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First-in-human study of the epichaperome inhibitor PU-H71: clinical results and metabolic profile

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Summary

Background—Molecular chaperone targeting has shown promise as a therapeutic approach in human cancers of various histologies and genetic backgrounds. The purine-scaffold inhibitor PU-H71 (NSC 750424), selective for Hsp90 in epichaperome networks, has demonstrated antitumor activity in multiple preclinical cancer models. The present study was a first in-human trial of PU-H71 aimed at establishing its safety and tolerability and characterizing its pharmacokinetic (PK) profile on a weekly administration schedule in human subjects with solid tumors refractory to standard treatments.

Methods—PU-H71 was administered intravenously over 1 h on days 1 and 8 of 21-day cycles in patients with refractory solid tumors. Dose escalation followed a modified accelerated design. Blood and urine were collected during cycles 1 and 2 for pharmacokinetics analysis.

Results—Seventeen patients were enrolled in this trial. Grade 2 and 3 adverse events were observed but no dose limiting toxicities occurred, thus the human maximum tolerated dose was not determined. The mean terminal half-life ($T_{1/2}$) was 8.4 ± 3.6 h, with no dependency to dose level. A pathway for the metabolic disposal of PU-H71 in humans was derived from microsome studies. Fourteen patients were also evaluable for clinical response; 6 (35%) achieved a best response of stable disease for >2 cycles, with 2 patients remaining on study for 6 cycles. The study closed prematurely due to discontinuation of drug supply.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conclusions—PU-H71 was well tolerated at the doses administered during this study (10 to 470 mg/m²/day), with no dose limiting toxicities.

Keywords

Hsp90; Epichaperome; PU-H71; Pharmacokinetics; Cancer

Introduction

Under conditions of cellular stress, the collection of molecules that support protein folding and macromolecule assembly—thus regulating cellular homeostasis—form an integrated functional network known as the epichaperome [1–3]. The 90-kDa heat shock protein (Hsp90), long known to stabilize and assist in the folding of client proteins involved in essential processes such as cell cycle progression, cell proliferation, and survival [4, 5], was recently revealed to be critical to epichaperome formation and function, and for promoting cell viability [3]. Among Hsp90 client proteins are transcription factors and oncoproteins such as P53, HIF-1, RAF, EGFR, HER2, MET, AKT and Bcl-2 [6]. Due to its role in maintaining protein stability, the expression of Hsp90 is upregulated in response to cellular stress and DNA damage, both hallmarks of malignant transformation [7–9]; and in turn, overexpression of Hsp90 has been linked to tumor cell proliferation [10], decreased survival in rats [11], and cancer progression [12]. For these reasons, Hsp90 is an attractive target for cancer therapeutics.

The two homologous cytosolic isoforms (Hsp90 α and Hsp90 β) are comprised of an N-terminal ATPase domain, a middle domain involved in client protein binding, and a C-terminal domain for dimerization [13]. Several Hsp90 inhibitors targeted to the ATP binding domain—such as the naturally occurring antibiotics geldanamycin and radicicol, and semisynthetic derivatives 17-N-Allylamino-17-demethoxygeldanamycin (17-AAG) and 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG)—have been developed and clinically evaluated; however, assessment of drug benefit has been hampered by poor agent solubility and high toxicity [14–19]. To circumvent toxicity issues, the National Cancer Institute (NCI) and the Chiosis research group at Memorial Sloan-Kettering Cancer Center have developed PU-H71, a synthetic epichaperome inhibitor derived from a purine scaffold and selective for Hsp90, that binds to the ATP-binding site of complexed Hsp90 and triggers conformational changes that lock the client protein-Hsp90 complex, leading to proteasome-mediated degradation of the client protein [20]. Post-translational modifications and molecular interactions with other chaperones, cochaperones, and client proteins induce biochemical changes in Hsp90 that increase its sensitivity to PU-H71 [21, 22]. In preclinical studies, PU-H71 showed high cytotoxicity against Bcl-6-dependent diffuse large B-cell lymphoma (DLBCL), hepatocellular carcinoma (HCC), and breast cancer cell lines through the induction of apoptosis, and caused downregulation of client oncoproteins with roles in cell differentiation, proliferation, survival, and invasiveness [23–25]. Furthermore, PU-H71 was shown to have potent and lasting growth inhibitory effects in mouse xenografts of triple negative breast cancer, lymphoma, myeloma, pancreatic, and hepatocellular carcinoma [23–25]. Based on these results, we conducted the first in-human trial of PU-H71 on a weekly, 2 weeks out of three (days 1 and 8 of a 21-day cycle) schedule (<http://ClinicalTrials.gov>

Identifier: NCT01581541) in patients with refractory solid tumors. The goal of this study was to determine the safety, tolerability and maximum tolerated dose (MTD) of PU-H71 in human patients with cancer, and to characterize the pharmacokinetic (PK) profile of the drug on a once weekly administration schedule. Patients and methods

Eligibility criteria

Patients (≥ 18 years of age) with histologically documented solid tumors that had progressed or recurred after at least one line of chemotherapy, or for which there was no acceptable standard therapy, were eligible for this study. Patients were required to have a life expectancy >3 months, an Eastern Cooperative Group (ECOG) performance status ≤ 2, and adequate organ and marrow function, defined as absolute neutrophil count ≥ 1500/μL, platelets ≥ 100,000/μL, total bili-rubin ≤ 1.5 X the institutional upper limit of normal (ULN), AST (SGOT) (i.e., aspartate aminotransferase) / ALT (SGPT) (i.e., alanine aminotransferase) ≤ 2.5 X institutional ULN, creatinine <1.5 X ULN or measured creatinine ≥ 60 mL/min for patients with clearance creatinine levels ≤ 1.5 X ULN. Previous therapy must have been completed at least 4 weeks prior to enrollment. Exclusion criteria included non-stabilized brain metastases and carcinomatous meningitis, pregnancy, and co-morbidity with clinically significant intercurrent illnesses such as symptomatic congestive heart failure, unstable angina pectoris or uncontrolled cardiac arrhythmia. The trial was conducted under a National Cancer Institute (NCI)-sponsored IND with institutional review board approval at the NCI Clinical Center; informed consent was obtained from all individual participants included in the study. Protocol design and conduct followed all applicable regulations, guidances, and local policies.

Study design

This was an open-label, single arm, first in-human trial of PUH71 in patients with advanced solid tumors. The agent was supplied by the Division of Cancer Treatment and Diagnosis, NCI. PU-H71 was administered by 1-h intravenous infusion once weekly for 2 weeks (days 1 and 8) followed by 1 week without treatment, in 21-day cycles. The starting dose of 10 mg/m²/day (DL1) was escalated following a modified Simon accelerated titration design 2B [26]. Beyond the proposed dose level 6 (110 mg/m²/day), dose escalation proceeded in increments of at least 33% above the previous dose level up until 470 mg/m²/day (DL11). During the accelerated phase of this study, one patient was to be assigned per dose level until the first instance of a dose-limiting toxicity (DLT) in cycle 1, or until two different patients at the same dose level experienced grade ≥ 2 toxicity in any cycle. At the first instance of grade 2 toxicity, except for ocular toxicity, two additional patients were to be treated at that dose; if no further grade ≥ 2 toxicities were observed, then accelerated dose escalation continued. Once the accelerated phase ended, further dose escalation proceeded as a traditional 3 + 3 design.

Toxicities were graded using Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Toxicities were required to resolve to grade 2 (grade 1 for ocular toxicity) or below prior to initiation of the next cycle. A DLT was defined as an adverse event that occurred during cycle 1, was thought to be related to study drug administration, and met one of the following criteria: grade ≥ 3 non-hematologic toxicities (except diarrhea, nausea,

vomiting without maximal supportive therapy; alopecia), grade 4 hematologic toxicities (except lymphopenia), and grade 2 ocular toxicity that did not resolve to grade 1 within 2 weeks. Occurrence of a DLT resulted in a dose reduction following resolution to grade 2. No more than 2 dose reductions were allowed per patient on study. The MTD was to be defined as the dose level at which no more than 1 in 6 patients experienced a DLT.

Safety and efficacy evaluations

Because of the potential for QTc prolongation with this agent, an electrocardiogram (ECG) was performed at baseline and 2–4 h after drug infusion on days 1 and 8 of cycle 1. All patients had a cardiac echo or multiple gated acquisition scan (MUGA) and serum cortisol level measured prior to treatment, and at the start of cycle 3. A baseline eye exam was also performed on all study participants; follow up exams were performed if clinically indicated. CT scans were performed at baseline, and tumor response was assessed every 2 cycles (6 weeks) based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [27].

Specimen collection

PK samples were analyzed at the Pharmacokinetics Laboratory, Frederick National Laboratory for Cancer Research. Blood samples for PK analysis were collected in 7-mL heparin-containing tubes before the start of infusion, 30 min after the start of infusion, 5 min before completion of infusion, and at 1.5, 2, 3, 4, 7, 10, and 24 h after the start of infusion on day 1 during cycle 1. Samples were also collected before drug dosing on day 8 of cycle 1, and on days 1 and 8 of cycle 2. Samples were then centrifuged at $1000 \times g$ for 10 min at 4 °C, and the resulting plasma was stored at –70 °C until analysis. Aliquots of urine were collected in 15-mL screw top tubes before treatment and every void post-treatment on day 1 of cycle 1. Additional 10-mL urine samples were collected pre-dose on day 8 of cycle 1, and on days 1 and 8 of cycle 2. The samples were stored at –70 °C before analysis.

Pharmacokinetics analyses

PU-H71 and its metabolites were quantitated by HPLC/MS. Briefly, 100 μ L plasma samples from patients were mixed with 300 μ L acetonitrile-containing 0.01 μ M D6-PUH71 (internal standard [ISTD], NSC 752199), thoroughly vortexed and centrifuged for 10 min. The resulting supernatant was recovered, evaporated to dryness, and then reconstituted in 100 μ L 25% acetonitrile/water, and 10- μ L aliquots were used for analysis. Separation of the PU-H71 and metabolites was accomplished on an Eclipse XDB C18 (Agilent, CA) column using 0.1% formic acid in water and methanol as mobile phase. The column effluent was introduced into the Vantage MS (Thermo Scientific, Palo Alto, CA) through a heated electrospray ionization source. For quantitation of PU-H71, the MS was operated in SRM mode, monitoring the transitions of m/z 513 to m/z 454 and m/z 174 for PUH71, and m/z 519 to m/z 460 and m/z 179 for the ISTD. Detector responses were determined by normalizing PU-H71 to ISTD peak areas. Standard curves were made by spiking control plasma (i.e., plasma from healthy volunteers) with known amounts of PUH71 and analyzed the same as patient samples. Response rates were found to be linear from 0.005 to 0.25 μ M. Patients samples with levels $>0.25 \mu$ M were reanalyzed after dilution with control plasma. Urine samples were analyzed after 1:10 dilution and spiking with ISTD. PU-H71

metabolites were analyzed by reinjection of reconstituted sample, with the MS operated in a full scan mode monitoring m/z 250–1000. The maximum concentration ($C_{\max}$) and time to reach $C_{\max}$ ($T_{\max}$) were determined by visual inspection of the concentration versus time data. Area Under the Curve (AUC) was estimated by trapezoidal rule calculations, and descriptive statistics were calculated with Microsoft Excel.

Investigation of PU-H71 metabolism

To identify the metabolic products of PU-H71, plasma samples from study participants were analyzed by liquid chromatography-mass spectrometry (LC-MS) using positive ion electrospray ionization and reverse phase chromatography in full-scan mode. The metabolic stability of PU-H71 was resolved using liver microsomes from mice, rats, dogs, and humans. One micromolar and 10 μ M samples of PU-H71 were incubated with 0.5 mg protein/mL liver microsomes or S9 fractions, along with 1 mM NADPH and 3.3 mM MgCl in 100 mM phosphate buffer at pH 7.4 in a 37 °C shaking water bath. For the O-methylation reactions, S-adenosyl methionine (SAM) was added at 0.1 mM. Aliquots of the microsomal or S9 reactions were processed similarly to plasma samples, and analyzed by LC/MS.

Results

Demographics, safety, and clinical outcome

Seventeen patients with advanced, refractory cancers that had progressed on previous therapies were enrolled in the study and evaluated for assessment of toxicities (Table 1). PU-H71 was generally well tolerated; the first grade 2 toxicity, signaling cessation of accelerated dose escalation, occurred at DL 6 (Table 2). There was a single grade 3 toxicity possibly related to PU-H71, vomiting, for a patient on DL11 (470 mg/m²). Due to cessation of the drug supply, patients could not be accrued at higher doses, and the MTD could not be determined.

As a secondary objective, fourteen patients were evaluated for clinical response to treatment by RECIST criteria; of the 3 of 17 patients that were not evaluable, two patients refused further treatment, and one started an alternative treatment. No patients demonstrated complete or partial response. Eight of the evaluable patients progressed early and received only 2 cycles of therapy. The remaining six evaluable patients (35%) had a best response of stable disease, with two completing 6 cycles of treatment at DL6 (110 mg/m²). Three patients with SD completed 4 cycles at DL4, DL7, and DL8 respectively; while one completed 3 treatment cycles at DL9 (Fig. 1). All six patients with SD eventually progressed on study. The median number of cycles administered was 2 (range 1–6) and median follow-up time was 42 days (range: 21–126 days).

Clinical pharmacokinetics

Sixteen patients gave blood samples for pharmacokinetics evaluation; mean PK parameters are summarized in Table 3. Mean plasma concentration-time curves per PU-H71 dose level after a single dose are presented in Fig. 2. The mean terminal half-life ($T_{1/2} \pm$ SD) was 8.4 ± 3.6 h, with a range of 2.7 to 19.8 h. No dependency of $T_{1/2}$ to dose level was established. The time ($T_{\max}$) when maximum plasma concentration ($C_{\max}$) was achieved occurred by the

end of the 1-h infusion for all patients. Total plasma exposure ($AUC_{0-\infty}$) and C_{max} dependency were observed with increasing dose levels (Fig. 3a and b). The fraction of total AUC ($\pm$ SD) during the infusion was $19.6\% \pm 11.3\%$. Elimination of the drug was investigated by analysis of an aliquot of the total urine collected in 24 h. This revealed that on average for all dose levels, $6.7 \pm 3.0\%$ of the given dose ($\pm$ SD) was recovered as free drug. Patient 3 (DL3, 40 mg/m^2) had unexpectedly high exposure, and only 1.9% of the free drug was recovered in this patient's urine sample (Fig. 3 and Table 3).

PU-H71 metabolism

To gain insight into the pathways involved in the metabolism of PU-H71, *in vitro* studies were conducted with human liver subcellular fractions. These experiments resulted in the generation of a metabolic scheme, displayed in Fig. 4. In human liver microsomal fractions, phase 1 reactions with PU-H71 included S-oxidation, N-dealkylation, de-iodination, and O-demethylation. The O-demethylation reaction, the most active phase 1 reaction, led to the formation of a catechol (501). Only with the addition of S-adenosyl methionine (SAM) to S9 fractions was the major phase 1 metabolite (open ring 515) observed, implicating a coupled action of catechol O-methyltransferase (COMT) in the metabolic reaction scheme (Fig. 4, dashed arrows).

Several major metabolites were found and measured simultaneously with the drug in plasma and urine samples from patients treated with the study drug: de-isopropyl PU-H71 (metabolite 471), de-methyl PU-H71 (501), open ring form of PU-H71 (515), de-iodo PU-H71 (387) and 2 glucuronides of the open ring PU-H71 (691-1 and 691-2). Representative ion mass tracings and plasma profiles of PU-H71 and its metabolites are shown in Figs. 5a and b. The catechol 501 had low abundance in patient plasma samples. The most abundant phase 2 metabolite (691-1) was a glucuronide of the product 515 of the COMT reaction. Generally, plasma concentrations of the metabolites mirrored that of the parent drug; however, none of the metabolites constituted more than 10% of PU-H71 concentration, indicating that metabolism of PU-H71 is a minor pathway in the elimination of the drug.

Discussion

Hsp90, activated by cellular stress, plays a crucial role in stabilizing oncogenic client proteins, thus promoting tumorigenesis [7, 28, 29]. Accordingly, tumor cells have higher expression of Hsp90 compared to normal cells—2- to 10-fold over-expression [30]—making Hsp90 a molecular target for cancer therapy [28, 31]. Furthermore, conformational differences between the housekeeping Hsp90 found in normal cells and the stress-activated Hsp90 facilitate selective targeting [9, 32].

The benzoquinone ansamycin antibiotic geldanamycin is a potent inhibitor of Hsp90 activity that binds to its ATP-binding pocket [33–35]. However, geldanamycin lacks stability *in vivo* as its benzoquinone moiety is quickly reduced by NADPH: quinone oxidoreductase (NQO1), mitigating its effect against Hsp90, and causing hepatotoxicity [36, 37]. Another natural product, radicicol, is similarly unsuitable for clinical use due to instability and poor solubility [8, 31, 38]. Using the structure of the natural antibiotic products geldanamycin or radicicol as a starting point, other compounds were later derived and evaluated in clinical

studies. Semi-synthetic geldanamycin derivatives, 17-N-allylamino-17-demethoxygeldanamycin (17-AAG, tanespimycin) and 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG, alvespimycin), exhibited improved toxicity profiles and characteristics compared to geldanamycin, but narrow therapeutic indices and lack of clinical response remained problematic [7, 8, 39, 40]. Compounds with resorcinol moieties, analogous to radicicol, have been more successful [8, 41–43].

In search of Hsp90 inhibitors with improved selectivity and pharmacological profiles, Chiosis et al. followed a structure-based, rational design approach, which led to the development of purine-based compounds [44–46]. Members of this drug class mimic the closed shape conformation of ATP/ADP when bound to Hsp90, and they have the added advantages of improved water solubility, low hepatotoxicity, and specificity to both open and closed conformations of Hsp90 in transformed cells [20, 45, 46]. Functional optimization yielded 6-amino-8-[(6-iodo-1,3-benzodioxol-5-yl)thio]-N-(1-methylethyl)-9H-purine-9-propanamine (PU-H71) [20]. Preclinical studies demonstrated that PU-H71 selectively inhibits multiple types of malignant cells with high potency compared to normal fibroblasts [47–49]. Furthermore, the iodide functional group makes it possible to track distribution and accumulation of PU-H71 in the body by positron emission tomography (PET) using a modified ^{124}I -PU-H71 [50].

PU-H71 has specific potency against oncogenic Hsp90, which acts as a nucleating site for the stress-induced formation of networks of chaperome proteins [32]. This process occurs in a variety in cancer cells irrespective of histology and genetic background, making epichaperome targeting via oncogenic Hsp90 an attractive therapeutic strategy for human cancers [3]. Based on the promising preclinical results [23, 25, 47, 49], we conducted the first in-human trial of PU-H71 on a once weekly schedule, 2 weeks out of 3 to evaluate the safety and tolerability of this agent. Concurrently, a study of this agent administered twice weekly, 2 weeks out of 3 to patients with advanced malignancies is also being conducted independently at the Memorial Sloan-Kettering Cancer Center (<http://ClinicalTrials.gov> identifier: NCT01393509) [51].

In the present study, we found PU-H71 to be generally well tolerated with a very low incidence of grade 2 adverse events attributable to the study drug, and a single instance of grade 3 toxicity (vomiting) possibly related to PU-H71 (Table 2). Drug exposure increased with dose level, consistent with the emergence of grade 2 toxicities at DL6 and higher. Nonetheless, the dose was safely escalated to 470 mg/m² (DL11) without occurrence of dose-limiting toxicities. One patient at DL3 appeared to have higher exposure than expected (Fig. 3a and b, grey circle). However, this patient did not experience any grade 2 toxicities, and patients at DL2 and DL4 had markedly lower drug exposures, leading us to believe that the PU-H71 plasma level measured for patient 3 was artificially high, possibly due to drawing of plasma samples through the same catheter used to administer PU-H71. This hypothesis is supported by the modest levels of PU-H71 recovered in the urine of this patient (Table 3), ruling out the possibility of an overdose. Furthermore, although all dose levels were below the MTD, the 5 patients on dose 266 mg/m² (DL9) reached PU-H71 peak plasma concentrations and exposures exceeding those that had produced activity in murine

xenograft models (corresponding to $C_{\max} > 3.4 \mu\text{M}$ and $\text{AUC}_{0-\infty} > 3115 \mu\text{M} \cdot \text{min}$ in humans) [25].

We present a metabolic scheme for PU-H71 based on the metabolic products recovered from liver microsome fractions (Fig. 4). In plasma samples from patients, the relative abundance of all metabolites did not exceed 10% of that of PU-H71 (Fig. 5), suggesting that metabolism is a minor pathway of elimination of the drug. Additionally, the potentially hepatotoxic and reactive catechol metabolite of PU-H71 (501) produced by cytochrome P450 was efficiently methylated by COMT activity, as evidenced by its lack of abundance in plasma samples compared to the COMT product 515, and glucuronides **691-1** and **691-2**.

To ascertain the effect of PU-H71 on its target, we had planned to conduct pharmacodynamics (PD) assessments of Hsp90 client proteins in biopsy samples from patients treated at the MTD. In both cell lines and xenograft studies in multiple cancer types, the antitumor activity of PU-H71 was associated with depletion of oncogenes, including AKT, EGFR, and RAF-1 [24, 25]. Therefore, ERK and AKT constitute ideal markers of Hsp90 inhibition because they directly interact with Hsp90, while also being downstream effectors of other Hsp90 client proteins [6]. However, this trial was closed prematurely in the dose-escalation phase due to the discontinuation of study drug supply. Therefore, the therapeutic index of PU-H71 was not resolved, and the lack of objective responses beyond stable disease suggests that at the once weekly schedule 2 weeks out of 3, higher doses or selection of patients based on epichaperome levels [3] would be required to achieve a more substantial therapeutic effect.

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Fig. 1.

Patient response. Six patients did not progress within the first 2 cycles, and thus had a best response of stable disease (SD; *blue*), although all 6 eventually progressed. Eight patients had progressive disease (PD; *red*) within the first 2 cycles; 3 patients were not evaluated (NE) or not evaluable per protocol (NP; *grey*). Dose levels (DLs) administered to each patient are shown. Patient diagnoses are listed in Table 1

Fig. 2.

Mean plasma concentration of PU-H71 per dose level. Following 45-min intravenous infusion of PU-H71 at the doses indicated, plasma levels of PU-H71 were measured and averaged for each dose level. DL1 = 10 mg/m²; DL2 = 20 mg/m²; DL3 = 40 mg/m² (*dashed grey line*); DL4 = 60 mg/m²; DL5 = 80 mg/m²; DL6 = 110 mg/m²; DL7 = 150 mg/m²; DL8 = 200 mg/m²; DL9 = 266 mg/m²; DL10 = 354 mg/m²; DL11 = 470 mg/m²

Fig. 3.

Plasma exposure to PU-H71. **(a)** The area under the plasma drug concentration-time curve ($AUC_{0-\infty}$) as a function of PU-H71 dose, representing total drug exposure. Linear regression coefficient: $R^2 = 0.72$. **(b)** Peak plasma level C_{max} as a function of PU-H71 dose. Patient 3 (DL3, 40 mg/m²; grey circle) exhibited higher than expected peak plasma concentration. Without patient 3 (grey circle), C_{max} demonstrated dose level dependency (linear regression coefficient $R^2 = 0.74$). Patient 7 at DL6 was not evaluable

Fig. 4.

Proposed pathway for human metabolism of PU-H71. PU-H71 metabolism scheme elucidated through in vitro experiments with microsomal incubation samples. Dashed arrows indicate the SAM-mediated O-methylation reaction catalyzed by catechol O-methyltransferase (COMT)

Fig. 5.

Representative plasma profiles of PU-H71 and its metabolites. **(a)** Representative ion mass tracings from an aliquot of patient plasma. Peaks corresponding to PU-H71, internal standard (ISTD; D6-PU-H71), and metabolites *471*, *691*, *515*, but not *501*, were observed.

(b) Relative metabolite levels for patient #12 were normalized against ISTD

Table 1Patient characteristics ($n = 17$)

Characteristic	n
Median age, years [range]	59 [19-77]
Gender	
Male	10
Female	7
Mean number of prior therapies [range]	7 [1-14]
Diagnosis (patient number)	
Malignant Hürthle cell tumor (pt 1)	1
Rectal adenocarcinoma (pt 2)	1
Adenocarcinoma, NOS (pts 3, 6, 7, 10, 11, 13)	6
Adenoid cystic carcinoma (pt 4)	1
Invasive poorly differentiated carcinoma (pt 5)	1
Metastatic adenocarcinoma (pt 8)	1
Hepatocellular carcinoma (pt 9)	1
Carcinoid tumor (pt 12)	1
Squamous cell carcinoma of the esophagus (pt 14)	1
Synovial sarcoma (pts 15, 17)	2
Non-small cell lung cancer (pt 16)	1

Pt patient, *NOS* not otherwise specified

Table 2

Adverse events by patient. Worst grade 2 adverse events possibly, probably, or definitely related to study drug for each patient

Adverse Event	Grade	10 mg/m ² (n = 1)	20 mg/m ² (n = 1)	40 mg/m ² (n = 1)	60 mg/m ² (n = 1)	80 mg/m ² (n = 2)	110 mg/m ² (n = 3)	150 mg/m ² (n = 1)	200 mg/m ² (n = 2)	266 mg/m ² (n = 1)	354 mg/m ² (n = 3)	470 mg/m ² (n = 1)
Anemia	2	-	-	-	-	-	1	-	-	-	1	-
AST increased	2	-	-	-	-	-	1	-	-	-	-	-
AV block first degree	2	-	-	-	-	-	-	-	-	-	1	-
Blood bilirubin increased	2	-	-	-	-	-	1	-	-	-	-	-
Fatigue	2	-	-	-	-	-	-	1	-	-	-	-
Headache	2	-	-	-	-	-	-	-	1	-	-	-
Lymphopenia	2	-	-	-	-	-	1	-	-	-	-	-
Nausea	2	-	-	-	-	-	-	-	-	-	-	1
Sinus bradycardia	2	-	-	-	-	-	-	-	-	-	1	-
Vomiting	3	-	-	-	-	-	-	-	-	-	-	1

AST aspartate aminotransferase, AV atrioventricular, n, total number of patients per dose level

Table 3

Pharmacokinetic parameters of PU-H71 per dose level following administration on day 1. For the dose levels at which multiple patients were accrued, data represent mean $\pm$ SD

Dose level	Number of patients	C _{max} (μM)	T _{1/2} (hours)	AUC _{0–20 h} (μM·min)	AUC _{0–∞} (μM·min)	Urinary excretion (%)	Clearance (L/h/kg)
1 (10 mg/m ²)	1	0.2	2.7	27	31	5.5	1.03
2 (20 mg/m ²)	1	0.3	10.5	74	100	na	0.63
3 (40 mg/m ²)	1	74.7	9.2	3845	3905	1.9	0.03
4 (60 mg/m ²)	1	1.3	6.1	273	301	8.8	0.63
5 (80 mg/m ²)	2	5.7 $\pm$ 0.1	6.7 $\pm$ 0.1	899 $\pm$ 32	1007 $\pm$ 32	6.7 $\pm$ 1.3	0.25 $\pm$ 0.01
6 (110 mg/m ²)	3	7.3 $\pm$ 2.4	7.6 $\pm$ 0.2	1186 $\pm$ 481	1375 $\pm$ 591	6.7 $\pm$ 4.6	0.28 $\pm$ 0.12
7 (150 mg/m ²)	1	8.7	7.2	1534	1771	8.1	0.27
8 (200 mg/m ²)	2	8.0 $\pm$ 4.5	7.1 $\pm$ 0.5	2090 $\pm$ 459	2477 $\pm$ 429	6.0 $\pm$ 4.2	0.26 $\pm$ 0.04
9 (266 mg/m ²)	1	7.8	10.2	3596	5449	5.4	0.15
10 (354 mg/m ²)	3	17.3 $\pm$ 8.5	11.5 $\pm$ 7.3	6866 $\pm$ 2876	10,815 $\pm$ 5499	8.6 $\pm$ 4.6	0.13 $\pm$ 0.09
11 (470 mg/m ²)	1	33.7	10.8	8568	12,151	5.7	0.12

Abbreviations: AUC_{0–24 h}, area under the serum concentration-time curve from dosing to 24 h after dosing; AUC_{0–∞}, area under the plasma concentration-time curve from dosing to infinity (total exposure); C_{max}, maximum serum concentration; T_{1/2}, terminal half-life; na, no urine specimen for patient 2 at 24 h