 2 or greater toxicity was observed. Once DLT has been identified, the cohort will be expanded to 6 patients at that dose level. Dose escalation will continue until MTD is defined, and 10 additional patients will be treated at the MTD.

Definition of MTD: MTD is defined as the highest dose level where a maximum of 1 of 6 patients experiences a DLT.

Response: Assessed according to RECIST.

PATIENT CHARACTERISTICS (N=38)

| | | | |
|--------------------|----------|----------------------|-------------------|
| Median Age (years) | 59.5 | ECOG PS 0-1 n(%) | 30 (100%) |
| Male n(%) | 16 (53%) | Median # prior chemo | 6 (range 1 to 14) |

ADVERSE EXPERIENCES

Across all dose levels, regardless of drug causality, nausea (50%), vomiting (47%) and asthenia (43%) were the most common adverse events, followed by pyrexia (33%), constipation and dyspnea (23%), diarrhea (20%), anemia (17%), anorexia (13%), and abdominal pain, fatigue, pain, athermalgia, back pain and muscle spasms (10%); most were Grade 1 or 2. One patient experienced G3 elevation of AST and ALT (C4D13) and one patient who started the study with baseline G2 anemia experienced G3 anemia (C1D8). DLco decreases were calculated in 11 (37%) of the treated patients. Of the 11, 8 were G2 or greater (1 at 53 mg/m², 4 at 74 mg/m² and 3 at 103 mg/m²). The patient at 53 mg/m² was asymptomatic with a G2 DLco on C1D8 and continued the study for a total of 6 cycles. All four patients at 74 mg/m² were discontinued due to the events though one was asymptomatic. Two of the patients at 103 mg/m² were discontinued due to their events while one patient had a G2 DLco on C2D8 which resolved within 2 weeks and was treated at a reduced dose of 74 mg/m² for one extra dose before discontinuing due to personal reasons.

SUMMARY OF RESULTS

| Dose (mg/m ² /Day) | No. Pts. Treated | DLT Events | No. Stable Disease (# cycles) |
|-------------------------------|---|--|---|
| 14 | 1 (colon) | None | 1 colon (2) |
| 19 | 1 (ovarian) | None | 0 |
| 27 | 3 (breast, renal, colon) | None | 1 ER- breast (4) 1 renal (4) |
| 38 | 3 (2 rectal, retroperitoneal) | None | 1 rectal (2.5) |
| 53 | 3 (sigmoid, mesothelioma, prostate) | None | 1 mesothelioma (6) |
| 74 | 6 (thyroid, colon, prostate, melanoma, liver, pancreas) | 1 asymptomatic G3 DLco (C2D1) | 1 prostate (6) 1 thyroid (6) |
| 103 | 3 (retroperitoneal, colon, renal) | 1 G4 pneumopathy* (C1D9); 1 G3 lung emboli/G3 DLco (C1D8) | 1 Renal (2.5) |
| 74 (MTD Cohort) | 10 (6 breast, 3 ovarian, 1 pancreas) | 1 G3 DLco w/ G2 Dyspnea (C1D8) 1 G3 DLco w/ G3 Dyspnea (C3D1) 1 G4 DLco w/ G3 Dyspnea (C4D8) | 1 TN Breast (4) 1 Ovarian (3) 1 ER/PR+ Breast (2.5) 1 Pancreas (3.5) |

*Pts erroneously treated while having G2 DLco, developed bilateral pulmonary infiltrates, resolved with steroids

MEAN AF PHARMACOKINETICS PARAMETERS

| Dose (mg/m ²) | Cycle | N | C _{max} (ng/mL) | T _{max} (hr) | AUC _{0-∞} (hr*ng/mL) | t _{1/2,2} (hr) | CL (L/hr/m ²) |
|---------------------------|-------|----|--------------------------|-----------------------|-------------------------------|-------------------------|---------------------------|
| 14 | 1 | 1 | 29 | 2.8 | NE | NE | NE |
| | 2 | 1 | 25 | 3.4 | NE | NE | NE |
| 19 | 1 | 1 | 64 | 2.5 | NE | NE | NE |
| | 2 | 1 | 64 | 3.0 | NE | NE | ME |
| 27 | 1 | 3 | 149 | 2.4 | 392 | 1.9 | 56 |
| | 2 | 3 | 181 | 2.0 | 448 | 1.9 | 49 |
| 38 | 1 | 3 | 146 | 2.4 | 520 | 4.4 | 65 |
| | 2 | 2 | 165 | 2.0 | 460 | 3.2 | 62 |
| 53 | 1 | 3 | 161 | 2.0 | 488 | 1.8 | 104 |
| | 2 | 2 | 169 | 2.5 | 4.1 | 1.7 | 97 |
| 74 | 1 | 14 | 481 | 2.4 | 1276 | 2.9 | 46 |
| | 2 | 9 | 390 | 2.7 | 1014 | 2.1 | 55 |
| 103 | 1 | 3 | 519 | 2.0 | 1388 | 1.9 | 64 |
| | 2 | 1 | 599 | 2.0 | 1624 | 1.4 | 45 |

CONCLUSION

- Pulmonary toxicity was found to be the Dose Limiting Toxicity. Some patients experiencing DLco decrease with or without corresponding dyspnea; and a few experienced pulmonary infiltrates which resolved after discontinuation of treatment with or without steroid administration.
- No major hematologic toxicity was observed.
- AF plasma concentrations were dose proportional, non-cumulative between cycles and the C_{max} obtained at the MTD dose (74 mg/m²/day) far exceeded concentrations required for in-vitro growth inhibition of a variety of cell lines such as MCF-7 (30 times the IC90)
- Stable disease of up to 6 cycles was observed in 40% of this heavily pretreated patient population is encouraging and merits further Phase II testing.