Mechanistic Evaluation of the Novel HSP90 Inhibitor NVP-AUY922 in Adult and Pediatric Glioblastoma

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Abstract

The dismal prognosis of glioblastoma (GB) indicates the urgent need for new therapies for these tumors. Heat shock protein 90 (HSP90) inhibitors induce the proteasome-mediated degradation of many oncogenic client proteins involved in all of the hallmark characteristics of cancer. Here, we explored the mechanistic potential of the potent synthetic diarylisoxazole amide resorcinol HSP90 inhibitor, NVP-AUY922, in adult and pediatric GB. In vitro antiproliferative potency (nanomolar range) was seen in both adult and pediatric human GB cell lines with different molecular pathologies. A cytostatic effect was observed in all GB lines; more apoptosis was observed at lower concentrations in the SF188 pediatric GB line and at 144 hours in the slower growing KNS2 GB line, as compared with the adult GB lines U87MG and SF268. In vitro combination studies with inhibitors of phosphoinositide 3-kinase/mammalian target of rapamycin (PI-103) or mitogen-activated protein/extracellular signal-regulated kinase (ERK) kinase (PD-0325901) supported the hypothesis that sustained inhibition of ERK up to 72 hours and at least temporary inhibition of AKT were necessary to induce apoptosis in GB lines. In athymic mice bearing established s.c. U87MG GB xenografts, NVP-AUY922 (50 mg/kg i.p × 3 days) caused the inhibition of ERK1/2 and AKT phosphorylation and induced apoptosis, whereas 17-AAG used at maximum tolerated dose was less effective. NVP-AUY922 antitumor activity with objective tumor regression resulted from antiproliferative, proapoptotic, and antiangiogenic effects, the latter shown by decreased microvessel density and HIF1α levels. Our results have established a mechanistic proof of concept for the potential of novel synthetic HSP90 inhibitors in adult and pediatric GB, alone or in combination with phosphoinositide 3-kinase/mammalian target of rapamycin and mitogen-activated protein/ERK kinase inhibitors. Mol Cancer Ther; 9(5); 1219–33. ©2010 AACR.

Introduction

Glioblastomas (GB) are highly invasive primary brain tumors with poor prognosis despite current therapies (surgery, radiotherapy, and chemotherapy; ref. 1). Targeted therapies, as single agents, have failed to offer long-term survival benefit despite objective initial responses (2). GB heterogeneity and a complex molecular pathology contribute to this lack of success. Thus, GB might be more efficiently eradicated by targeting multiple signaling pathways or different tumor features (proliferation, resistance to apoptosis, angiogenesis, and invasion) simultaneously.

The molecular chaperone heat shock protein 90 (HSP90) regulates the conformation, stability, and function of many critical oncogenic client proteins that are essential in maintaining the malignant transformation and in increasing the survival, growth, and invasive potential of cancer cells (3). HSP90 inhibitors induce proteasome-mediated degradation of these client proteins (3). 17-Aminomido-17-demethoxygeldanamycin (17-AAG, tanespimycin), the first-in-class HSP90 inhibitor to enter clinical trials, has shown early signs of clinical activity in adult and pediatric patients with different tumor types (4–6). Antiproliferative, anti-invasive, and proapoptotic effects have been observed in in vitro adult GB (aGB) cells with ansamycin benzoquinone HSP90 inhibitors, such as 17-AAG, and with the structurally unrelated natural product HSP90 inhibitor radicicol (7–11). 17-AAG was also shown to target the glioma stem cells that may initiate tumor recurrences (12). Synergistic interactions have been reported between HSP90 inhibitors and anti-GB therapies, such as radiotherapy (12), SN38 (13), LY294002 (14), and gefitinib (15). However, ansamycin benzoquinones
present limitations (e.g., suboptimal solubility, cumbersome formulation, and extensive metabolism; ref. 3). In particular, low activity of the NAD(P)H:quinone oxidoreductase 1 (NQO1/DT-diaphorase) is a factor in intrinsic (16) and acquired resistance to 17-AAG in GB cells (17).

The synthetic pyrazole/isoxazole resorcinol class of HSP90 inhibitors (18–20) offer advantages over 17-AAG, including independence from NQO1 metabolism, PgP insensitivity, and favorable aqueous solubility (21, 22). One member of this series, NVP-AUY922, has recently entered phase II clinical trials in adult patients (22). Interestingly, NVP-AUY922 and related agents retain full activity in GB lines rendered resistant to 17-AAG (17). In addition, we have been unable to generate resistance to NVP-AUY922 in GB lines by using a continuous drug exposure protocol that did induce 17-AAG resistance (17).

The aim of the present study was to evaluate the mechanistic potential of NVP-AUY922 in both aGB and pediatric human GB (pGB) models. We show that NVP-AUY922 exhibits a potent anti-GB activity both in in vitro cell culture systems and also in in vivo subcutaneous (s.c.) human GB models driven by different genetic abnormalities, from both adult and pediatric origins. We show that by depleting client proteins involved in the main GB oncogenic pathways, NVP-AUY922 exhibited cytostatic, proapoptotic, and antiangiogenic effects, with more extensive apoptosis in the pGB lines studied. We also provide evidence to support the hypothesis that proapoptotic effects of NVP-AUY922 depend on the inhibition of both extracellular signal-regulated kinase (ERK) and AKT phosphorylation. Taken together, our results have established a mechanistic proof of concept for the potential of novel synthetic HSP90 inhibitors in aGB and pGB, both alone or in combination with phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) and mitogen-activated protein (MEK) inhibitors.

**Materials and Methods**

**GB cell lines.** Human GB cell lines from adult (U87MG and SF268) and pediatric (SF188 and KNS42) patients were obtained and grown as previously published (17).

**Drugs and compounds.** HSP90 inhibitors were either purchased or prepared as described (17). The dual PI3K/mTOR inhibitor PI-103 and the MEK inhibitor PD-0325901 were provided by Piramed Ltd. and the Dundee University, United Kingdom, respectively.

**Growth inhibition studies.** Growth inhibition was determined using the sulforhodamine B assay (SRB; ref. 16). Briefly, 1 × 10³ cells were seeded into 96-well microtiter plates and allowed to attach for 36 hours (2 × 10³ cells for KNS42). Compounds at a range of concentrations were added in quadruplicate wells for 6 days (at least three doubling times) in a volume of 200 µL per well. The IC₅₀ was calculated as the drug concentration that inhibits cell proliferation by 50% compared with controls.

**Cell viability, cell cycle, and apoptosis analysis.** Cell count and cell cycle status were determined as described (21), involving the trypan blue exclusion method and DNA content analysis using propidium iodine staining and flow cytometry, respectively, on the total cells population (attached and detached cells). Sub-G₁ population quantification by flow cytometry and poly ADP ribose polymerase (PARP) and caspase cleavage by immunoblotting was used to confirm apoptosis. The antibody C-2-10 (Clontech) recognizing both the 116-kDa native PARP and the 85-kDa apoptosis-related cleavage product was used together with anti-caspase antibodies recognizing both full-length inactive pro-caspases and active cleaved caspase-3, caspase-7, caspase-8, and caspase-9. Antibodies are listed in Supplementary Data (Supplementary Table S1).

**Combination studies.** Cells were treated with increasing concentrations of drugs either alone or in combination at their equipotent molar ratio concomitantly. Effects on cell number were determined by SRB assay. The results were analyzed using the median effect analysis method (23) and by deriving the combination index (CI), which was calculated at equipotent combined drug concentrations that inhibit growth at 50% (ED₅₀). Exclusive CI values were used to analyze combinations with agents sharing a similar mechanism of action (HSP90 inhibitors, PI-103 and PD-0325901).

**Western blot analysis.** Procedures for cell lysate preparation and Western blotting were as described (21). Immunodetection was carried out using antibodies listed in Supplementary Data (Supplementary Table S1). Densitometry was done on the Western blots by the Image Quant Pro software. Results are provided for repeat experiments (n = 3). SEMs for repeat experiments were typically 18% to 20% of the mean.

**Tumor xenograft efficacy studies.** Animal procedures were carried out within the guidelines set out by The Institute of Cancer Research’s Animal Ethics Committee and in compliance with national guidelines (24).

Human U87MG aGB cells (2 × 10³) were injected s.c. in the flanks of eight female NCr athymic mice per group. Animals were treated for 2 weeks with vehicle, NVP-AUY922, or 17-AAG once tumors reached 8 mm in mean diameter. Tumor volumes and body weights were measured thrice weekly. Therapeutic activity was determined according to the number of complete responses, partial responses (regression, >50% decrease), stable disease, and tumor-free survivors at day 120. Tumor growth delay was calculated as the difference between treated and control groups in the median time to reach a median tumor volume five times greater than the initial volume as described (25). Progression-free survival probabilities were calculated using the Kaplan-Meier method.

**Biomarker analysis by electrochemiluminescent immunoassay and vascular endothelial growth factor analysis by ELISA.** Pharmacodynamic biomarker studies were done on tumor tissues harvested 24 and 48 hours after a 3-day treatment course. Frozen samples
were lysed, homogenized, and analyzed for protein content as described (26).

Expression of selected proteins (HSP72, HIF1α, and phosphorylated and total AKT, ERK, and MET) was determined by electrochemiluminescent immunoassay (MesoScale Discovery MSD) as described (26).

Vascular endothelial growth factor (VEGF) expression was analyzed using a Human Quantikine VEGF ELISA kit (R&D Systems). Briefly, tumor lysates were titrated to determine the optimum amount of protein for the control samples to be within the linear range of the assay. Sixteen micrograms of protein were loaded per well and the protocol was followed as per the manufacturer’s instructions for serum or plasma samples. Results (pg/mL) were calculated by subtracting readings at 570 nm from the readings at 450 nm (to correct for optical imperfections in the plate) and were determined from the standard curve.

**Immunohistochemistry, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling and microvessel density analysis.** Five-micrometer-thick sections of formalin-fixed, paraffin-embedded tumors were prepared. Proliferation index (mouse anti-human Ki67 antibody staining, clone MIB-1, DAKO; 1/300 dilution) and apoptosis (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, In situ Cell Death Detection kit, AP, Roche Diagnostics GmbH) were determined as described (25). Eight fields per section at ×40 objective magnification, randomly selected, were counted.

Mouse blood vessels were stained with rat anti-mouse CD34 antibody (Abcam; dilution 1/50) and microvessel density was measured as described (27). Five fields per section at ×5 objective magnification, randomly selected, were analyzed with the Image-Pro Plus 5.0 software (Media Cybernetics).

**Statistical analysis.** All values are mean ± SD of at least three independent experiments, unless otherwise stated. Statistical significance was calculated by a two-tailed paired t test. Statistical significance between in vivo treatment groups and controls in their time to reach five times the initial tumor volume was estimated by a two-tailed nonparametric Kruskal-Wallis test. P < 0.05 was considered statistically significant. For Kaplan-Meier curves, differences in progression-free survival were tested for statistical significance using a log-rank test; P < 0.05 was considered statistically significant.

**Results**

**NVP-AUY922 induces potent cell growth inhibition in adult and pediatric GB cell lines.** The molecular characteristics of the human GB cell lines used in this study are described in the Supplementary Data (Supplementary Fig. S1) and in previous work (17, 28). Our earlier studies (22) have shown that NVP-AUY922 exhibits potent activity in a range of cancer cell lines of different origins. NVP-AUY922 exhibited strong antiproliferative effects against both human aGB (U87MG and SF268) and pGB lines (SF188 and KNS42) with IC50 values by SRB assay in the nanomolar range: 7.8 ± 1.2, 6.1 ± 1.5, 7.6 ± 2.2, and 4.8 ± 1.2 nmol/L, respectively (Table 1). Two analogues of NVP-AUY922 (VER-50589 and VER-49009) with lower potency against HSP90 (21) showed 8- and 82-fold increases in IC50 values, respectively.

Sensitivity to HSP90 inhibitors of different chemical classes, as determined by SRB assay, was independent of adult versus pediatric GB cell origins. Cellular potency, as measured by the mean IC50 for the four GB lines tested, is higher for NVP-AUY922 than for 17-AAG by a factor of 1.8 in U87MG, 2.0 in SF268, 1.5 in SF188, and 6.2 in KNS42 (Table 1). These differences are generally modest, with the exception of KNS42 in which the difference is much greater. When compared with radicicol and the purine BIIB021, NVP-AUY922 is 5- to 9-fold more potent, respectively. NQO1 expression can play a role in the sensitivity of cancer cell lines to 17-AAG (16). In a panel of 13 aGB and pGB cell lines, there was no clear relationship

**Table 1. Cell growth inhibition effect of HSP90 inhibitors as measured by SRB in GB cell lines**

<table>
<thead>
<tr>
<th>IC50 (nmol/L)</th>
<th>Adult cell lines</th>
<th>Pediatric cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U87MG</td>
<td>SF268</td>
</tr>
<tr>
<td>NVP-AUY922</td>
<td>7.8 ± 1.2</td>
<td>6.1 ± 1.5</td>
</tr>
<tr>
<td>VER-50589</td>
<td>57.1 ± 20.8</td>
<td>28.0 ± 10.8</td>
</tr>
<tr>
<td>VER-49009</td>
<td>587.8 ± 99.3</td>
<td>414.2 ± 20.3</td>
</tr>
<tr>
<td>Radicicol</td>
<td>44.9 ± 4.9</td>
<td>32.4 ± 12.0</td>
</tr>
<tr>
<td>17-AAG</td>
<td>13.9 ± 2.0</td>
<td>12.4 ± 0.6</td>
</tr>
<tr>
<td>17-DMAG</td>
<td>3.7 ± 1.3</td>
<td>3.2 ± 1.4</td>
</tr>
<tr>
<td>BIIB021</td>
<td>75.1 ± 47.0</td>
<td>80.5 ± 39.0</td>
</tr>
</tbody>
</table>

**NOTE:** Values are mean ± SD from at least three independent experiments. IC50 values of NVP-AUY922, its less potent analogues VER-50589 and VER-49009, the natural product radicicol, the ansamycin benzoquinones 17-AAG and 17-DMAG, and the synthetic purine scaffold HSP90 inhibitor BIIB021, in two adult (U87MG and SF268) and two pediatric (SF188 and KNS42) GB lines.
between response to 17-AAG and NQO1 expression (Supplementary Fig. S1C; Table 1); this likely indicates additional factors being involved in sensitivity, as noted for other tumor types (16, 29). However, in contrast to our results with 17-AAG (17), NVP-AUY922 exhibited comparatively high potency in the low NQO1-expressing pGB line KNS42 (Supplementary Fig. S1C; Table 1). In addition, in the UW479 pGB cell line that shows undetectable NQO1 expression and is the most resistant to 17-AAG (IC_{50} 99.0 nmol/L), NVP-AUY922 is 62-fold more potent (IC_{50} 1.6 nmol/L; Supplementary Fig. S1C). The enhanced activity of NVP-AUY922 in GB cell lines resistant to 17-AAG through reduced NQO1 expression was shown in our previous studies (17).

**NVP-AUY922 exhibits a potent cytostatic effect due to either G1 or G1-G2 arrest, particularly in pediatric GB cell lines.** We previously showed that 17-AAG causes G1 and G2-M cell cycle arrest and induces cytostasis and apoptosis in various cancer cell lines (30). Here, direct measurement of viable cell counts showed that both NVP-AUY922 and 17-AAG (at 5x IC_{50} concentrations by SRB assay; Table 1) caused a cytostatic effect up to 48 hours posttreatment in all cell lines (Fig. 1). At 72 hours, in aGB lines (U87MG and SF268) or in the SF188 pGB line treated with 17-AAG, cells regrew in the presence of the drug. In the pGB lines, the cytostatic effects were sustained for at least 144 hours in KNS42 cells with both inhibitors, whereas NVP-AUY922 reduced viable cell numbers in SF188 cells from 72 hours.

Next, we studied the effects of NVP-AUY922 and 17-AAG on cell cycle distribution and apoptosis markers up to 144 hours (Fig. 2). Because of space constraints, results are shown for cell cycle distribution (Fig. 2A), and PARP and caspase cleavage (Fig. 2C) up to 72 hours, which was the most informative period. Data for the sub-G1 peaks are shown up to 144 hours (Fig. 2B). Both NVP-AUY922 and 17-AAG decreased the S-phase population (<10% of cycling cells) and induced either a G1 or G2 arrest, depending on the cell line and the HSP90 inhibitor (Supplementary Fig. S2A; Fig. 2A). In all GB lines, both HSP90 inhibitors consistently depleted G1-S transition proteins together with the HSP90 client protein CDK4 and its partner cyclin D1, whereas a decrease in the expression of the HSP90 client protein CDC2 was observed only in cells arresting in G2 (Supplementary Fig. S2C and Tables S2A and B; ref. 9).

**NVP-AUY922 induces apoptotic cell death, particularly in the pediatric GB cell lines.** NVP-AUY922–induced apoptosis, as shown by an increased sub-G1 population (Supplementary Fig. S2B; Fig. 2B and cleaved PARP (Supplementary Fig. S2C and Tables S2A and B; Fig. 2C), was more predominant in the pGB cell line SF188 compared with the aGB cell lines studied here. At 48 and 72 hours, the apoptotic population in NVP-AUY922–treated SF188 pGB cells represented 37% and 29% of events, respectively (Fig. 2B), with correspondingly high levels of cleaved PARP (Supplementary Table S2A; Fig. 2C). Apoptosis with NVP-AUY922 was greater than with 17-AAG in the SF188 pGB line (Supplementary Table S2A; Fig. 2B and C). In KNS42 pGB cells, which have the longest doubling time, PARP cleavage was observed from 48 hours, whereas the sub-G1 population increase occurred later at 144 hours.

**Figure 1.** Potent cell growth inhibition induced by NVP-AUY922 in adult and pediatric GB cell lines, compared with 17-AAG. Effect on absolute viable cell count (trypan blue–excluding cells) in GB lines treated with 5x IC_{50} concentrations (determined by SRB assay) of NVP-AUY922 (○) and 17-AAG (▲), compared with nontreated controls (▪). Points, mean of at least three independent experiments; bars, SD.
Figure 2. Cell cycle and proapoptotic effect of NVP-AUY922 and 17-AAG in adult and pediatric GB cell lines. The adult (U87MG) and the pediatric (SF188) GB lines were treated with 5× IC_{50} concentrations (determined by SRB assay) of either NVP-AUY922 or 17-AAG at time 0 and harvested at indicated time points. A, cell cycle profile at representative time points determined by DNA content analysis using propidium iodine staining and flow cytometry. B, sub-G₁ population as a percentage of total events, as determined by flow cytometry. Columns, mean of at least three independent experiments; bars, SD. C, immunoblotting analysis of G₁-S (CDK4 and cyclin D1) and G₂-M (CDC2) cell cycle transition proteins, PARP and caspase cleavage (●, full-length fragments; - , cleaved fragments). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. Results are representative of at least three independent experiments. Densitometry data corresponding to the Western blots in C and repeat experiments that gave similar results are shown in Supplementary Table S2A (n = 3; the SEM expressed as a percent of mean was calculated and found to have an overall mean value of 20.3%).
hours (21% of events) with both HSP90 inhibitors (Supplementary Fig. S2B). In contrast to the pGB lines, the apoptotic population never exceeded 12% of events in aGB lines regardless of the HSP90 inhibitor used (Supplementary Fig. S2; Fig. 2).

As with 17-AAG, NVP-AUY922 exposure resulted in a time-dependent decrease in uncleared PARP (Fig. 2C, upper band). NVP-AUY922 also caused an increase in cleaved PARP (Fig. 2C, lower band) in both U87MG and SF188 cells. It was more difficult to detect cleaved PARP in U87MG cells, which are less prone to apoptosis in response to HSP90 inhibitors (Fig. 2B), especially with 17-AAG (Fig. 2C, left, lower band); however, a 2-fold increase in cleaved PARP in response to 17-AAG was detected by densitometry (Supplementary Table S2A; Supplementary Table S2A and B; Fig. 2C). The activation of caspase-7 and caspase-8 (extrinsic pathway) was observed with both HSP90 inhibitors. Additional activation of caspase-3 (mitochondrial pathway) occurred in SF268 cells (Supplementary Table S2A; Fig. 2C), with a greater intensity in the case of NVP-AUY922 than with 17-AAG (Supplementary Fig. S2C and Table S2A and B; Fig. 2C).

NVP-AUY922 induces the molecular signature of HSP90 inhibition in GB cell lines. Figure 3 shows a set of Western blots for the expression of a number of HSP90 client proteins and stress response biomarkers in response to NVP-AUY922 and 17-AAG over a time course up to 72 hours in U87MG, SF268, SF188, and KNS42 cell lines. Effects seen were very reproducible (n = 3); Fig. 3 is an example of a representative experiment and quantitative data averaged over the three repeats are shown in Supplementary Table S2B; SEM values were typically 18% of the mean.

Figure 3A shows data for HSP90α, HSP90β, HSP72, and HSP27 as markers of the HSF1-dependent stress response (3, 31). At 5× IC50 antiproliferative concentrations of NVP-AUY922, levels of HSP72 and HSP27 were reproducibly (n = 3) increased after 8 hours in all cell lines (Supplementary Table S2B; Fig. 3A), except for HSP27 in SF268 cells in which this protein was not expressed (17). Stress protein induction was concentration-dependent and occurred from 1× IC50 (lower than the concentration required to deplete HSP90 client proteins; data not shown). Levels of the α and β forms of HSP90, which are the targets of NVP-AUY922 (22), were moderately increased (Supplementary Table S2B; Fig. 3A), as shown in other cell types with 17-AAG (32). Overall, these results show a typical HSF1 stress response to NVP-AUY922 treatment in the aGB and pGB lines.

NVP-AUY922 consistently (n = 3) depleted the main growth factor receptor tyrosine kinases (RTK) expressed in GB, namely wild-type epidermal growth factor receptor (EGFR), and the client proteins IGF1R, PDGFRα, PDGFRβ, C-MET, and C-KIT (Supplementary Table S2B; Fig. 3B). It can be seen that the extent of, delay in, and duration of RTK depletion were dependent on the RTKs and the individual cell lines concerned. As previously reported (33), ERBB2 was the most sensitive client protein in the hierarchy of responses; it was depleted as early as 8 hours but recovered rapidly in SF268 cells. In this aGB line, a transient early increase in the expression of some RTKs (C-MET and PDGFRα) was also observed at 8 hours followed by the depletion of these client proteins, as reported for other clients (34).

Pathways downstream of RTKs were also reproducibly (n = 3) and quantifiably (Supplementary Table S2B) inactivated by NVP-AUY922 (Fig. 3C). Although not a client protein, NVP-AUY922 decreased the level of phosphorylated ERK1/2 (p-ERK) from 8 hours as a result of depletion of the upstream client proteins such as C-RAF and RTKs. p-ERK inhibition occurred in SF268 cells, whereas no C-RAF depletion and an increase in C-MET and PDGFRβ levels were observed. NVP-AUY922 depleted phosphorylated AKT (p-AKT) more rapidly and to a greater extent than total AKT, as previously published (30). The client protein AKT was depleted to a greater extent and duration in pGB lines when compared with aGB lines (Fig. 3C).

In most cases, all client proteins and downstream effectors were depleted or inactivated by 24 hours and recovered by 48 hours posttreatment. Interestingly, no recovery was observed in p-AKT, AKT, and p-ERK in the pGB lines after NVP-AUY922 treatment (Fig. 3). Recovery of these proteins was observed in aGB lines and in the pGB line SF188 treated with 17-AAG (Supplementary Table S2C; Fig. 3C), which seemed to correlate with cell regrowth (Fig. 1B). No recovery of p-AKT and p-ERK was observed in KNS42 cells (Supplementary Table S2C; Fig. 3C), consistent with the prolonged cytostatic effect and cell death observed after the treatment with both HSP90 inhibitors in this cell line (Supplementary Fig. S2A and B).

These results suggest that sustained inhibition of p-AKT and p-ERK might be implicated in the prolonged cytostatic and proapoptotic effects of HSP90 inhibition, particularly in the pGB lines.

The proapoptotic effect of HSP90 inhibition in GB cell lines is concentration dependent and is associated with sustained inhibition of the ERK pathway. Decreased cell viability and increased apoptosis induced by NVP-AUY922 and 17-AAG were concentration dependent in both U87MG aGB (Fig. 4) and SF188 pGB cells (Supplementary Fig. S3). However, the concentration required to induce apoptosis was lower for NVP-AUY922 than for 17-AAG in both GB lines (10× and 20× IC50 for U87MG and 5× and 15× IC50 for SF188, respectively) and lower in SF188 compared with U87MG cells (Supplementary Fig. S3; Fig. 4). Thus, HSP90 inhibitor–induced apoptosis depended on both the drug used and the intrinsic characteristics of the particular GB cells.
Interestingly, in U87MG cells, apoptosis was observed when simultaneous inhibition of p-AKT and p-ERK persisted at 72 hours (Supplementary Table S2D; Fig. 4C). When both p-AKT and p-ERK recovered or when p-ERK only (15× IC₅₀ of 17-AAG) recovered at 72 hours, no major increase in apoptosis was observed. In SF188 cells, partial recovery in p-AKT levels (15× IC₅₀ of 17-AAG) did not impair HSP90 inhibitor–induced apoptosis (Supplementary Fig. S3C and Table S2E). Thus, these correlative studies showed that apoptosis occurred when both AKT and ERK pathways were inhibited and seemed to require sustained p-ERK inhibition for up to 72 hours.

Combination studies with HSP90, PI3K/mTOR, and MEK inhibitors in the U87MG aGB line. In view of the relatively low level of apoptosis in U87MG cells treated with NVP-AUY922 and to further explore the role of PI3K/mTOR and mitogen-activated protein kinase (MAPK) pathways in GB apoptosis with this agent, we used the dual PI3K/mTOR inhibitor PI-103 and the

![Figure 3. Molecular signature of HSP90 inhibition induced by NVP-AUY922 in adult and pediatric GB cell lines. Western blots of cell lysates from the adult (U87MG and SF268) and the pediatric (SF188 and KNS42) GB lines treated with 5× IC₅₀ concentrations (determined by SRB assay) of NVP-AUY922 over 72 hours. A, induction of the HSF1-dependent stress response. B, depletion of the main RTKs involved in GB oncogenesis. C, inhibition of RTK downstream pathways by NVP-AUY922 compared with modifications induced by 5× IC₅₀ concentrations of 17-AAG. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. Densitometry data corresponding to the Western blots shown here and repeat experiments that gave similar results are shown in Supplementary Table S2B (n = 3; the SEM expressed as a percent of mean was calculated and found to have an overall mean value of 17.7%).](image-url)
MEK inhibitor PD-0325901. When used singly, both PI-103 and PD-0325901 induced G1 arrest (data not shown; ref. 35) without apoptosis (Supplementary Table S2F; Fig. 5). PI-103 decreased p-AKT levels at early time points (data not shown; ref. 35) with a recovery at 72 hours, whereas PD-0325901 induced the sustained depletion of p-ERK (Supplementary Table S2F; Fig. 5). Cross-talk between signaling pathways led to increased p-ERK and p-AKT by PI-103 and PD-0325901 treatment, respectively (Supplementary Table S2F; Fig. 5).

Having defined the pathway modulation by PI-103 and PD-0325901, we next examined the effect of combination using SRB measurements and median effect analysis (23). Concomitant PI-103/PD-0325901 combination treatment of U87MG cells gave an additive effect (CI = 1; Table 2) and also decreased p-AKT and p-ERK levels compared with those observed with each agent alone, whereas total ERK, total AKT, and HSP72 expression remained unchanged. However, no apoptosis was observed (Fig. 5). Thus, the combinatorial inhibition of PI3K/mTOR and MAPK pathways was not sufficient to induce apoptosis in the U87MG GB line, despite additive antiproliferative activity.

Next, when either PI-103 or PD-0325901 was combined with HSP90 inhibitors in U87MG cells, synergism with 17-AAG was observed (CI = 0.8 with PI-103 and CI = 0.5 with PD-032501; Table 2). In combination with NVP-AUY922, additivity (CI = 1.0) was seen with...
PD-0325901 and slight antagonism was observed with PI-103 (CI = 1.1; Table 2) based on SRB assay data. A higher level of apoptosis occurred when U87MG cells were treated with NVP-AUY922 combined with either PI-103 or PD-0325901, with a sub-G1 population representing 14% and 25% of events, respectively (compared with <2% in controls, \(P < 0.01\)). This was associated with PARP and caspase-3 cleavage (Supplementary Table S2F; Fig. 5). These combinations involving NVP-AUY922 induced similar levels of p-ERK inhibition as with PI-103/PD-0325901 combination; in addition, p-AKT was depleted to a greater extent (Supplementary Table S2F; Fig. 5). When 17-AAG was combined with PD-0325901, apoptosis was induced despite an incomplete p-AKT inhibition at early time points (data not shown) and p-AKT levels remained comparable with the nontreated control at 72 hours (Supplementary Table S2F; Fig. 5). These results suggest that sustained PI3K/mTOR pathway inhibition was not necessary for the apoptotic process. Conversely, the recovery of p-ERK seen with the 17-AAG/PI-103 combination led to a prolonged cytostatic effect without apoptosis (Supplementary Table S2F; Fig. 5), indicating that sustained p-ERK depletion was likely necessary to induce apoptosis.

In the case of SF188 pGB cells, different results were obtained with the combination studies compared with

**Figure 5.** Proapoptotic effects of combined treatments with HSP90 inhibitors and PI3K/mTOR inhibitor (PI-103) or the MEK inhibitor (PD-0325901) in the aGB cell line U87MG. Cell viability and analysis of the sub-G1 population by flow cytometry. Immunoblotting (PARP and caspase-3 cleavages; ▫, full-length fragments; ▴, cleaved fragments) and inhibition of ERK/AKT phosphorylation. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. Results are representative of at least three independent experiments. Densitometry data corresponding to the Western blots in A and repeat experiments that gave similar results (n = 3) are shown in Supplementary Table S2D. Columns, mean of three independent experiments (n = 3); bars, SD. Corresponding results for SF188 cells are provided in Supplementary Data (Supplementary Fig. S3).
the U87MG aGB cells (Supplementary Fig. S4 and Table S2G). The PI-103/ PD-0325901 combination was synergistic and was able to induce apoptosis in SF188 cells despite only an early and temporary decrease in p-AKT (at 24 hours), whereas p-ERK depletion persisted for up to 72 hours (Supplementary Fig. S4C and Table S2G). This strong apoptotic effect (as shown by decreased cell viability, sub-G₁ population of >30%, and PARP and caspase-3 cleavage) was also observed when SF188 cells were treated for 72 hours with NVP-AUY922 and apoptosis was further increased when NVP-AUY922 was combined with either PI-103 or PD-0325901 (Supplementary Fig. S4B). In these combined treatments, p-AKT and p-ERK were reduced to undetectable levels up to 72 hours (Supplementary Fig. S4C and Table S2G). In contrast to NVP-AUY922, PI-103 and PD-0325901 combinations with 17-AAG did not induce apoptosis in SF188 cells (Supplementary Fig. S4B).

Taken together, the above data show that increased apoptosis is seen in U87MG and SF188 cells with combinations of NVP-AUY922 plus either the PI3K/mTOR inhibitor PI-103 or the MEK inhibitor PD-0325901; in some cases, this increase in apoptosis was observed despite antagonism being seen using median effect analysis, which is based on an SRB readout. The correlative biomarker results suggest that both sustained reduction of p-ERK levels and at least temporary depletion of p-AKT were necessary to induce apoptosis in the two GB cell lines studied. Depending on the GB line, this dual p-AKT and p-ERK depletion was either sufficient (SF188) or required additional factors, as yet unidentified, which are modified by HSP90 inhibition (e.g., in U87MG, depletion of other client proteins) to maximize the apoptotic outcome.

**NVP-AUY922 induces tumor regression through antiproliferative, proapoptotic, and antiangiogenic effects in adult human U87MG GB tumor xenografts.** Next, to explore therapeutic effects against GB cells in vivo, NVP-AUY922 and 17-AAG were studied in mice bearing established s.c. U87MG aGB xenografts (Fig. 6). 17-AAG was administered at the maximum tolerated dose (MTD; 80 mg/kg i.p., 5 days per week on days 1-5 and 8-12). Tumor growth inhibition with stable disease, regression (>50% decrease), or progression (>25% decrease) was observed in seven of eight animals (Fig. 6A). A greater antitumor effect was observed with NVP-AUY922 at a lower dose than the MTD (50 mg/kg i.p, 5 d/w for 2 weeks, MTD = 75 mg/kg daily dosing), with two complete responses, two partial responses and four stable disease (n = 8 animals). At the end of the treatment (day 12), the tumor volume of the NVP-AUY922–treated group had decreased to 69% of the initial volume (P < 0.001). Tumor regression persisted after cessation of the treatment and two animals were free of tumor at 120 days. Thus, NVP-AUY922 and 17-AAG treatment led to significant tumor growth delay of 20 and 15 days, respectively (P < 0.001; Fig. 6A). Analysis of these results using the Kaplan-Meier curves gave very similar values for progression-free survival to the tumor growth delay values given above (Supplementary Fig. S5).

The therapeutic effects were concordant with the molecular signature of HSP90 inhibition observed after a 3-day treatment course (Table 3). Induction of the HSF1 stress response was observed at 24 hours, which then decreased 48 hours posttreatment. The depletion of representative HSP90 client proteins (AKT, HIF1α, and MET) and inhibition of their activation (p-AKT and p-MET) and of the downstream RAS/RAF/MAPK pathway (p-ERK) were maintained even at 48 hours posttreatment, particularly with NVP-AUY922.

The antitumor activity of NVP-AUY922 was sustained by antiproliferative, proapoptotic, and antiangiogenic

| Table 2. Exclusive Combination Index (CI) values calculated at equipotent combined compound concentrations that inhibit U87MG aGB cell growth by 50% as determined by the median effect analysis method |
|-----------------|-----------------|-----------------|
| (CI)            | PI 103          | 17-AAG          | NVP-AUY922 |
| PI-103          | -               | 0.8 ± 0.1       | 1.1 ± 0.3   |
| PD-0325901      | 1.0 ± 0.2       | 0.5 ± 0.2       | 1.0 ± 0.5   |

**NOTE:** Values are means ± SD from at least 3 independent experiments. CI < 0.9, synergy; CI = 1, additivity; and CI > 1.1, antagonism.

**Table 3. In vivo biomarker modulation in s.c. U87MG aGB human tumor xenografts in mice treated with 17-AAG and NVP-AUY for 3 days, as described in Fig. 6**

<table>
<thead>
<tr>
<th>T/C (%)</th>
<th>17-AAG</th>
<th>NVP-AUY922</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>HSP72</td>
<td>344</td>
<td>246</td>
</tr>
<tr>
<td>P-ERK</td>
<td>87</td>
<td>89</td>
</tr>
<tr>
<td>ERK</td>
<td>128</td>
<td>89</td>
</tr>
<tr>
<td>P-AKT</td>
<td>78</td>
<td>70</td>
</tr>
<tr>
<td>AKT</td>
<td>52*</td>
<td>68</td>
</tr>
<tr>
<td>HIF1α</td>
<td>144</td>
<td>90</td>
</tr>
<tr>
<td>P-MET</td>
<td>33*</td>
<td>36</td>
</tr>
<tr>
<td>MET</td>
<td>44</td>
<td>45</td>
</tr>
<tr>
<td>VEGF</td>
<td>55</td>
<td>33*</td>
</tr>
</tbody>
</table>

**NOTE:** Biomarkers were determined using protein quantification by electrochemoluminescent immunoassay (Meso-scale Discovery) except for VEGF, which was determined by ELISA. Results are given as the percentage of protein level in the treated group compared with control group at the corresponding time point (% T/C). Significant †, P < 0.05 and †, P < 0.01
Figure 6. In vivo effects of NVP-AUY922 and 17-AAG in established adult U87MG GB s.c tumor xenografts. Control mice received vehicle only (10% DMSO, 5% Tween 20, and 85% saline as control for NVP-AUY922; and 43% ethanol, 33% propylene glycol, and 24% cremaphor as control for 17-AAG). Treated mice received either 80 mg/kg i.p. once daily 17-AAG (MTD) or 50 mg/kg once daily NVP-AUY922 (below the MTD). A, antitumor activity of a 2-week treatment once daily, 5 days per week on days 1-5 and 8-12 by either NVP-AUY922 (▾) or 17-AAG (▴), compared with vehicle controls (▪). ▲, days of drug administration. B, apoptosis was assessed by caspase-3 cleavage (immunoblotting; ▼, full-length inactive fragments; −, cleaved active fragments) and DNA fragmentation analysis (TUNEL). Representative images are shown at ×40 objective magnification for each treatment group at 24 or 48 hours after a 3-day treatment course. Two panels are shown for the NVP-AUY922 group as distribution of TUNEL-positive cells throughout each sample were unequal. Values shown are mean of percentage of positive cells in eight fields of view. C, microvessel density analysis by immunohistochemistry staining in mouse endothelial cells by rat anti-CD34 antibody. The histograms show results expressed as the sum of vessel areas in the fields of view (×5 objective magnitude). Significant *P < 0.05 and **P < 0.01 (*). Above the histograms, the values show percent treated over control (% T/C) for the drug treatment.
effects. The proliferation index (percentage of Ki67-positive cells) was significantly decreased 24 hours post-NVP-AUY922 treatment compared with controls (36 ± 3% and 41 ± 2%, respectively, P < 0.05; data not shown). Apoptosis was shown by a significant increase in TUNEL-positive cells and caspase-3 cleavage at 48 hours post-NVP-AUY922 treatment (Fig. 6B) and was associated with significant depletion of p-ERK, p-AKT, and AKT (Table 3). A significant decrease in microvessel density was observed with NVP-AUY922 (Fig. 6C), which might be explained, at least in part, by p-AKT, AKT, and HIF1α depletion (Table 3) and also by VEGF receptor 2 depletion (27). The levels of VEGF were decreased significantly to 33% of control at 48 hours after treatment with 17-AAG (P < 0.05) but there was no significant effect with NVP-AUY922 (P > 0.05). Inhibition and depletion of the client protein MET, which has been implicated in GB cell migration (36), was also observed (Table 3).

Discussion

GB is a tumor in which efficient treatment options are limited and new therapies with novel mechanisms of action are urgently required. Natural product HSP90 inhibitors and their derivatives have shown interesting in vitro properties against aGB (7–11), but few data have been published using in vivo human GB models (12, 22), in pGB models (17), or with new synthetic HSP90 inhibitors that are devoid of the significant limitations of 17-AAG (22).

The new, potent synthetic diarylisoxazole amide resorcinol HSP90 inhibitor NVP-AUY922 exhibits potent activity in a range of cancer cell lines of different origins (20, 22). Here, the effects of NVP-AUY922 and other structurally dissimilar HSP90 inhibitors (6) were evaluated mechanistically in human adult (U87MG and SF268) and pediatric (SF188 and KNS42) GB cell lines. Our results showed that they potently inhibited cell proliferation in all lines tested. Comparing the IC50 measured by SRB assay for NVP-AUY922 versus 17-AAG, NVP-AUY922 is more potent than 17-AAG by a factor of 1.8 in U87MG, 2.0 in SF268, 1.5 in SF188, and 6.2 in KNS42. Thus, overall, it can be concluded that NVP-AUY922 showed a modest increase in antiproliferative potency, with a greater differential in the pGB KNS42 cell line. NVP-AUY922 was also more potent than the purine-scaffold HSP90 inhibitor BIIb021 (10 to 23-fold).

Through the combinatorial consequences of HSP90 inhibition, this potent growth-inhibitory effect was obtained in GB models exhibiting different genetic abnormalities that are among the most common in primary and secondary aGB, as well also in pGB models (28). Interestingly, cellular fate after NVP-AUY922 treatment differed between the pGB and aGB lines tested. A cytostatic effect was observed in all GB lines; apoptosis was detected at lower concentrations in the SF188 pediatric GB lines and also at 144 hours in the slower growing KNS42 pediatric GB line, compared with the aGB lines U87MG and SF268. Given that only four cell lines were used here, further studies are recommended to explore the universality of the tendency toward greater apoptotic responses in pGB cell lines. The interesting differences between the pGB and aGB lines shown here are consistent with several lines of clinical and molecular evidence suggesting that pGB are different from aGB (28, 37). Consequently, drug testing results obtained from aGB models may not be directly applicable to pGB. Thus, it is crucial to include pGB models in preclinical anti-GB drug development (28).

As observed in other tumor types (22) and in aGB models with ansamycin benzoquinones (12), NVP-AUY922 exhibited in vitro antiproliferative (cytostatic) and proapoptotic effects, associated with the molecular signature of HSP90 inhibition (induction of HSP72 and HSP27, and depletion of multiple HSP90 client proteins relevant to GB), and were time and concentration dependent. NVP-AUY922 depleted the main RTKs involved in GB oncogenesis (EGFR, IGF1R, PDGFRα, β, C-KIT, and MET) in an RTK-dependent and cell line–dependent manner, and inhibited and depleted downstream proteins in both the PI3K (e.g., AKT) and RAS/RAF/MAPK (e.g., C-RAF) pathways with efficient inhibition of the downstream MAPK effector, p-ERK. However, correlative studies showed that depletion of RTKs alone did not seem to explain apoptosis in our GB lines. Rather, our results suggest that AKT and ERK inhibition seemed to play a key role in the NVP-AUY922–induced apoptosis seen in pGB lines. We therefore analyzed the relationship between modifications of the AKT/ERK pathways and apoptosis under several conditions of HSP90 inhibition and attempted to dissect the effect of each pathway by using PI3K/mTOR or MEK inhibitors alone and in combination with HSP90 inhibitors in the GB lines. We showed that prolonged inhibition of ERK that was associated with the temporary early inhibition of AKT seemed to be the minimum required to induce GB cell apoptosis. In the aGB line U87MG, additional factors modified by HSP90 inhibition, yet to be identified, must also be necessary. The relationship between AKT/ERK inhibition and NVP-AUY922–induced apoptosis was further confirmed by enhanced antitumor activity in mice bearing established s.c. U87MG xenografts treated with NVP-AUY922, compared with 17-AAG.

Both AKT and ERK pathways are activated in most aGB patients (2) as well as in a subtype of pGB of particularly poor prognosis that is genetically different from aGB (38). Concomitant activation of both AKT and ERK pathways are necessary to induce GB formation in mice, with oncogenesis being further increased in INK4a/ADP ribosylation factor–deficient mice, which harbor disruption of p53 and RB pathways (39, 40). Our data indicated that either AKT or ERK inhibition alone did not lead to apoptosis, whereas concomitant AKT and ERK inhibition was sufficient to induce apoptosis in SF188 cells and was necessary but not sufficient in U87MG cells. The kinetics
of depletion of these signaling pathways seemed to be important. Note, however, that neither NVP-AUY922-induced changes in the cyclinD1/CDK4 (Supplementary Fig. S2C; Fig. 2C) or p53/p14/HDM2 (data not shown) oncogenic pathways, nor did TP53 mutation correlate with NVP-AUY922–induced apoptosis, as observed in other tumor types (41). Thus, further studies are required to determine which additional factors are important to induce apoptosis in GB lines.

Because GB is the cumulative result of multiple oncogenic changes, targeted therapies are readily bypassed through the activation of collateral pathways, as observed when we treated GB cells with either a MEK or a dual PI3K/mTOR inhibitor alone. In addition, cross-talk between various RTKs and activation of the downstream PI3K pathway through PTEN loss participate in the lack of responsiveness in GB to anti-EGFR drugs (42–44). Thus, the current trend in GB phase I/II trials is to combine targeted agents to overcome existing or induced combinatorial drivers of malignancy. The capacity of HSP90 inhibitor such as NVP-AUY922 to inhibit several RTKs and downstream pathways is thus an interesting and attractive feature in GB and indicates considerable therapeutic potential, as confirmed herein. Even greater activity might be anticipated by combining HSP90 with other molecularly targeted agents to ensure maximum blockade of oncogenic signaling pathways. 17-AAG was previously reported to cooperate with anti-EGFR (15) and anti-PI3K therapies (14) to induce apoptosis in GB cells. Here, we observed that combining NVP-AUY922 with either an PI3K/mTOR or a MEK inhibitor was more efficient in inducing apoptosis in GB cells compared with treatment targeting the PI3K/mTOR and MEK signaling proteins (PI-103/PD-0325901) either alone or in combination with each other in the absence of the HSP90 inhibitors. The combination of NVP-AUY922 with these agents was also superior in terms of apoptosis induction when compared with the combination of 17-AAG with these same agents. Finally, combined treatments with HSP90 and MEK inhibitors had a greater proapoptotic effect than the combined treatments with HSP90 and PI3K/mTOR inhibitors. Consistent with this, GB initiation in INK4a/ADP ribosylation factor null mice was obtained by additional activation of ERK pathway but not by additional AKT activation alone (39, 40). It should be noted that with some combinations (NVP-AUY922/PI-103 in U87MG cells, and NVP-AUY922/PI-103 and NVP-AUY922/PD-0325901 in SF188 cells), the increased apoptosis was seen despite antagonism being shown by median effect analysis. Such an outcome is possible because the median effect analysis is based on an SRB assay readout, which is sensitive to both cell cycle arrest and apoptotic effects. Further studies are required to determine the consequences of the antiproliferative and apoptotic effects of the combinations with respect to tumor response in vivo.

In our in vivo studies, NVP-AUY922 antitumor activity was shown against s.c. U87MG GB xenografts. Tumor growth delay and progression-free survival was associated with antiproliferative and proapoptotic effects and with clear HSP90 biomarker modulation, including ERK/AKT inhibition, in the GB xenografts, as we also observed in vitro. The robust in vivo molecular responses also correlated with other therapeutic properties of NVP-AUY922, in particular its antiangiogenic effect. Progression of GB requires activation of angiogenesis, which depends on the upregulation of VEGF through AKT at early stages (45) and involves the hypoxia/HIF1α pathway at the latest stages (46), both being present in advanced GB tumors in patients. NVP-AUY922 was shown to inhibit in vitro endothelial cell functions associated with angiogenesis and to deplete VEGF receptor 2 and VEGF receptor 3 in human tumor xenografts (22). We observed that NVP-AUY922 depleted both p-AKT and HIF1α and decreased microvessel density, attesting to its antiangiogenic properties in this model. There may be a contribution to the antiangiogenic effect of 17-AAG through VEGF depletion but this was not detected with NVP-AUY922. Decreased HIF1α levels under NVP-AUY922 treatment might be the consequence of proteasomal degradation of this HSP90 client protein (10) or reflect the inhibition of different regulatory factors including AKT and ERK, which stabilize HIF1α protein and enhance HIF1α transcriptional activity (47). In addition, in vivo depletion of HSP90 client proteins AKT, HIF1α, and MET is consistent with the potential anti-invasive effects of NVP-AUY922. Anti-invasive effects of HSP90 inhibition were reported in vitro with the natural product HSP90 inhibitors (radicicol and geldanamycin) and 17-AAG in GB (10, 48) and with NVP-AUY922 in other tumor types (22, 25). This might be important in GB, as migrating cells are more resistant to cell death and lead to GB recurrences and mortality (49).

Interestingly, the same level of in vivo efficacy seen with a dose of NVP-AUY922 below the MTD was not achieved at the MTD of 17-AAG. This latter benzoquinone ansamycin HSP90 inhibitor is metabolized by NQO1, a known determinant of acquired resistance to this drug in GB cells (17). We also previously observed NQO1 downregulation after 10 days of treatment with 17-AAG in this in vivo aGB model (17), which might contribute to decreased efficacy compared with NVP-AUY922.

In conclusion, GB are tumors in which acceptable treatment options are limited and our results suggest that HSP90 inhibitors may have therapeutic potential in both adult and pediatric patients. Our studies with NVP-AUY922 described herein provide a mechanistic rationale and proof of principle that new synthetic HSP90 inhibitors might represent promising candidates as anti-GB agents. Although the mechanism of action of NVP-AUY922 is generally very similar to that of 17-AAG, this new agent may have potential advantages with respect to increased proapoptotic effects seen here in some settings and independence from NQO1 metabolism (17), together with improved formulation and potential for reduced hepatotoxicity due to the absence of
the quinone moiety. At this stage, our antitumor efficacy studies have been restricted to human GB xenografts grown in the s.c. site. Having established proof of concept, it is now clearly important to further evaluate the therapeutic activity of HSP90 inhibitors against GB tumors grown in the intracranial location. This requires HSP90 inhibitors that have appropriate blood-brain barrier penetration. Importantly, a very recent article has described the promising therapeutic effects of a blood-brain barrier–permeable synthetic HSP90 inhibitor against intracerebrally implanted U87MG GB xenografts (50). Thus, such HSP90 inhibitors may represent interesting potential new agents for evaluation against GB in the clinic, alone or in association with other targeted therapies.

Disclosure of Potential Conflicts of Interest

P. Workman and his group received research funding on the development of HSP90 inhibitors from Vernalis Ltd. and intellectual property from this program was licensed to Vernalis Ltd. and Novartis. N. Gaspar, S. Sharp, C. Jones, A. Pearson, S. Gowar, S. Popov, S. Eccles, and P. Workman are employees of The Institute of Cancer Research, which has a commercial interest in HSP90 inhibitors under development by Novartis Ltd. and PI3K inhibitors being developed by Genentech.

P. Workman has been a consultant to Novartis and S. Eccles is a consultant for Vernalis. P. Workman was a scientific founder of, Scientific Advisory Board member of, and consultant to and received research funding from Piramed Pharma, acquired by Roche.

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References


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