Pharmacokinetic-Pharmacodynamic Relationships for the Heat Shock Protein 90 Molecular Chaperone Inhibitor 17-Allylamino, 17-Demethoxygeldanamycin in Human Ovarian Cancer Xenograft Models

Udai Banerji, Michael Walton, Florence Raynaud, Rachel Grimshaw, Lloyd Kelland, Melani Valenti, Ian Judson, and Paul Workman

Abstract

Purpose: To establish the pharmacokinetic and pharmacodynamic profile of the heat shock protein 90 (HSP90) inhibitor 17-allylamino, 17-demethoxygeldanamycin (17-AAG) in ovarian cancer xenograft models.

Experimental Design: The effects of 17-AAG on growth inhibition and the expression of pharmacodynamic biomarkers c-RAF-1, CDK4, and HSP70 were studied in human ovarian cancer cell lines A2780 and CH1. Corresponding experiments were conducted with established tumor xenografts. The variability and specificity of pharmacodynamic markers in human peripheral blood lymphocytes (PBL) were studied.

Results: The IC50 values of 17-AAG in A2780 and CH1 cells were 18.3 nmol/L (SD, 2.3) and 410.1 nmol/L (SD, 9.4), respectively. Pharmacodynamic changes indicative of HSP90 inhibition were demonstrable at greater than or equal the IC50 concentration in both cell lines. Xenograft experiments confirmed tumor growth inhibition in vivo. Peak concentrations of 17-AAG achieved in A2780 and CH1 tumors were 15.6 and 16.5 μmol/L, respectively, and there was no significant difference between day 1 and 11 pharmacokinetic profiles. Reversible changes in pharmacodynamic biomarkers were shown in tumor and murine PBLs in both xenograft models. Expression of pharmacodynamic biomarkers varied between human PBLs from different human volunteers but not within the same individual. Pharmacodynamic biomarker changes consistent with HSP90 inhibition were shown in human PBLs exposed ex vivo to 17-AAG but not to selected cytotoxic drugs.

Conclusion: Pharmacokinetic-pharmacodynamic relationships were established for 17-AAG. This information formed the basis of a pharmacokinetic-pharmacodynamic-driven phase I trial.

Heat shock protein 90 (HSP90; ref. 1) is a highly conserved intracellular molecular chaperone regulating posttranslational folding of client proteins. In addition to its important role in cellular homeostasis, HSP90 is also a critical player in the response of cells to heat shock, antigen presentation, and buffering genetic mutations at the protein level (2–4). Of particular relevance to cancer is that the list of HSP90 client proteins (5) includes a large number that play important roles in malignancy, including ERB-B2 (6), BCR-ABL (7), estrogen receptors (8), androgen receptors (9), c-RAF-1 (10), CDK4 (11), AKT (12), mutant p53 (13), MET (14), h-TERT (15), and HIF1-α (16). Furthermore, because these oncogenic client proteins are involved in multiple hallmark traits of malignancy, such as deregulated signal transduction, cell cycle progression, apoptosis, immortalization, angiogenesis, invasion, and metastasis (17), inhibition of HSP90 would be expected to deliver a powerful combinatorial blockade on the cancer phenotype (18). Geldanamycin and its analogue 17-allylamino, 17-demethoxygeldanamycin (17-AAG) are able to compete with ATP at the nucleotide binding site in the NH2-terminal domain of HSP90 (19–22). Inhibition of the essential ATPase activity of HSP90 by these agents leads to the degradation of multiple client proteins by the ubiquitin-proteasome pathway (23), resulting in cell cycle arrest and apoptosis (24).

Whereas geldanamycin showed promising preclinical activity in vitro, it was found to be too hepatotoxic in animal models and could not be tested in humans (25). 17-AAG is structurally similar to geldanamycin, maintains its antitumor activity, and has a better toxicity profile and therapeutic index. As a result of this, it is the first anticancer drug of its class to enter clinical trials (26–30).
Conventionally, phase I trials of anticancer agents have focused mainly on the end points of toxicity, tumor response, and more recently, the pharmacokinetic variables of the drug. Early evaluation of the new generation of molecularly targeted therapies requires the assessment of pharmacodynamic biomarkers that provide proof of concept for molecular target inhibition and facilitate the rational selection of the dose and schedule for phase II trials (31, 32). Understanding the pharmacokinetic-pharmacodynamic relationship is particularly important, allowing the construction of a pharmacologic audit trail that links pharmacokinetic and pharmacodynamic variables to target modulation, biological responses, and potentially, to clinical outcome (33–35). Inclusion of pharmacodynamic end points in phase I trials of molecular targeted agents and their incorporation into the decision-making is disappointingly infrequent (36). This is due in part to the fact that appropriate molecular biomarkers are often not identified and validated in time to have a significant effect on phase I clinical trials.

Cell growth inhibition studies. Cells were seeded into 96-well microtiter plates and 24 hours later were exposed to increasing concentrations of 17-AAG for 96 hours. Thereafter, the plates were treated with 10% trichloracetic acid and stained with 0.4% sulforhodamine B (Sigma) in 1% acetic acid. Sulforhodamine B was solubilized by 10 mmol/L Tris-HCl and the absorbance measured at 540 nm using a Titertek multiscan plate reader (Flow Laboratories, Hampshire, United Kingdom).

Isolation of human and murine peripheral blood leukocytes. Whole blood was collected in heparinized tubes (vacutainer, BD, Franklin Lakes, NJ) and diluted 1:1 with 0.9% saline. The diluted blood was layered over a ficoll gradient (Lymphoprep, Neegard, Norway) and centrifuged at 400 × g for 30 minutes. The lymphocytes were then isolated from the plasma-ficoll interface. PBLs were resuspended in 0.9% NaCl and centrifuged at 600 × g to obtain cell pellets.

Western blotting. Cell pellets were lysed in lysis buffer [0.1% NP40, 50 mmol/L HEPES (pH 7.4), 250 mmol/L NaCl, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 20 μg/mL leupeptin, 1 mmol/L DTT, 1 mmol/L EDTA, 1 mmol/L NaF, 10 mmol/L β-glycerophosphate, and 0.1 mmol/L sodium orthovanadate]. Samples (50 μg of protein) were denatured in Laemmli loading buffer [10% glycerol, 5% β-mercaptoethanol, 2% SDS, 62.5 mmol/L Tris (pH 6.8), and 0.05% bromophenol blue], resolved by SDS-PAGE, and transferred onto 0.45-μm polyvinylidene difluoride membranes (Millipore Corp., Billerica, MA). Membranes were blocked in casein blocking buffer [150 mmol/L NaCl, 10 mmol/L Tris base, 0.25 mmol/L thimerosal, 0.5% casein]. They were then probed with the following primary antibodies: c-RAF-1 (SC-133, 1:500, Santa Cruz Biotechnology, Santa Cruz, CA), HSP70 (SPA-810, 1:2,000, Stressgen Biotechnologies, Victoria, Canada), LCK (06-583, 1:5,000, Upstate Biotechnology, Lake Placid, NY), CDK4 (SC-260, 1:1,000, Santa Cruz Biotechnology), and glyceraldehyde-3-phosphate dehydrogenase as the loading control (MAB-374, 1:5,000 Chemicon International, Inc., Temecula, CA). Visualization of the bound primary antibody was done by probing with horseradish peroxidase–conjugated secondary antibodies (1:1,000, Amersham Biosciences, Buckinghamshire, United Kingdom) and exposure to enhanced chemiluminescence reagent (Supersignal, Pierce, Rockford, IL) using film (Hyperfilm, Amersham Biosciences). A semiquantitative analysis of the protein expression in Western blots was carried out using Image Quant 5 (Molecular Dynamics, Amersham Biosciences, Sunnyvale, CA). Levels of protein expression were normalized to glyceraldehyde-3-phosphate dehydrogenase.

Pharmacokinetic analysis. The high-performance liquid chromatography system consisted of a P4000 quaternary pump with degasser, an AS3000 autosampler, and a U 6000LP UV detector set at 330 nm (Thermo Separation Products, Milton Keynes, United Kingdom). A Supelcosil LC18 column was protected with a Supelguard guard column (Supelco, Sigma-Aldrich, Dorset, United Kingdom) and kept at 40°C during the analysis. Mobile phase consisted of a gradient of acetonitrile in water. Plasma samples were extracted by solid phase extraction on 1 mL Bondelut C18 columns (Amersham Biotechnology, Buckinghamshire, United Kingdom). Following a wash with 2 mL of water, samples were eluted with 1% formic acid in water, evaporated to dryness, reconstituted in 150 μL mobile phase, and 30 μL were injected. Tumor and liver samples were homogenized at 1 g/mL and 100 μL extracted with 1 mL ethyl acetate. Following sample drying, the residue was dissolved in 150 μL mobile phase and 0.5 μL injected onto the system. Calibration curves were produced from 50 to 5,000 ng/mL for plasma and 50 to 10,000 ng/mL for tissues by spiking 17-AAG and 17-AG into control plasma or tissue homogenate. Acceptance criteria were established according to published guidelines (42). All samples were added to 30 μL internal standard (5 mg/mL α-naphthoflavone). Pharmacokinetic variables were derived from WinNonlin noncompartmental analysis version 3.2 (Pharsight, Inc., Cary, NC). Previously described statistical tests were used to compare areas under the curves (43).

Human tumor xenografts. The CH1 and A2780 human ovarian lines were grown as s.c. xenografts in female athymic nude mice (nu/nu) by KRAS and chemotherapy-resistant CH1 ovarian cancer cell line (41) was obtained from The Institute of Cancer Research tissue culture bank. Both lines were grown in 10 mmol/L Tris-HCl and the absorbance measured at 540 nm using a Titertek multiscan plate reader (Flow Laboratories, Hampshire, United Kingdom).

Materials and Methods

Drugs. 17-AAG and the vehicle 2% egg phospholipid vehicle were kindly supplied by the Developmental Therapeutics Division of The National Cancer Institute (Rockville, MD). Carboplatin and paclitaxel were sourced from Sigma (Poole, United Kingdom).

Cell culture and assay. The A2780 ovarian cancer cell line was obtained from the American Type Culture Collection (Rockville, MD) and the CH1 ovarian cancer cell line (41) was obtained from The Institute of Cancer Research tissue culture bank. Both lines were grown as monolayers in DMEM (Sigma) containing 10% FCS (Life Technologies, Paisley, United Kingdom), 10% heat-inactivated FCS (Life Technologies), and 1% nonessential amino acids (Life Technologies) in 6% CO2. Human lymphocytes were obtained from volunteers after they signed an informed consent and were studied in suspension culture in RPMI 1640 (Sigma) with 20% heat-inactivated FCS (Life Technologies).
passage of ~2-mm-diameter pieces of solid tumor. Established tumors were treated at an average diameter of 4 to 6 mm corresponding to a mean volume of 55.8 mm$^3$ (SD, 27.6). Once tumors were established, animals were randomized into groups for therapy with either control vehicle (10% DMSO, 90% egg phospholipid) or 17-AAG. Animals were dosed once daily on days 0 to 4 and 7 to 11 as used previously (44).

Tumor growth was assessed thrice weekly and tumor volumes were calculated according to the formula: volume = \( (ab^2 \pi)/6 \), where \( a \) and \( b \) are orthogonal diameters and \( a \) is the longest diameter. Tumor volumes were then expressed as a proportion of the volume at the start of treatment (relative tumor volume). Thus, in the results shown (Fig. 2 and Fig. 5A-B), the relative tumor volume at the start of the experiment is, by definition, unity. The efficacy of the drug was then determined by the growth delay (difference in time, in days, for treated versus control tumor volumes to reach 10$^4$ and 7 to 11. Tumor growth delays were calculated from the growth delay (difference in time, in days, for treated versus control tumor volumes to reach 10$^4$ pretreatment volume). In addition, to correct for the differences in growth rate between the two tumors, specific growth delays were calculated by dividing the growth delays in days by the tumor doubling time. All animal procedures were approved by the Institute of Cancer Research Ethics Committee and were carried in compliance with the United Kingdom Coordinating Committee on Cancer Research Guidelines on the Welfare of Animals in Experimental Neoplasia (45).

### Results

**In vitro growth inhibition in human ovarian cancer cells.** The ability of 17-AAG to inhibit the growth of A2780 and CH1 human ovarian cancer cells *in vitro* was determined by 96-hour sulforhodamine B assays. A2780 cells were found to be considerably more sensitive to 17-AAG than CH1 cells (Fig. 1A). The IC$_{50}$ values for growth inhibition were 18.3 nmol/L (SD, 2.3) and 410.4 nmol/L (SD, 9.38), respectively, representing a difference of 22-fold.

**In vitro pharmacodynamic changes in human ovarian cancer cells.** Both A2780 and CH1 cells were exposed to increasing concentrations of 17-AAG (0.2 $\times$ IC$_{50}$, IC$_{50}$, 5 $\times$ IC$_{50}$, 10 $\times$ IC$_{50}$) for 24 hours. There was a concentration-dependent depletion of the client proteins c-RAF-1 and CDK4 and induction of the cochaperone HSP70 at 24 hours in both cell lines (Fig. 1B). No pharmacodynamic changes were seen at concentrations below IC$_{50}$ (Fig. 1B), suggesting that pharmacodynamic biomarker changes occurred at pharmacologically relevant drug exposures. It is important to emphasize that pharmacodynamic changes occurred at much lower absolute concentrations for A2780 compared with CH1 cells. Thus, the pharmacodynamic changes are reflective of the respective drug sensitivities of the two ovarian cancer cell lines.

**In vivo growth inhibition of human ovarian cancer xenografts.** The ability of 17-AAG to inhibit the growth of established tumors was investigated in nude mice bearing A2780 and CH1 human ovarian cancer cell lines grown as s.c. xenografts. Mice with established tumors were treated with a total of 10 doses of 17-AAG (80 mg/kg i.p.) given once on days 0 to 4 and 7 to 11. Tumor growth delays were calculated from the time to reach 10$^4$ their original tumor volume on day 0 before the first dose of 17-AAG or vehicle control. 17-AAG inhibited the growth of both xenografts (Fig. 2). Growth delays were 13.9 and 10.3 days for A2780 and CH1 xenografts, respectively. The corresponding specific growth delays, corrected for differences in doubling time between the two tumor lines, were 7.4 and 3.6, respectively. Thus, CH1 tumor xenografts were less sensitive than A2780 xenografts *in vivo* as well as *in vitro*.

### Pharmacokinetic profile of 17-AAG in human ovarian cancer xenograft models. A single dose of 17-AAG (80 mg/kg) was administered i.p. to mice bearing A2780 or CH1 xenografts. Concentrations of 17-AAG and its principle metabolite 17-AG (44) were determined over a period of 24 hours. The results are shown in Fig. 3 and pharmacokinetic variables are listed in Supplementary Table 1. The extent of exposure of the A2780 and CH1 tumors to 17-AAG was very similar. For example, areas under the curves for A2780 and CH1 tumors were 52.6 and 50.1 pmol/L hour, respectively. However, exposures above the respective IC$_{50}$ values were much greater in A2780 compared with CH1 tumors (Fig. 3). 17-AAG remained in the tumors longer than in the plasma (Fig. 3; Supplementary Table 1) The metabolite 17-AG, known to be active (44), was present in the tumor and plasma for a longer period than 17-AAG.

Pharmacokinetic analysis was also carried out on A2780 xenograft-bearing mice which received multiple doses of 17-AAG (80 mg/kg i.p. X10 doses) given once a day on days 0 to 4 and 7 to...
The plasma and tumor areas under the curves of 17-AAG were determined on days 1 and 11 (Fig. 4) and were not significantly different ($Z = 0.03$ for plasma and $Z = 0.8$ for tumors).

Pharmacodynamic markers in ovarian cancer xenograft models. Mice bearing A2780 and CH1 xenografts were treated with the same therapeutic schedule discussed above. Pharmacodynamic changes were studied in both human tumor and murine PBLs on days 0, 4, 11, 13, 16, 18, and 20. c-RAF-1, CDK4, and HSP70 were examined in tumors and PBLs and the additional T cell–specific client protein biomarker LCK was studied in PBLs. Day 0 measurements were made immediately before the start of treatment, day 4 and 11 measurements were made during treatment, and days 13 to 20 represented the posttreatment recovery phase. In A2780 xenografts and the corresponding mouse PBLs, there was a depletion of client proteins c-RAF-1 and CDK4 on day 4 during treatment, corresponding to the period of tumor growth inhibition (Fig. 5A). Typical values for the depletion of c-RAF-1 (assessed by semiquantitative densitometry) in tumors and PBLs were 5.7- and 6.5-fold, respectively. The corresponding figures for the depletion of CDK4 in tumors and PBLs were 3.0- and 1.8-fold, respectively. During the recovery phase, both kinase biomarkers returned to levels of expression similar to control values, except CDK4 in tumors that remained depleted (3.5-fold). The cochaperone HSP70 was induced in both tumor and PBLs on day 4 (1.6- and 11.4-fold increase in tumor and PBLs, respectively). In addition, LCK showed depletion (2.8-fold) in PBLs on day 4, recovering by day 20. In general, the molecular signature of the HSP90 inhibitor was similar in A2780 tumor and PBLs (Fig. 5A).

Figure 5B shows that pharmacodynamic markers in CH1 tumor xenografts also exhibited a reversible depletion of the client protein c-RAF-1 (2.8-fold) and induction of HSP70 (8.8-fold) on day 4 corresponding to the period of tumor growth delay. CDK4 was not informative in CH1 xenografts, due to lower levels of expression. Murine PBLs in CH1 tumor-bearing mice showed similar changes to those described in the CH1 tumors (data not shown). The levels of HSP70 expression on day 4 in PBLs from CH1 tumor-bearing xenografts showed a 15.7-fold increase compared with an 8.8-fold increase in tumor.

Expression of pharmacodynamic markers in human peripheral blood lymphocytes. Before the start of a phase I clinical trial of 17-AAG, we studied the expression of the proposed pharmacodynamic markers in human PBLs. PBLs from eight healthy volunteers were sampled and the expression of pharmacodynamic biomarkers c-RAF-1, HSP70, and LCK were investigated. Expression varied considerably across the eight samples studied (data not shown). However, PBLs sampled from individual volunteers at different time points showed little variation (Fig. 6A).

Specificity of pharmacodynamic markers in human peripheral blood lymphocytes. We exposed human PBLs ex vivo for 24 hours to 17-AAG and two cytotoxic chemotherapeutic agents (paclitaxel and carboplatin) that are commonly used in the treatment of ovarian cancer. Concentrations that were achievable clinically (26, 27, 46) were chosen. Western blot analysis revealed that the molecular signature of HSP90 inhibition (i.e., c-RAF-1, LCK, and CDK4 depletion along with HSP70 induction) occurred in PBLs treated with 17-AAG but not paclitaxel or carboplatin (Fig. 6B).

Discussion

The primary objective of this study was to validate pharmacodynamic biomarkers for inhibition of HSP90 by 17-AAG and to establish pharmacokinetic-pharmacodynamic relationships in a human ovarian cancer xenograft model. The major intended use of these biomarkers was to obtain clinical proof of concept for the inhibition of the HSP90 molecular chaperone in subsequent phase I studies. It was also intended that an understanding of pharmacokinetic-pharmacodynamic relationships would be useful to facilitate the conduct of the phase I trial, as well as the rational selection of a dose and schedule for the phase II study. It should be noted that it was not necessarily expected that the pharmacokinetic-pharmacodynamic variables would be predictive of tumor sensitivity, because many other downstream determinants of response may be important (39).
We first established the concentrations of 17-AAG that inhibited the growth of the two human ovarian cancer cell lines A2780 and CH1. A2780 cells were considerably more sensitive to 17-AAG than CH1 cells (Fig. 1). Because the former cell line exhibits much higher expression of the quinone reductase enzyme NQO1 or DT-diaphorase (47), this observation is in agreement with our previous demonstration of the importance of NQO1 expression in the sensitivity of cancer cells to 17-AAG (44). The 22-fold difference in the sensitivity of the A2780 and CH1 ovarian cancer cells to 17-AAG makes them useful for the study of pharmacodynamic biomarkers.

Having defined the pharmacologically relevant concentrations for each ovarian cancer cell line, we then studied the response of pharmacodynamic markers to growth inhibitory concentrations. In agreement with previous work, we showed that the serine-threonine kinases c-RAF-1 (10) and CDK4 (11) were depleted by 17-AAG, whereas HSP70 was induced when HSP90 was inhibited (2, 39). Although other biomarkers have been studied (2, 39), this particular group was selected because it is relevant to most if not all tumor types, which is an advantage in a phase I trial where a range of cancers are included. In addition, a molecular signature (2) in which certain biomarkers were decreased while others increased might prove more informative and robust than relying on a single marker alone. The results obtained in the A2780 and CH1 ovarian cancer cells in vitro support the use of the selected proteins as pharmacodynamic biomarkers of HSP90 inhibition. However, before their use in our phase I trial, we wished to validate the proposed molecular signature in an in vivo tumor xenograft setting. To do this, the effects of 17-AAG were evaluated in the same two human ovarian cancer cell lines A2780 and CH1. Tumors were grown s.c. and treated when they had become established with a mean volume of 55.8 mm³. A2780 xenografts were more sensitive than CH1 xenografts, corresponding to their differential sensitivity in vitro (Fig. 2). The extent of this difference was less than that observed in cell culture (Fig. 1), an effect that was also seen in human colon cancer models where it was attributed to the fact that the metabolite 17-AG is produced in vivo, sensitivity to which is not dependent on DT-diaphorase (44).

Next, we studied the concentrations of the drug that were achievable using an active dosing schedule in vivo. Consistent with tumor xenograft responses, concentrations of 17-AAG known to inhibit cells in vitro were achieved in both plasma

![Fig. 3. Pharmacokinetics of 17-AAG. Mice bearing A2780 and CH1 human ovarian cancer xenografts were treated with a single dose of 17-AAG (80 mg/kg i.p.). A, 17-AAG concentrations in plasma, tumor, and liver in A2780 xenograft-bearing mice. The concentrations of 17-AAG achieved in the tumor remains above the IC₅₀ for A2780 in vitro of 18 nmol/L for over 24 hours. B, concentrations of the active metabolite 17-AG achieved in plasma, tumor, and liver. C, additive concentrations of 17-AAG and its active metabolite 17-AG in plasma, tumor, and liver. D-F, corresponding data in CH1 xenograft-bearing mice.](image-url)
and tumor. Concentrations of the drug and its metabolite were maintained for a considerably longer period in the tumor compared with plasma, similar to previously published data (48). This may be one of the potential reasons why 17-AAG is able to achieve a safe therapeutic index and may be related to the preferential binding to the superchaperone complex in malignant cells (49, 50). The extent of exposure to 17-AAG was similar in absolute terms for A2780 and CH1 ovarian xenografts. However, A2780 cells have a much lower IC\textsubscript{50} for inhibition of proliferation than CH1 cells (see Results). Therefore, exposure levels above the respective IC\textsubscript{50} values were much greater for the more sensitive A2780 compared with the more resistant CH1 model (Fig. 3). Thus, the exposures were consistent with the differences in in vitro sensitivities of the two ovarian cancer xenografts. As mentioned above, the major metabolite 17-AG is an active HSP90 inhibitor (44) and this constitutes an additional factor in pharmacokinetic-pharmacodynamic considerations. Thus, it is important to note that 17-AG also achieves high concentrations in both tumor and plasma and has a longer half-life than 17-AAG. Hence, 17-AG may contribute to the activity of 17-AAG in vivo, and this reduces the differential sensitivity of A2780 and CH1 tumor xenografts compared with the in vitro setting. Our pharmacokinetic data are not dissimilar to those published in CD2F1 mice treated with 17-AAG at 60 mg/kg (48). In addition, we established for the first time that, with the prolonged repeat dosing schedule used here, the drug concentrations achievable in plasma and tumor did not vary with repeat dosing. This has clinical significance as a variety of schedules of administration are being pursued in phase I clinical trials (26–30, 51).

As mentioned, pharmacodynamic markers are important in establishing that the drug under study is indeed inhibiting the proposed molecular target and is thus likely to be acting by the mode of action ascribed to it. In addition to providing proof of concept for the proposed molecular mechanism, pharmacokinetic-pharmacodynamic relationships are useful in building a pharmacological audit trail and can be used to improve decision making in phase I clinical trials, in particular to optimize the dosing schedule (35). It would be advantageous if the pharmacodynamic biomarkers were predictive of response, but this is not essential. As mentioned above, other downstream factors are likely to play a role in sensitivity (39). For a discussion of the different categories of biomarkers, see refs. (32, 34, 36, 52). Nevertheless, it was important to examine the biomarkers in relation to the respective sensitivities of the ovarian cancer lines used.

Our experiments show that there was a concentration-dependent response in the pharmacodynamic changes that occurred when both A2780 and CH1 human ovarian cancer cells were exposed to 17-AAG in vitro and the pharmacodynamic changes did not occur at concentrations below IC\textsubscript{50} levels (Fig. 1B). It is important to emphasize that the biomarkers all exhibited changes at concentrations around the respective IC\textsubscript{50} values, indicating a relationship with tumor cell sensitivity in the two ovarian cancer models (Fig. 1B).

The pharmacodynamic changes in A2780 xenografts and murine PBLs occurred during 17-AAG treatment when tumor growth was inhibited. Recovery of pharmacodynamic markers to pretreatment levels during tumor regrowth was seen with the exception of CDK4 in A2780 tumors, where client protein depletion persisted for reasons that are not clear. Similar pharmacodynamic changes were noted in CH1 xenografts, although CDK4 showed low levels of expression and was therefore not informative in this model. It is interesting to note that the increase in expression of HSP70 on day 4 was 15.7-fold in the corresponding PBLs and 8.8-fold in CH1 xenografts versus 1.6-fold in the A2780 xenografts versus 11.6-fold in the CD2F1 mice treated with 17-AAG at 60 mg/kg (48). In addition to providing proof of concept for the proposed molecular mechanism, Pharmacodynamic changes in xenograft tissue following 17-AAG administration in melanoma, prostate, and breast cancer xenografts have been published previously (53–55). However, the present study is to our knowledge the first carried out specifically to relate pharmacokinetic-pharmacodynamic changes in xenograft tissue to effects of 17-AAG on tumor growth. This is also the first publication to examine pharmacodynamic changes in murine PBLs and their relationship to pharmacodynamic changes and growth inhibitory effects in tumor xenografts treated with a HSP90 inhibitor. The extent of depletion of...
client proteins was generally of a similar order in both ovarian tumor xenografts and PBLs (Fig. 5A and B). No conclusions can be made concerning the relationship between biomarkers and the extent of inhibition of tumor xenograft growth and indeed this was not the objective of the study. However, as mentioned, the in vitro data clearly showed that the biomarker changes do correspond to the different sensitivities of the A2780 and CH1 models (Fig. 1). Further studies are required in different animal models to evaluate more fully the relationship between tumor growth inhibition and biomarker changes. At this point, we recommend the use of the biomarkers as pharmacodynamic end points to provide evidence of HSP90 inhibition.

Whereas pharmacodynamic changes in tumors provide important proof of concept that the drug is inhibiting the target in cancer tissue, there is a need for serial tumor biopsies that has ethical and logistical implications and which can slow down recruitment for phase I clinical trials. PBLs are a source of easily accessible normal tissue. Whereas we did show that the nature of the pharmacodynamic changes in PBLs was generally similar to those in the tumor in the xenograft models, albeit with HSP70 induction being generally greater in PBLs due to the a lower baseline levels (see above), certain issues needed to be clarified before their use in a phase I clinical trial. These concerned the variability and specificity of the markers used.

Both interindividual and intraindividual expression of pharmacodynamic markers were studied in PBLs from normal volunteers. There was considerable variation in the level of expression of these biomarkers between individuals. However,
the expression of the markers was consistent within the same individual (Fig. 6A). We therefore concluded that we could use these pharmacodynamic makers in a phase I trial with patients acting as their own control.

The next important issue to be addressed was the specificity of the pharmacodynamic biomarkers. We have previously shown that human PBLs when exposed ex vivo to 17-AAG exhibit induction of HSP70 (40). In this present study, we showed that the pharmacodynamic biomarkers c-RAF-1, LCK, CDK4, and HSP70 showed altered expression on exposure of PBLs to 17-AAG but not to paclitaxel or carboplatin (Fig. 6B). This finding strengthens the case for using the previously mentioned pharmacodynamic biomarkers to study 17-AAG as a single agent or in combination with other cytotoxic agents. We have also studied pharmacodynamic changes in the HCT116 colon cancer cell line in response to a wide array of cytotoxic agents (melphalan, topotecan, 5-fluorouracil, mitomycin C, vincristine, daunorubicin, cisplatin, and paclitaxel) at equitoxic concentrations to 17-AAG. We found that the pharmacodynamic changes occurred only when the cells were exposed to HSP90 inhibitors 17-AAG and radicicol but not with the other cytotoxic agents (data not shown). There is evidence that histone deacetylase inhibitors can produce the molecular signature attributed to inhibition of HSP90. This is thought to be due to increased acetylation and hence inhibition of the chaperone (56).

A phase I trial of 17-AAG has been carried out at our institution (26, 27). The pharmacokinetic-pharmacodynamic relationships discussed here have been important in developing biomarkers in tumor and normal tissue to aid dose escalation and scheduling in the trial. As recently reviewed by Parulekar and Eisenhauer (36), use of pharmacokinetic-pharmacodynamic end points in trials of molecular therapeutics over the last 5 years has been disappointingly low. In our experience, the timely identification and validation of pharmacodynamic biomarkers and the establishment of pharmacokinetic-pharmacodynamic relationships has been crucial to the successful implementation into our phase I study.

In conclusion, 17-AAG is a novel HSP90 inhibitor that is the first in its class to enter clinical trials. We have identified and validated a group of molecular biomarkers for HSP90 inhibition, consisting of c-RAF-1, CDK4, and HSP70 as well as LCK for use in PBLs. These markers comprise a molecular signature that should be applicable to a wide range of human tumors. We have established pharmacokinetic-pharmacodynamic relationships in human ovarian cancer xenografts that occur at the same time as tumor growth inhibition and that are reproducible, robust, specific, and informative. We showed that changes in molecular pharmacodynamic biomarkers occurred in both tumor and PBLs at doses and concentrations of 17-AAG that are required for therapeutic activity. Thus, c-RAF-1, HSP70, and CDK4 are pharmacodynamic markers that can be used to evaluate 17-AAG in tumor biopsies and additionally LCK can be used as a pharmacodynamic marker in PBLs. The pharmacokinetic-pharmacodynamic end points described in this article can be used to construct a pharmacologic audit trail for HSP90 inhibitors and can, in particular, be used to provide proof of concept for target inhibition and to guide dosing in early clinical trials. Although designed to show evidence of target inhibition in phase I studies and help in the selection of dose and schedule in phase II, it is possible that changes in the molecular signature of HSP90 inhibition may be predictive of therapeutic activity, at least in some settings. However, it is likely that additional factors will play a role downstream of client protein depletion and HSP70 up-regulation (39). Of particular interest is that the induction of HSP70 and HSP90 family members by 17-AAG (40) may have an antiapoptotic effect (57).
The results discussed in this article gave us confidence to use the pharmacokinetic-pharmacodynamic end points described here in the phase I clinical trial carried out at our institution (26, 27). We proposed a pharmacokinetically-pharmacodynamically driven strategy in which pharmacokinetics only would be carried out until the plasma levels were in the therapeutic range as defined by in vitro IC_{50} values and pharmacokinetic properties reported herein. At that point, pharmacodynamic biomarkers would be measured in PBLs. Only when pharmacodynamic changes were seen in PBLs would tumor biopsies be taken. This was not only more ethically acceptable, but it reduced considerably the number of biopsies that needed to be carried out in the phase I study. This strategy was implemented and proved to be successful. The preclinical studies reported here informed the choice of timing of tumor biopsies, with 24 hours after treatment being selected. Both the nature and changes in biomarkers seen in PBLs and tumor tissue in the phase I trial (26, 27) were similar to those reported here in the ovarian cancer models. The biomarkers used were selected to be as broadly applicable as possible across the multiple tumor types that are represented in phase I trials. Not all biomarkers were detectable in all patients, but the panel selected ensured that an interpretable result could be obtained in all cases. Pharmacodynamic changes seen in tumor tissue were also reflected in PBLs. The results suggested that HSP90 is inhibited in patients’ tumors for somewhere between 1 and 5 days. Interestingly, there was evidence of more sustained modulation of CDK4 as seen in the present xenograft studies (Fig. 5A). The pharmacodynamic data suggested that a schedule in which 17-AAG is administered more often than once a week may be needed to provide continuous inhibition of HSP90. A daily schedule (5 of 7 days every 3 weeks) gave much lower plasma drug levels and leaves the target inhibited for 2 weeks (28). Other trials are evaluating administration of twice or thrice a week schedules (58).

Finally, it was worth pointing out that the results of present studies and those of previous human tumor xenograft investigations (44, 53–55) have indicated that a cytostatic response, equivalent to disease stabilization rather than regression, is likely to be the key to 17-AAG administration. In agreement with this, we saw two cases of disease stabilization in patients with advanced metastatic malignant melanoma treated in our phase I clinical trial (26, 27). This expectation of disease stabilization rather than regression has been important in designing phase II trials of 17-AAG. Indeed, this concept has underpinned the design of a phase II study in metastatic malignant melanoma being carried out at our institution.

References

14. Webb CP, Hoss CD, Koochepour S, et al. The gelda-

namicyns are potent inhibitors of the hepatocyte growth factor/scatter factor-met-urokinase plasmino-
29. Grem JL, Morrison G, Guo XD, et al. Phase I and pha-
cromatic analysis of 17- (allylamino)–17-demethoxy-
33. Garrett MD, Workman P. Discovering novel chemo-

www.aacrjournals.org 7031 Clin Cancer Res 2005;11(19) October 1, 2005 Downloaded from clinicanres.aacrjournals.org on April 9, 2017. © 2005 American Association for Cancer Research.


Pharmacokinetic-Pharmacodynamic Relationships for the Heat Shock Protein 90 Molecular Chaperone Inhibitor 17-Allylamino, 17-Demethoxygeldanamycin in Human Ovarian Cancer Xenograft Models

Udai Banerji, Michael Walton, Florence Raynaud, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/11/19/7023

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2006/02/09/11.19.7023.DC1

Cited articles
This article cites 52 articles, 27 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/11/19/7023.full.html#ref-list-1

Citing articles
This article has been cited by 41 HighWire-hosted articles. Access the articles at:
/content/11/19/7023.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.