The Hsp90 chaperone complex as a novel target for cancer therapy
M. P. Goetz, D. O. Toft, M. M. Ames & C. Erlichman*

Background: Heat shock protein 90 (Hsp90) is responsible for chaperoning proteins involved in cell signaling, proliferation and survival. 17-allylamino-17-demethoxygeldanamycin (17-AAG) is an anticancer agent currently in phase I trials in the USA and UK. It represents a class of drugs, the benzoquinone ansamycin antibiotics, capable of binding and disrupting the function of Hsp90, leading to the depletion of multiple oncogenic client proteins.

Materials and methods: Studies were identified through a PubMed search, review of bibliographies of relevant articles and review of abstracts from national meetings.

Results: Preclinical studies have demonstrated that disruption of many client proteins chaperoned by Hsp90 is achievable and associated with significant growth inhibition, both in vitro and in tumor xenografts. Following an overview of the mechanism of action of ansamycin antibiotics and the pathways they disrupt, we review the current clinical status of 17-AAG, and discuss future directions for combinations of traditional antineoplastics with 17-AAG.

Conclusions: 17-AAG represents a class of drugs capable of affecting multiple targets in the signal transduction pathway involved in tumor cell proliferation and survival. Early results from phase I studies indicate that 17-AAG administration results in an acceptable toxicity profile while achieving in vivo disruption of client proteins.

Key words: 17-AAG, heat shock protein, Hsp90

Introduction
Despite tremendous advances in the understanding and treatment of cancer, current cancer chemotherapy for patients with solid tumors remains unsatisfactory [1]. Although classic anticancer agents that target DNA have led to cures in a few solid tumors, the prognosis for most patients with neoplastic disease is still dismal [2].

A growing understanding of the molecular, genetic and biochemical changes that occur during the process of carcinogenesis, progression and metastasis has resulted in the focus of drug development shifting from empirical therapy towards therapeutics that act on specific molecular targets responsible for the malignant phenotype [3]. Currently, areas of emphasis in new drug development include: regulators of signal transduction (the HER receptor family, Ras, Raf and MEK kinases) [4–7]; regulators of cell survival (Bcl-2 and its homologs) [8]; oncogenic proteins such as bcr/abl [9]; cell-cycle regulating proteins such as the cyclins, the cyclin-dependent kinases and inhibitors of cyclin-dependent kinases [10]; and proteins involved in the tumor angiogenesis, such as matrix metalloproteinases [11] and endothelial growth factor receptors [12].

A complex, interactive network of signaling pathways regulates cell proliferation and survival. Therefore, disruption of only one or two targets may not abrogate the malignant phenotype in most solid tumors of adults. This is suggested by the promising results with trastuzamab. Among the 20% of breast cancer patients with Her2 overexpression (2+ or 3+), only 35% of this subset of patients demonstrate a clinical response [13]. Since HER2 belongs to the epidermal growth factor (EGF) family of receptors, it is possible that other members of this receptor family become important in tumor cell proliferation when HER2 is blocked. Alternatively, other receptors may substitute for Her2-mediated signaling when this pathway is inhibited. For example, a recent report suggests that resistance to trastuzamab may be related in part to overexpression of IGF-I receptor [14].

Because of the redundancy in the pathways for tumorigenesis and the multiple interactive signaling routes, significant obstacles exist for the development of effective selective targeted approaches. The benzoquinone ansamycin antibiotics (BA), herbimycin, geldanamycin and 17-allylamino-17-demethoxygeldanamycin (17-AAG), represent a class of drugs capable of affecting multiple targets in the signal transduction pathway involved in tumor cell proliferation and survival. This class of drugs is characterized by its ability to specifically bind to and disrupt the function of the chaperone protein heat shock protein 90 (Hsp90), leading to the depletion of...
multiple oncogenic client proteins [15, 16]. 17-AAG is the first drug of this class to reach clinical trials. Following an overview of the mechanism of action of ansamycin antibiotics and the pathways they disrupt, we review the current clinical status of 17-AAG, and discuss future directions for combinations of traditional anti-neoplastics with 17-AAG.

**Heat shock proteins**

The heat shock response was first described in 1962 [17], and heat shock proteins (HSPs) are named for their increased synthesis after heat shock that is contrary to the reduced synthesis of most cellular proteins under these conditions. In addition to heat, these proteins are modulated by nutrient deprivation, and oxidative and other stresses where protein denaturation might otherwise occur [18, 19]. Many HSPs form multimolecular complexes that act as molecular chaperones binding other proteins, denoted as client proteins. These complexes play a regulatory role in the fate of proteins in several different ways including: folding of proteins in the cytosol, endoplasmic reticulum and mitochondria; intracellular transport of proteins; repair or degradation of proteins partially denatured by exposure to various environmental stresses; control of regulatory proteins; and refolding of misfolded proteins [18, 19]. HSPs differ in their cellular localization and functions and mammalian HSPs have been classified into several families according to their molecular size: Hsp90, Hsp70, Hsp60 and Hsp40, and the small HSPs such as Hsp27 [18, 19] (Table 1).

**Hsp90**

Hsp90 is one of the most abundant cellular chaperone proteins. It functions in a multi-component complex of chaperone proteins that may include p60Hop, p50Cd37, Hsp40/HDJ2, p23, Hsp70 and one of a variety immunophilins [20, 21]. It accounts for 1–2% of total protein in unstressed cells and increases to 4–6% of cellular proteins under stress. Unlike other chaperones, Hsp90 distinguishes itself in that most of its known clients are protein kinases or transcription factors involved in signal transduction [20–22].

<table>
<thead>
<tr>
<th>Name</th>
<th>Localization</th>
<th>Function</th>
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<tbody>
<tr>
<td>Hsp104</td>
<td>Cytoplasm</td>
<td>Releases proteins from aggregates</td>
</tr>
<tr>
<td>Hsp90α,β</td>
<td>Cytoplasm</td>
<td>Prevents protein aggregation, helps protein stabilization and protein trafficking, facilitates activation of many regulated proteins</td>
</tr>
<tr>
<td>GRP94</td>
<td>Endoplasmic reticulum</td>
<td>Quality control of protein processing in the endoplasmic reticulum</td>
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<tr>
<td>TRAP/Hsp75</td>
<td>Mitochondria</td>
<td>Unknown</td>
</tr>
<tr>
<td>Hsp70</td>
<td>Cytoplasm</td>
<td>Prevents protein aggregation, helps protein folding</td>
</tr>
<tr>
<td>GRP78, BiP</td>
<td>Endoplasmic reticulum</td>
<td>Protein import and folding in the endoplasmic reticulum</td>
</tr>
<tr>
<td>Hsp60, chaperonins</td>
<td>Cytoplasm and mitochondria</td>
<td>Prevents protein aggregation, helps protein folding</td>
</tr>
<tr>
<td>Hsp40/HDJ2</td>
<td>Cytoplasm</td>
<td>Helps protein folding as a co-chaperone of Hsp70</td>
</tr>
<tr>
<td>Hsp27</td>
<td>Cytoplasm</td>
<td>Prevents protein aggregation, may have role in cell growth and differentiation</td>
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<table>
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<tr>
<th>αHsp90,αHsp70,αHsp60</th>
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These include ligand-dependent transcription factors such as steroid hormone receptors, ligand-independent transcription factors such as MyoD, tyrosine kinases such as p185erbB2 (Her-2/neu), serine/threonine kinases such as Cdk-4 and Raf-1, and mutant transcription factors such as p53.

In humans, there are two Hsp90 isoforms in the cytosol, Hsp90α and Hsp90β. These proteins are closely related. They are both induced by stress and no differences in their activities have been identified [23]. Hsp90 is a phosphorylated homodimer containing two to three covalently bound phosphate molecules on each monomer.

Hsp90 contains a highly conserved ATP binding domain near its N-terminus and the chaperoning activity of Hsp90 requires both the binding and hydrolysis of ATP at this site [24–26]. A second nucleotide binding site appears to be present near the C-terminus, but this is less well characterized [27–29]. The C-terminus is also the main region for dimer interaction and for the binding of p60Hop and immunophils. The binding of ATP at the N-terminal site alters the conformational state of Hsp90 and affects its interactions with client proteins and co-chaperones. BA compete with ATP/ADP in the nucleotide binding pocket, resulting in disruption of the Hsp90 function and degradation of Hsp90 client proteins by the ubiquitin-dependent proteasome pathway [15, 16].

Although there are numerous known client proteins for Hsp90, the Hsp90 binding process has only been studied with a few of these proteins. A model for the binding of Hsp90 to the progesterone receptor (PR) is shown in Figure 1. This is a multiprotein process that occurs in steps. Initial recognition of the PR is performed by Hsp40 and Hsp70 both binding the PR in a cooperative step that involves ATP hydrolysis by Hsp70 [30]. This results in the assembly of a large complex where the proteins HIP and p60Hop bind to Hsp70 and Hsp90 is recruited through its interaction with p60Hop. The final step appears to involve the loss of much of the Hsp70, Hip and p60Hop and the recruitment of two Hsp90 binding proteins p23 and one of the immunophilin proteins. This step requires the interaction of Hsp90 with ATP and the resulting conformational changes produce a PR with an active
hormone-binding site. This complex is dynamic and is continuously being assembled and disassembled. Upon binding hormone, the PR assumes a conformation that is no longer recognized by the chaperone machinery.

Hsp90 levels are regulated in part through the binding of heat shock factor-1 (HSF). HSF is a transcription factor that is normally bound to Hsp90 and is inactive. Under stress conditions, HSF separates from Hsp90 and is phosphorylated by protein kinases, forming trimers that enter the nucleus to bind heat shock elements in the promoter region of the Hsp90 gene and other HSP genes. After further phosphorylation of HSF, Hsp90 mRNA is transcribed and leaves the nucleus entering the cytosol where a new Hsp90 chaperone complex is assembled.

**Hsp90 client proteins**

Hsp90 is an important chaperone for a vast array of client proteins. Because many of its client proteins are involved in cell signaling, proliferation and survival, Hsp90 is a potential target of anticancer therapy. Hsp90-associated proteins can be categorized into three general groups: protein kinases, transcription factors/polymerases and a miscellaneous group (Table 2). Although it is beyond the scope of this review to describe all of the client proteins chaperoned by Hsp90, some of these proteins are of major interest in oncology and are outlined below. More complete lists of client proteins with references have been provided in recent reviews [22, 31].

**Protein kinases**

1. Her-2 (p185erbB2) is a receptor tyrosine kinase overexpressed in a significant proportion of malignancies, including breast, ovarian, prostate and gastric cancers, and is associated with a poor prognosis [32, 33]. The product of the erbB2 gene (Her-2) binds to Hsp90 and its endoplasmic reticulum homolog, Grp94 [34]. Treatment with BA leads to disruption of these complexes, resulting in rapid polyubiquitination of the transmembrane protein followed by its proteasome-dependent degradation [35].

2. Akt kinase plays an important role in the control of pathways that regulate proliferation and apoptosis [36]. Recent findings have implicated Akt in cancer progression because it stimulates cell proliferation and suppresses apoptosis. In tumor cells in which Akt is activated by the upstream protein Her-2, ansamycin treatment leads to degradation of Her2 and a rapid loss of Akt activity [37]. Furthermore, recent data suggest that occupancy of the Hsp90 pocket by ansamycins results in a reduction in Akt half-life and protein expression secondary to Akt ubiquitination and proteasomal degradation [38].

3. The transforming protein of Rous sarcoma virus, p60v-src, is a tyrosine kinase known to be complexed with Hsp90 during transport to the cell membrane. v-src serves as a prototype of an oncogene family that induces cellular transformation by nonregulated kinase activity. BA were first identified as inhibitors of p60v-src tyrosine kinase activity that reversed the transformed phenotype of cells driven by v-src family members [39]. However, subsequent studies have shown that tyrosine kinase inhibition is indirect and that BA treatment results in Hsp90 disruption, leading to kinase degradation [15].

4. Cdk4/cyclin D complexes play an essential role during progression through the G1 phase of the cell cycle by phosphorylating the retinoblastoma protein [40]. The mammalian homolog of the yeast cell cycle control protein Cdc37, p50cdc37, assembles with Cdk4 in high molecular weight complexes that also contain Hsp90 [41]. Pharmacological disruption of Hsp90 with BA results in loss of association of Cdc37 with Cdk4, resulting in a reduction in the half-life of newly synthesized Cdk4 [41].

5. Raf-1 is part of a conserved signal transduction pathway that transmits signals from cytosolic and transmembrane tyrosine kinases to mitogen-activated protein kinases [40]. Raf-1 kinase

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**Table 2. Partial listing of Hsp90-bound proteins of major interest in oncology**

<table>
<thead>
<tr>
<th>Category</th>
<th>Proteins</th>
</tr>
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<tbody>
<tr>
<td>Transcription factors/polymerases</td>
<td>Progesterone receptor, Estrogen receptor, Androgen receptor, P53 mutant, Hypoxia-inducible factor-1α, Telomerase</td>
</tr>
<tr>
<td>Signaling protein kinases</td>
<td>v-src, c-src, c-Raf, MEK, Focal adhesion kinase (FAK), p210^Src, ErbB2, CDK4, Epidermal growth factor receptor, Akt kinase, Other proteins bound to Hsp90, Nitric oxide synthase, Cystic fibrosis transmembrane conductance regulator, Centrin/centrosome, Actin, Tubulin</td>
</tr>
</tbody>
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**Figure 1.** A model for the binding of heat shock protein 90 to the progesterone receptor.
associates with the Hsp90 chaperone complex containing p50Cdc37, as well as the more recently described Hsp90N (which does not associate with p50Cdc37), leading to Raf-1 stabilization and inhibiting its proteasome-dependent degradation. Disruption of Hsp90 function also inhibits Raf-1 signaling, in part by preventing newly synthesized Raf protein from reaching the plasma membrane [42] and possibly by inhibiting the assembly of a Raf-1/MEK1 signaling unit [43].

6. P21O

The BCR–ABL fusion protein is the result of the reciprocal exchange of genetic material between the long arms of chromosomes 9 and 22 [44, 45]. The result is unregulated tyrosine kinase activity that leads to the chronic phase of chronic myelogenous leukemia [46] and is present in a subset of patients with acute lymphoblastic leukemia [47]. The chimeric BCR–ABL exists in a complex with Hsp90, and treatment with BA causes degradation of BCR–ABL while restoring the sensitivity of BCR–ABL-expressing cells to traditional chemotherapeutic agents [48].

Transcription factors/polymerases

1. Steroid hormone receptors are the best-characterized examples of an Hsp90-dependent signaling pathway [20, 21]. Steroid hormone receptors are known to be complexed with HSPs including Hsp90 [21]. Although Hsp90 is not required for steroid hormone receptor synthesis, it is required to maintain the receptor in a conformation capable of binding hormone [49]. Geldanamycin (GA) mediates disruption of Hsp90 complexes and prevents the assembly of the complexes required to maintain hormone receptors in their mature, ligand-binding configuration [16]. In the case of the PR, androgen receptor and glucocorticoid receptor, drug-mediated disruption of Hsp90 function results in a rapid loss of both high affinity hormone binding activity and ligand induced gene activation.

2. Mutated p53: p53 is a tumor suppressor protein that acts in the nucleus to effect cell cycle arrest and apoptosis. p53 is mutated or absent in approximately half of all human cancers [50]. Researchers have shown that most mutated forms of p53 require at least transient interaction with the Hsp90 complex to achieve the conformation of the mutated protein [51]. Blagosklonny et al. [52] demonstrated that treatment of several breast cancer, prostate cancer and leukemia cell lines with BA results in the destabilization of mutated p53 with no influence on wild-type p53 levels. This in turn led to restoration of normal transcriptional activity of the wild-type protein in tumor cells previously heterozygous for mutant p53.

3. Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor that controls the expression of many genes, the protein products of which play an important role in tumor growth, including angiogenesis, glucose transport and glycolysis [53]. HIF-1α is expressed in the majority of metastases and late-stage tumors and is bound by Hsp90 [54, 55]. Treatment with GA prevents heat and hypoxia-induced HIF-1α expression [56], and in renal cell carcinoma cell lines, promotes HIF-1α ubiquitination and proteasome-mediated degradation independent of the von Hippel–Lindau tumor suppressor gene [57].

Benzoquinone ansamycins

The BA antibiotic family is characterized by linkage of a quinone moiety to a planar macrocyclic ansa bridge structure (Figure 2). Geldanamycin, considered the prototype for this class, was first purified in 1970 from the broth of Streptomyces hygroscopicus var. geldanus var. nova, Gelandamycin and herbimycin were initially identified as agents that revert transformation by v-src and exhibit potent antitumor activity [39]. This group of compounds was shown to have potent antitumor activity against human cancer cells in culture and in xenografts [58]. Subsequent studies revealed that inhibition of c-Src catalytic activity, the expected mechanism of action, was not responsible for antitumor activity. Instead, BA specifically bind to Hsp90 [15], alter its function [15, 16] and promote the proteolytic degradation of substrate proteins [15, 33, 59]. These drugs bind to a highly conserved 25 kDa N-terminal domain of Hsp90, a binding site for ATP [24–26], resulting in disruption of the Hsp90 chaperone complex.

Animal toxicology studies of geldanamycin revealed significant toxicities, including liver (elevation of transaminases), elevations in creatinine phosphokinase, lactic dehydrogenase and blood urea nitrogen, which precluded the use of geldanamycin in humans [60]. More recently, 17-AAG was synthesized and evaluated. It is a geldanamycin analog in which an allyl amino group replaces the methoxy in the 17 position (Figure 2). Preclinical studies have demonstrated that 17-AAG has a similar mechanism of action to geldanamycin but with a significantly improved toxicity profile. 17-AAG has entered into clinical trials in the USA and UK.

Current clinical investigations with 17-AAG

Four of five clinical trials have been presented to date involving two different schedules of dose administration. One schedule involved a once weekly intravenous administration (days 1, 8 and 15) while the other was a daily ×5 schedule. Both schedules were repeated every 3 weeks. Early data suggest that the daily ×5 schedule may be more toxic.

We have reported our experience in 20 patients utilizing a 90 min infusion on days 1, 8 and 15 of a 3 week cycle in patients with advanced solid tumors [61]. Treatment doses were escalated starting at 15 up to 431 mg/m²/day using an accelerated titration
design. Dose limiting toxicity was noted in two patients at the 431 mg/m²/dose and included the following grade 3 toxicities: liver (bilirubin and aspartate aminotransferase), fatigue, nausea/vomiting and anemia. The most common grade 1 and 2 toxicities were anorexia, nausea, anemia and diarrhea. Pharmacokinetic analysis of plasma samples drawn on day 1 (n = 9) revealed that the median clearance was 412 ml/min/m² (range 208–4885). The C_{max} increased linearly with dose and the t_{1/2} was 166 ± 115 min. Formation of the active metabolite, 17-amo-geldanamycin (17-AG), was detected at all dose levels. Increases in Hsp70 levels in peripheral blood mononuclear cells (PBMCs) have been observed with treatment.

Banerji et al. [62] reported data on 22 patients utilizing a weekly intravenous schedule. The starting dose was 10 mg/m²/week and doses were doubled until they reached 320 mg/m²/week, following which the dose was escalated by 40% to 450 mg/m²/week due to grade 3 nausea and vomiting in two of six patients treated at 320 mg/m²/week. Pharmacokinetic analysis showed a linear increase of AUC with dose. The drug was extensively distributed [volume of distribution (V_{D}) = 186 l] and cleared [clearance (Cl) 47.3 l/h]. The C_{max} at the highest dose level was 16.71 µM. Analysis of pharmacodynamic markers in PBMCs at 450 mg/m²/week, showed a reduction in the expression of Raf-1 between 24–48 h and Hsp70 induction at 24–48 h. Pre- and post-treatment tumor biopsies in four patients (three at 320 mg/m²/week and one at 450 mg/m²/week) showed an induction of Hsp70 (four of four patients), CDK4 depletion (three of four) and Raf-1 depletion (one of four).

Munster et al. [63] reported data from 16 patients utilizing an intravenous infusion schedule (daily ×5 days repeated every 21 days). Dose limiting toxicities of diarrhea, thrombocytopenia and transient transaminitis were seen at 80 mg/m². Pharmacokinetic analysis revealed t_{1/2} of 90 min for the parent compound with peak levels of 2700 nM. The biologically active metabolite, 17-AG, had a t_{1/2} of 105 min with peak levels of 607 nM.

Agnew et al. [64] reported updated results from a phase I trial utilizing the same schedule of a daily 1 h infusion for 5 days every 3 weeks in adult patients with solid tumors. An accelerated dose design was utilized with one patient entered at each of the first four dose levels (10–28 mg/m²), while 15 were entered at the two highest levels (40 and 56 mg/m²). Dose limiting toxicity was found to be reversible grade III hepatotoxicity (two of five patients) manifested as transaminits at a dose of 56 mg/m². The maximum tolerated dose was 40 mg/m², at which three of five evaluable patients had reversible grade 2 hepatotoxicity. At 56 mg/m², the terminal half-life of 17-AAG was 2.3-fold shorter than that of 17-AG (3.8 versus 8.6 h). The C_{max}, Cl and V_{D} for 17-AAG and 17-AG were 2080 and 770 nM, 19.9 and 30.8 l/h/m², and 92 and 203 l/m², respectively.

**Metabolism**

The in vitro metabolism of 17-AAG by mouse and human hepatic preparations has been studied [65]. Metabolic activity, requiring an electron donor (NADPH), resided predominantly in the microsomal fraction and resulted in three metabolites with molecular weights of 545, 601 and 619 kDa, consistent with 17-AG, an epoxide and a diol, respectively. Cytochrome P450 3A4 has been implicated as the responsible isoenzyme after the observation that human microsomal metabolism of 17-AAG was inhibited by ketoconazole [65]. We have also documented 17-AAG metabolism by the related CYP isoform CYP3A5 [61]. The metabolite 17-AG has been reported to have biological activity equivalent to the parent compound, 17-AAG, as determined by the ability to decrease p185erbB2 [66]. Furthermore, 17-AG accounts for a significant proportion of total activity, with a mean AUC 85% of that for 17-AAG [61].

In addition to CYP3A, other metabolizing enzymes may be important in determining cellular sensitivity to 17-AAG. This was suggested when researchers showed that expression of NQO1 in human colon and ovarian cancer cell lines, the National Cancer Institute panel of 60 human tumor cell lines and xenografts correlated with 17-AAG induced growth inhibition [67]. Interestingly, this was not observed with the metabolite 17-AG or for geldanamycin. Known functional polymorphisms in NQO1 may be one explanation for the wide range of sensitivity that is seen in vitro between cell lines exposed to 17-AAG, and may contribute to clinical activity and toxicity. Furthermore, because the activity of the metabolite 17-AG is not dependent on NQO1 status, it is possible that CYP3A pharmacogenetics will affect the importance of NQO1 on cell sensitivity by determining the relative proportion of 17-AG and the parent compound.

**Future clinical investigations**

Much of the original focus in the development of geldanamycin derivatives such as 17-AAG has centered on the ability of derivatives to deplete the erbB2 gene product (p185) [66, 68]. It follows that single-agent studies utilizing 17-AAG are warranted in tumor groups such as breast, ovarian and gastric cancers, where over-expression of the erbB2 oncogene has been linked to poor prognosis and drug resistance [69, 70].

17-AAG may also be effective in colon cancer, where preclinical data have been published demonstrating the ability of 17-AAG to induce cell-line dependent cytostasis and apoptosis [71]. This finding correlated with depletion of N-ras, Ki-ras and c-Akt, as well as c-Raf-1. Interestingly, 17-AAG induced a G2/M arrest in three of the four colon cancer cell lines, despite the expression of Rb. This finding is in contrast with another study, which demonstrated that in the presence of Rb, BA-treated cells arrested in G1, rather than G2/M as occurred with cells that lack the Rb gene product [72].

The evaluation of 17-AAG as a single agent may be also indicated in hormone-responsive tumors such as breast, endometrial and prostate cancer, where steroid hormone receptors are an important target. This is substantiated by the recent work of Bagatell et al. [73], who showed that treatment of breast cancer cells with geldanamycin depleted the estrogen and progesterone hormone receptors as well as delayed the growth of estrogen-responsive tumor xenografts. This mechanism of action is unique, in that depletion of estrogen receptor levels is independent of ligand binding. Because one of the known mechanisms of resistance to antiestrogens involves mutation of the estrogen receptor, 17-AAG and related compounds may allow clinicians to continue
to target the estrogen receptor in breast cancers resistant to anti-
estrogens.

In addition, evidence is accumulating that disruption of Hsp90 in hormone-responsive prostate cancer may also prove beneficial. This was recently demonstrated when investigators used GA to disrupt both hormone-binding activity and androgen receptor protein stability in prostate cancer cell lines, resulting in loss of androgen-induced gene activation [74]. The progression of human prostate cancer is often linked to a high level of androgen receptor expression or mutations [75–77]. In a manner analogous to the estrogen receptor, 17-AAG may also be effective in hormone refractory prostate cancer whereby depletion of the androgen receptor independent of ligand binding may allow treatment of androgen-independent prostate cancer [78].

**Combination chemotherapy**

Many new small molecules and antibodies designed to inhibit specific targets are currently in clinical trial. However, it is increasingly clear that despite specific and potent *in vivo* inhibition of a specific molecular target, cancer cells are adept in the activation and upregulation of other signaling mechanisms that may lead to drug resistance. This finding has prompted investigators to determine whether the combination of ‘non-specific’ cell cytotoxics, such as anthracyclines and/or taxanes, in combination with targeted treatments (e.g. trastuzamab) might result in additive or synergic cell killing. In the case of trastuzamab, a multicenter phase III trial recently reported that in patients with metastatic erbB2-expressing breast cancer, trastuzamab in combination with chemotherapy is more effective than trastuzamab alone (median survival 25.1 versus 20.3 months) [5].

Although all of the work thus far has been preclinical, there is mounting evidence that the combination of select cytotoxics with 17-AAG might result in synergic cell killing. Munster et al. [79], after reporting that 17-AAG causes an Rb-dependent G1 arrest and apoptosis [72], presented evidence for synergy in Rb competent cell lines treated with paclitaxel followed by 17-AAG. This synergy, however, was dependent on the schedule of administration. In Rb competent cells, the reverse sequence of drug exposure seemed to protect from taxane-induced cell death by blocking cells in G1, whereas cells with mutated Rb did not show schedule dependence. In contrast to paclitaxel, administration of 17-AAG with doxorubicin resulted in synergy independent of schedule and Rb status.

Our laboratory has demonstrated sequence-independent synergy for the combination of GA and cisplatin in both A549 lung cancer and HCT-8 colon cell lines. However, in the case of gemcitabine, we have found sequence-dependent synergy in both cell lines when GA and gemcitabine are combined. We are currently evaluating this combination in an ongoing phase I study.

**Other Hsp90 inhibitors**

In addition to BA, the unrelated antibiotics radicicol and novobiocin have been shown to target Hsp90, leading to reduced levels of multiple downstream targets [80–82]. Knowledge of the crystal structure of Hsp90 has also allowed researchers to develop other Hsp90 inhibitors that bind to the Hsp90 ATP/ADP pocket, leading to the degradation of targets such as HER2 and Akt *in vitro* and in tumor xenografts [83, 84]. Other approaches include the synthesis of immunoconjugates in which an anti-Her2 monoclonal antibody is coupled with GA [85], as well as the synthesis of geldanamycin heterodimers where the selective phosphoinositide 3-kinase inhibitor LY294002 is linked to geldanamycin [86]. Although each of these compounds has shown promise *in vitro*, sometimes leading to more potent disruption and degradation of Hsp90 client proteins, it remains to be seen whether these compounds will offer greater clinical benefit than 17-AAG while maintaining an acceptable toxicity profile.

The BA 17-AAG represents a class of drugs designed to inhibit the function of Hsp90, leading to the degradation of multiple client proteins important in cell signaling, growth and survival. Preclinical work has demonstrated that disruption of many of these client proteins is achievable and associated with significant growth inhibition, both *in vitro* and in tumor xenografts. Early results from phase I studies indicate that weekly administration of 17-AAG results in acceptable toxicity but that daily ≤5 administration is associated with dose limiting hepatotoxicity. Clinical studies exploring alternative schedules of 17-AAG and combinations with standard cytotoxics are being explored.

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**References**


