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Development and application of Hsp90 inhibitors

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Heat shock protein 90 has emerged as an important target in several diseases. The present review will discuss our understanding of the role played by Hsp90 in regulating and maintaining the transformed phenotype in cancers and neurodegenerative diseases, as well as recent findings on its roles in fungal and viral infections. It will also update the reader on the preclinical development and clinical translation of Hsp90 inhibitors.

Introduction

As we have currently come to understand, heat shock protein 90 (Hsp90) is a key component of a multichaperone complex with important roles in the development and progression of pathogenic cellular transformation [1–4]. Its wide-ranging functions result from the ability of Hsp90 to chaperone several 'client proteins' that play a central pathogenic role in human diseases including cancer, neurodegenerative diseases, and viral infections. At the time of its discovery, however, it was difficult to imagine Hsp90 as a potential therapeutic target. The chaperone is highly abundant in most tissues, comprising over 1% of the total cellular content even in the absence of stress. Further, genetic knockout of Hsp90 is lethal in eukaryotes and over 100 kinases and transcription factors, many of which are crucial to normal cellular growth and survival, have been shown to interact functionally with Hsp90. The initial interpretation of these findings was that a therapeutic window would be difficult, if not impossible, to achieve with Hsp90 inhibitors. This unfavorable view regarding the potential of Hsp90 as a therapeutic target has changed only after the discovery of geldanamycin (GM) (Figure 1). GM was found as part of a phenotypic screen focused on the identification of agents capable of inducing the morphologic reversion of *v*-src-transformed 3T3 cells [5]. It was initially hypothesized that GM was a direct inhibitor of *v*-SRC activity. Whitesell and colleagues later clarified that GM's biologic activity was actually attributable to its ability to bind to and inhibit Hsp90 [6]. Co-crystallization studies demonstrated that GM binds to an ATP/ADP pocket in the N-terminal domain of Hsp90 and that it inhibits Hsp90 function by preventing full chaperone cycling. In cells exposed to GM, unprocessed chaperone-client protein complexes accumulate within the cell leading to recruitment of E3 ubiquitin ligases that target Hsp90 clients for degradation in the proteasome [7,8].

Much of our understanding of the role of Hsp90 in promoting malignant transformation has been derived from studies using GM and other structurally diverse Hsp90 inhibitors as biologic probes [9]. A surprising observation derived with these agents was that cancer cells were significantly more sensitive to Hsp90 inhibition than non-transformed cells [1–6,9]. This finding raised the possibility that Hsp90 inhibitors may possess an exploitable therapeutic index and prompted the testing of this strategy using xenograft and transgenic cancer models. These studies, which have used primarily 17-allylamino-17-demethoxy-geldanamycin (17AAG) (Figure 1), a GM derivative with a more favorable profile, but also synthetic small molecule Hsp90 inhibitors, clearly demonstrate that Hsp90 inhibitors possess potent anti-cancer activity at non-toxic doses [10–12].

Several models have been proposed to explain the selective sensitivity of cancer cells to Hsp90 inhibition [13–16]. One model, akin to the 'oncogene addiction' model proposed by Weinstein [13], focuses on the exclusive dependence of some transformed cells on a sensitive Hsp90 client protein. In this model, degradation of a specific Hsp90 client in the appropriate genetic context,

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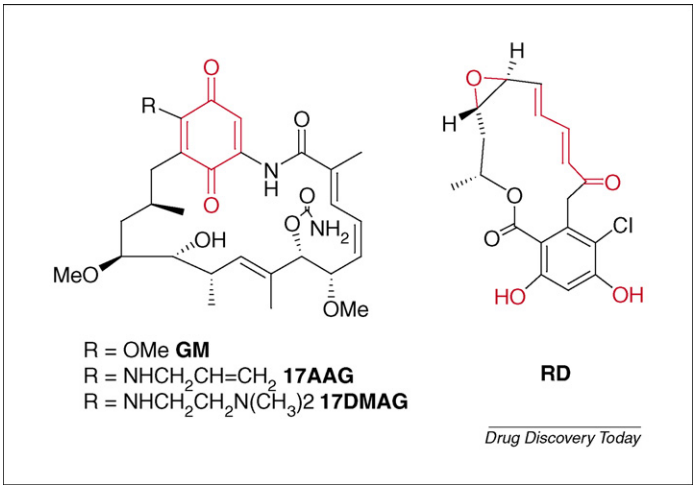

FIGURE 1

Chemical structures of the natural product Hsp90 inhibitors. Structural elements that lead to *in vivo* instability and/or to off-target toxicities are depicted in red.

for example, BRAF in a melanoma cell with V600E mutant BRAF or Bcr-Abl in chronic myeloid leukemia (CML) [1–4], results in apoptosis and/or differentiation, whereas its degradation in normal cells leads to little to no effect. As Hsp90 interacts with a large number of oncogenic kinases and transcription factors, this model has been used to justify the clinical development of Hsp90 inhibitors in a broad range of tumor types (Table 1). The importance of this model to the clinical development of Hsp90 inhibitors is that it highlights the relevance of patient selection in the design of Hsp90 inhibitor trials. Unfortunately, only limited animal studies have been performed with these inhibitors to date and, therefore, the ability of 17AAG or other Hsp90 inhibitors to degrade most putative Hsp90 clients *in vivo* has yet to be explored in relevant animal model systems.

A complementary 'Hsp90 addiction model' is based upon the hypothesis that Hsp90 is limiting in many tumor cells because of their increased load of mutated and unfolded proteins [14,15]. An increased requirement for Hsp90 chaperone function in tumors cells may be due to the overexpression of mutated Hsp90 clients or amplification of clients such as HER2. The hypoxic, low pH, and low nutrient conditions found in many tumors may further increase the number of denatured proteins found in tumors, and thus the need for Hsp90 chaperone function. In support of this hypothesis, Kamal *et al.* have shown that Hsp90 in tumor cells is found entirely in an active complex with co-chaperones, whereas most Hsp90 in normal tissues resides in a free, uncomplexed, or latent state [16]. These data suggest that the sensitivity of some cancer cells to Hsp90 inhibition may not be attributable to their reliance on any individual Hsp90 client or clients, but rather to their increased dependence upon Hsp90 chaperone function. This may explain why therapies that further stress the chaperone system such as anti-angiogenic agents, proteasome inhibitors, cytotoxic chemotherapies, and radiation may broadly synergize with Hsp90 inhibitors even in tumors in which Hsp90 inhibitors alone have little or no activity [1–3].

Finally, the selective sensitivity of transformed cells for Hsp90 inhibitors may be partly due to the selective accumulation of these drugs in cancer cells [15]. The selective accumulation of Hsp90 inhibitors in tumors has been reported with compounds that display little structural homology and diverse solubility profiles. One possible explanation for this result is that the binding affinity of these compounds may be higher for tumor-derived Hsp90 than recombinant Hsp90 or Hsp90 derived from non-transformed cells because of the higher abundance of activated, co-chaperone bound Hsp90 found in tumors [15,16].

Application of Hsp90 inhibitors in the treatment of cancer patients (Table 2)

Though useful as biologic probes for studying the role of Hsp90 in mediating transformation, the natural products GM and radicicol (RD) (Figure 1) have several pharmacologic limitations, including poor solubility, limited *in vivo* stability, and off-target toxicities that have precluded their use as drugs [9]. GM proved too toxic for human use [17] but 17AAG (Figure 1), a carbon-17 substituted derivative, retains activity against Hsp90 but with a more favorable toxicity profile [18]. 17AAG entered human clinical testing in 1999 and has been evaluated in phase 1 trials using weekly, twice weekly (days 1, 4), daily ×5 (21 day cycle), and daily ×3 (14 day cycle) schedules [19–24]. In these trials, the toxicity of 17AAG was dose dependent and schedule dependent with hepatic toxicity being more prominent with daily administration schedules. 17AAG has

TABLE 1

Selected Hsp90 clients

Client	Target disease	References
HER2	Breast cancer	[45]
Raf-1/mutant BRAF	Melanoma	[46,47]
Mutant EGFR	Non-small cell lung, glioblastoma	[48,49]
c-Kit	GIST	[50]
c-Met	Gastric, lung, glioblastoma	[51]
HIF-1α	Renal cancer	[52,53]
Zap70	CLL	[54]
Bcr-Abl, mBcr-Abl	CML	[55,56]
Flt-3	AML	[57]
IGF-1R/Akt	Myeloma	[58]
NMP-ALK	Lymphoma	[59]
Akt	SCLC	[60]

TABLE 2

Hsp90 inhibitor trials in cancer

Drug	Sponsor	Adminstration	Status
17AAG (DMSO/EPL formulation)	NCI/Kosan	Intravenous	Phase 1/2
KOS-953 (17AAG, tanespimycin)	Kosan	Intravenous	Phase 1/2
CNF-1010 (17AAG)	Biogen Idec	Intravenous	Phase 1
IPI-504	Infinity	Intravenous	Phase 1
KOS-1022 (17DMAG, alvespimycin)	Kosan	Intravenous	Phase 1
KOS-1022 (17DMAG, alvespimycin)	Kosan	Oral	Phase 1
CNF-2024	Biogen Idec	Oral	Phase 1
SNX-5422	Serenex	Oral	Phase 1

limited solubility and, therefore, in order to formulate this drug for human use, the Cancer Therapy Evaluation Program (CTEP) that sponsored the initial phase 1 trials developed a dimethyl sulfoxide (DMSO), egg-phospholipid vehicle. Notably, many of the toxicities (anorexia, odor) observed in the phase 1 setting were probably attributable to the DMSO in this formulation. Pharmacokinetic studies incorporated into these phase 1 trials suggest that serum concentrations significantly greater than those required for depletion of Hsp90 clients in cell culture and xenograft model systems could be achieved with acceptable toxicity. Peripheral blood mononuclear cell studies and limited tumor biopsies showing degradation of Raf-1 and upregulation of Hsp70 suggest that at least partial target modulation was achieved [19,23]. However, minimal efficacy (primarily stable disease in melanoma, renal, and prostate cancers) was observed in the phase 1 trials and no patients in these trials achieved either a complete or partial response by Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Notably, these phase 1 trials were not enriched with those patients predicted by the preclinical experience to be most likely to respond (for example, patients with HER2-amplified breast cancer).

Development of second generation Hsp90 inhibitors

On the basis of more recent clinical experience, the limited efficacy observed in the initial phase 1 trials of 17AAG was probably due to a lack of patient enrichment for those most likely to benefit and suboptimal target inhibition due to the requirement for intravenous dosing and the off-target toxicities of 17AAG and its DMSO formulation. These findings have catalyzed future efforts directed at both improving the delivery of 17AAG and identifying novel scaffolds with improved pharmacologic and toxicity profiles.

Improved formulations and chemical derivatives of 17AAG

The first second generation formulation of 17AAG to enter the clinic was a cremaphor-based (DMSO-free) formulation (KOS-953) developed by Kosan Pharmaceuticals (http://www.kosan.com). In a phase 1 trial of KOS-953 and trastuzumab, 25 patients were enrolled at four dose levels (225–450 mg/m^2) and treated with both agents on a weekly schedule [25]. Using this weekly schedule, hepatotoxicity was minimal and no bone marrow suppression was seen. In this trial, one patient with trastuzumab-refractory HER2-positive breast cancer had a confirmed partial response by RECIST criteria. Three additional patients with HER2-amplified breast cancer had tumor regressions of 25, 22, and 21%, respectively. Though preliminary, these data are the most convincing to date to suggest that Hsp90 represents a useful therapeutic target for human cancer. A phase 2 trial of this combination is currently accruing patients with trastuzumab-refractory, HER2-amplified metastatic breast cancer. A phase 1 trial of the KOS-953 formulation of 17AAG in combination with bortezomib was concurrently initiated in patients with multiple myeloma [26]. On this trial, 18/41 patients demonstrated a response (CR + PR + MR) to the combination. This included 10/27 patients who had previously received bortezomib (3/11 of which were defined by the investigators as being definitively bortezomib refractory) [27]. Notably, the 17AAG formulation being used on this study was recently changed from the cremaphor-based formulation to a suspension formulation that does not require steroid premedication.

Conforma Therapeutics (currently Biogen Idec) performed a phase 1 trial of an oil-in-water nanoemulsion of 17AAG (CNF-1010) [28]. In this trial, CNF-1010 was administered twice weekly for three consecutive weeks, followed by a one week break (four-week cycles). The pharmacokinetics of 17AAG using the nanoemulsion formulation were comparable to those of the NCI-DMSO-based formulation and no formulation-associated toxicities were observed. HER2 plasma-extracellular domain (ECD) was measured as a pharmacodynamic marker of Hsp90 inhibition in this trial. A decline in HER2 plasma-ECD was observed at doses greater than 83 mg/m^2. Three minor responses, all at doses at or above 83 mg/m^2, were observed in patients with melanoma, gastric, and duodenal cancers.

In a different approach, Infinity Pharmaceuticals has developed a reduced form of 17AAG (IPI-504) that, when isolated as a hydrochloride salt, is water soluble [29]. *In vivo* and *in vitro*, IPI-504 interconverts with 17AAG existing in a pH and enzyme-mediated dynamic redox equilibrium. Promising preliminary results of a phase 1 trial of IPI-504 in patients with relapsed, refractory gastrointestinal stromal tumors (GIST), and other advanced soft tissue sarcomas were recently reported [30]. Patients were treated on this trial using a day 1, 4, 8, 11 every 21 day schedule. To date, dose escalation has reached 400 mg/m^2, far above the level achievable with the NCI DMSO formulation. No responses by RECIST criteria have been reported, but 4 of 18 evaluable patients achieved a partial response by European Organization for Research and Treatment of Cancer (EORTC) positron emission tomography (PET) criteria and 11/18 achieved at least stable disease. Enrollment on this study continues using a twice-weekly continuous schedule.

A second generation GM derivative, 17-dimethylaminoethylamino-17-desmethoxygeldanamycin (17DMAG) (Figure 1) has also entered phase 1 clinical testing (http://clinicaltrials.gov/). 17DMAG has several potential advantages over 17AAG, including water solubility and much greater oral bioavailability. Trials of oral and intravenously administered 17DMAG are currently ongoing at several centers. Promising early results were recently reported in a Phase 1 trial of 17DMAG in patients with chemotherapy refractory acute myelogenous leukemia in which 3 of 17 patients had a complete response to therapy [31].

A major obstacle to the clinical development of this class of inhibitors has been the inability to assess quantitatively, as a function of time, the effect of 17AAG on Hsp90 function in patients. Several of the phase 1 trials incorporated pre-treatment and post-treatment tumor biopsies [19–24]. The sum of the experience is that target modulation can be achieved with 17AAG at non-toxic doses, but it remains unknown which Hsp90 clients can effectively be degraded at non-toxic doses, the magnitude of target degradation and the durability of the effect. Notably, as most tumor biopsies were obtained in patients with melanoma, the pharmacodynamic effect of 17AAG on some of the most sensitive Hsp90 clients, such as HER2, is yet to be explored. As a more detailed pharmacodynamic assessment of individual client proteins is crucial in the planning of phase 2 trials of these compounds, significant effort is currently being directed toward developing more useful pharmacodynamic markers. One novel approach currently being assessed is the use of radiolabeled antibody fragments to measure, non-invasively, changes in HER2 expression by PET imaging [32].

Hsp90 inhibitors based on novel scaffolds

Preclinical data with non-ansamycin Hsp90 inhibitors suggest that the dose-limiting hepatotoxicity of 17AAG may be partly 'off target' and attributable to the chemical reactivity of its benzoquinone group (depicted in red, Figure 1) and not a direct consequence of Hsp90 inhibition. For these reasons, Hsp90 inhibitors that lack a quinone moiety may be more efficacious and less toxic than 17AAG. Finally, expression of P-glycoprotein (P-gp) or loss or mutation of the NQO1 gene that is required for the bio-reduction of 17AAG to the more potent hydroquinone has been proposed as mechanisms of *de novo* or acquired resistance to 17AAG [33]. These potential drawbacks prompted the search for Hsp90 inhibitors with improved 'drug-like' characteristics. In one approach, Chiosis *et al.* took advantage of the unique shape adopted by ATP and ADP when bound to Hsp90 and designed small molecule Hsp90 inhibitors based on a purine-scaffold (PU3, Figure 2) [34]. While bound to Hsp90, the regulatory nucleotides take on a bent shape found only in ATPases belonging to the GHKL family, and called the Bergerat fold (G = DNA gyrase subunit B, H = Hsp90, K = histidine kinases, and L = MutL). In fact, when inside the Hsp90 pocket, both GM and RD take on the bent, C-shaped conformation mimicking ADP binding. This fold is not observed in the high-affinity binding sites of kinases (which adopt a P-loop motif) or in other chaperones such as Hsp70 (which adopts an actin fold), suggesting that is possible to discover compounds of high selectivity for Hsp90 over other ATPases by identifying those that specifically bind to Hsp90 via the N-terminal ATPase pocket [35]. Others, such as Workman and co-workers, have screened large libraries of compounds to identify Hsp90 inhibitors based on the pyrazole-scaffold (CCT018159, Figure 2) [36]. These are ATPase inhibitors as well. Serenex has used a chemoproteomics technology platform to discover a distinct, but not yet disclosed, chemical scaffold (http://www.serenex.com).

CNF-2024 (Biogen Idec) and SNX-5422 (Serenex), two orally available small molecule Hsp90 inhibitors developed on these novel scaffolds, have now entered phase 1 clinical trial. CNF2024, a purine-scaffold Hsp90 inhibitor, developed initially by Conforma Therapeutics, entered clinical evaluation, in patients with advanced cancers, in October 2005. Following the acquisition of Conforma, its development is proceeding under the new owner, Biogen Idec (http://www.biogenidec.com). Phase I, dose-escalation studies of CNF2024 administered orally are reported in patients with relapsed B cell chronic lymphocytic leukemia (CLL) and advanced solid tumors or lymphomas.

Serenex, Inc. announced in May 2007 the initiation of a phase 1 trial for SNX-5422. This phase 1 dose-escalation study will evaluate the safety, pharmacokinetic, and pharmacodynamic properties of SNX-5422 (http://www.serenex.com).

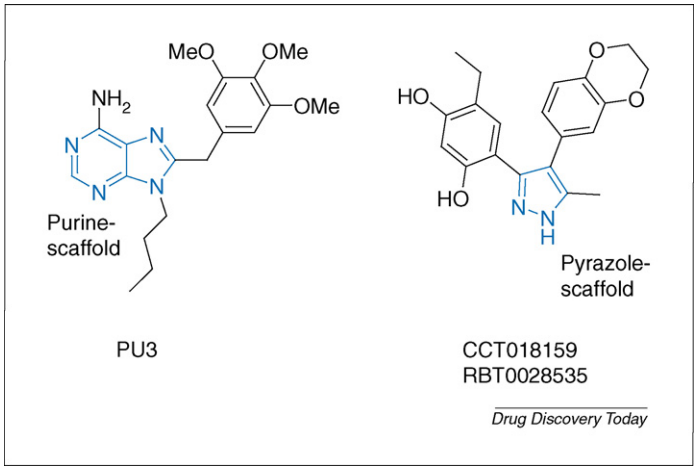

FIGURE 2

The chemical structure of first identified Hsp90 inhibitors of the purine-scaffold, PU3 [34], and the pyrazole-scaffold, CCT018159 [36]. The molecular scaffolds are depicted in blue.

Translation of Hsp90 inhibitors to other diseases

Neurodegenerative diseases

The important functions played by Hsp90 in maintaining the functional stability and viability of cells under a transforming pressure may be clinically exploitable in other pathogenic transformations, such as neurodegeneration. Tauopathies, such as Alzheimer's disease (AD) and frontotemporal dementia (FTD), are neurodegenerative diseases in which transformation is characterized by abnormalities in the protein tau leading to an accumulation of hyperphosphorylated and aggregated tau toxic species [37,38]. Recent evidence suggests a role for Hsp90 in allowing and sustaining the accumulation of such toxic tau aggregates [37,38]. Specifically, Luo *et al.* have shown that the stability of both p35, a neuronal protein that activates cdk5 through complex formation leading to aberrant tau phosphorylation and that of mutant but not wild type tau protein is maintained in tauopathies by Hsp90. Inhibition of Hsp90 by a purine-scaffold Hsp90 inhibitor in both cellular and mouse models of tauopathies led to reduction of the pathogenic activity of these proteins and resulted in elimination of aggregated tau [37]. Dickey *et al.* have additionally proposed a central role for the Hsp90 complex in the pathogenesis of tauopathies and have shown that a purine-scaffold Hsp90 inhibitor promoted selective decrease in ptau species in a mouse model of tauopathy [38]. They also demonstrated that the Hsp90 complex in affected areas of AD brain, where pathologic protein accumulation is found postmortem, has a significantly higher binding affinity (approximately 1000-fold) for small molecule Hsp90 inhibitors than does Hsp90 derived from unaffected brain tissue from the same patients or from brain tissue of control cases. These findings complement the previous observations made by Waza *et al.* in bulbar muscular atrophy (SBMA). These authors demonstrated that androgen receptor (AR), a pathogenic gene product in SBMA, is one of the Hsp90 client proteins. Administration of 17AAG to a SBMA transgenic mouse model ameliorated motor impairments without detectable toxicity, by reducing amounts of monomeric and aggregated mutant AR. Mutant AR was preferentially degraded in the presence of 17AAG in both cells and transgenic mice as compared to wild-type AR [39].

Collectively, these findings implicate an important role for Hsp90 in the development of Alzheimer's disease and other neurodegenerative diseases and suggest that Hsp90-interfering drugs may represent a potential novel class of drugs to promote the survival of neurons. They also imply that, because of their specificity for high-affinity Hsp90, small molecule Hsp90 inhibitors may selectively target neurodegenerative disease processes without toxicity toward normal tissues.

Candida albicans infections

NeuTec Pharma, which Novartis acquired in mid-2006 to expand its portfolio of compounds for hospital-acquired fungal and bacterial infections (http://www.novartis.com.), has developed Mycograb, a human genetically recombinant antibody that binds to fungal Hsp90. The antibody disables fungal defense mechanism and makes fungi more susceptible to medicines such as amphotericin B. Mycograb has been granted Orphan Drug status in Europe and the US for use against invasive fungal infections, including invasive candidiasis. This life-threatening fungal infection, which is due to the Candida species, has a high mortality rate. In clinical trials, the combination of Mycograb plus amphotericin B demonstrated clear superiority over amphotericin B monotherapy, considered the standard of care [40].

Viral replication

Recently, the chaperone Hsp90 was identified as an essential factor in the folding and maturation of picornavirus capsid proteins [41]. Pharmacologic inhibition of Hsp90 impaired the replication of poliovirus, rhinovirus, and coxsackievirus in cell culture. Unlike other anti-viral approaches, anti-Hsp90 treatment did not yield drug-resistant viruses. *In vivo*, Hsp90 inhibitors drastically reduced poliovirus replication in infected animals without the emergence of drug-resistant escape mutants. Collectively, these propose Hsp90 inhibition as a novel general anti-viral strategy that is refractory to development of drug resistance.

Conclusions

Many cells under a transforming pressure, whether malignant, neurodegenerative, or infective, co-opt Hsp90 to maintain the stability and folding of aberrant, transformation-driving proteins, and to regain a 'pseudo-stable' state. As these effects occur in a cell-specific and transformation-specific manner, one may envision that by inhibition of one protein – Hsp90 – biologic activity may be obtained in a wide-range of diseases.

The ability of Hsp90 inhibitors to affect, simultaneously, multiple transforming molecules and pathways is a unique and therapeutically attractive feature of targeting this chaperone. These findings suggest that Hsp90 inhibitors might provide a broader, more effective anti-cancer, anti-neurodegenerative, and anti-infectious therapy than molecules targeting single, activated, but dispensable signaling molecules that are the focus of most current drug discovery efforts. Moreover, the apparent increased requirement for Hsp90 activity in at least cancer and neurodegenerative diseases suggests the real possibility of an exploitable therapeutic index for this approach.

Although the impact Hsp90 inhibitors may have in cancer treatment is apparent, one may not overlook potential issues. The chaperone was suggested to have a role in the maturation of the cardiac potassium channel hERG [42]; however, no evidence that hERG is, in fact, degraded at non-toxic doses in preclinical animal systems has substantiated these findings. Nor have QTc prolongations on clinical trials been conclusively attributed to Hsp90. Metastasis to the bone was reported for 17AAG in a mouse model [43]. This is an important observation, but it is difficult to realize its clinical significance, as the breast cancer model it was obtained in, MDA-MB-231, is not particularly sensitive to 17AAG. A regulatory feedback induction of Hsp70, observed with Hsp90 inhibition, was suggested to diminish their potential anti-cancer activity, although the effect seems to be tumor type dependent [4]. In spite of these potential drawbacks, we predict a bright future for Hsp90 inhibitors in the treatment of diseases. Owing to an intrinsic role in maintaining cellular transformation, cells would theoretically be less likely to mount resistance to Hsp90 inhibition by modifying the target itself. Resistance to 17AAG has been reported in hormone refractory breast cancer cells. These 17AAG-resistant cells remained sensitive to the chemically distinct RD (Figure 1), suggesting that resistance was not due to a change in Hsp90 structure or biology but was probably attributable to drug efflux or metabolism mechanisms [44], emphasizing the importance of developing second generation Hsp90 inhibitors of diverse chemical structures and modes of action, and the need of bringing to clinic an array of such agents. In addition to pursuing next generation Hsp90 inhibitors, it will also be crucial to determine the best manner in which to use the available inhibitors in order to achieve the greatest benefit from inhibiting the target in a disease-specific manner.

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