Review

Molecular Cancer Research

Maximizing the Therapeutic Potential of HSP90 Inhibitors

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Abstract

HSP90 is required for maintaining the stability and activity of a diverse group of client proteins, including protein kinases, transcription factors, and steroid hormone receptors involved in cell signaling, proliferation, survival, oncogenesis, and cancer progression. Inhibition of HSP90 alters the HSP90-client protein complex, leading to reduced activity, misfolding, ubiquitination, and, ultimately, proteasomal degradation of client proteins. HSP90 inhibitors have demonstrated significant antitumor activity in a wide variety of preclinical models, with evidence of selectivity for cancer versus normal cells. In the clinic, however, the efficacy of this class of therapeutic agents has been relatively limited to date, with promising responses mainly observed in breast and lung cancer, but no major activity seen in other tumor types. In addition, adverse events and some significant toxicities have been documented. Key to improving these clinical outcomes is a better understanding of the cellular consequences of inhibiting HSP90 that may underlie treatment response or resistance. This review considers the recent progress that has been made in the study of HSP90 and its inhibitors and highlights new opportunities to maximize their therapeutic potential. *Mol Cancer Res; 13(11); 1445–51. ©2015 AACR.*

Introduction

Molecular chaperones are responsible for the assembly and correct folding of polypeptide chains into their oligomeric structures (1), with aggregation or degradation of proteins occurring when a functional three-dimensional protein structure is not met. Heat-shock proteins (HSPs) comprise a group of molecular chaperones that are upregulated under stress to prevent the denaturation and inappropriate aggregation of proteins, in order to maintain protein homeostasis (2). HSPs also serve a vital role under non–stress-related conditions in a myriad of housekeeping functions, including signal transduction, proliferation, apoptosis, and protein trafficking (1, 2). The 90 kDa heat shock protein, HSP90, is one of the most abundant and highly conserved chaperones, accounting for 1% to 2% of all cellular proteins and increasing by up to 10-fold under physiologic stress (2). The discovery of HSP90 as the molecular target of natural anticancer products geldanamycin (GM) and radicicol (RD) in the mid-1990s sparked widespread interest in the inhibition of HSP90 as a strategy for the treatment of cancer (3, 4). Here, we review progress that has been made in the clinical development of HSP90 inhibitors and highlight exciting new opportunities that exist to improve clinical outcomes through research into the molecular actions of these inhibitors and their cellular target.

HSP90 Structure and Function

Presently, four isoforms of HSP90 have been identified, which differ in their cellular localization. The two major cytoplasmic isoforms are HSP90α (inducible) and HSP90β (constitutively expressed) that are approximately 86% homologous (5). Although these isoforms display generally overlapping roles, they vary in expression between embryonic and adult tissues, with some evidence for different roles under stress conditions (6); HSP90α plays a cytoprotective role and is fast to respond, whereas HSP90β has been linked to a slower response involving cellular adaptation (6). The HSP90 family also includes paralogs found within the endoplasmic reticulum (GRP94; ref. 7) and mitochondria (TNF receptor–associated protein 1; TRAP1; refs. 6, 8).

HSP90 relies on its ability to bind and hydrolyze ATP in order to effectively regulate the maturation of its so-called "client" proteins through a conformationally dynamic ATPase-driven cycle, controlled by an orchestrated set of interactions with a range of co-chaperones (9, 10). Different classes of HSP90 client proteins progress through this cycle in different ways, involving interactions with distinct co-chaperone proteins. A simplified version of the cycle, outlining the minimum requirements for client maturation, is shown in Fig. 1. All forms of HSP90 naturally exist as obligate homodimers comprising two identical monomers, each with three distinct functional domains (9, 10). The C-terminal dimerization domain contains a conserved pentapeptide sequence (MEEVD) that is the primary binding site for a specific set of tetratricopeptide repeat (TPR) domain-containing co-chaperone proteins, which aid in the progression of client proteins through the HSP90 cycle (10). The middle domain binds both client and co-chaperone proteins and is believed to assist HSP90

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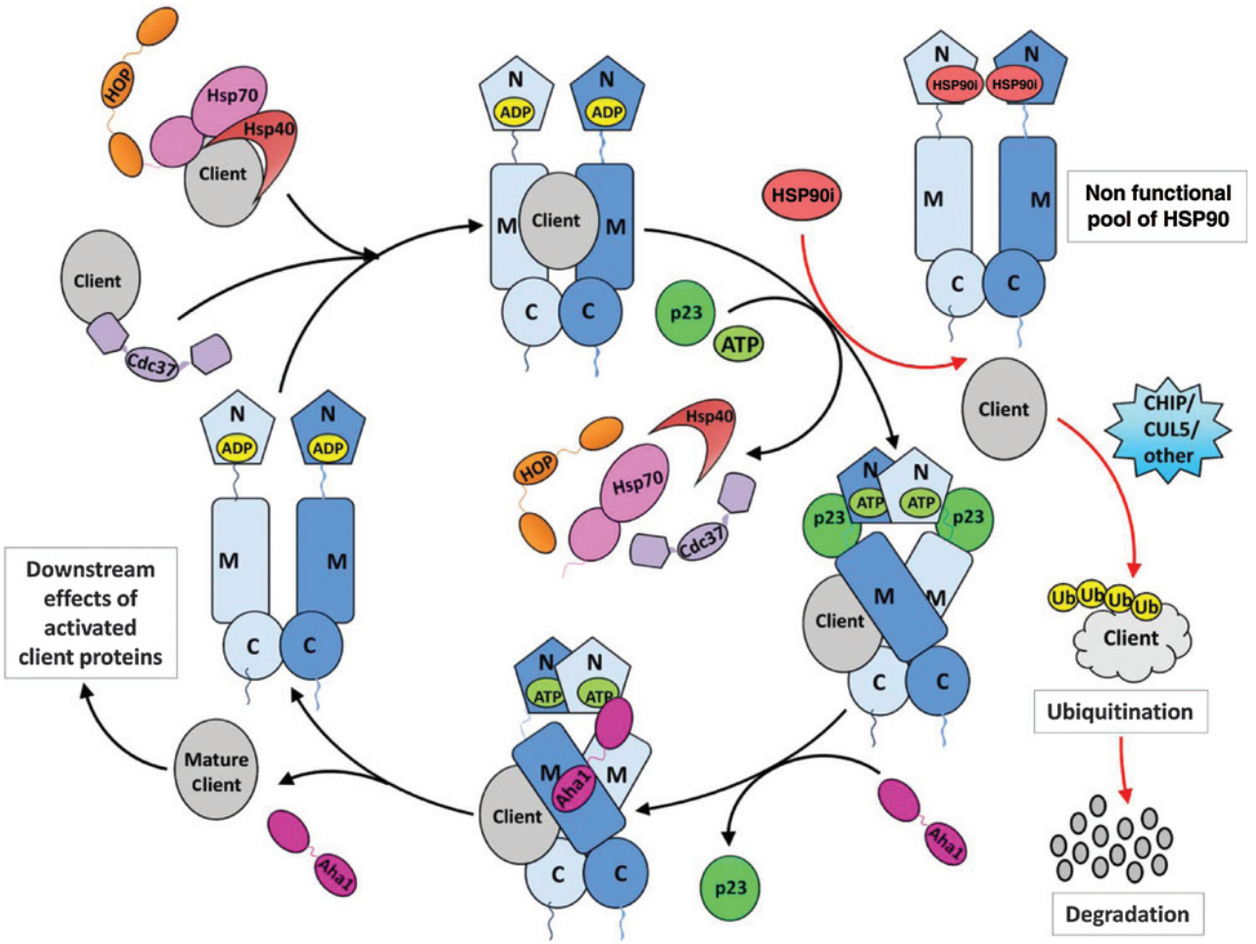

Figure 1.
The chaperone cycle of HSP90 and Effects of HSP90 inhibition. As new client proteins are synthesized, they are rapidly bound by HSP40, independent of ATP and other proteins. HSP40 then associates with HSP70, allowing HSP70 to bind the client, thus forming the HSP70–HSP40 chaperone complex. Certain client proteins, such as transcription factors, are delivered to the ADP-bound "open" form of HSP90 by the HSP70–HSP40 chaperone complex via the TPR-containing co-chaperone **H**SP70-HSP90 **O**rganizing **P**rotein (HOP). The role of HOP in this complex is to reversibly link HSP70 to HSP90 through the MEEVD peptide to allow transfer of client proteins to HSP90. Client binding leads to conformational changes within HSP90 causing it to conform to an ATP-bound "closed" state in association with the co-chaperone, p23. p23 preferentially binds the ATP-bound state of HSP90 and stimulates dissociation of the HSP70–HSP40 complex from HSP90. Kinases are delivered to HSP90 through an alternative method that involves a complex with the co-chaperone **C**ell-**D**ivision **C**ycle **37** homolog (Cdc37). Cdc37 delivers client kinases via interactions with the ATP lid of HSP90's N-terminal domain and the highly conserved glycine-rich loop of the protein kinases N-lobe. These and other co-chaperone interactions induce conformational changes in client proteins that allow their maturation and activation. For all clients, the cycle ends with HSP90 binding the co-chaperone **A**ctivator of **H**SP90 **A**TPase 1 (Aha1), which causes stimulation of ATP hydrolysis by HSP90, leading to release of the mature and active client protein. Inhibition of HSP90 by small-molecule inhibitors acting at the N-terminal nucleotide site blocks ATP binding and hydrolysis, leading to arrest of the chaperone cycle, loss of co-chaperones from the chaperone complex, inhibition of client activity, and ubiquitin-dependent proteasomal-dependent degradation of the client.

in binding to specific clients. The N-terminal nucleotide-binding domain defines HSP90 as a member of the gyrase-HSP90-histidine kinase-MutL (GHKL) family of homodimeric ATPases that trap ATP in their nucleotide-binding domain, involving dimerization of their N-termini (11).

HSP90 as a Therapeutic Target in Cancer

HSP90 is known to facilitate the stabilization and activation of over 300 client proteins (see www.picard.ch/downloads/HSP90interactors.pdf for an updated client protein list). A surprisingly large number of HSP90 client proteins play crucial roles in oncogenic signaling, and in establishing the hallmark traits of malignancy, including proliferation, evasion of apoptosis, immortalization, invasion, angiogenesis, and metastasis (12). Inhibition of HSP90 leads to rapid inhibition of client protein activity, followed by the ubiquitin-mediated proteasomal degradation of client proteins and culminating in the simultaneous depletion of multiple oncoproteins, combinatorial downregulation of signals propagated through numerous oncogenic signaling pathways, and modulation of all aspects of the malignant phenotype. Cancer cells are particularly sensitive to HSP90 inhibition because they are "addicted" to the oncogenic processes that drive malignancy and therefore rely on HSP90 for chaperoning and maintenance of these oncogenic pathways (13). Cancer cells also rely on HSP90 to stabilize mutated, fused, and overexpressed oncoproteins, such as vSRC, HER2, BCR-ABL, B-RAF, and ELM4-ALK (12, 14). HSP90 itself is commonly overexpressed in cancer

cells, and highly-cited evidence suggests it is present in a very active, multichaperone complex (15). More recently, attention has begun to focus on the role of secreted HSP90 in driving cancer cell invasion and metastasis (16, 17). Invasive cancer cells have been shown to secrete HSP90-α, which in turn activates the proinvasive protein matrix metalloproteinases, thereby contributing to increased cancer cell migration (18, 19).

Advances in the Clinical Development of HSP90 Inhibitors

Association with a plethora of signal transduction and other pathways has positioned HSP90 as a promising target for cancer treatment and one of the most actively pursued by drug discovery groups in both academia and industry (12, 20). The majority of HSP90 inhibitors that are currently available, and all that have been clinically assessed, bind to the nucleotide-binding pocket of the N-terminal domain and block the processing of client proteins by preventing ATP binding and hydrolysis (21, 22). This action thwarts completion of the HSP90 chaperone cycle, and clients are subsequently targeted for proteosomal degradation by E3 ubiquitin ligases, including **c**arboxyl terminus of the **H**sc70-**i**nteracting **p**rotein (CHIP; refs. 23, 24) and Cullin-RING ligase Cullin-5 (CUL5; ref. 25). Inhibitors of HSP90 are capable of degrading essentially all HSP90 clients, including oncogenic fusion proteins and transcription factors, along with mutated and active forms of serine/threonine and tyrosine kinases (13, 14). A recent study has provided new insight into the molecular sequelae following HSP90 inhibition, revealing surprisingly complex effects involving CUL5, including rapid loss of signaling output from the client protein as well as dissociation of co-chaperones from the client complex after binding of inhibitors to HSP90 (26). Both of these events occur prior to the eventual degradation of the client and involve CUL5. It is likely that these early molecular events may be equally important determinants of sensitivity to HSP90 inhibition, along with the client degradation that occurs much later.

The first HSP90 inhibitor identified was the bacterial-derived benzoquinone ansamycin GM, following a screen for compounds capable of reversing v-SRC oncogene transformation (4). GM was shown to bind the ATP-binding site in the N-terminus of HSP90 (21, 27), resulting in destabilization of the SRC client protein. GM was never evaluated in the clinic because of its poor "drug-like" properties and toxicity (28); thus, the first-in-class HSP90 inhibitor to enter the clinic was the GM analogue 17-allylamino-demethoxygeldanamycin (17-AAG; tanespimycin; ref. 29). Tanespimycin was tested in both solid and hematologic malignancies in more than 30 clinical trials (phase I/II), both as a single agent and in combination with either chemotherapy or targeted drugs (reviewed in ref. 30). Early phase I trials of tanespimycin were disappointing, with only modest activity noted in some tumor types (12). The limited success of single-agent tanespimycin has been attributed, at least in part, to suboptimal inhibition of target client proteins, most likely owing to insufficient drug dose or frequency of administration, variable pharmacokinetics, suboptimal formulation, and dose-limiting toxicities, including hepatotoxicity. However, promising activity was seen in a phase II study in $HER2^+$ breast cancer (31). Additional limitations with tanespimycin are the susceptibility to multidrug resistance mechanisms, such as p-glycoprotein–mediated efflux, together with polymorphic-reductive metabolism of the benzoquinone by the enzymes NQO1/DT-diaphorase or CYP3A4 (32). Although quinone metabolism increases the drug's HSP90 inhibitory potency, it likely contributes to the observed liver toxicity and may represent a mechanism of primary and acquired resistance (33).

Another GM analogue 17-(dimethylaminotheyl-amino)-17-demethoxygeldanamycin (17-DMAG; alvespimycin; KOS-1022), developed by Kosan and the NCI, displayed improved pharmacologic properties compared with tanespimycin, including increased water solubility, better oral bioavailability, and less dependence on NQO1/DT-diaphorase metabolism (32, 34). Objective tumor responses were observed in castration-resistant prostate cancer, melanoma, acute myeloid leukemia, and in combination with trastuzumab in $HER2^+$ metastatic breast cancer (35, 36). However, the clinical development of tanespimycin and alvespimycin was halted in 2008, a decision that may have involved commercial considerations (37). Still in clinical development is the soluble hydroquinone hydrochloride salt of tanespimycin, IPI-504 (retaspimycin) developed by Infinity Pharmaceuticals. Overall, GM derivatives provided critical proof-of-concept that HSP90 is a relevant target for cancer therapy, and allowed clinical validation of pharmacodynamic biomarkers that are still used in subsequent clinical trials (29, 38, 39). Their pharmacologic limitations, however, have prompted the subsequent development of rationally designed synthetic HSP90 inhibitors.

Two leading small-molecule classes of HSP90 inhibitors have progressed to clinical development. The first class contains the ATP site-binding resorcinol moiety also present in RD whereas the second class is the purine scaffold series (12, 40). Synthetic second-generation HSP90 inhibitors have generally greater potency, the potential to achieve more-prolonged target inhibition, in some cases with oral administration and blood–brain barrier penetration, together with reduced hepatotoxicity owing to replacement of the quinone moiety. Their toxicity profile is also more favorable, with ocular and gastrointestinal toxicity and fatigue being the most frequent side effects. Among the most advanced compounds are resorcinol-based agents AUY922 (luminespib; Vernalis, formerly Novartis) and AT13387 (onalespib; Astex Pharmaceuticals), currently in phase II, and STA-9090 (ganetespib, Syntha Pharmaceuticals), currently in phase III clinical development.

Luminespib is an isoxazole resorcinol derivative of a lead compound identified through a high-throughput screen, with potent preclinical efficacy observed in a range of tumor types (41). Phase II studies in molecularly prespecified cohorts, such as EGFR-mutated and ALK-rearranged non–small cell lung cancer (NSCLC) and $HER2^+$ breast cancer (refractory to standard anti-HER2 regimen), have demonstrated promising activity, with response rates ranging from 10% to 25% (42, 43). Recently, promising early-stage antitumor activity has also been observed in NSCLC patients with EGFR exon 20 insertions—a rare subtype (4%) of EGFR mutations that are refractory to EGFR-specific tyrosine kinase inhibitors (NCT01854034; ref. 44). Luminespib is currently in phase II testing in advanced ALK-positive NSCLC (NCT01752400).

Onalespib is a potent resorcylic dihydroxybenzamide discovered through fragment-based drug screening against the ATP-binding domain of HSP90 (45) with a long duration of action in preclinical models (46). Phase I single-agent activity of onalespib was observed in an imatinib-resistant metastatic

gastrointestinal stromal tumor with a cKit mutation (47). Preliminary evidence of activity of onalespib in combination with the ALK inhibitor crizotinib was reported in a phase I study in ALK-rearranged metastatic NSCLC previously treated with crizotinib (response rate 16%). A phase II randomized study of onalespib in combination with crizotinib versus crizotinib alone in ALK-positive NSCLC (NCT01712217) is ongoing. A phase I study of olanespib in melanoma in combination with the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib (NCT02097225) is also under way.

Ganetespib is a resorcinol-containing triazolone agent that has been assessed in various phase I and II studies for both solid and hematologic malignancies and is currently in phase III clinical development in combination with the taxane chemotherapeutic docetaxel in NSCLC (NCT01798485). Its most promising single-agent activity was reported in heavily pretreated NSCLC patients, particularly those with tumors harboring ALK rearrangement with durable response observed in 50% of patients (48). Results from a phase I study evaluating the combination of ganetespib and crizotinib in ALK-rearranged metastatic NSCLC not previously treated with crizotinib were also recently reported (67% response rate). Final results from a phase II study evaluating ganetespib in molecularly stratified breast cancer patients are awaited (ENCHANT-1 Trial; NCT01677455).

Enhancing the Therapeutic Potential of HSP90 Inhibition

Despite the recognition of HSP90 as an important anticancer target with pleiotropic effects on many oncogenic client proteins, HSP90 inhibitors have not to date demonstrated their predicted level of clinical efficacy. Some of the key objectives that, if achieved, would further realize the full therapeutic potential of HSP90 inhibition are summarized in the following sections.

Molecular stratification: not all HSP90 clients are equally important

Clinical activity demonstrated in specific molecular backgrounds, e.g., HER2 amplification in breast cancer and ALK rearrangements in NSCLC, suggests that HSP90 inhibitors may have their major clinical impact in specific tumor types wherein the driver oncoprotein or fusion protein is a highly sensitive HSP90 client, as is the case for HER2 and translocated ALK (12). Malignancies in which buffering of proteotoxic stress is essential for survival, e.g., multiple myeloma, might also be more sensitive to HSP90 inhibition. In other cancer types, such as prostate cancer, HSP90 inhibition appears to be less effective despite the fact that the respective oncogenic constituents of such cancers, including the androgen receptor, are among HSP90's clientele (49). This suggests that not all client proteins of HSP90 are equally sensitive or therapeutically important. The demonstrated hierarchy of clients in terms of dependence on HSP90 and elucidation of the HSP90 interactome in malignant cells (50, 51) will likely have major impact for further clinical development of HSP90 inhibitors and allow rational prioritization of target cancers for clinical investigation and of appropriate patient populations most likely to benefit.

Minimizing the heat shock response

The heat shock response (HSR), mediated by activation of heat shock factor 1 (HSF1), is an ancient, highly conserved mechanism that protects organisms against various adverse environmental and pathologic conditions that damage cellular proteins. Under such conditions, HSF1 is released from an inhibitory complex with HSP90 and induces transcriptional upregulation of numerous prosurvival proteins, including HSP70, HSP40, and HSP27, which can in turn limit the activity of HSP90 inhibitors (39, 52–54). Importantly, this occurs not only in tumor cells but also in other cells of the tumor microenvironment that play critical roles in tumor cell behavior and response or resistance to therapeutics. A recent report of the tumor-promoting effects of HSF1 in cancer-associated stromal cells (55) provides a further reminder that the potential ramifications of HSP90 inhibition in nontumor cells must be considered. Silencing of HSF1, HSP70, or HSP27 significantly increases cell sensitivity to HSP90 inhibition and induction of apoptosis in cancer cells (53), (56–58). Efforts are under way to identify and validate inhibitors of HSF1 and HSP70, and to explore their combination with HSP90 inhibitors (59), and recent work has implicated inhibition of mTOR signaling as a novel strategy by which induction of HSPs can be blocked, by inhibiting nuclear translocation of HSF-1 (60). Alternatively, c-terminal inhibitors of HSP90, such as novobiocin, and its analogues appear to be associated with significantly less HSF1 activation than N-terminal inhibitors, for reasons that are not yet known, but these are yet to enter clinical evaluation (61).

Optimizing dosing and schedule through pharmacodynamic biomarkers

An important clinical application of the HSR is as a pharmacodynamic biomarker for HSP90 inhibition (38, 39). Induction of HSP70 (also denoted as HSP72) measured in isolated human peripheral blood mononuclear cells has been widely used to monitor efficacy of HSP90 inhibitors in clinical trials (29). However, it is well established that induction of the HSR by HSP90 inhibitors occurs at lower concentrations than does depletion of client proteins, and in clinical trials of 17-DMAG in patients with advanced malignancies, HSP70 levels measured in peripheral blood mononuclear cells showed no correlation with clinical response (62, 63). Therefore, direct evaluation of the client protein depletion and modulation of downstream signaling pathway(s) of interest, coupled with evaluation of biologic effects (e.g., increased apoptosis, reduced proliferation), may be more helpful to identify optimal dose and schedule to move forward in early stages of clinical development, following the concept of the Pharmacological Audit Trail (64).

Dissecting and exploiting the complex molecular and cellular response to HSP90 inhibition

Despite many years of research on HSP90 inhibitors, the detailed responses to HSP90 blockade are still not fully understood at the molecular and cellular levels. As described above, HSP90 chaperone function is regulated by complex interactions with co-chaperone proteins. Co-chaperones can affect the ATPase rate of HSP90 and recruitment of client proteins, exhibit chaperone function on their own, or play a role in client protein localization (65). Levels of expression of co-chaperones may have diverse effects on HSP90 activity and can play a role in cellular response and/or resistance to HSP90 inhibition, and the consequences are likely different for different client proteins (66–68). HSP90 chaperone function is also affected by posttranslational modification, including phosphorylation and acetylation; understanding these modifications could provide a mechanism to enhance activity or reveal mechanisms underlying resistance

(69). In addition, the fate of client proteins upon HSP90 inhibition is influenced by the U3 ubiquitin ligases CHIP and CUL5. Therefore, targeting co-chaperones, or other HSP90 downstream interactors involved in regulation of HSP90 chaperone complex, is a logical strategy that may be therapeutically beneficial, especially when combined with HSP90 inhibitors. However, the molecular complications of targeting co-chaperones has been highlighted recently for the kinase-selective co-chaperone cdc37 (70), and there are likely to be significant challenges with the demanding druggability of the protein–protein interactions involved.

Use of combinatorial strategies

Recent molecular insights into the function of HSP90 and its inhibitors, detailed above, provide compelling evidence that the best way to exploit HSP90 as a therapeutic target will be in combination with other anticancer agents. In this regard, HSP90 inhibitors have been found to potentiate the activity of various chemotherapeutic agents, radiotherapy, and other molecularly targeted agents in a variety of preclinical cancer models. Although the clinical efficacy of such combinations is still under investigation, HSP90 inhibitors as single agents exert predominantly cytostatic effects in most preclinical models, and combination with other, more molecularly targeted, agents may be required to enhance tumor-selective killing *in vivo* (13). Modulating a driver oncoprotein with a one-two punch, using a combination of drugs that directly inhibit its function (e.g., kinase activity) together with overall protein level, could be particularly damaging for the cancer cell. This concept has been highlighted in a recent report showing that ATP-competitive kinase inhibitors not only suppress enzymatic activity but also block access of kinase clients to the HSP90-cdc37 chaperone machinery, resulting in client degradation (71). This finding provides new mechanistic insight into the action of protein kinase inhibitors, while also raising the exciting possibility that simultaneous treatment with both a kinase inhibitor and an HSP90 inhibitor may not only enhance the suppression of kinase activity (26) but also potentiate the depletion of driver oncoproteins. An important question to address is whether combination of an HSP90 inhibitor with relevant molecularly targeted agents could either prevent the emergence of, or overcome, treatment resistance to the latter. This idea has recently received important credence in preclinical settings of estrogen receptor–dependent breast cancer, mutant BRAF melanoma, MET-driven renal and gastric cancer, and NSCLC (72–76). Although combinatorial approaches are attractive, clinical failures of some combinations in recent years highlight the need to proceed only based on the soundest biologic rationale coupled with robust Pharmacological Audit Trail biomarker studies and an awareness of potential for increased side effects (77, 78).

Conclusions and Future Perspectives

Since the first HSP90 inhibitor entered clinical studies in the 1990s, there have been several distinct agents evaluated. Although none yet have received FDA approval, several have shown promising pharmacologic and clinical activities. The development of the first-generation geldanamycins was hampered by formulation issues and significant toxicities, at least some of which are likely to be off-target effects. To a large extent, the most serious limitations have been overcome by second-generation synthetic inhibitors that are now in the clinic and tolerability is acceptable. Our understanding of the molecular mode of drug action has also improved considerably. Clinical studies have shown the most promising results in malignancies that are most strongly addicted to particular HSP90 clients with especially high dependency on the chaperone, such as $HER2^{+}$ breast cancer and EML4-ALK–positive NSCLC, with new clinical data also adding EGFR exome 20 insertion mutants in NSCLC to the list. There may be new opportunities in BRAF-mutant melanoma and hematologic cancers—including multiple myeloma owing to deregulated proteostasis and leukemias driven by HSP90 clients (e.g., BCR-ABL in chronic myeloid leukemia). Here, we have highlighted a number of approaches to better realize the full potential of HSP90 inhibitors. Using HSP90 inhibitors up front in combination with molecularly targeted agents is a particularly attractive strategy, as it can be rapidly implemented in the clinic and has the exciting potential to overcome the major clinical problem that we face today: namely cancer evolution and drug resistance.

Disclosure of Potential Conflicts of Interest

R. Ferraldeschi is Director Translational Research at Astex Pharmaceuticals. P. Workman reports receiving commercial research grant support from Vernalis; has ownership interest (including patents) in Chroma Therapeutics; is a consultant/advisory board member for Novartis and Nuevolution; and has provided expert testimony for ICR. No potential conflicts of interest were disclosed by the other authors.

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