Hsp90 Molecular Chaperone Inhibitors: Are We There Yet?

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Abstract

Heat shock protein (Hsp) 90 is an ATP-dependent molecular chaperone that is exploited by malignant cells to support activated oncoproteins, including many cancer-associated kinases and transcription factors, and it is essential for oncogenic transformation. Originally viewed with skepticism, Hsp90 inhibitors are now being actively pursued by the pharmaceutical industry, with 17 agents having entered clinical trials. Investigators established Hsp90′s druggability using the natural products geldanamycin and radicicol, which mimic the unusual ATP structure adopted in the chaperone's N-terminal nucleotide-binding pocket and cause potent and selective blockade of ATP binding/hydrolysis, inhibit chaperone function, deplete oncogenic clients, and show antitumor activity. Preclinical data obtained with these natural products have heightened interest in Hsp90 as a drug target, and 17-allylamino-17-demethoxygeldanamycin (17-AAG, tanespimycin) has shown clinical activity (as defined by Response Evaluation Criteria in Solid Tumors) in HER2+ breast cancer. Many optimized synthetic, small-molecule Hsp90 inhibitors from diverse chemotypes are now in clinical trials. Here, we review the discovery and development of Hsp90 inhibitors and assess their potential. There has been significant learning from studies of the basic biology of Hsp90, as well as translational drug development involving this chaperone, enhanced by the use of Hsp90 inhibitors as chemical probes. Success will likely lie in treating cancers that are addicted to particular driver oncogene products (e.g., HER2, ALK, EGFR, and BRAF) that are sensitive Hsp90 clients, as well as malignancies (especially multiple myeloma) in which buffering of proteotoxic stress is critical for survival. We discuss approaches for enhancing the effectiveness of Hsp90 inhibitors and highlight new chaperone and stress-response pathway targets, including HSF1 and Hsp70. *Clin Cancer Res; 18(1); 64–76. ©2012 AACR.*

Introduction

Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone that regulates the late-stage maturation, activation, and stability of a diverse range of client proteins (defined as proteins with demonstrated binding to Hsp90 whose steady-state level declines upon treatment with Hsp90 inhibitors, usually as a result of proteasome-mediated degradation; see http://www.picard.ch/downloads for a curated list), many which are involved in signal transduction and other key pathways that are especially important in malignancy (1). Although it is highly expressed in normal cells, where it helps to maintain protein homeostasis, Hsp90 is exploited by cancer cells for at least 2 purposes: (i) to support the activated or metastable (e.g., labile) forms of oncoproteins, including many kinases and transcription factors, that are mutated, translocated, amplified, or overexpressed in malignancy; and (ii) to buffer cellular stresses induced by the malignant lifestyle (Fig. 1; refs. 2 and 3). Hsp90 itself is often overexpressed (4) and present in an activated multichaperone complex in cancer cells (5), and it is now regarded as essential for malignant transformation and progression (2, 3).

When the concept of targeting Hsp90 in cancer was first promulgated in the early 1990s, it was viewed with considerable skepticism by the pharmaceutical industry. This was primarily because it was unprecedented to propose targeting a housekeeping protein that is abundantly expressed in normal cells, and there was a perceived risk that Hsp90 inhibition might therefore generate unacceptable toxicity. Thus, the early clinical development of Hsp90 inhibitors was undertaken by the U.S. National Cancer Institute, a small number of academic nonprofit groups, and a few small biotechnology companies. The degree to

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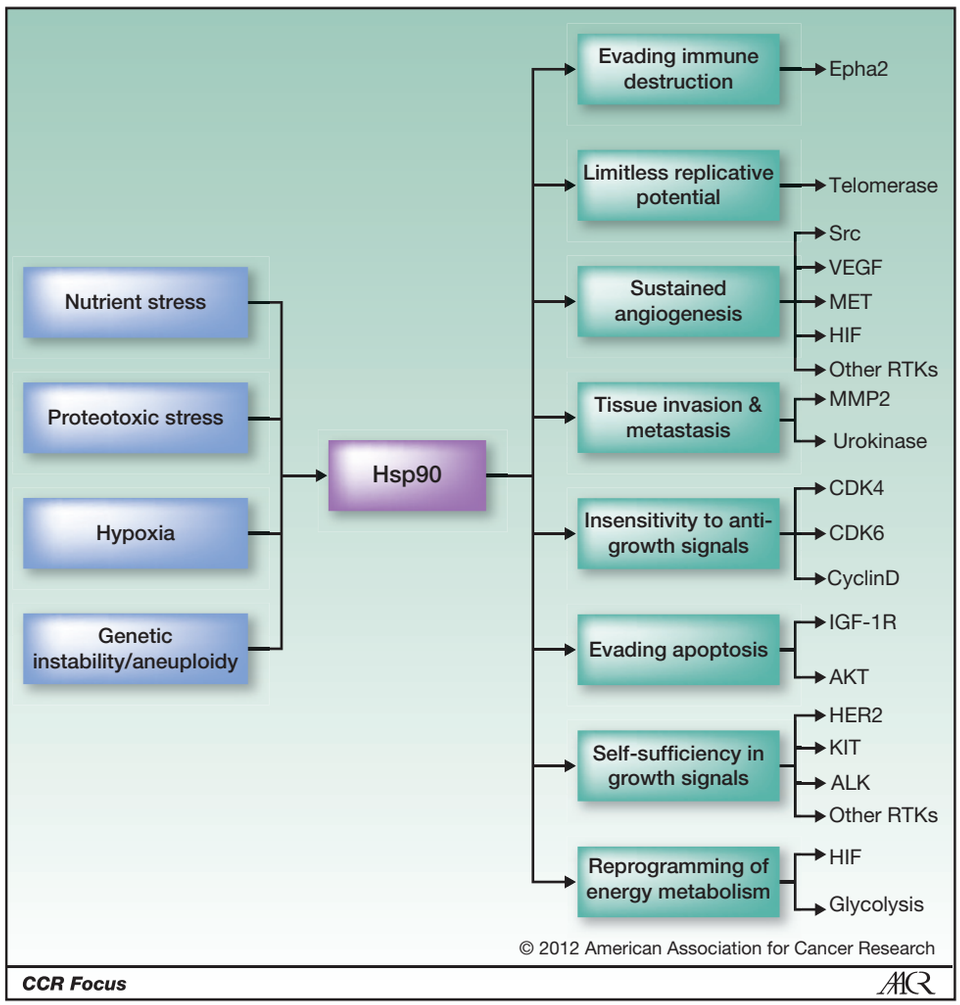

Figure 1. Hsp90 serves as a buffer against the many environmental stresses that cancer cells must endure and overcome. To accomplish this, the molecular chaperone regulates numerous signaling proteins and pathways (shown on the right).

which opinion has changed is shown by the fact that Hsp90 is now one of the most actively pursued cancer drug targets by the pharmaceutical industry, with 17 agents having entered clinical trials (6). An impressive growth in interest in Hsp90 is evident in both the academic and patent literature. Although currently no Hsp90-targeting agents have been approved for human use, clinical activity has been achieved with several drugs in multiple tumor types, and potential routes to regulatory approval are becoming apparent.

In parallel with recent preclinical and clinical therapeutic developments, researchers have made considerable progress in elucidating the molecular, cellular, and organismal contributions of Hsp90 (1–3). Experience gained over the last several years in both the basic biology and the translational drug development around Hsp90, enhanced by the use of Hsp90 inhibitors as chemical probes, has helped us understand how we can best achieve clinical success through inhibition of the molecular chaperone. As part of this *CCR Focus* section ["Drug Development: What Experience Has Taught Us" (7–10)], we provide an update on both therapeutic and relevant fundamental investigations of Hsp90, and we illustrate productive synergies between the two. We also examine future prospects for Hsp90 inhibitors in the clinic and highlight new follow-on targets. We conclude that future success will most likely come from the use of chemically optimized Hsp90 inhibitors from the many that are now available, to treat cancers that especially depend on particular driver oncogene products that are sensitive Hsp90 clients, as well as those malignancies (best exemplified by multiple myeloma) in which buffering of proteotoxic stress is critical for survival. Finally, we discuss approaches to enhance the effectiveness of Hsp90 inhibitors, and we highlight new chaperone and stress-response pathway targets, including HSF1 and Hsp70.

Target Validation, Chemical Tools, Drug Discovery, and Clinical Development

Researchers established the druggability of Hsp90 using the natural products radicicol and geldanamycin (Fig. 2).

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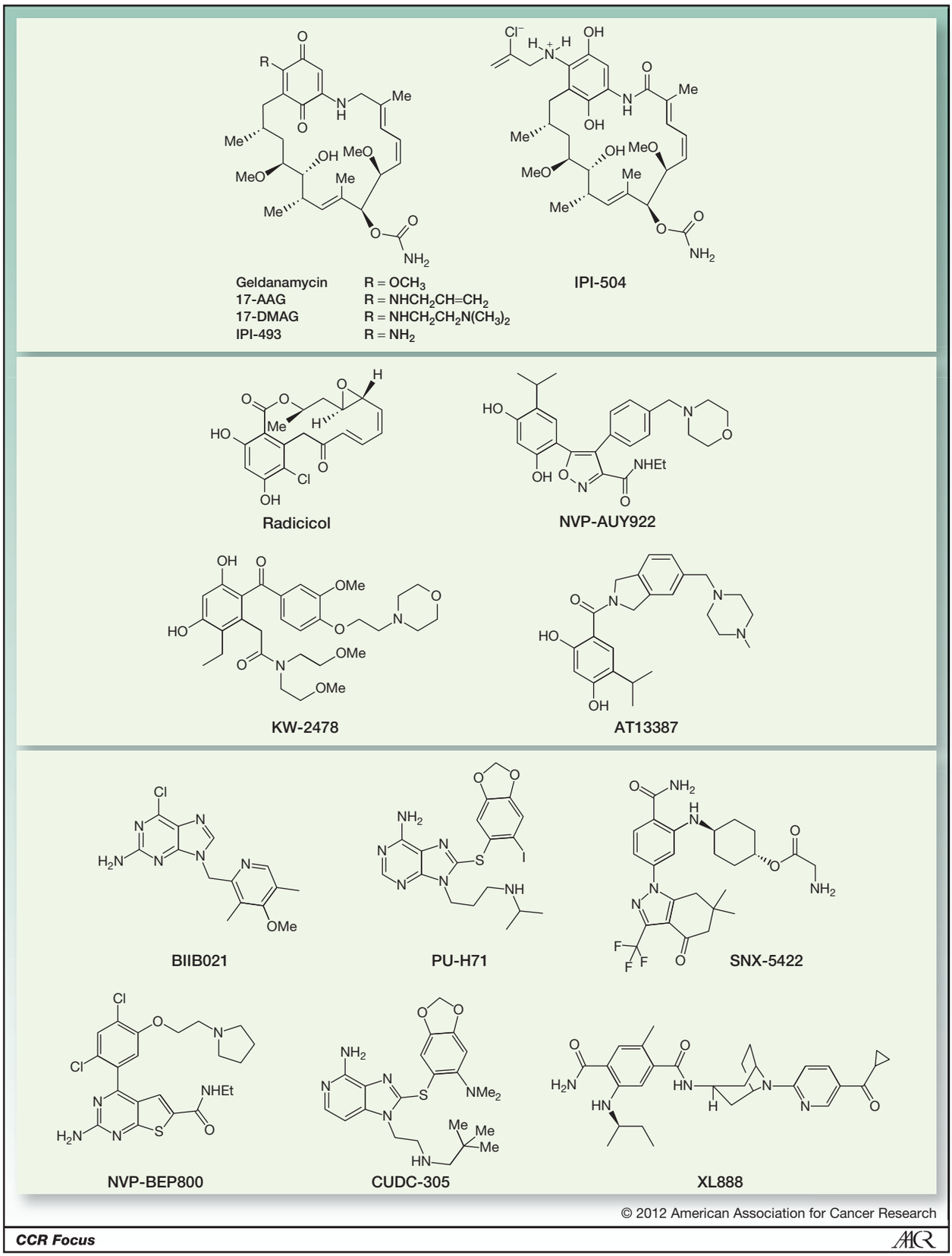

Figure 2. Chemical structures of selected Hsp90 inhibitors discussed in this article.

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These products were isolated in 1953 and 1970, respectively, and were shown to have biologic activity by a then unknown mechanism (11). A critical observation for the Hsp90 field was the demonstration in 1994 that Hsp90 is the molecular target of geldanamycin, and that Hsp90 inhibition by this agent prevents formation of the complex between Hsp90 and its client protein SRC, resulting in SRC destabilization and explaining the reversal of SRC transformation (12). Several years later, radicicol was also shown to target Hsp90 (13, 14). Another breakthrough was the discovery in the late 1990s that geldanamycin and radicicol mimic the relatively unusual structure that ATP adopts in the deep, N-terminal, nucleotide-binding pocket of Hsp90, exploiting the topologically distinct Bergerat fold that is characteristic of the small GHKL (Gyrase, Hsp90, Histidine Kinase, MutL) subgroup within the ATPase/kinase superfamily, thereby leading to potent and selective inhibition of ATP binding and hydrolysis (15, 16), and in turn to the depletion of oncogenic clients through ubiquitin-mediated proteasomal degradation (17, 18).

These natural products provided important chemical probes that proved invaluable to the Hsp90 field, enabling its function to be queried in detail and validating Hsp90 as a druggable target (19). Moreover, although geldanamycin and radicicol proved too toxic and unstable/reactive for clinical use, they each provided the chemical basis for drugs that subsequently entered the clinic. The first to progress to clinical trials was the better-tolerated geldanamycin analog 17-allylamino-17-demethoxygeldanamycin (17-AAG, KOS-953, tanespimycin; Fig. 2). In addition, radicicol's resorcinol Hsp90-binding unit was recapitulated in many subsequent small-molecule Hsp90 inhibitors (see below). Thus, as is often the case in biomedical research, Hsp90 inhibitors owe a deep debt to the world of natural products.

In phase I studies with tanespimycin, investigators demonstrated proof-of-mechanism for target inhibition using a validated pharmacodynamic biomarker signature of client protein depletion and HSF1-dependent Hsp induction (20, 21). Tanespimycin also showed evidence of clinical activity in various malignancies, most impressively to date in phase I and II studies in HER2+, trastuzumab-refractory breast cancer where objective Response Evaluation Criteria in Solid Tumors (RECIST) responses were seen on a weekly schedule of 450 mg/m^2 (22–24). This activity is attributed to the extreme sensitivity of HER2 as an Hsp90 client and its role as a driver oncoprotein in this patient population. The most common side effects with weekly and other schedules were predominantly grade 1 or 2 diarrhea, fatigue, nausea, headache, neuropathy, and transaminitis, and these effects were readily managed with pre-/supportive medications.

However, despite the promising results seen in HER2+ breast cancer and multiple myeloma, where encouraging activity was observed in combination with the proteasome inhibitor bortezomib (25, 26), development of tanespimycin was subsequently discontinued. It has been speculated that termination by the company that supplied tanespimycin (Bristol-Myers Squibb) may have been related to production/formulation and patent expiry concerns (refs. 24 and 27; http://www.myelomabeacon.com/news/2010/07//tanespimycin-development-halted/). Although prolonged disease stabilization was achieved in phase I studies of tanespimycin in various additional tumor types expressing particular Hsp90 client proteins, no complete or partial tumor responses were seen (28, 29). This limited activity has been attributed to suboptimal inhibition of the target client proteins, most likely owing to insufficient drug dose or frequency of administration. In fact, consistent with the clinical data, an inspection of animal model studies in relevant tumors (e.g., ovarian, colon, breast, melanoma, prostate, and lung tumors with appropriate client proteins and addictions) shows a similar pattern of growth inhibition or cytostatic arrest rather than tumor regression in response to single-agent tanespimycin (21, 30–34). This is also consistent with cell-cycle arrest predominating over apoptosis induction in cell culture models (35). Another limitation with tanespimycin is the requirement for reductive metabolism of the benzoquinone by NQO1/DT-diaphorase. Although this process increases the drug's inhibitory potency, it is hypothesized to contribute to the observed liver toxicity, and it clearly allows a mechanism of intrinsic and acquired drug resistance caused by low NQO1 expression (30, 36, 37).

Another geldanamycin analog, alvespimycin (17-dimethylaminoethylamino-17-demethoxygeldanamycin, 17-DMAG; Fig. 2) is much less sensitive to and dependent on NQO1, and it has improved formulation and pharmacokinetic properties. Of interest, a recent study with alvespimycin showed one complete response in castrate-refractory prostate cancer (CRPC), as indicated by prostate-specific antigen levels and confirmed by computed tomography, as well as stable disease in other patients with CRPC, chondrosarcoma, and renal cancer (38). Activity in CRPC was likely related to depletion of the androgen receptor, an Hsp90 client protein, as seen in preclinical models (33). There seems to be no further development of either 17-DMAG or IPI-493 (the 17-amino analog and metabolite of tanespimycin), which is similarly less dependent on NQO1 (ref. 39; Fig. 2). Still in clinical development, however, is the soluble stabilized hydroquinone form of 17-AAG, IPI-504 (retaspimycin hydrochloride; Fig. 2). Evaluation of this drug in gastrointestinal stromal tumor, which is usually driven by the Hsp90 client protein KIT, was stopped because of higher than expected hepatic toxicity, probably due to the combined effects of the drug and metastatic liver disease (39). However, encouraging activity has been seen in non–small cell lung cancer (NSCLC) patients with oncogenic anaplastic lymphoma kinase (ALK) gene rearrangements, the products of which are Hsp90 clients, and ongoing clinical evaluations in this setting include genetic stratification (40).

The trailblazing proof-of-concept work with geldanamycin analogs stimulated the race to discover synthetic

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small-molecule Hsp90 inhibitors that could overcome some or all of the limitations of this class, such as by allowing the use of doses and schedules that provide sufficiently sustained client depletion while sparing the liver toxicity hypothesized to be caused by quinone metabolism (41, 42) and by avoiding the P-glycoprotein–mediated efflux seen with tanespimycin (30). A large number of new Hsp90 inhibitors that do not suffer from these constraints are now in clinical development. Examples of disclosed chemical structures are shown in Fig. 2.

Investigators seeking to discover new Hsp90 drug candidates have benefited greatly from structure-based design using available X-ray crystal structures of Hsp90, and initial chemical matter has frequently emerged from high-throughput, fragment, or virtual screening (39, 43). For example, success was achieved early on with (i) the purine scaffold series, which was based initially on the prototype PU3 (44, 45) and led to the clinical candidates BBIIB021 (CNF-2024) and BIIB028 (structure not disclosed), as well as PU-H71, now in phase I clinical trial (46); and (ii) the resorcylic pyrazoles and isoxazoles [based on CCT018159 (47)], which led to NVP-AUY922/VER52296 (48), the resorcylic dihydroxybenzamide AT13387 (49), and the structurally related KW-2478 (50). A diverse range of chemical scaffolds have since emerged, as illustrated by the publication of 40 to 70 patents per year from 2005 to 2010, covering purines and resorcinols as well as pyrimidines, aminopyridines, azoles, and other chemotypes (Fig. 2; ref. 51). These include SNX-5422, which is a prodrug of the active benzamide SNX-2112, the precursor of which was identified through a purine-based proteomic screen (52); the orally active thienopyrimidine NVP-BEP800/VER-82576, which was derived with the use of a combined fragment-based and *in silico* approach (53); the 8-arylthiopurine CUDC-305, which is orally bioavailable, blood-brain barrier–permeant, and active in an orthotopic brain tumor model (54); and the *N*-aryltropane XL888 (55). Another promising agent currently in multiple clinical trials is STA-9090 [ganetespib (56)]. Although its full structure is undisclosed, it is thought to be a resorcinol-containing triazole, for which a phosphate prodrug is also being developed (39, 56).

Of importance, in cases in which the cocrystal structure of a drug bound to the N-terminal domain of Hsp90 has been determined, the inhibitors all seem to exploit the same core network of water-mediated hydrogen-bonding interactions exploited by geldanamycin and radicicol (as well as ATP/ADP) to anchor the drugs into the base of the N-terminal nucleotide-binding pocket of the chaperone (16, 57, 58). This is illustrated in Fig. 3 for the current clinical drug NVP-AUY922. The various new agents have the potential to be administered more frequently and to achieve a higher maximum dose (and hence better/more-prolonged target inhibition), in some cases with oral administration and blood-brain barrier penetration. New drugs also lack the significant hepatotoxicity that was limiting for members of the geldanamycin chemotype, consistent with this side effect being related to the quinone in those agents (see above). Encouraging early clinical data have been reported concerning these agents' pharmacodynamic and antitumor activities in diverse malignancies, again with the expected client protein and genetic profiles, including breast, NSCLC, and rectal cancer, as well as in melanoma and leukemia (40, 59–65).

Biological Insights and Therapeutic Implications

As we learn more about the role of Hsp90 in modulating signaling networks and the sensitivity of various client proteins to Hsp90 inhibition, a better understanding of Hsp90 biology has already educated and will continue to inform the ongoing clinical development of Hsp90 inhibitor-based therapy, in part by supporting the correct choice of tumor types and revealing additional molecular targets whose inhibition synergizes with Hsp90 inhibition, as discussed below.

Hsp90 helps to coordinate cellular and organismal responses to environmental stresses. For the fruit fly *Drosophila melanogaster*, this may involve the ability to survive in a habitat where temperature fluctuations are common. For the protozoan parasite *Plasmodium falciparum*, this certainly involves successful adaptation to a life cycle that includes more than one host, as well as dramatically different local environments and temperatures. For cancer cells, unavoidable environmental perturbations include nutrient stress, proteotoxic stress, hypoxia, inherent genetic instability/aneuploidy, and even the necessity to avoid attack by the host immune system (Fig. 1). Hsp90 is able to buffer these stresses, thereby allowing cancer cells to survive and indeed to flourish in an inhospitable environment. As described above, it does this by modulating the stability and/or activity of numerous proteins comprising nodal points in multiple signaling pathways that foster survival (3).

Whereas specific Hsp90-dependent pathways that promote unlimited growth, survival in low-oxygen conditions, and escape from apoptosis and that overcome nutrient deprivation by fostering angiogenesis have been known for some time, recent data suggest that Hsp90 inhibition promotes enhanced host natural killer cell–mediated tumor killing (66). Further, the receptor tyrosine kinase ephrin receptor A2 (EphA2) was recently identified as an Hsp90 client (67). EphA2 is abundantly expressed in a broad range of cancers and is recognized as a self-protein by the host, thereby limiting the ability of CD8+ T cells to recognize and kill the tumor. Hsp90 inhibitor–dependent degradation of tumor cell EphA2 significantly improves the *in vitro* antitumor activity of host CD8+ T cells, suggesting that Hsp90 inhibition may have a beneficial impact on host tumor surveillance (68). It remains to be explored whether Hsp90 inhibitors might enhance the activity of therapeutic vaccines that rely on

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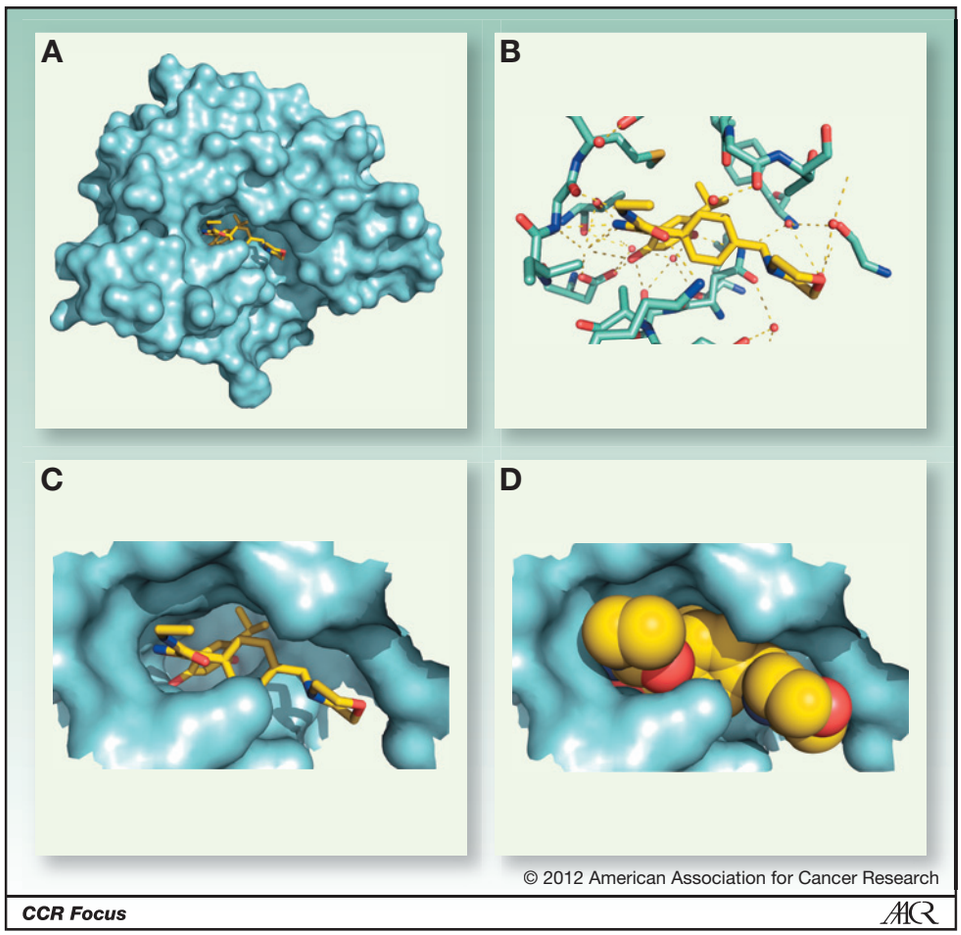

Figure 3. X-ray cocrystal structure of the clinically evaluated resorcylic isoxazole drug NVP-AUY922 bound to the N-terminal domain of human Hsp90α. The structure was obtained at 2.0 Å. PDB code 2VCI (48). A, a view of the surface of the entire human N-terminal domain of Hsp90α (in blue) with the resorcylic isoxazole inhibitor NVP-AUY922 (see Fig. 2 for chemical structure) bound in the deep ATP pocket. B, a more detailed view of NVP-AUY922 in the ATP pocket. The same core network of hydrogen-bonding interactions that are exploited by ATP/ADP, geldanamycin, and radicicol, including water-mediated ones, are used to anchor this drug and other new synthetic inhibitors into the base of the N-terminal nucleotide-binding pocket. The core structure of the drug is shown in yellow and protein residues are in green. Some of the hydrogen-bonding interactions (shown as dotted yellow lines) involve water molecules (red spheres). In this view, the resorcinol ring is on the left-hand side, pointing deep into the base of the pocket, and the 2 resorcinol hydroxyl groups make key hydrogen-bond interactions. The isoxazole ring and morpholine ring (the latter on the far right) are viewed side on. The amide substitution on the isoxazole ring extends out of the plane toward the viewer. All of these structural elements make additional hydrogen-bonding interactions in the ATP pocket. C, the position of the drug is the same as in panel B, but the hydrogen-bonding interactions have been removed and the protein surface is included (colored light blue). The manner in which the resorcinol ring (on the left, with the 2 hydroxyl group oxygen atoms colored red) binds deep into the base of the nucleotide binding pocket is clearly visible, and the isopropyl group (on the resorcinol ring and pointing to the right) can be seen binding in a shallow hydrophobic pocket. The morpholine ring points out of the solvent channel on the right-hand side. Again, the isoxazole ring amide projects out of the plane. Also clear in this representation is that the NVP-AUY922 molecule does not bind in the flat orientation depicted in the chemical structure representation (Fig. 2). Rather, it adopts a twisted conformation that fits the unusual topology of the binding pocket. These features are generally characteristic of Hsp90 N-terminal inhibitors, and they combine to give high levels of potency and selectivity for the target. D, the solvent-accessible surface of the drug illustrates how effectively the drug fills the nucleotide-binding pocket.

activating the host immune system to recognize and attack cancer cells.

Cancer cells are inherently genetically unstable, and aneuploidy is a hallmark of cancer. A recent study identified the Hsp90 inhibitor tanespimycin as being among a small group of compounds that show enhanced efficacy toward aneuploid cancer cell lines *in vitro* (69). Several colorectal cancer cell lines with high-grade aneuploid karyotypes were shown to be significantly more sensitive to the Hsp90 inhibitor compared with colorectal cell lines with near-euploid karyotypes. A similar differential sensitivity was seen in aneuploid lung cancer cell lines. Of importance, the sensitivity of these cells to Hsp90 inhibition was independent of p53 status, although in some cellular contexts, p53 status has been reported to influence response (70).

The greater sensitivity of aneuploid cancer cells to Hsp90 inhibition may reflect enhanced proteotoxic stress associated with aneuploidy, in part as a consequence of accumulating abundant misfolded proteins (69). Thus, it is

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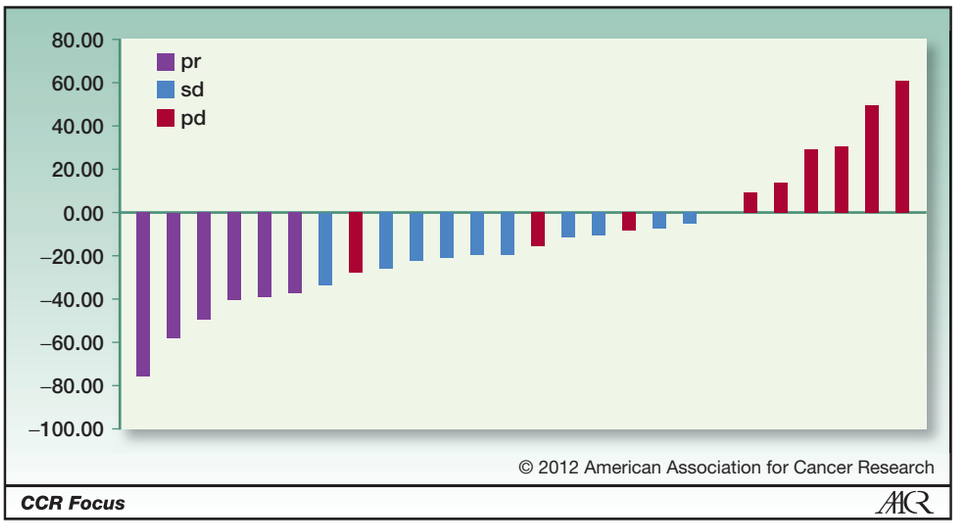

Figure 4. Patients with metastatic HER2+ breast cancer whose disease had previously progressed on trastuzumab received weekly treatment with tanespimycin at 450 mg/m^2 intravenously and trastuzumab at a conventional dose. Therapy was continued until disease progression occurred. The primary endpoint was response rate by RECIST. The overall response rate in evaluable patients was 22% and the clinical benefit rate (CR + PR + SD) was 59%. Data are depicted as a waterfall plot with best response (%) indicated on the *y*-axis. Partial response (pr) is depicted by purple bars, stable disease (sd) is depicted by blue bars, and disease progression (pd) is depicted by red bars. Data used with permission from Modi et al. (19).

not surprising that Hsp90 inhibitors synergize with proteasome inhibitors in multiple myeloma, a cancer in which the proteasome degradation machinery is taxed to the utmost. As described earlier in this review, the combination of tanespimycin and the proteasome inhibitor bortezomib has been associated with durable responses in heavily pretreated patients with multiple myeloma, including those with bortezomib-refractory disease (25, 26, 71, 72). Additional Hsp90 inhibitors are being evaluated in this setting (50, 73, 74).

Some Hsp90 clients are known tumor-driver proteins. Would tumors that are addicted to (i.e., dependent on) these clients be most likely to show clinical responses to Hsp90 inhibitors? The strong relationship between the client-driver protein dependence on Hsp90 and the potential clinical efficacy of Hsp90 inhibition is illustrated by 2 powerful examples. The first is the tyrosine kinase receptor HER2, which was reported 15 years ago to be an Hsp90 client that is extremely sensitive (compared with most other clients) to chaperone inhibition (17, 75). Numerous preclinical data have shown Hsp90 inhibitors to be efficacious in HER2+ breast cancer xenograft studies (76). It is worth reemphasizing that Hsp90 inhibitors combined with trastuzumab showed clinical activity in HER2+ breast cancer patients in whom tumor progression was seen on trastuzumab alone. In the first phase II study to definitively show RECIST-defined responses for this drug in solid tumors, the overall response rate was 22% and the clinical benefit rate (complete response + partial response + stable disease) was 59% (Fig. 4; refs. 22–24).

The second example is the mutated (rearranged) tyrosine kinase ALK, which is perhaps the only Hsp90 client that is as or more sensitive than HER2 to chaperone inhibition (77). ALK rearrangements, particularly the EML4-ALK fusion protein, are found in a small subset (~4%) of NSCLC patients. In a recent nonrandomized, phase II study of the Hsp90 inhibitor retaspimycin in patients with molecularly defined NSCLC, an overall response rate of 7% was observed. However, of the 3 patients in the study whose tumors harbored ALK rearrangements, 2 had partial responses and the third experienced prolonged stable disease (40). Results of an open-label phase II study of another Hsp90 inhibitor, ganetespib (STA-9090), in a similar patient population (advanced NSCLC) were reported at the ASCO 2011 Annual Meeting (65). Of the 62 patients in the study, 8 had tumors with ALK rearrangements, and 4 of these had durable, objective responses.

These 2 examples suggest that client protein sensitivity to Hsp90 inhibition may be a key contributor to Hsp90 inhibitor clinical efficacy, but only in cases where the tumor is addicted to the client. Hsp90 inhibition may also represent an effective strategy to overcome or prevent tyrosine kinase inhibitor (TKI) resistance (78–81). A recent study (82) showed acquired resistance to the ALK inhibitor crizotinib in an NSCLC patient whose cancer relapsed after 5 months of treatment. A molecular analysis of the resistant tumor cells revealed 2 novel mutations in the *EML4-ALK* gene, one of which (L1196M) conferred resistance to crizotinib. The study showed that these crizotinib-resistant tumor cells remained addicted to ALK signaling but retained sensitivity to the Hsp90 inhibitor tanespimycin. Indeed, the mutated ALK from these cells was efficiently depleted by tanespimycin. Similarly, in an NSCLC patient whose tumor initially responded to crizotinib and who remained on the drug

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for 1 year before progressing, a further tumor response was noted after treatment with ganetespib (65).

Chronic myelogenous leukemia in patients treated with the Abl TKI imatinib eventually becomes resistant to the drug due to development or outgrowth of Bcr-Abl drug-resistant mutations, the most common being T315I. This Bcr-Abl mutant is resistant to all first- and second-line TKIs tested, but it remains sensitive to Hsp90 inhibition [Bcr-Abl is a highly dependent Hsp90 client (83)]. These examples suggest that, at least in certain circumstances, the combination of an Hsp90 inhibitor with a TKI may suppress or delay the occurrence of drug-resistant mutations.

In addition to the amplification of and dependence on client proteins such as HER2 or rearranged ALK as predictors of tumor response to Hsp90 inhibitors, overexpression of Hsp90 itself has been identified as an independent prognostic factor in breast cancer (4). This may represent a clinical example of nononcogene addiction to stress protection pathways, and it may explain the Hsp90 inhibitor efficacy of triple-negative breast cancer in preclinical models (46). Hsp90 expression levels are also inversely related to long-term survival in NSCLC patients (84), suggesting that stratification of patients' tumors based on Hsp90 expression, as well as on expression of oncogenic clients, may select for a population that might be more responsive to Hsp90 inhibitors.

Future Prospects: What Next?

Several approaches are being investigated to enhance cancer cell sensitivity to Hsp90 inhibitors, including targeting Hsp90 cochaperone proteins, HSF1 and its transcriptional targets, and posttranslational modifiers of Hsp90. Modulating cochaperone expression affects cancer cell sensitivity to Hsp90 inhibitors. For example, deletion of p23 in yeast results in hypersensitivity to the Hsp90 inhibitors geldanamycin and radicicol, whereas p23 overexpression protects yeast from these agents (85). Because this cochaperone is reported to be overexpressed in cancer (86), pharmacologic inhibition of p23 may increase cancer cell sensitivity to Hsp90-targeted drugs. Similarly, silencing of the cochaperones Aha1 and Cdc37, which are also overexpressed in cancer, sensitizes tumor cells to tanespimycin (87–90). Therefore, targeting these proteins or their interaction with Hsp90 may provide a therapeutic benefit when combined with Hsp90 inhibitors (90, 91).

Activation of the heat shock transcription factor HSF1 occurs uniformly in response to the Hsp90 inhibitors currently under clinical evaluation. Although this response is useful for providing pharmacodynamic biomarkers, when it occurs in tumor cells it is generally considered to limit the activity of Hsp90 inhibitors because HSF1-dependent transcriptional induction of Hsp70, Hsp27, and to some degree Hsp90 itself, protects cancer cells from apoptosis. Indeed, cells in which HSF1 has been knocked out are much more sensitive to Hsp90 inhibitors compared with their wild-type counterparts (92). Likewise, silencing of either Hsp70 or Hsp27 has been shown to dramatically increase cancer cell sensitivity to Hsp90 inhibition and induction of apoptosis (93, 94). Efforts are underway to identify and validate inhibitors of HSF1, Hsp70, and Hsp27 and to explore their combination with Hsp90 inhibitors (95–99).

Hsp90 is subject to several posttranslational modifications that affect chaperone function. For example, the tyrosine kinase WEE1, an Hsp90 client, directly phosphorylates the chaperone on a conserved tyrosine residue in Hsp90's N-terminal domain (100). Phosphorylation at this site positively affects the ability of Hsp90 to chaperone a number of cancer-related kinases, including HER2, SRC, CRAF, CDK4, and WEE1 itself. Of importance, WEE1 phosphorylation of Hsp90 negatively affects tanespimycin binding, and silencing or pharmacologic inhibition of WEE1 sensitizes prostate and cervical cancer cells to Hsp90 inhibition. These data suggest that a more detailed understanding of Hsp90 phosphorylation and perhaps additional posttranslational modifications could provide new strategies to enhance the efficacy of Hsp90 inhibitors and at the same time reveal novel resistance mechanisms [see Mollapour and Neckers (101) for a thorough review of the latest information on this topic].

Although to date the vast majority of drug development efforts have focused on targeting the N-domain ATP binding site of Hsp90, a second druggable site has been identified in the C-domain of the protein (102, 103). Coumarin antibiotics, such as novobiocin, are the prototypic inhibitors that interact at this site. Recently, investigators have made significant advances in improving the affinity of these compounds for Hsp90 and have shown their ability to induce apoptosis in cancer cells, in some cases with superior efficacy compared with tanespimycin (104, 105). One potential benefit of these drugs is that some of the C-terminal inhibitors seem to be associated with significantly less-robust HSF1 activation than is characteristic of N-terminal inhibitors (106). Existing data strongly support further medicinal chemistry optimization and preclinical evaluation of C-terminal Hsp90 inhibitors.

The relative importance of the 4 Hsp90 isoforms (Hsp90α and Hsp90β in the cytoplasm and the nucleus, GRP94 in the endoplasmic reticulum, and TRAP1 in the mitochondria) for cancer remains poorly understood. Although current inhibitors most potently interact with Hsp90α and β isoforms (e.g., see ref. 41), the impact of isoform selectivity on the therapeutic index and toxic effects of these inhibitors should be explored in greater detail.

The critical role of Hsp90 in promoting cellular and organismal survival in response to environmental stress cannot be disputed. Thus, although the protein has been validated as a *bona fide* molecular target in cancer and the inhibitors are generally well tolerated in the clinic, it should be remembered that this chaperone also helps to maintain normal cellular homeostasis and has certain functions in

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specific cellular contexts that one would not want to inhibit, at least on a long-term basis. For example, prolonged Hsp90 inhibition may have a deleterious effect on DNA mutation frequency in germ cells, where Hsp90 is required for suppression of transposon activity (107, 108). Hsp90 inhibition may increase transposon mobility via loss of activity of the arginine methyltransferase PRMT5, an Hsp90 client (109).

Hsp90 also positively regulates at least one transcription factor with tumor suppressor activity, namely, interferon regulatory factor 1 (IRF1). Loss of IRF1 cooperates with Ras mutation to transform cells, and *IRF1* is deleted in certain cancers (110). Hsp90 inhibition is associated with inhibition of IRF1 transcriptional activity and IRF1 protein degradation (111).

LATS1 and LATS2 kinases are both clients of Hsp90 and are degraded upon Hsp90 inhibition (112). Both kinases positively regulate the Hippo tumor suppressor pathway, and LATS-deficient mice are prone to develop ovarian cancers and sarcomas (113, 114). LATS1 activity was disrupted in Hsp90 inhibitor-treated ovarian cancer cell lines *in vitro* and in ovarian cancer xenograft tumors obtained from mice treated with the Hsp90 inhibitor tanespimycin (112).

Finally, low-penetrant mutations of the retinoblastoma tumor suppressor gene give rise to retinoblastoma proteins that retain ~50% of wild-type tumor suppressor activity. However, these mutant retinoblastoma proteins interact with and depend on Hsp90 for stability, and they are inactivated and destabilized in cells exposed to the Hsp90 inhibitor geldanamycin (115). Thus, Hsp90 inhibitor treatment of patients harboring these low-penetrant retinoblastoma mutant alleles, which normally are characterized by reduced or absent tumor formation, could phenocopy retinoblastoma null mutations that are invariably associated with early-onset multifocal retinoblastoma.

Taken together, these data show that several tumor suppressor pathways may be deregulated following Hsp90 inhibition. They further emphasize that the cumulative impact of an Hsp90 inhibitor on both the individual and the cancer cell is multifactorial and will almost certainly be influenced by the duration of treatment, the disparate sensitivity to Hsp90 inhibition of the various client proteins present in normal and cancer cells, the dependence of the particular cancer on the continued expression of one or more of these clients, and the local environmental context in which Hsp90 inhibition occurs. Nonetheless, with these caveats in mind, the promising clinical responses that continue to be seen with several Hsp90 inhibitors in a number of molecularly defined cancers certainly support the continued therapeutic development of these agents.

Conclusions

Considerable progress has been made both in understanding the basic structure–function biology of Hsp90 and in translating this knowledge into anticancer drug therapies. Studies of tanespimycin showed a proof-of-mechanism for Hsp90 inhibition in the clinic and proof-of-concept for therapeutic activity, most impressively in breast cancer. The view has been expressed that termination of development of this drug was premature and was not a result of scientific/clinical considerations (24, 27). Additional promising signs of activity have been seen in various molecularly relevant tumor types (e.g., in prostate cancer, where ongoing addiction to the androgen receptor client protein is clear) and in other cancers in which Hsp90 client kinases are key drivers. On the other hand, to date, no Hsp90 inhibitor has received marketing approval.

So are we there yet? Although targeting Hsp90 in cancer patients to achieve a significant therapeutic benefit is still a work in progress, we have certainly learned from experience. A number of highly potent and pharmaceutically improved Hsp90 inhibitors that avoid some of the drawbacks of the first-generation inhibitors, as discussed above, are now in clinical trial. In the short term, success will most likely come from using these enhanced agents to treat cancers that are addicted to particular amplified, mutated, or translocated driver oncogene products that are highly sensitive client proteins of Hsp90, such as HER2, ALK, EGFR, BRAF, and perhaps also androgen and estrogen receptors. Additional cancers that will likely prove sensitive to Hsp90 inhibitors include malignancies in which buffering of proteotoxic stress is essential for cancer cell survival, as exemplified by multiple myeloma. In the long term, realizing the full therapeutic potential of Hsp90 inhibitors may require concomitant inhibition of Hsp70 isoforms or blockade of HSF1. In addition, tumor stratification by client protein status and Hsp90 expression level, more complete genetic profiling of tumors as a basis for patient selection, and judicious exploration of biologically informed drug combinations, including Hsp90 inhibitors combined with agents that directly block the function of a given Hsp90 oncoprotein client, will help guide the field to a more efficacious use of these molecularly intriguing and therapeutically promising drugs.

Disclosure of Potential Conflicts of Interest

P. Workman is an employee of the Institute of Cancer Research, which has a commercial interest in Hsp90 inhibitors and operates a reward to inventors program. Intellectual property regarding Hsp90 inhibitors developed at the Institute of Cancer Research is licensed to Vernalis and Novartis. P. Workman has served as a consultant to Novartis, was a scientific founder of Chroma Therapeutics, and is the current chairman of the Chroma Therapeutics Scientific Advisory Board. L. Neckers disclosed no potential conflicts of interest.

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Len Neckers and Paul Workman

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