# REQUEST FOR PROJECT TEAM APPLICATIONS
# for Conducting Clinical Trials Using AT13387 (NSC 749712)

The Cancer Therapy Evaluation Program (CTEP) is accepting Project Team Applications (PTAs) for a project using AT13387, a non-ansamycin synthetic small molecule inhibitor of heat shock protein 90 (HSP90) being developed by CTEP as an anticancer agent in collaboration with Astex Pharmaceuticals, Inc., a subsidiary of Otsuka Pharmaceuticals. HSP90 facilitates the posttranslational folding and stabilization of a distinct set of proteins, called HSP90 client proteins, which includes several oncogenes. Inhibition of HSP90 leads to the degradation of its client proteins and thereby disrupts signaling pathways necessary for cellular growth and proliferation. AT13387 has been shown to inhibit cell growth and survival in multiple tumor cell lines; activity has been demonstrated in several tumor xenograft models including breast, colon, acute myeloid leukemia (AML) (Investigator's Brochure, 2012), non-small cell lung cancer (NSCLC), gastrointestinal stromal tumors (GIST), melanoma, and prostate cancer (Investigator's Brochure, 2013). AT13387 has been studied in five clinical trials in patients with solid tumors/GIST/prostate/NSCLC, when administered as monotherapy or in combination on different intermittent schedules, *i.e*., once weekly (QW) for 3 weeks (QWx3), twice weekly (2QW) several days apart (2QW) for 3 weeks (2QWx3), or twice weekly on 2 consecutive days for 3 weeks (QDx2 for QWx3). At present, CTEP is interested in developing phase 1/1b combination trials with the intent to take these combinations into phase 2 trials with AT13387 in the following histologies:

- Head and neck cancer, either frontline with radiation or with chemotherapy or targeted agents in frontline or at recurrence
- Epidermal growth factor receptor (EGFR) targeted therapy-resistant NSCLC in combination with paclitaxel or frontline combination with EGFR inhibitor to prevent/delay development of resistance
- Triple negative breast cancer (TNBC) or receptor-positive hormone refractory breast cancer as a single-agent study or in combination with a taxane, possibly in the maintenance phase after adjuvant therapy
- ALK+ lymphoma

Additionally, CTEP would be interested in investigations of biomarkers, including genomic biomarkers that may predict for response to treatment.

**Background/Rationale**

HSP90 is a ubiquitously expressed and highly abundant molecular chaperone involved in the functional stabilization and the final maturation of a large number of client proteins, which are central players in key signal transduction pathways; many of which are also directly implicated in cancer progression (Citri *et al.* 2006, Woodhead *et al*., 2010). Adenosine triphosphate (ATP) binds to the N-terminal domain of HSP90, and the hydrolysis of ATP catalyzes the conformational maturation of HSP90 client proteins. Inhibition of ATP binding to HSP90 has been shown to induce the degradation of client proteins and has the potential to prevent signaling through multiple growth pathways and receptors simultaneously, thereby overcoming established methods of treatment resistance. HSP90 and other molecular chaperones are induced in response to a variety of stress factors, including heat, heavy metals, hypoxia, and acidosis (Whitesell and Lindquist, 2005). HSP90 is also expressed at levels 2- to10-fold higher in tumor cells than in normal cells, and overexpression of HSP90 has been correlated with decreased survival in breast cancer (Pick *et al.*, 2007). Increased constitutive levels of HSP90 have also been implicated in proliferation of leukemia cells (Yufu *et al*., 1992).

Mechanism of Action

AT13387 is a small molecule inhibitor of HSP90 that binds to the ATP site on the N-terminal domain of HSP90 with high affinity (dissociation constant [$K_d$] = 0.71 nM) (Investigator's Brochure, 2013). *In vitro* studies demonstrated that AT13387 induces down-regulation of key client proteins and causes growth arrest and apoptosis in a range of tumor cell lines. Antagonism at the ATP site of HSP90 caused by an HSP90 inhibitor leads to the inhibition of chaperone function and promotion of the degradation of its client proteins.

**Nonclinical Studies of AT13387**

*In Vitro* and *In Vivo* Activity

AT13387 potently inhibits cell proliferation across a wide panel of tumor cell lines (lung, melanoma, prostate, and GIST) with 50 of the 59 cell lines tested having half maximal inhibitory concentration ($IC_{50}$) values <100 nM (Investigator's Brochure, 2013). Treatment of tumor cells with AT13387 led to the degradation or depletion of multiple known HSP90 client proteins (*e.g.*, AKT, EGFR, BRAF, CRAF, EML4-ALK, and c-Kit). Antitumor activity correlated with reduced levels of phosphorylated proteins (phospho-AKT, phospho-ERK, phospho-cKit) and increased levels of HSP70. AT13387 has been evaluated and found active in multiple human cancer xenograft models in nude or severe combined immunodeficiency (SCID) mice, including breast (Her-2 positive BT474), colon (HCT116), AML (FLT3-ITD-positive MV-411) (Investigator's Brochure, 2012), NSCLC (mutant EGFR NCI-H1975 and ALK-driven crizotinib-sensitive NCI-H2228), melanoma (vemurafenib-sensitive A375 and SK-MEL-28, and vemurafenib-resistant A2058), GIST (imatinib-sensitive GIST-PSW and imatinib-resistant GIST-430), and prostate (hormone-resistant androgen receptor positive 22RV1) cell lines (Investigator's Brochure, 2013). AT13387 inhibited the growth of subcutaneous (SC) xenografts when administered in several different intermittent schedules. A comparison of several studies suggested that either QW or 2QW administration (Days 1 and 4) required less total compound, was better tolerated than more frequent schedules, and still significantly inhibited xenograft growth. The combination of paclitaxel (20 mg/kg) and AT13387 (55 mg/kg) achieved complete tumor growth inhibition during the 3-week dosing period in NCI-H1975 xenografts (Lyons *et al*., 2008). The combination of oral imatinib (50 mg/kg) and AT13387 (70 mg/kg intraperitoneally [IP]) caused tumor regression in GIST-PSW and enhanced inhibition compared to either agent alone in GIST-430 (Investigator's Brochure, 2013). The combination was well tolerated with no observable additive toxicities. AT13387 caused prolonged knockdown of HSP90 client proteins in cells and tumors with a half-life of 38-75 hours in tumor xenografts in mice, suggesting potential for prolonged effect after a single dose.

Pharmacokinetics

After intravenous (IV) administration to mice, rats, and dogs and IP administration to mice, AT13387 displayed a short plasma half-life ($t_{1/2}$) in mice and rats (1-3 hours) and a moderate $t_{1/2}$ in dogs (11 hours) in spite of high plasma clearance (CL) (Investigator's Brochure, 2013). Volume of distribution ($V_d$) was greater than total body water indicating distribution of AT13387 into tissues. In the tumor xenograft models, AT13387 was extensively distributed to tumor tissue and cleared from it at a considerably slower rate than from plasma, blood or muscle (normal tissues) (Lyons *et al*., 2008). Slow drug clearance from the tumor was consistent with AT13387-induced prolonged pharmacodynamics effects observed in the tumor. The *in vitro* intrinsic CL of AT13387 determined in isolated intact hepatocytes was high across all the species tested (mouse, rat, dog, and human; scaled values ranged from 35 mL/min/kg in human to 184 mL/min/kg in rat) (Investigator's Brochure, 2013). Binding of AT13387 to plasma proteins was moderate and comparable across all species tested, ranging from 77.2% in dog plasma to 90.1% in mouse plasma. The blood:plasma distribution in mouse, rat, dog, and human whole blood ranged from 0.8 to 5.0, indicating approximately equal distribution between the plasma and cellular fraction. The potential for AT13387 to inhibit cytochromes P450 (CYP) 1A2, 3A4, 2D6, 2C9, and 2C19 was assessed, and results indicated an $IC_{50}$>10 μM, suggesting a low potential for clinically significant drug-drug interactions mediated by these enzymes. Glucuronidation, sulphation, and N-oxidation appear to be routes of metabolism for AT13387 based on *in vitro* studies of cryopreserved hepatocytes as well as metabolites detected in samples *in vivo*.

Safety Pharmacology

No adverse central nervous system (CNS) effects were observed in the rat with AT13387 doses up to 200 mg/kg in males and 125 mg/kg in females, compared with control vehicle-treated animals (Investigator's Brochure, 2013). In a cardiovascular and respiratory (CV/R) safety study in beagle dogs, a dose-related increase in heart rate was observed at 4 mg/kg and above, from 10 minutes after the start of infusion, which peaked at the end of infusion and returned to control levels by approximately 5 hours after the end of infusion. The increased heart rate observed at 15 mg/kg was associated with a concomitant decrease in blood pressure

(systolic, diastolic, and mean arterial). AT13387 had no significant effect on QT interval or $QT_c$ at any dose level tested.

Toxicology

The toxicity profile of AT13387 was evaluated in rats and dogs (Investigator's Brochure, 2013). Clinical pathology changes suggestive of adverse effects in the bone marrow, kidney, and liver were observed for both species. Histopathologic changes were only seen in dogs and included testes, gallbladder, bone marrow (sternum), kidneys, and thymus. The overall effects observed in surviving rats and dogs were transient and reversible, with the exception of the testicular lesions observed in dogs at high doses, for which the recovery period of 14 days was not sufficient. In the definitive rat study, no unambiguous target organs of toxicity were identified, and the no-observed-adverse-effect level (NOAEL) was estimated to be 50 mg/kg/dose given 2QW for 3 weeks (2QWx3). A dose severely toxic to 10% of rodents ($STD_{10}$) could not be confidently determined in rats, although safety was established in this species. In dogs dosed via a peripheral vein, a clear dose-effect relationship was established for AT13387. The nominal highest non-severely toxic dose (HNSTD) was 3 mg/kg/dose given 2QWx3 and the NOAEL was taken to be 1 mg/kg/dose on the same schedule.

**Clinical Studies of AT13387**

Five clinical studies of AT13387 are active (four studies sponsored by Astex Therapeutics, Inc., and one by CTEP) (Investigator's Brochure, 2013). AT13387 has been administered as a 1-hour IV infusion at various intermittent dosing schedules:

- QWx3: once weekly for 3 weeks (days 1, 8, 15) on a 4-week cycle
- 2QWx3: twice weekly several days apart for 3 weeks (days 1, 4, 8, 11, 15, 18) on a 4-week cycle
- QDx2 for QWx3: twice weekly on 2 consecutive days for 3 weeks (days 1, 2, 8, 9, 15, 16) on a 4-week cycle.

**Table 1: Dosing regimens evaluated in the clinical studies**

| Study | Patient population | Treatment and schedule | Treated pts (*) of a total planned accrual |
|---|---|---|---|
| AT13387-01 (monotherapy) Ph1 dose escalation study | Metastatic solid tumors | Part 1: AT13387 (10, 20, 40, 80, and 120 mg/m$^2$) IV 2QWx3 (28-day cycle)<br>or<br>Part 2: AT13387 (150, 180, 220, 260, and 310 mg/m$^2$) IV QWx3 (28-day cycle) | 63 of 63 |
| AT13387-02 (monotherapy and combination) Ph2 randomized study | Unresectable and/or metastatic malignant GIST after progression with ≤3 TKIs | AT13387 (120, 150, 180, and 220 mg/m$^2$) IV QWx3 (28-day cycle)<br>+<br>Imatinib: 400 mg QD | 26 of 48 |
| AT13387-03/ CTEP 8828 (monotherapy) Ph1 dose escalation study | Refractory solid tumors | AT13387 (20, 40, 80, 120, 160, and 210 mg/m$^2$) IV QDx2 for QWx3 (28-day cycle)<br>Dose expansion at MTD (160 mg/m$^2$) | Completed accrual (31of 37) |
| AT13387-04 (monotherapy and combination) Ph1/2 randomized study | CRPC no longer responding to abiraterone | Ph1: AT13387 (220 mg/m$^2$) IV QWx3 (28-day cycle)<br>or<br>AT13387 (120 mg/m$^2$) IV QDx2 for QWx3 (28-day cycle)<br><br>Ph2: Best regimen for AT13387 from Ph1<br>+<br>Abiraterone 1000 mg PO QD + steroids 5 mg PO BID | 48 of 164 |

| Study | Patient population | Treatment and schedule | Treated pts (*) of a total planned accrual |
|---|---|---|---|
| AT13387-05 (monotherapy and combination) Ph1/2 randomized study | ALK+ NSCLC | Part A (Ph1): AT13387 (150, 180, and 220 mg/m$^2$) IV QWx3 (28-day cycle)<br>+<br>Crizotinib 250 mg PO BID<br><br>Part B (Ph2, randomized):<br>Single agent crizotinib<br>*vs.*<br>AT13387+crizotinib at MTD<br><br>Part C (Ph2, randomized):<br>Single agent AT13387<br>*vs.*<br>AT13387+crizotinib at MTD | open to accrual, total of 228 pts anticipated |

pts(*): patients treated as of February 2013
Ph: phase; IV: intravenous; QWx3: once weekly for 3 weeks; 2QWx3: twice weekly several days apart for 3 weeks; ; QDx2 for QWx3: twice weekly on 2 consecutive days for 3 weeks; GIST: gastrointestinal stromal tumor; TKI: tyrosine kinase inhibitor; QD: once daily; CRPC: Castration-resistant prostate cancer; NSCLC: non-small cell lung cancer; PO: orally; BID twice daily; ALK+: anaplastic lymphoma kinase positive

The MTD and recommended phase 2 dose (RP2D) for various dosing schedules are as follows:

- 2QWx3 (Days 1, 4, 8, 11, 15, 18) regimen in study AT13387-01: 120 mg/m$^2$/dose after a dose-limiting toxicity (DLT) of reversible grade 3 visual impairment (fuzzy vision, green hue, and peripheral flashing) with grade 3 changes in electroretinogram experienced by one patient (Mahadevan *et al*., 2012)
- QDx2 for QWx3 (Days 1, 2, 8, 9, 15, 16) regimen in study AT13387-03 (CTEP 8828): 160 mg/m$^2$/dose the DLT being elevated transaminases (Investigator's Brochure, 2013)
- QWx3 (Days 1, 8, 15) regimen in study AT13387-01: 260 mg/m$^2$/dose after the DLT defined as the combined effect of moderate toxicities mostly grade 2 diarrhea, nausea, vomiting, fatigue, and systemic infusion reactions (Mahadevan *et al*., 2012)

Pharmacokinetics

Pharmacokinetic (PK) data are available from two studies: AT13387-01 and AT13387-02 (Investigator's Brochure, 2013). Plasma sampling for the AT13387 PK was carried out on Days 1 and 18 (for the 2QW schedule) on Cycle 1 or on Days 1 and 15 (for the QW schedule) in AT13387-01. The PK of AT13387 showed dose-dependent increase in area under the plasma concentration-time curve from time 0 to the last data point ($AUC_{0-t}$) and maximum concentration ($C_{max}$) over doses ranging from 10 to 310 mg/m$^2$/dose. The PK of AT13387 showed inter-individual variability of 2–5-fold for $AUC_{0-t}$ and $t_{1/2}$ but up to 9-fold for $C_{max}$ within the cohorts. The $t_{1/2}$ was dose-independent with mean cohort values ranging from 6.6 to 11.5 hours. Maximum $t_{1/2}$ observed for any individual profile was 14 hours. Plasma CL of AT13387 was independent of dose (range 0.96–1.45 L/hr/kg). Less than 5% of the administered dose was recovered in the urine during 48 hours post-dose. The $V_d$ was high (9.8-22.9 L/kg). Preliminary investigations into the metabolism of AT13387 in humans have shown that the two isomeric O-glucuronide conjugates of AT13387 are detectable in the urine of patients dosed at 120 mg/m$^2$.

In AT13387-02, patients received IV infusion doses of AT13387 QW in combination with daily oral doses of imatinib (Investigator's Brochure, 2013). The plasma PK of AT13387 was assessed on Day 8 of the study (second dose of AT13387). Patients in Cohort 1 of AT13387-02 received doses of 180 mg/m$^2$: the principal mean PK parameters derived from six patients are summarized in Table 2.

**Table 2: Preliminary Summary of PK Parameters for AT13387**

| Treatment (180 mg/m$^2$/dose AT13387) | Study | $AUC_{0-t}$ (hr.ng/mL) | | $t_{1/2}$ (hr) | | $C_{max}$ (ng/mL) | |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD |
| AT13387 only | AT13387-01 | 2376 | 362 | 7.0 | 1.0 | 923 | 176 |
| AT13387 + imatinib | AT13387-02 | 3371 | 1180 | 6.1 | 1.0 | 1019 | 382 |

$AUC_{0-t}$: area under the plasma concentration-time curve from time 0 to the last data point; $t_{1/2}$: half-life; $C_{max}$: maximum concentration; SD: standard deviation

The mean $AUC_{0-t}$ value obtained in AT13387-02 was greater, and showed wider inter-patient variability, than the mean $AUC_{0-t}$ for the same dose level in AT13387-01: values for $t_{1/2}$ and $C_{max}$ of AT13387 were similar in the 2 studies. Combination treatment with imatinib did not seem to significantly alter the PK of AT13387.

Efficacy

In AT13387-01, the best response to treatment in the heavily pretreated patient population was in 3 patients with GIST who received the QW regimen: 1 partial response (PR) of 113 days duration (220 mg/m$^2$/dose) and stable disease (SD) that persisted for ≥6 months in 2 patients (335 and 231 days duration; 260 and 220 mg/m$^2$/dose, respectively) (Investigator's Brochure, 2013, Mahadevan *et al*., 2012). Two other patients had SD that persisted for ≥6 months, one with metastatic melanoma (217 days duration; 2QW at 80 mg/m$^2$/dose) and one with adenoid cystic carcinoma of the right parotid (184 days duration; QW at 260 mg/m$^2$/dose). Preliminary analysis of the 10 patients with NSCLC enrolled in this study showed that four (40%) had SD (duration 47-85 days).

In AT13387-02, the best responses to date have been one PR (180 mg/m$^2$/dose) and four patients with SD (one with 150 mg/m$^2$/dose and three with 180 mg/m$^2$/dose QWx3). In the CTEP study AT13387-03, five patients achieved SD after 2 cycles of AT13387 given QDx2 for QWx3; one of these patients with hepatocarcinoma is still in SD after 6 cycles of therapy (personal communication by Dr. Kummar, January 8, 2014)

Safety

As of January 9, 2013, a total of 87 patients had been treated with AT13387 (Investigator's Brochure, 2013). Six deaths were reported (three were due to disease progression, one to metastatic colon cancer and two to pneumonia). All were considered not related to AT13387 by principal investigators; although in one case (grade 5 metastatic colon cancer), the contribution of AT13387 could not be completely ruled out. Other serious adverse events (SAEs) (that did not lead to death) were reported for 23/87 patients (26%). The only SAEs reported for >1 patient were blood creatine phosphokinase (CPK) increased and electrocardiogram QT prolonged (two patients each, 2%). Other SAEs considered related to study treatment were reported for 11/87 patients (13%). No related SAE was reported from patients receiving less than 120 mg/m$^2$/dose AT13387. Most of the patients (7/11) with related SAEs recovered from the event by last follow-up. The most common AEs were diarrhea (73%); fatigue (49%); nausea (44%); abdominal pain, decreased appetite, and dizziness (30% each); anemia and vomiting (28% each); and dry mouth (27%). The most common related AEs were diarrhea (67%), nausea (37%), and fatigue (35%). A common AE is visual impairment, including peripheral flashes (photopsia), blurred or double vision, floaters, color distortion and dimness, difficulties with light/dark accommodation, tunnel vision or other field defects, halos, apparent movement of stationary objects, and complex disturbances. Symptoms are generally grade 1, intermittent, reversible, and transient, lasting a few seconds to a few minutes and occurring on 1-3 days/cycle. Two cases of renal AEs (one grade 4, another grade 2) were reported in combination with imatinib. $QT_c$ prolongation (asymptomatic, grade 2, in one patient) occurred in combination with imatinib. Decreased left ventricular ejection fraction (LVEF) (grade 2, reversible, in one patient) was also observed.

Pharmacodynamics/Biomarkers

Inhibition of HSP90 by HSP90 inhibitors leads to activation of HSP70; therefore, an increase in the HSP70 level after administering such an inhibitor can serve as a molecular signature of HSP90 inhibition

(Investigator's Brochure, 2013). Increases in HSP70 in plasma were detected at all AT13387 dose levels in Study AT13387-01 (Mahadevan et al., 2012).

**CTEP's Plans for AT13387's Development**

CTEP is interested in developing AT 13387 in histologies driven by client proteins of HSP 90. PI3K/RTK/RAS are major targets for genomic alterations and molecular targeted therapy in head and neck small cell cancer (HNSCC) and NSCLC. Targeting HSP90 demonstrates antitumor activity in HNSCC and NSCLC in preclinical studies. Preclinical testings have shown potential for targeting genomic alterations with HSP 90 inhibitors and in combination with taxanes and radiation. Other histologies with possible client protein targets that are of interest are TNBC/hormone refractory ER+ breast cancer, EGFR refractory NSCLC and ALK+ lymphoma.

CTEP is requesting investigators to assist in the development of a variety of phase 1/1b combination trials with the intent to take these combinations into phase 2 trials with AT13387 in the following histologies:

- In patients with head and neck cancer, either frontline with radiation or with chemotherapy or targeted agents in frontline or at recurrence
- Epidermal growth factor receptor (EGFR) targeted therapy-resistant NSCLC in combination with paclitaxel or frontline combination with EGFR inhibitor to prevent/delay development of resistance
- Triple negative breast cancer (TNBC) or receptor-positive hormone refractory breast cancer as a single-agent study or in combination with a taxane, possibly in the maintenance phase after adjuvant therapy
- ALK+ lymphoma

Applicant may want to include in their application any collaborative translational work (or capabilities) relevant to the above development plans.

Correlative Studies of Interest to CTEP

Correlative laboratory studies may additionally be proposed. Any correlative laboratory studies should include the method and rationale. CTEP would be interested in investigations of biomarkers, including genomic biomarkers that may predict for response to treatment.

Questions regarding this request for applications may be addressed to Alice Chen, M.D., Senior Clinical Investigator, Investigational Drug Branch, CTEP, DCTD, NCI (phone: 240-276-6565; FAX: 240-276-7894; e-mail: chenali@ctep.ncil.nih.gov).

PTAs should be sent to the PIO at the address below by **5:00 PM Eastern Time (ET), March 16, 2014**. The most recent version of the PTA form, available on the CTEP Website (http://ctep.nih.gov), must be used. PTAs should be submitted electronically to:

Protocol and Information Office (PIO), CTEP/DCTD/NCI
E-mail: pio@ctep.nci.nih.gov
Phone: (240) 276-6535

**References**


Citri, A., D. Harari, G. Shohat, *et al.* (2006). Hsp90 recognizes a common surface on client kinases. *J Biol Chem*. 281:14361-14369.

Investigator's Brochure. (2012). AT13387. Version 7. Astex Pharmaceuticals, Inc. Dublin, CA and Cambridge, UK.

Investigator's Brochure. (2013). AT13387. Version 8. Astex Pharmaceuticals, Inc. Dublin, CA and Cambridge, UK.

Lyons, J., B. Graham, M. Reule, *et al*. (2008). AT13387, A Fragment-Derived Clinical Candidate is Active in Lung Cancer and Melanoma Models. *The 20th EORTC-NCI-AACR Symposium.* 147(Poster).

Mahadevan, D., D. M. Rensvold, S.E. Kurtin, *et al*. (2012). First in human phase I study – results of a second-generation non-ansamycin heat shock protein 90 (HSP90) inhibitor AT13387 in refractory solid tumors. *ASCO Meeting Abstracts*. 30:3028.

Pick, E., Y. Kluger, J. Giltnane, *et al.* (2007). High HSP90 expression is associated with decreased survival in breast cancer. *Cancer Res*. 67:2392-2397.

Whitesell, L., and S. L. Lindquist. (2005). HSP90 and the chaperoning of cancer. *Nat Rev Cancer*. 5(10):761-772.

Woodhead, A.J., H. Angove, M.G. Carr, *et al.* (2010). Discovery of (2,4-dihydroxy-5-isopropylphenyl)-[5-(4-methylpiperazin-1-ylmethyl)-1,3-dihydroisoindol-2-yl]methanone (AT13387), a novel inhibitor of the molecular chaperone Hsp90 by fragment based drug design. *J Med Chem*. 53:5956-5969.

Yufu, Y., J. Nishimura, and H. Nawata. (1992). High constitutive expression of heat shock protein 90α in human acute leukemia cells. *Leuk Res.* 16:597-605.