First-in-Human Phase I Dose-Escalation Study of the HSP90 Inhibitor AUY922 in Patients with Advanced Solid Tumors

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Abstract

Purpose: A phase I study was conducted with the primary objective of determining the maximum tolerated dose (MTD) of AUY922 in patients with advanced solid tumors. Secondary objectives included characterization of the safety, pharmacokinetic, and pharmacodynamic profiles.

Patients and Methods: Patients with advanced solid tumors received 1-hour i.v. infusions of AUY922 once a week in a 28-day cycle. An adaptive Bayesian logistic regression model that employed observed dose-limiting toxicities (DLT) in the first treatment cycle was used to guide dose-escalation decisions, with the established MTD to be used in phase II studies.

Results: One hundred and one patients were enrolled and explored at doses in the range of 2 to 70 mg/m². DLTs occurred in 8 patients (22–70 mg/m²) and included diarrhea, asthenia/fatigue, anorexia, atrial flutter, and visual symptoms. At 70 mg/m², the AUY922 concentration achieved was consistent with active concentrations in a range of xenograft models. There was evidence of target inhibition in peripheral blood mononuclear cells (HSP70 induction) and tumor (client protein depletion and reduction of metabolic activity by 18F-FDG PET). The recommended phase II dose (RP2D) of 70 mg/m² was proposed on the basis of toxicity and pharmacokinetic and pharmacodynamic profiles.

Conclusions: At the RP2D of 70 mg/m², AUY922 exhibited acceptable tolerability, and phase II single-agent and combination studies have been initiated in patients with HER2-positive breast, gastric, and non–small cell lung cancers. Clin Cancer Res; 19(13); 3671–80. ©2013 AACR.

Introduction

HSPs are a family of molecular chaperones that are ubiquitous in normal and cancer cells and are essential to the folding of a wide variety of oncogenic client proteins (1–4). HSP90 client proteins influence the hallmark traits of cancer, including self-sufficiency in growth signals, evasion of apoptosis, tissue invasion, and angiogenesis (5). The inhibition of HSP90 leads to misfolding of client proteins and degradation through the ubiquitin proteasome pathway (6). HSP90 inhibitors target the postulated dependence of cancer cells on mutated or amplified oncoproteins, such as EGFR receptor (EGFR) or human epidermal growth factor receptor 2 (HER2; refs. 7–10). In addition, the dependence of key signaling proteins such as RAF and AKT on the HSP90 chaperone complex can lead to a combinatorial blockade of signal transduction (1, 2, 6). These properties suggest HSP90 inhibitors have potential as an anticancer treatment in a wide range of cancers, both as single agents and in combination with other anticancer agents (6). Although increased expression of HSP90 is associated with poor prognosis in some tumors (11–13), predictive biomarkers of response to HSP90 inhibition are more likely to be linked to mutated or amplified oncogenic drivers (1, 2, 6).

The first generation of HSP90 inhibitors were geldanamycin derivatives, important in establishing a proof of concept that it was possible to achieve clinical responses related to HSP90 inhibition in a clinical setting. However, geldanamycin derivatives have disadvantages, including dependence on NAD(P)H:quinone oxidoreductase (DT diaphorase) activity.
Translational Relevance

HSP90 is a molecular chaperone that is critical to the folding and function of client proteins. Inhibition of HSP90 leads to degradation of the unfolded client proteins by the ubiquitin proteasome pathway. Key oncopgenic client proteins include HER-2, EGFR, BRAF, ALK, VEGFR, HIF-1, AKT, BCR-ABL, MET, SRC, and CDK4. AUY922 is a potent second-generation isoxazolyl resorcinol-based HSP90 inhibitor and does not exhibit hepatotoxicity and metabolic liabilities due to a quinone moiety, as seen with previous geldanamycin-based HSP90 inhibitors. AUY922 is active across a wide range of human cancer models in vitro and in vivo. This phase I study, key to the early development of AUY922, uses a Bayesian design to define a tolerable dose and is underpinned by pharmacokinetic and pharmacodynamic biomarker studies. The dose and schedule from this study are being used to assess AUY922 in molecularly stratified phase II studies.

difficult formulation, and hepatotoxicity (14–17). These clinical limitations of the geldanamycins have prompted the design and discovery of new, second-generation synthetic HSP90 inhibitors with greater potency, reduced hepatotoxicity, and reduced dependence on DT-diaphorase. AUY922 (5-(2,4-dihydroxy-5-isopropyl-phenyl)-N-ethyl-4-[4-(morphismolinomethyl)phenyl]isoxazole-3-carboxamide) is a highly potent, isoxazole-based, non-geldanamycin HSP90 inhibitor that inhibits the ATPase activity of HSP90 (18, 19). AUY922 has nanomolar efficacy against a wide range of human cancer cell models in vitro and also inhibits tumor progression in a variety of tumor models in vivo (18–22).

Tissue distribution experiments in BT474 tumor-bearing mice showed that AUY922 was present in the tumor for at least 1 week, with the tumor exposure being approximately 47-fold higher than that in plasma, and significant tumor growth inhibition and good tolerability were observed when AUY922 was administered once per week in the xenograft model (21). Subsequent preclinical toxicology studies therefore used a weekly i.v. schedule. This phase I dose-escalation study aimed to determine the MTD of AUY922 given as a 1-hour infusion once a week in patients with advanced solid tumors. Secondary objectives included characterization of the pharmacokinetic and pharmacodynamic profiles of the drug.

Patients and Methods

Patient selection

Adult patients ages 18 years or older with advanced solid tumors who had progressed on standard treatment and who had adequate hepatic and renal function were eligible (see Supplementary Data for more detailed inclusion and exclusion criteria). In contrast with other targeted agents, such as BRAF or phosphoinositide 3-kinase inhibitors, HSP90 inhibitors inhibit a wide range of signal transduction pathways, and thus no patient-selection strategies based on molecular markers were considered in the dose-escalation component of this phase I trial.

This trial was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonization. All studies were conducted after approval by the local Human Investigations Committees. Written informed consent was obtained from all patients before screening.

Study design and treatment plan

AUY922 was administered as a 1-hour i.v. infusion once a week, and a treatment cycle was considered to be 28 days. An adaptive Bayesian logistic regression model (BLRM) with overdose control was used to model the relationship between dose and the probability of a patient experiencing a dose-limiting toxicity (DLT; ref. 23). According to the BLRM-guided dose escalation, a minimum of 3 patients were assigned sequentially to a cohort, and 2 evaluable patients not experiencing DLT or clinically relevant toxicity were considered sufficient for dose-escalation decisions. A minimum of 3 patients was required if DLT or clinically relevant grade 2 or higher toxicity occurred. The maximum intercohort dose escalation permitted was 100%. If clinically relevant toxicities of grade 2 or higher were observed in more than 1 patient, dose escalation was limited to 50%. Multiple cohorts could be sequentially enrolled at a dose level to better estimate the DLT rate as well as to better assess, for example, lower-grade safety events, activity, etc. The starting dose for AUY922 was 2 mg/m², based on a fraction of the maximum tolerated dose (MTD; 1 mg/kg) determined during preclinical toxicology studies in dogs, which were the most sensitive species treated. On the basis of the preclinical toxicity in dogs, the median probabilities of DLTs were set to 0.1% at 2 mg/m² and 33% at 28 mg/m². The aim of the study was to achieve a posterior probability that the true DLT rate was in the range of 16% to 33% for the selected dose. AUY922 was administered using a weekly i.v. schedule. The protocol-defined MTD was the highest dose of AUY922 given for at least 3 doses out of 4 in the first treatment cycle that was expected to produce DLT in less than 33% of patients. The MTD (or if not reached, the proposed recommended phase II dose, RP2D) cohort was expanded with additional patients to a minimum of 22 patients for a 90% probability of detecting an adverse event (AE) with an incidence rate of 10%. The study intent-to-treat (ITT) population consisted of all patients who received at least 1 dose of study drug.

Toxicity assessments

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria Version 3.0 (CTC). A DLT was defined as the occurrence of a clinically relevant drug-related AE or abnormal laboratory value assessed as clinically relevant and occurring less than 28 days after the first dose of AUY922 in cycle 1. Hematologic, renal, and hepatic functions were measured weekly before infusion. In
addition, electrocardiographic (ECG) assessments were carried out on days of infusion during cycle 1 and coinciding with pharmacokinetic sampling. Following reports of grade 1 to 3 visual symptoms after weekly administration of AUY922 at dose levels of 40 mg/m² or more, standard ophthalmologic assessments were carried out during baseline and at the time of reported visual symptoms and/or after discontinuation from the study in affected patients. Standard assessments included visual acuity, intraocular pressure, slit lamp tests, dilated fundus tests, and color vision (Ishihara plate) tests. Additional assessments or tests were conducted as clinically indicated, including invasive ophthalmologic assessments such as electroretinograms, where appropriate.

Pharmacokinetics and pharmacodynamics
Pharmacokinetic evaluation was conducted by analyzing AUY922 and its phenolic glucuronide metabolite BJP762 in blood using liquid chromatography and tandem mass spectrometry (see Supplementary Data for details). A non-compartamental analysis was conducted (using WinNonlin Version 5.2) to determine pharmacokinetic parameters such as area under the blood concentration–time curve (AUC), maximum (peak) blood drug concentration \(C_{\text{max}}\), and the elimination half-life \(T_{1/2}\) for AUY922 and BJP762, as appropriate.

Peripheral blood mononuclear cell (PBMC) samples were taken from all patients on cycles 1 and 2 to determine HSP70 induction. The level of expression of HSP70 was quantitated by ELISA (details in Supplementary Data). Summary statistics for HSP70 induction in PBMCs by scheduled time point and treatment group, including percentage of change from baseline, were determined. Pre- and posttreatment tumor samples were also obtained in 2 patients. The HSP70 induction and client protein depletion (AKT) were studied using immunohistochemical techniques.

All patients with expected high \(^{18}\text{F}-\text{fluorodeoxyglucose (FDG)}\) tracer uptake (non-prostate cancer; tumor \(\geq 2 \text{ cm}\)) underwent \(^{18}\text{F}-\text{FDG PET}\) at baseline and \(\pm 1\) days after the infusions on day 1 of cycles 2 and 3. Metabolic response by central radiologic review was determined according to predefined criteria (24).

Response assessments
Efficacy of AUY922 was assessed using computed tomography or MRI of all tumor lesions and applying adapted Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.0 (25).

Drug supply and administration
AUY922 was prepared using a 5 mg/mL solution in aqueous glucose, diluted in 5% dextrose or sucrose to a maximum i.v. infusion volume of 500 mL, under aseptic conditions and protected from light to prevent the photo-labile drug from decomposition. AUY922 was administered by i.v. infusion over a 1-hour period.

Results
Patient characteristics
A total of 101 patients received AUY922 in 9 treatment groups at doses of 2 to 70 mg/m² (28 patients received AUY922 at 70 mg/m²). Patients (41% male, 81% Caucasian) were of mean age 57 years, and 99 (98%) patients had a World Health Organization Performance Status of 0 or 1. The most common tumors treated were colon, breast, and ovarian cancer (details in Supplementary Table S1). All patients were evaluable for safety and efficacy analyses.

Study duration
The study started on July 23, 2007 (first patient/first visit), and the RP2D was declared on September 4, 2009 (after 25 months). A further RP2D expansion was carried out, and no further patients were added to the study as of July 18, 2011 (last patient/first dose). The study was completed on November 19, 2011 (last patient/subject completed).

Dose escalation and toxicity
There were 8 DLTs, all of which were grade 3 (Table 1), and occurred in one of 11 patients at 22 mg/m² (atrial flutter), two of 16 patients at 40 mg/m² (dyspnea, anorexia, fatigue, and diarrhea), two of 18 patients at 54 mg/m² (anorexia, dyspnea, and fatigue), and 8 of 20 patients at 60 mg/m² (diarrhea).

### Table 1. Summary of dose-limiting toxicities (maximum tolerated dose–determining analysis set)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose received [no. of infusions (study day)]</th>
<th>Specific event</th>
<th>CTC grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22 mg/m² [3 infusions (day 15)]</td>
<td>Atrial flutter/fibrillation</td>
<td>3 (both)</td>
</tr>
<tr>
<td>2</td>
<td>40 mg/m² [2 infusions (day 14)]</td>
<td>Anorexia, dyspnea, and fatigue*</td>
<td>3 (all)</td>
</tr>
<tr>
<td>3</td>
<td>40 mg/m² [1 infusion (day 2)]</td>
<td>Diarrhea</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>54 mg/m² [3 infusions (day 16)]</td>
<td>Asthenia*</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>54 mg/m² [1 infusion (day 2)]</td>
<td>Diarrhea</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>70 mg/m² [3 infusions (day 16)]</td>
<td>Visual symptoms</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>70 mg/m² [3 infusions (day 19)]</td>
<td>Visual symptoms</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>70 mg/m² [3 infusions (day 16)]</td>
<td>Diarrhea</td>
<td>3</td>
</tr>
</tbody>
</table>

*Patient 2 had approximately 20-lb (9.1-kg) weight loss before study start; *Patient 4 had grade 1/2 asthenia at baseline.
Table 2. Incidence of adverse events (all grades, >10%) potentially related to AUY922

<table>
<thead>
<tr>
<th>Adverse events (preferred term), n (%)</th>
<th>Dose, mg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 3)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0</td>
</tr>
<tr>
<td>Night blindness</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Photopsia</td>
<td>0</td>
</tr>
<tr>
<td>Blurred vision</td>
<td>0</td>
</tr>
<tr>
<td>Asthenia</td>
<td>0</td>
</tr>
<tr>
<td>Visual impairment</td>
<td>0</td>
</tr>
<tr>
<td>Anemia</td>
<td>0</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>0</td>
</tr>
</tbody>
</table>

Patients who experienced more than one occurrence of the same event are only counted once within each category.

(asthenia and diarrhea), and three of 24 patients at 70 mg/m² (diarrhea and visual symptoms). The most common toxicities potentially related to AUY922 were diarrhea (58%), nausea (40%), fatigue (30%), night blindness (23%), vomiting (23%), and asthenia (12%), as shown in Table 2. Overall, 22 (22%) patients had one dose delay, and 12 (12%) patients had more than one dose delay. A total of 27 patients (27%) experienced dose delay due to an AE, and 8 (8%) patients discontinued due to an AE. The diarrhea seen as a DLT resolved on stopping trial medication, and grade 1 to 2 diarrhea was well controlled with loperamide and typically lasted for 1 to 3 days. Atrial flutter was seen as a DLT in just one patient (at 22 mg/m²); ECG recordings did not reveal a prolonged QTcF, and echocardiographic assessment did not show a drop in ejection fraction in this patient. One patient discontinued from the study after the first dose of AUY922 due to a prolonged QTcF at 40 mg/m². The QTcF prolongation was attributed to electrolyte disturbance worsened by diarrhea and resolved following electrolyte replacement. Neither the prolongation of QTcF nor diarrhea met the criteria for a DLT. The event was not reported as a DLT. Overall, 23 of 98 patients (23%) had newly occurring QTcF of more than 450 ms following treatment; newly occurring QTcF of more than 480 ms was rare (2%).

Visual AEs potentially related to AUY922 occurred in 43% of patients and displayed a clear dose-related increase. Visual AEs were grades 1 or 2 in 41 of 101 patients (41%) and grade 3 in 2 of 101 patients (2%) and were variously described as night blindness, photopsia, blurred vision, and visual impairment. Instances of visual toxicity of less than grade 3 severity did not interfere with activities of daily living and were reversible when study drug was interrupted or discontinued. No grade 4 or irreversible visual toxicities were observed. There was no consistent dose relationship for asthenia and fatigue reported as a DLT at 40 and 54 mg/m², but a relationship to study drug exposure could not be excluded in these patients.

Upon observation of 2 DLTs in 7 patients at 40 mg/m², a standard 3 + 3 approach would lead to declaration of the MTD as 28 mg/m² (the DLTs occurred before all 7 patients had completed their first cycle of treatment, which would have led to the declaration of a nontolerable dose and dose reduction for all other patients who had not completed the first cycle of treatment at the lower dose). The BLRM supported continued dosing at 40 mg/m² on the basis of the assessment that at 40 mg/m² the probability of a true DLT rate above 33% was less than 0.25. Additional cohorts were then recruited at 40 mg/m² and, subsequently, at 54 and 70 mg/m², where the final RP2D was declared. Additional patients were recruited at these higher doses to better understand the visual AE profile prior to RP2D selection. Notably, the additional information (DLT and additional data) supported the final RP2D recommendation of 70 mg/m² from the BLRM, 2.5 times higher than the 28 mg/m² recommendation from a standard 3 + 3 algorithm. The estimated DLT rate from the BLRM at the RP2D was 15.1% with a very small probability of unacceptable toxicity, 0.006 (Table 3).

Pharmacokinetic studies

Pharmacokinetic parameters are presented (pharmacokinetic blood parameters for the 2–70 mg/m² cohorts) in Table 4. The mean concentration–time profiles of AUY922 in the blood are presented in Fig. 1A. Mice bearing BT474 xenografts showed significant reduction in tumor growth when treated with AUY922, 25 mg/kg once a week. The average efficacious plasma concentration in this preclinical xenograft model was estimated to be approximately 20 ng/mL (21). In addition, pharmacodynamic changes on depletion of key client proteins via HSP90 inhibition in vitro were seen at concentrations of approximately 20 ng/mL in preclinical models (19). Trough concentrations higher than 20 ng/mL were seen in the patients treated at dose levels of 40 mg/m² or more.
in this clinical trial (Fig. 1A). AUY922 was well distributed (volume of distribution 1,628 L) and had a $T_{1/2}$ of 120 hours at 70 mg/m². A dose-dependent increase in exposure to AUY922 was observed (Fig. 1B and C); additionally, no drug accumulation was observed for AUY922 and BJP762 in blood after repeat once-weekly doses. Mean $C_{\text{max}}$ increased from 73.8 to 1,277.9 ng/mL, and mean AUC₀–inf increased from 1,755.7 to 13,456.7 h·ng/mL in the 2-mg/m² and 70-mg/m² dose cohorts, respectively. A similar profile was observed for the metabolite BJP762 (Table 4). Peak concentrations occurred at the end of 1-hour infusion for both AUY922 and its metabolite BJP762, suggesting rapid biotransformation from parent AUY922 to BJP762. In vitro studies showed that distribution of AUY922 to blood cells from plasma was concentration dependent and saturable. Pharmacokinetic modeling indicated that the dose-dependent clearance and volume distribution of AUY922 in blood observed in the present study was attributed to the concentration-dependent blood cell binding. Pharmacokinetics is linear in plasma over the dose range of 2 to 70 mg/m² investigated.

### Pharmacodynamic analyses

The cochaperone protein HSP70 was induced following treatment with AUY922. The highest HSP70 change by treatment group (for the PBMC biomarker analysis subset; $n = 61$) is represented in Fig. 2A. HSP70 induction occurred in a dose-dependent manner at dose levels 4 through to 40 mg/m², and induction remained similar at the higher dose levels, reaching a plateau at 54 mg/m². Evaluation of HSP70 induction in PBMCs and PET scans were carried out at different time points. As a model of induction of HSP70 related to time was not available, analysis of the correlation of HSP70 induction in PBMCs and metabolic response on PET was not done. In 2 patients, pre- and posttreatment tumor biopsy samples were studied for HSP70 induction and depletion of AKT, and the findings were suggestive of target engagement in tumor tissue (Fig. 2B). $^{18}$F-FDG-uptake data for cycle 2 day 1 and cycle 3 day 1 were available for 98 patients across dose levels 2 to 70 mg/m². At the 4 highest dose levels, a best overall response across cycles 2 to 3 of partial metabolic response [PMR, ≥25% reduction in sum of standardized uptake values ($sSUV_{\text{max}}$)] and stable metabolic disease (SMD) was observed in 11 of 69 (including 4/9 lung cancer patients) patients (16%) and in 22 of 69 patients (32%), respectively. An incremental increase in the proportion of patients achieving PMR when comparing the 3 highest dose levels is shown in Fig. 2C.

### Antitumor activity

All 101 patients were evaluable, and of these, none had a complete or partial response by adapted RECIST Version 1.0.

### Decisions regarding recommended phase II dose

The MTD of AUY922 was not reached in this study. Following completion of the final 70-mg/m² cohort of the dose-expansion phase of the study, the BLRM, subject to overdose control and protocol criteria, would have allowed escalation to a dose of 90 mg/m². At that time, the probability of unacceptable toxicity for the 70 mg/m² dose was estimated to be 0.006. However, dose escalation was not recommended due to the potential risk of visual toxicity. Other factors taken into consideration were that pharmacokinetic profiles were consistent with those of doses active in preclinical models, and evidence indicates that target inhibition in pharmacodynamic and PET assays was achieved. The recommended phase II dose was thus 70 mg/m².

### Discussion

Multiple HSP90 inhibitors are in clinical development as single agents and in combination with other anticancer agents (26, 27). Geldanamycin-based HSP90 inhibitors have shown activity in EML4–ALK [echinoderm microtubule-associated-protein-like 4 (EML4); anaplastic lymphoma kinase (ALK)]-dependent NSCLC (28), and in combination with targeted agents such as trastuzumab in HER2-amplified breast cancer (29); however, hepatotoxicity

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**Table 3.** Summary of observed dose-limiting toxicity rates and estimates from the Bayesian model

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>No. of patients treated</th>
<th>Number of patients evaluable for DLT assessment</th>
<th>Number of DLTs observed</th>
<th>Observed DLT rate (%)</th>
<th>Estimated DLT rate from BLRM</th>
<th>Estimated risk that true DLT rate exceeds 33% from BLRM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>2.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>22</td>
<td>12</td>
<td>11</td>
<td>1</td>
<td>9.1</td>
<td>4.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>28</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>6.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>40</td>
<td>16</td>
<td>16</td>
<td>2</td>
<td>12.5</td>
<td>8.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>54</td>
<td>19</td>
<td>18</td>
<td>2</td>
<td>11.1</td>
<td>11.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>70</td>
<td>28</td>
<td>24</td>
<td>3</td>
<td>12.5</td>
<td>15.1</td>
<td>0.006</td>
</tr>
</tbody>
</table>

**Discussion**

Multiple HSP90 inhibitors are in clinical development as single agents and in combination with other anti-cancer agents (26, 27). Geldanamycin-based HSP90 inhibitors have shown activity in EML4–ALK [echinoderm microtubule-associated-protein-like 4 (EML4); anaplastic lymphoma kinase (ALK)]-dependent NSCLC (28), and in combination with targeted agents such as trastuzumab in HER2-amplified breast cancer (29); however, hepatotoxicity in this clinical trial (Fig. 1A). AUY922 was well distributed (volume of distribution 1,628 L) and had a $T_{1/2}$ of 120 hours at 70 mg/m². A dose-dependent increase in exposure to AUY922 was observed (Fig. 1B and C); additionally, no drug accumulation was observed for AUY922 and BJP762 in blood after repeat once-weekly doses. Mean $C_{\text{max}}$ increased from 73.8 to 1,277.9 ng/mL, and mean AUC₀–inf increased from 1,755.7 to 13,456.7 h·ng/mL in the 2-mg/m² and 70-mg/m² dose cohorts, respectively. A similar profile was observed for the metabolite BJP762 (Table 4). Peak concentrations occurred at the end of 1-hour infusion for both AUY922 and its metabolite BJP762, suggesting rapid biotransformation from parent AUY922 to BJP762. In vitro studies showed that distribution of AUY922 to blood cells from plasma was concentration dependent and saturable. Pharmacokinetic modeling indicated that the dose-dependent clearance and volume distribution of AUY922 in blood observed in the present study was attributed to the concentration-dependent blood cell binding. Pharmacokinetics is linear in plasma over the dose range of 2 to 70 mg/m² investigated.

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Sessa et al.

Table 4. Summary of pharmacokinetic parameters (mean ± SD) for blood AUY922 and metabolite BJP762 by treatment group (pharmacokinetic/C6 analysis subset) during cycle 1 of day 1 for all doses (2–70 mg/m²)

<table>
<thead>
<tr>
<th>Dose, mg/m²</th>
<th>n (n = 16)</th>
<th>AUY922 AUC (0–inf) (h*ng/mL)</th>
<th>AUY922 AUC(0–C₁₀) (h*ng/mL)</th>
<th>AUY922 Vₘₐₓ (L)</th>
<th>AUY922 CL (L/h)</th>
<th>AUY922 T₁/₂ (h)</th>
<th>BJP762 AUC (0–inf) (h*ng/mL)</th>
<th>BJP762 AUC(0–C₁₀) (h*ng/mL)</th>
<th>BJP762 Vₘₐₓ (L)</th>
<th>BJP762 CL (L/h)</th>
<th>BJP762 T₁/₂ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>54 (n = 17)</td>
<td>1445 ± 5382</td>
<td>1093 ± 6739</td>
<td>1953 ± 9276</td>
<td>1217 ± 6125</td>
<td>1255 ± 6305</td>
<td>2606 ± 1245</td>
<td>2396 ± 1284</td>
<td>259 ± 946</td>
<td>214 ± 1278</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>7</td>
<td>22 (n = 9 )</td>
<td>1756 ± 3904</td>
<td>1207 ± 4333</td>
<td>1953 ± 9276</td>
<td>1217 ± 6125</td>
<td>1255 ± 6305</td>
<td>2606 ± 1245</td>
<td>2396 ± 1284</td>
<td>259 ± 946</td>
<td>214 ± 1278</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>22</td>
<td>28 (n = 8 )</td>
<td>834 ± 2725</td>
<td>52 ± 493</td>
<td>2199 ± 14020</td>
<td>31 ± 1171</td>
<td>92 ± 6739</td>
<td>210 ± 1245</td>
<td>195 ± 1174</td>
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<td>92 ± 6739</td>
<td>31 ± 1171</td>
</tr>
<tr>
<td>40</td>
<td>20 (n = 7 )</td>
<td>834 ± 2725</td>
<td>52 ± 493</td>
<td>2199 ± 14020</td>
<td>31 ± 1171</td>
<td>92 ± 6739</td>
<td>210 ± 1245</td>
<td>195 ± 1174</td>
<td>31 ± 1171</td>
<td>92 ± 6739</td>
<td>31 ± 1171</td>
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<tr>
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<td>834 ± 2725</td>
<td>52 ± 493</td>
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<td>31 ± 1171</td>
<td>92 ± 6739</td>
<td>210 ± 1245</td>
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<td>31 ± 1171</td>
<td>92 ± 6739</td>
<td>31 ± 1171</td>
</tr>
<tr>
<td>70</td>
<td>11 (n = 3 )</td>
<td>834 ± 2725</td>
<td>52 ± 493</td>
<td>2199 ± 14020</td>
<td>31 ± 1171</td>
<td>92 ± 6739</td>
<td>210 ± 1245</td>
<td>195 ± 1174</td>
<td>31 ± 1171</td>
<td>92 ± 6739</td>
<td>31 ± 1171</td>
</tr>
</tbody>
</table>

Abbreviations: AUY922, maximum blood concentration; CL, apparent drug clearance; Cₘₐₓ, apparent volume of distribution during the terminal phase.

Clinical Cancer Research

has been a concern in both the early and late stages of development of these agents (15, 30).

AUY922 is a second-generation, non–geldanamycin iso-
xazole HSP90 inhibitor, and is the first in this class to report full results of a phase 1 study. AUY922 has shown an acceptable safety profile at dose levels of 2 to 70 mg/m², and a recommended dose was determined as 70 mg/m² i.v. on a once-weekly schedule. The most common AEs (mostly grade 1–2) included diarrhea, nausea, and fatigue, which were considered manageable, comparable in type with those reported with other HSP90 inhibitors, and probably on-target effects (31). Visual AEs were reported, which were most frequent at the higher (40–70 mg/m²) dose levels, although typically grade 1 to 2, and were reversible following interruption or discontinuation of study treatment. Visual disturbances have been reported with other geldana-
nycin and non-geldanamycin HSP90 inhibitors (32–34). These effects may be attributable to HSP90 inhibitor–induced photoreceptor degeneration and possibly related to tissue distribution of water-soluble agents facilitating a high retina/plasma concentration ratio, as well as the retinal elimination profile (35). It remains to be clarified whether visual disturbances represent on-target or off-tar-
target effects of non-geldanamycin resorcinol-based deriva-
tives. A relatively large number of patients were treated in this study, including 28 patients at 70 mg/m², and no grade 4 or irreversible eye toxicity was observed. One of the strengths of the BLRM is that it offers a superior estimation of the true MTD compared with a 3 + 3 dose-escalation algorithm (36). Although the usual limit of a 3 + 3 design is 6 patients, additional patients were recruited at some dose levels during the study in order to investigate specific questions about the safety profile of the compound. Although these data gave greater understanding of the risk of retinal and cardiac events, the additional DLT data collected were incorporated in the Bayesian model and used to improve the estimate of the recommended dose. The predicted MTD on the basis of the most sensitive preclinical model (dog) was 20 mg/m². DLTs were seen at dose levels of 22 mg/m² and above; however, cautious dose escalation facilitated by the Bayesian trial design allowed higher doses of AUY922 to be evaluated than would have been recommended by a standard dose-escalation model and gave an optimal recommended safe dose to take forward in subsequent studies, 2.5-times higher than that from the 3 + 3 algorithm. After completion of the MTD expansion arm, a total of 28 patients had been enrolled to the 70 mg/m²-dose group, and 24 of these patients were evalua-
tble for the BLRM. At this point, the BLRM estimated the probability of unacceptable toxicity to be 0.006, and 70 mg/ m² was the dose considered safe to take forward in phase Ib and phase II trials.

AUY922 was well distributed, with a terminal T₁/₂ of 120 hours at 70 mg/m², which is longer than that of geldanamycin-based HSP90 inhibitors such as 17-AAG (5 hours; ref. 15), and it lends itself to weekly dosing. Furthermore, in vivo models suggest accumulation of AUY922 in xenograft tumor tissue compared with plasma,
as has been seen with other HSP90 inhibitors (19). The pharmacodynamic studies, including a study of PBMCs, tumor biopsies, and PET scans, suggest target engagement, thus completing key elements of the pharmacologic audit trail (37).

DLTs were observed across a range of doses (22–70 mg/m²); however, recurrent DLTs such as diarrhea and visual symptoms were seen above the dose level of 40 mg/m². The induction of HSP70 in surrogate tissue occurred at 28 mg/m² and was not significantly higher at 70 mg/m². The reduction in SUVmax on PET scans occurred at 40 mg/m² and was greater at 70 mg/m², but this is not a specific marker of HSP90 inhibition. These findings, in addition to pharmacokinetic data, suggest that a therapeutic window of AUY922 may theoretically extend to as low as 40 mg/m². It is possible that reduced dose levels could be used if patients do not tolerate a dose of 70 mg/m² on chronic dosing, or in a trial where there is overlapping toxicity when AUY922 is explored in combination with a second anticancer agent.

The absence of clinical responses may reflect the fact that patients were not molecularly prioritized before entry into the phase I trial. Given the challenges in managing toxicity in previous HSP90 inhibitors, this study focused on using the BLRM design to obtain reliable estimates of toxicity before recommending a phase II dose. Multiple phase II studies in molecularly prespecified cohorts such as HER2-amplified breast cancer and EGFR-mutated NSCLC were prospectively planned, and are ongoing, to specifically address the proof of concept that HSP90 inhibitors are effective in these subsets of patients. These trials have documented single-agent
activity of AUY922 in HER-2 amplified breast cancer and EGFR-mutant and ALK-rearranged NSCLC (38, 39). In conclusion, given the safety, pharmacologic, and pharmacodynamic profile, 70 mg/m² was recommended as the dose to be taken forward in phase Ib and phase II studies.

Figure 2. A, highest change in HSP70 induction by treatment group (PBMC biomarker subset; n = 61). B, modulation of HSP90 client protein PI3K in a tumor biopsy from a patient with ER⁰ breast cancer after AUY922 (70 mg/m²) treatment. A, pretreatment. B, posttreatment. C, best metabolic response change by ¹⁸F-flurodeoxyglucose PET from baseline to start of cycle 2 or cycle 3. Patients are ranked by change in sSUVmax. Asterisk denotes patients who attained a PMR.
Disclosure of Potential Conflicts of Interest

K.N. Bhalla received commercial research support from Novartis Pharmaceuticals. C. Britten has a commercial research grant and is a consultant/advisory board member of Novartis. V.A. Papadimitrakopoulou is a consultant/advisory board member of Genentech and Merck. M. Akimov is employed (other than primary affiliation; e.g., consulting) as a clinical program leader in Novartis Pharma AG. C. Quadri is employed (other than primary affiliation; e.g., consulting) as a clinical program leader in Novartis Pharma AG and has an ownership interest (including patents) in Novartis Pharma. H. Lu is employed (other than primary affiliation; e.g., consulting) as a senior fellow in Novartis. U. Banerji is employed by The Institute of Cancer Research, received payments as part of the rewards to inventors scheme, and has other commercial research support from Novartis Pharmaceuticals UK Ltd. Dr. Banerji has also received honoraria from the speakers’ bureau from Novartis, and is a consultant/advisory board member of Novartis. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Sessa, V.A. Papadimitrakopoulou, U. Banerji

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Sessa, G.I. Shapiro, K.N. Bhalla, C. Britten, M.M. Mita, T.J. Pluard, M. Akimov, C. Quadri, H. Lu, S.M. Bailey, S. Chica, U. Banerji


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Cristiana Sessa, Geoffrey I. Shapiro, Kapil N. Bhalla, et al.


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