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Phase I dose escalation study of the PKC ι inhibitor aurothiomalate for advanced non-small cell lung cancer, ovarian cancer and pancreatic cancer

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Abstract

Objective—Protein kinase C ι (PKC ι) is overexpressed in non-small cell lung (NSCLC), ovarian and pancreatic cancers where it plays a critical role in oncogenesis. The gold compound aurothiomalate (ATM) has been shown to inhibit PKC ι signaling and exhibits potent anti-tumor activity in preclinical models. We sought to determine the maximum tolerated dose (MTD) of ATM.

Methods—We conducted a phase I dose escalation trial of ATM in patients with NSCLC, ovarian or pancreatic cancer. Patients received ATM IM weekly for three cycles (cycle duration 4 weeks) at 25 mg, 50 mg or 75 mg in a 3+3 design. The dose was not escalated for individual patients. Blood samples were analyzed for elemental gold levels. Patients were evaluated every four weeks for toxicity and every eight weeks for response.

Results—Fifteen patients were enrolled in this study. Six patients were treated at 25 mg, 7 patients at 50 mg, and 2 at 75 mg. There was 1 dose limiting toxicity at 25 mg (hypokalemia), one at 50 mg (urinary tract infection), and none at 75 mg. There were 3 grade 3 hematologic toxicities. The recommended MTD of ATM is 50 mg. Patients received treatment for a median of 2 cycles (range 1-3). There appeared to be a dose-related accumulation of steady-state plasma concentrations of gold consistent with linear pharmacokinetics.

Conclusions—In summary, this phase I study was successful in identifying ATM 50 mg IM weekly as the MTD. Future clinical investigations targeting PKC ι are currently in progress.

Keywords

protein kinase C ι ; aurothiomalate; non-small cell lung cancer; ovarian cancer; pancreatic cancer

Introduction

Protein kinase C (PKC) isoenzymes play key regulatory roles in many cellular functions including control of the proliferation, motility and survival of human cancer cells [1]. The atypical protein kinase C ϵ (PKC ϵ) is overexpressed in, and plays a critical role in, the oncogenic growth of non-small cell lung cancer (NSCLC) [2], ovarian cancer[3], pancreatic cancer[4], and colon cancer [5]. In lung squamous cell carcinoma and serous ovarian cancers, overexpression of PKC ϵ is often the result of tumor-specific *PRKCE* gene amplification[2]. The function and oncogenic activity of PKC ϵ is dependent upon a NH₂ terminal regulatory Phox and Bem1p (PB1) domain that mediates specific protein-protein interactions[6]. A fluorescence resonance energy transfer assay was developed to detect inhibition of PB1-PB1 domain interactions between PKC ϵ and Par6, a key adapter protein that is required for the oncogenic activity of PKC ϵ [6]. The gold compounds aurothioglucose (ATG) and aurothiomalate (ATM) were found to inhibit PB1-PB1 domain interactions between PKC ϵ and Par6 and therefore block downstream activation of Rac1, a key effector of PKC ϵ -Par6-dependent oncogenic signaling [6]. Mechanistically, ATM was found to directly bind to a specific cysteine residue unique to the PKC ϵ PB1 domain and thereby inhibit binding of Par6 [7]. Furthermore, mutation of this key cysteine residue confers resistance to ATM-mediated inhibition of transformed growth, demonstrating that the anti-tumor activity of gold salts are due to their ability to disrupt the PKC ϵ -Par6 interaction[7]. ATM and ATG have been used for the treatment of rheumatoid arthritis for years[8], but novel agents are now used more commonly for that disorder. Though ATG and ATM are thought to interact with NF- κ B as well as other cellular proteins, their exact mechanism of action as anti-inflammatory agents remains uncertain[9, 10].

Since PKC ϵ is overexpressed in many tumor types and its expression is required for tumorigenicity, we sought to determine at what dose ATM, a selective inhibitor of PKC ϵ , was safe for use in patients with NSCLC, ovarian cancer or pancreatic cancer by conducting a phase I dose escalation trial. Additionally, we sought to determine early evidence of clinical activity of ATM in these cancer types.

Methods

Eligibility

Patients with pathologically proven advanced NSCLC, ovarian cancer or pancreatic cancer that were able to provide informed consent were eligible for this trial. Furthermore, patients had to be 18 years of age or older, consent to blood draws, and have an estimated life expectancy greater than 12 weeks. Subjects were required to have an absolute neutrophil count (ANC) $\geq 1500/\mu\text{L}$, a platelet count $\geq 100,000/\mu\text{L}$, total bilirubin $\leq$ two times the upper limit of normal (ULN), AST $\leq 3\times$ ULN, ALT $\leq 5\times$ ULN, creatinine $\leq 1.2\times$ ULN and a hemoglobin $\geq 9\text{g/dL}$. Women of childbearing potential were required to have a negative pregnancy test. Exclusion criteria included known potentially curable cancer, ECOG performance status ≥ 3 , uncontrolled infection, failure to recover from effects of previous chemotherapy treatments, NYHA classification $\geq$ III, symptomatic or worsening CNS metastases, and known allergy to ATM. Pregnant women, nursing women, and subjects who were unwilling to employ adequate contraception were also excluded. Subjects who received chemotherapy, immunotherapy, biologic therapy, or radiation therapy within three weeks of registration, or mitomycin C and nitrosurea within six weeks of registration, or radiation to more than 25% of their bone marrow were also excluded.

Study Design

After registration, patients underwent hypersensitivity testing with 10 mg of ATM intramuscularly one week prior to initiation of cycle one. Subjects sensitive to ATM were replaced; those without sensitivity to ATM proceeded to dose assignment. In cohort I, three patients were to be treated at each dose level of ATM at 25 mg, 50 mg or 75 mg and observed for a minimum of four weeks before new subjects were treated. Patients received ATM intramuscularly weekly for up to three cycles (cycle duration was four weeks) then once every four weeks until a cumulative dose of 1 gram of ATM was reached. Doses were not escalated in any individual patient. In cohort II, up to nine additional patients with pancreatic adenocarcinoma were allowed to enroll in an expansion cohort to more fully assess toxicities and perform translational and pharmacokinetic studies.

Assessment

Dose limiting toxicity (DLT) was defined as any grade 4 ANC for 5 days, grade 4 hemoglobin or platelet count $<25,000/\mu\text{L}$, serum creatinine two times baseline, or any grade 3 non-hematologic event as determined by the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Additionally, any toxicity requiring therapy to be held for four weeks or more was considered a DLT.

The maximum tolerated dose (MTD) was defined as the dose level below the lowest dose that induced DLT in at least one-third of patients (at least 2 of a maximum of 6 new patients). Three patients were assigned to each dose level then observed for at least four weeks to assess toxicity before new patients were enrolled. If no DLT was observed, three new patients were accrued to the next higher dose level. If a DLT was seen in two or three of three patients at a given dose level, then the next three patients were treated at the next lower dose level. If DLT was seen in one of three patients treated at a given dose level, up to three additional patients were enrolled and treated at the same dose level. If DLT was seen in at least one of these additional three patients the MTD was exceeded, and the MTD was defined as the previous dose unless only three patients were treated at that dose level. In that case, three additional patients were treated at that lower dose level.

Patients were evaluated for response every eight weeks with computed tomography using modified response evaluation criteria in solid tumors (RECIST) criteria. The best overall response was recorded from the start of treatment until the time of disease progression or recurrence. Blood samples were collected at baseline and prior to therapy on weeks 3, 5, 7, 9 and 11 to determine steady state levels of total serum gold.

The primary end point of this trial was MTD of ATM in advanced NSCLC, advanced ovarian cancer and advanced pancreatic adenocarcinoma. Secondary endpoints included a description of toxicities associated with ATM, any preliminary evidence of biologic activity of ATM, correlations of PKC ζ expression and anti-tumor effects of ATM, associations of clinical toxicity and response with steady state levels.

Statistics

Basic statistics were used to describe patient characteristics, MTD, toxicities, and overall best response.

Results

Seventeen patients were enrolled into this trial between February 14, 2007 and December 10, 2010 (Table 1). There were sixteen patients enrolled into the initial dose escalation cohort, and one patient enrolled into the expansion cohort. Two patients from cohort one

were replaced due to sensitivity to ATM. The median age of patients treated in this trial was 62 years (range 47-74). All patients had prior surgery for their underlying malignancy, and almost half (7/15) had received prior radiotherapy. Patients received a median of 2 cycles of therapy (range 1-3) and discontinued treatment primarily due to progression of disease (Table 2).

All patients were evaluable for DLT. Six patients were treated at dose level one. One of these patients experienced a grade 3 DLT (hypokalemia). Six patients were treated at dose level two, and one of these patients experienced a grade 3 DLT (urinary tract infection). Neither of the two patients at dose level three experienced a DLT (Table 3). Due to the concern of repeated dosing at dose level three, the MTD recommended for the expansion cohort was dose level 2, 50mg of ATM. The one patient treated in cohort two at dose level two experienced a DLT (hypokalemia) (Table 3).

There were no grade four toxicities seen in this trial. There were three grade three hematologic toxicities in cohort one: lymphopenia (2) and decreased CD4 count (1). There were also three non-hematologic grade three toxicities: urinary tract infection in cohort one (1), and one instance of hypokalemia (2) in each cohort (Table 4).

Two of 14 treated patients in cohort I had stable disease for one cycle as their best response (Table 2). All other patients, including the one in cohort two, had progressive disease at reassessment or declined further therapy. The two patients with stable disease did not experience any grade 3 or 4 toxicities.

Levels of elemental gold in plasma were measured to reflect ATM levels. There appeared to be a dose-related accumulation of steady-state plasma concentrations of gold with concentrations exceeding 20 μ M after one month of therapy with 75 mg of ATM and after 2 months of therapy with 50 mg of ATM (Figure 1).

Discussion

We recommend a 50 mg dose level of ATM for future study. Treatment with ATM was complicated by hematologic toxicities. We also observed two instances of hypokalemia, and a urinary tract infection. The best response observed in two of the fifteen treated patients was stable disease for one cycle.

PKC ι is overexpressed in NSCLC[2], ovarian cancer[3] and pancreatic cancer[4], and plays a critical role in tumorigenesis[11]. ATM forms a thio-gold adduct with a cysteine at position 69 of PKC ι which is within thePB1 domain binding interface between PKC ι and its binding partner Par6 thus hindering interaction of PKC ι with Par6[7]. This study was not designed to determine the efficacy of ATM, and we observed only limited anti-tumor activity with ATM. Since these patients were not stratified based on PKC ι expression, it is possible that the patients in this study had tumors that did not overexpress PKC ι or whose tumors were not driven by PKC ι . In this regard, our pre-clinical studies in non-small cell lung cancer indicate that PKC ι expression is positively associated with response to ATM both in vitro and in vivo[12]. Additionally, since these patients were heavy pre-treated with multiple prior chemotherapies, they likely have developed multi-drug resistance that may have conferred resistance to ATM. It is possible that the doses of ATM utilized in this trial were not sufficient to result in adequate inhibition of tumor-associated PKC ι . However, the serum gold levels achieved in these studies were at or above the levels necessary to achieve potent anti-tumor effects, and effective inhibition of tumor PKC ι signaling, in pre-clinical NSCLC xenograft models[13], suggesting that sufficient drug levels were achieved using this regimen.

In summary, this phase I study was successful in identifying ATM 50 mg IM weekly as the maximally tolerated dose. In this heavily pre-treated group of patients, stable disease was the best response. Future clinical investigations targeting PKC ι are currently in progress.

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Figure 1.
Elemental Gold Plasma Concentrations
Serum gold concentrations from patients undergoing therapy with ATM. The shaded area indicates the calculated IC_{50} range for ATM in the panel of NSCLC cells used in the pre-clinical studies in vitro and in vivo.

Table 1

Treated Patient Characteristics

	Overall (n=15)	Cohort I (n=14)	Cohort II (n=1)
Age (median, range)	62 (47-74)	59 (47-74)	67
Gender (number, %)			
Male	6 (40%)	5 (35.7%)	1 (100%)
Female	9 (60%)	9 (64.3%)	
Primary Tumor			
Lung	10	10	
Ovarian	4	4	
Pancreas	1		1
Prior Treatments (number, %)			
Radiation Therapy	7 (46.7%)	7 (50%)	
Surgery	15 (100%)	14 (100%)	1 (100%)

Table 2

Treatment summary

	Overall (n=15)	Cohort I (n=14)	Cohort II (n=1)
Cohort I			
Dose level 1 *		6	
Dose level 2		6	
Dose level 3		2	
Cohort 2			
Dose level 2 *			1
Number of cycles received, median (range)	2 (1-3)	2 (1-3)	1
Best Response			
SD	2 (13.3%)	2 (14.3%)	
PD	13 (86.7%)	12 (85.7%)	1 (100%)
Reason for discontinuation			
Progressive disease	14(82.4%)	13 (81.3%)	1 (100%)
Declined further treatment	1 (5.9%)	1 (6.3%)	

* Starting dose for cohort

Table 3

Dose limiting toxicity summary

Cohort 1		
Dose level	No. Patients	DLTs
1	6	1
2	6	1
3	2	0
Cohort 2		
Dose level	No. Patients	DLTs
2	1	1

This table represents a summary of DLTs by dose level for patients who were not sensitive to ATM.

Table 4

Overall toxicities

Toxicity	Grade	
	2	3
Decreased CD4 count		1
Anemia	2	
Lymphopenia	3	2
Neutropenia	1	
Leukopenia	1	
Urinary tract infection		1
Hypophosphatemia	1	
Hypokalemia		2
Cough	1	
Dyspnea	1	
Prolonged aPTT	1	
Fatigue	1	
Alopecia	1	
Hyperpigmentation	1	
Dysphagia	1	

There were no grade four toxicities reported. The only toxicity reported in cohort two was a grade three hypokalemia. All other reported toxicities are from cohort one.