Review

HSP90 as a platform for the assembly of more effective cancer chemotherapy

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A B S T R A C T

Since initial discovery of the first HSP90 inhibitor over a decade and a half ago, tremendous progress has been made in developing potent and selective compounds with which to target this chaperone in the treatment of cancers. These compounds have been invaluable in dissecting how HSP90 supports the dramatic alterations in cellular physiology that constitute the malignant phenotype and give rise to the clinical manifestations of diverse cancers. Unfortunately, single agent activity for HSP90 inhibitors has been disappointingly modest against recurrent, refractory cancers in most of the clinical trials that have been reported to date. This problem could be due to pharmacological limitations of the first-generation inhibitors that have been most extensively studied. But we suggest it may well be intrinsic to the target itself. This review will focus on how the utilization of HSP90 by cancer cells might be targeted to enhance the activity of other anticancer drugs while at the same time limiting the ability of advanced cancers to adapt and evolve drug resistance; the net result being more durable disease control. A better understanding of these fundamental issues will surely make the ongoing clinical development of HSP90 inhibitors as anticancer drugs less empiric, more efficient and hopefully more successful. This article is part of a Special Issue entitled: Heat Shock Protein 90 (HSP90).

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“It is not enough to know that changes in DNA can in some unknown way cause a change in phenotype; we need to know at least in outline how phenotypes respond to particular changes in DNA. It is this third pillar, an understanding of the organism’s response to genetic change, that is our subject here and the resolution to Darwin’s dilemma.”

M.W. Kirschner and J.C. Gerhart

The Plausibility of Life, 2005

1. Introduction

Over the last three decades, great progress has been made in understanding the molecular mechanisms that drive carcinogenesis. In a wide range of different cancers, hundreds of specific genes and proteins have been implicated and the biochemical functions of many of these defined. Unfortunately, potent and selective agents designed to target specific oncoproteins such as HER2 in breast cancer [1], the fusion kinase BCR–ABL in chronic myeloid leukemia [2] and the EGFR in non-small cell lung cancer [3,4] are proving less efficacious than had been hoped due to the intrinsic complexity of molecular oncogenesis and the frequent emergence of resistance, especially in advanced disease. Much of the same can be said of older conventional chemotherapeutics that target DNA metabolism and cellular replication through a variety of distinct mechanisms. Although drug efflux pumps can play a role in acquired resistance to these agents, specific target-related mechanisms are often the primary culprit.

Within the array of drugs and macromolecules now advancing through the process of clinical development, compounds that target the functions of Heat Shock Protein 90 (HSP90) are unique. As a multifunctional molecular chaperone, HSP90 regulates the post-translational stability and function of a distinct but diverse set of “client” proteins known to be critically involved in oncogenesis. Much of the current enthusiasm driving the discovery and development of HSP90 inhibitors has been generated by their potential to accomplish what many molecularly targeted anticancer therapies do not: the simultaneous disruption of multiple signaling pathways critical to tumor cell growth and survival. Such a combinatorial attack on the oncogenic clients of HSP90 has been proposed to represent a “rational approach” to addressing the heterogeneity and complexity of the numerous genetic defects characteristic of most clinical cancers [5].

Unfortunately, single agent activity for HSP90 inhibitors has been disappointingly modest against recurrent, refractory cancers in the Phase I and Phase II clinical trials that have been reported to date. This problem could be due to pharmacological limitations of the
first-generation inhibitors that have been most extensively studied. But we suggest it may well be intrinsic to the target itself. In this review, we will examine the concept that the best way to exploit HSP90 as a therapeutic target will be in combination with other anticancer agents. Analogous to the cellular role HSP90 plays as a platform for the assembly of multi-protein chaperone complexes, we suggest HSP90 inhibition can serve as a platform for the assembly of specific multi-drug chemotherapeutic regimens that will more effectively control disparate cancers (Fig. 1). This approach leverages what is known about the adaptive roles that HSP90 plays in rendering cells and organisms more robust to lethal challenges. In cancers, such challenges can arise as a result of the harsh tumor microenvironment, therapeutic interventions and alterations to normal physiology driven by malignant transformation. In buffering the phenotypic expressions of genetic variation and sculpting the architecture of entire signaling networks, HSP90 plays an essential role in enabling tumor progression without being an oncogene in the classical sense of driving the process itself. Perhaps not terribly surprising then, HSP90 inhibitors as single agents have exerted predominantly cytostatic effects in most tumor models and clinical trials to date.

To provide a somewhat different perspective from the other reviews in this special issue, we will focus on how the utilization of HSP90 by cancer cells might be targeted to enhance the activity of other anticancer drugs while at the same time limiting the ability of advanced cancers to adapt and evolve drug resistance; the net result being more durable disease control. Rather than provide definitive answers, our goal is to stimulate further discussion and investigation by translational scientists. Addressing these fundamental issues will surely make the ongoing clinical development of Hsp90 inhibitors as anticancer drugs less empiric, more efficient and hopefully more successful.

2. Basic insights

2.1. Enhancing the activity of drugs with disparate anticancer mechanisms

Decades of careful investigation encompassing many different fields have provided a wealth of biochemical and structural knowledge about HSP90 and its interaction with its client proteins [6]. Nevertheless, our understanding of HSP90 function and how it is altered in various cancers remains far from complete. In particular the consequences of inhibiting HSP90 function for cellular processes other than mitogenic signaling, such as protein homeostasis, energy metabolism, chromatin re-modeling and DNA repair are just beginning to get much deserved consideration [5]. Drug-induced changes in these aspects of physiology have also been largely ignored in the clinical development of HSP90 inhibitors which has largely been driven by a “super kinase inhibitor” orientation in guiding the selection of specific disease histologies for inclusion in Phase II studies [7]. This strategy has led to some notable successes in patients with tumors driven by activating mutations (e.g. EML4–ALK in non-small cell lung cancer) and gene amplification (e.g. HER2 in breast cancer). A broader view, however, is required in planning how best to combine HSP90 inhibitors with other chemotherapeutics. In this regard, HSP90 inhibitors serve as an excellent paradigm for thinking more generally about therapeutic interventions as “perturbagens” that modulate not just their immediate target, but rather the function of entire cellular systems to generate their desired therapeutic effects [8–10].

2.1.1. Conventional chemotherapeutics

In cell culture models, combining HSP90 inhibitors with cytotoxic agents has the broad ability to increase their anticancer activity by a host of molecular mechanisms that are still being elucidated. In the case of alkylating agents and antimetabolites, sensitization appears to involve HSP90 inhibitor-mediated depletion of CHK1 and WEE1 to abrogate S and G2/M cell cycle checkpoint controls in a p53-independent manner [11]. Disruption of these checkpoints is also thought to underlie sensitization to topoisomerase I inhibitors such as irinotecan [12]. Similarly, HSP90 inhibitors have been shown to act as radio-sensitizers in pre-clinical models, again independent of p53 and probably related to impairment of cell cycle checkpoints and DNA repair mechanisms [13]. Instead of checkpoint disruption, sensitization to etoposide and other topoisomerase II poisons is reported to occur through release of a repressive interaction between HSP90 and the topoisomerase leading to an increase in active enzyme and the ability of etoposide to induce more DNA damage [14]. Interestingly, cisplatin, which adds DNA, may also attack reactive cysteines in the C-terminus of HSP90 and impair its chaperone function [15]. When used in combination with classical N-terminal-binding inhibitors, this could result in more profound inhibition of the chaperone’s functions. An added advantage could be that cisplatin exposure also blocks the compensatory up-regulation of heat shock protein expression induced by classical HSP90 inhibitors [16,17]. The mechanism remains undefined, but inhibiting this cytoprotective response has been proposed as a strategy to increase the anticancer activity of HSP90 inhibitors [18]. Whether it would also increase their systemic toxicity is not clear.

In the case of microtubule poisons, mitotic catastrophe is exacerbated by HSP90 inhibition in cell lines with defects in the function of BRCA1 [19] or RB [20]. Interference with the role of HSP90 in centrosome organization presumably underlies this effect [21,22]. In an interesting twist, the microtubule poison docetaxel has been reported to impair HSP90 chaperone function by causing its dissociation from tubulin, thereby stimulating proteasome-mediated degradation of the chaperone itself. The extent to which this novel activity contributes to the established anticancer activity of docetaxel in patients is unknown [23]. It is intriguing, however, that the clinical activity of classical cytotoxic agents such as cisplatin and docetaxel may well be mediated or at least enhanced to some degree by their effects on HSP90 function.

Overlaid on mechanisms specific to different chemotherapeutic classes, HSP90 inhibitors are well recognized to disrupt the function of AKT and other key survival signaling molecules [16,24–27]. This activity can lower the set-point for induction of programmed cell death by both conventional and targeted agents. It may also directly counteract the anti-apoptotic effects of commonly occurring oncogenic lesions such as loss of PTEN function that contribute to drug resistance [28,29]. Indeed, a recent pharmacokinetic–pharmacodynamic study of the HSP90 inhibitor PF4942847 found that of all HSP90 clients examined, inhibition of AKT was the biomarker most predictive of anti-tumor activity in a human breast cancer xenograft model [30].

2.1.2. Kinase inhibitors

Many potent drugs designed to selectively inhibit specific oncogenic kinases are now in clinical development for the treatment of a variety of cancers. Some are proving highly active, inducing impressive responses in cancers with the relevant underlying molecular pathology. Some have even become the standard of care in certain malignancies such as the BCR–ABL inhibitor imatinib in chronic myeloid leukemia (CML) [31]. Unfortunately, all are proving less efficacious than had been hoped. In advanced disease, responses are often profound, but temporary [32]. The mechanisms that underlie both initial sensitivity and acquired resistance to kinase inhibitors vary with the specifics of tumor type and drug, but at least two general principles are emerging. First, in genetically unstable tumor cell populations, drug pressure can efficiently select for mutations in the targeted kinase that diminish drug binding but preserve oncogenic activity. Second, activation of by-pass signaling pathways can allow cancer cells to rapidly become less dependent on the drug target for growth and survival, so-called oncogenic switching [33]. To address the first problem, next generation kinase inhibitors have been synthesized based on structural insights provided by isolation of drug-resistant mutants.
The ATPase cycle of HSP90 can also be inhibited by acetylation of lysine residues within the protein. Histone deacetylase 6 (HDAC6) co-purifies with Hsp90 and HDAC6 knockdown promotes the depletion of several known Hsp90 clients [40]. Thus, it appears that HDAC inhibitors such as vorinostat (suberoylanilide hydroxamic acid) which are currently undergoing clinical evaluation based on their ability to alter chromatin structure and gene expression may also exert anticancer activity in part through HSP90 inhibition [41, 42]. Preclinical data demonstrate that combined exposure to HDAC inhibitor and a classical N-terminal HSP90 inhibitor results in more profound compromise of HSP90 chaperone activity and greater anticancer activity [43].

Beyond its stabilization of specific oncogenic clients, Hsp90 plays a role in maintaining protein homeostasis in the cell by cooperating with the ubiquitin–proteasome system to degrade a wider range of misfolded proteins [44]. When combined with an inhibitor of the proteasome, Hsp90 inhibitors can overload the protein degradation machinery and drive induction of apoptosis in susceptible cell types. Presumably due to enormous flux through the secretory pathway of immunoglobulin-secreting myeloma cells, this tumor type is very sensitive to dual inhibition of HSP90 and the proteasome [45]. The role of HSP90 in chaperoning components of the phosphoinositol 3-kinase (PI-3K)–mTOR pathway such as AKT has prompted studies combining Hsp90 inhibitors with rapamycin and other compounds targeting this pathway to increase their anticancer activity [46–48].

2.2. Limiting the emergence of target-related resistance

2.2.1. Resistance as an evolutionary process

Efforts to improve the efficacy of current chemotherapeutic regimens are plagued by the inescapable genetic heterogeneity and evolvability of human cancers [49, 50]. It is crucial to bear in mind that cancers, while monoclonal in origin, are characterized by high rates of mutation and frequent aneuploidy [51]. They are not static multigenic diseases with a limited, definable set of targets. Instead, clinical cancers are heterogeneous populations, among which arise cells that are ever better adapted to evading host defense mechanisms, to populating new environments, and to resisting therapeutic attacks. The total number of genomic alterations per typical carcinoma cell is estimated to be roughly 10,000 even though only 5–10 specific genetic alterations are sufficient for malignancy [52, 53]. As cancers evolve along multiple pathways, driven by intense selective pressures in the host, they exploit a cache of pre-existing and newly acquired genetic variation to do so. Viewed in this light, cancer evolution within an individual host is driven by the very same pressures of natural selection that drive the macroevolution of organisms and populations in nature.
How does HSP90 interface with this evolutionary perspective on tumor progression and what might be the consequences of inhibiting it on the emergence of drug resistance? As a direct consequence of its protein chaperoning activity, HSP90 permits polymorphic variants of critical signaling molecules and transcription factors to retain uniform “wild type” biochemical activity. This “buffering” at the protein level by HSP90 funnels complex developmental processes into discrete, well-defined outcomes despite underlying genotypic variation, and it appears to be essential for the robust expression of uniform phenotypes under basal conditions [54,55]. Under stressful conditions, however, some unstable client proteins of HSP90 are likely to become even more unstable. This problem creates an increased demand for HSP90 to facilitate the refolding of its usual client proteins as well as new, mutant or stress-denatured clients. The accumulated genetic variation in certain individuals can thereby exceed the buffering capacity of HSP90 and produce diverse, but genotype-specific phenotypes [56]. Effects on the manifestation of underlying genetic variation have been demonstrated for HSP90 inhibition in a variety of metazoan organisms ranging from fruit flies [57] to plants [58] and zebrafish [59]. Revealing previously hidden genetic variation makes it available at the phenotypic level for natural selection to enhance the survival of distinct genotypes within a population [60]. Recently additional mechanisms have been described by which HSP90 and other chaperones can impact phenotypic diversity. These include effects on the heritability of epigenetic traits [55], the tolerance of protein structures to mutation [61] and even the activity of regulatory genetic elements [62].

Viewed from an evolutionary perspective, a tumor can be viewed as a large, genetically and epigenetically heterogeneous population of cells [63]. We propose that HSP90 acts as a biochemical buffer at the protein level for this extensive heterogeneity to maintain cell viability and limit phenotypic variation, a process known in developmental biology as canalization. During the natural history of cancer progression, however, canalization of the malignant phenotype could break down when HSP90 capacity is exceeded as a result of normal aging, an increasing load of mutant and/or misfolded oncoproteins, or the hostile tumor microenvironment—or more likely, all these factors in concert. Epigenetic instability and phenotypic diversity within the tumor cell population would increase and accelerate the emergence of invasive, metastatic and drug-resistant biology [64,65]. Such an evolutionary view of tumor progression fits well with the clinical behavior of many malignancies and suggests that durable control of clinical cancers is likely to be achieved only by limiting their ability to adapt and evolve [49]. How HSP90 inhibitors might impact tumor evolution is not known. As would be expected, clinical trials of all the investigational HSP90 inhibitors in development have been restricted to patients with advanced, refractory malignancies.

Most cancers progress from benign, relatively well-differentiated tumors to increasingly invasive and metastatic cancers characterized by profound genomic instability and the accumulation of numerous genetic alterations [66]. The role of HSP90 might shift as cells move through the initiation phase to the progression phases of tumorigenesis. If so, inhibiting HSP90 could have profoundly different effects on early stage lesions versus advanced cancers. It could be important to assess the status of the HSP90 reservoir in a particular tumor prior to initiating therapy with an inhibitor. The level of HSP90 protein in non-small-cell lung cancers has recently been shown to

### Table 1

HSP90 inhibitor clinical trials.

<table>
<thead>
<tr>
<th>Class</th>
<th>Compound</th>
<th>Route</th>
<th>Manufacturer</th>
<th>Phase</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geldanamycin derivative</td>
<td>Tanesipimycin (17-AAG)</td>
<td>IV</td>
<td>BMS</td>
<td>II/III</td>
<td>No longer being developed.</td>
</tr>
<tr>
<td></td>
<td>Alvespimycin (17-OMAG)</td>
<td>IV</td>
<td>Kosan/BMS</td>
<td>I</td>
<td>Multiple phase I studies completed, including in combination with trastuzumab.</td>
</tr>
<tr>
<td></td>
<td>Retaspimycin (IPI-504)</td>
<td>IV</td>
<td>Infinity</td>
<td>II/III</td>
<td>Multiple phase I/I trials completed in castrate resistant prostate cancer, breast cancer, NSCLC. Phase III trial in GIST terminated for toxicity. Randomized phase II of docetaxel +/−IPI-504 in NSCLC ongoing (NCT01362400).</td>
</tr>
<tr>
<td>Resorcinol scaffold</td>
<td>ABI-010 (albinomannano-particle 17-AAG)</td>
<td>IV</td>
<td>Abraxis</td>
<td>I</td>
<td>No active trials listed in <a href="http://www.clinicaltrials.gov">www.clinicaltrials.gov</a></td>
</tr>
<tr>
<td></td>
<td>IPI-493 (17-AG)</td>
<td>PO</td>
<td>Infinity</td>
<td>I</td>
<td>Two phase I studies completed. Development halted as drug exposure was inferior to retaspimycin</td>
</tr>
<tr>
<td></td>
<td>Ganetespib (STA-9090)</td>
<td>IV</td>
<td>Synta</td>
<td>I</td>
<td>Multiple ongoing phase II trials in castrate resistant prostate cancer (NCT01270880), breast cancer (NCT01273896), hematologic malignancies (NCT0084872), esophagagogastic cancer (NCT0167114), pancreatic cancer (NCT01227018), melanoma (NCT01200238), SCLC (NCT01173523), NSCLC (NCT01312225), GIST (NCT01305919). Ongoing randomized Phase II/III of docetaxel +/−IPI-504 in NSCLC ongoing (NCT01348126).</td>
</tr>
<tr>
<td>Other small molecule</td>
<td>SNX-5422</td>
<td>PO</td>
<td>Pfizer</td>
<td>I</td>
<td>Several ongoing phase I in solid tumors (NCT00878423, NCT01245218, NCT0146102), Ongoing randomized phase II +/−imatinib in GIST (NCT01294202).</td>
</tr>
<tr>
<td></td>
<td>AT13387</td>
<td>IV</td>
<td>Pfizer</td>
<td>I</td>
<td>Development halted due to excessive ocular toxicity.a</td>
</tr>
<tr>
<td></td>
<td>BIIB021*</td>
<td>PO</td>
<td>Biogen Idec</td>
<td>I</td>
<td>Multiple phase I trials in solid tumors and CLL completed. Phase I combination studies in breast cancer with trastuzumab, exermestane no longer recruiting. Phase I no longer recruiting. No other trials listed in clinicaltrials.gov.</td>
</tr>
<tr>
<td></td>
<td>BIIB028</td>
<td>KW</td>
<td>BIogen Idec</td>
<td>I</td>
<td>Phase I in multiple myeloma, CLL NHL completed. Phase I/II trial in combination with bortezomib for myeloma ongoing (NCT01063907).</td>
</tr>
<tr>
<td></td>
<td>2478</td>
<td>IV</td>
<td>Kyowa Hakko</td>
<td>I/II</td>
<td>Phase I terminated. No active trials listed in clinicaltrials.gov.</td>
</tr>
<tr>
<td></td>
<td>XLR88</td>
<td>PO</td>
<td>Exelixis</td>
<td>I</td>
<td>Phase I studies ongoing in advanced solid tumors (NCT01064809; NCT00879905)</td>
</tr>
<tr>
<td></td>
<td>NVP-HSP90</td>
<td>PO</td>
<td>Novartis</td>
<td>I</td>
<td>Phase I no longer recruiting. No other trials listed in clinicaltrials.gov.</td>
</tr>
<tr>
<td></td>
<td>MPM-3100</td>
<td>PO</td>
<td>Myriad</td>
<td>I</td>
<td>Single agent, solid tumors (NCT01288403)</td>
</tr>
<tr>
<td></td>
<td>DS-2248</td>
<td>PO</td>
<td>Daichi Sankyo</td>
<td>I</td>
<td>Single agent, advanced solid tumors/lymphoma (NCT01168752).</td>
</tr>
<tr>
<td></td>
<td>Debio 932</td>
<td>PO</td>
<td>Debiopharm</td>
<td>I</td>
<td>Single agent, advanced solid tumors/lymphoma (NCT01393509).</td>
</tr>
<tr>
<td></td>
<td>PU-H71</td>
<td>IV</td>
<td>Memorial</td>
<td>I</td>
<td>Sloan-Kettering</td>
</tr>
</tbody>
</table>

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AHA1 and CDC37 has been shown to affect the sensitivity of cancer caspofungin in resistant clinical isolates of P. fumigatus more or less likely to derive clinical benefit whether patients with evidence of activation in their tumors are clinically significant. HSF1 status will be monitored in a soon-to-open clinical trial involving patients with metastatic breast cancer to determine the role(s) of HSP90 in cancer evolution as well as the development of drug resistance.

2.2.2. Lessons from fungi

Direct evidence supporting a critical role for HSP90 in buffering genetic variation and enabling the evolution of drug resistance is emerging from experimental models involving fungi. Many fundamental biological processes and molecular pathways are conserved between human cancer cells and these rapidly proliferating eukaryotic organisms including an absolute requirement for HSP90 function to accommodate the load of a paracrine chaperone machinery [70,71]. HSF1 status will be monitored in a soon-to-open clinical trial involving patients with metastatic breast cancer to determine whether patients with evidence of activation in their tumors are more or less likely to derive clinical benefit from HSP90 inhibition.

By acutely re-drawing the genotype–phenotype map and limiting the ability to adapt and respond, HSP90 inhibition could prevent the outgrowth of resistant clones. On a cautionary note, however, compromising HSP90 function alone in advanced malignancies, especially a sub-lethal extent could work to reveal their underlying genetic diversity and increase epigenetic instability, thereby accelerating the process of cancer initiation and malignant progression in highly unpredictable ways. The potential of kinase inhibitors to drive the development of secondary skin tumors has been demonstrated in recent clinical trials of vemurafenib and sorafenib [72,73]. No such events have been reported so far in the development of HSP90 inhibitors, but work is underway by us and others to better define the role(s) of HSP90 in cancer evolution as well as the development of drug resistance.

2.2.3. Acquired resistance to HSP90 inhibitors

While the frequent emergence of target-related resistance has been seen during the clinical development of many kinase inhibitors, no drug-resistant HSP90 mutations have been reported in patients with advanced cancers, even after prolonged exposure. Pre-clinical studies have shown that reduced expression of the oxidoreductase NQO1 can confer resistance to quinone-containing ansamycin HSP90 inhibitors such as 17-AAG, but no cross-resistance to other classes of inhibitor has been seen [79]. Ansamycin-based inhibitors are also substrates for P-glycoprotein-mediated drug efflux which is a concern in cancer cells of multi-drug resistance (MDR) phenotype [80]. A naturally occurring single amino acid change in the ATP-binding pocket of HSP90 from the fungus Humicola fuscoatra has been reported to confer resistance to radicicol and other resorcinol-based synthetic HSP90 inhibitors [81]. Such a mutation has not been reported in any pre-clinical or clinical studies to date. Compensatory responses and epistatic alterations in other components of the HSP90-based chaperone machinery could lead to diminished drug sensitivity, but this mode of functional resistance may be quite dynamic and will be difficult to document in the clinical setting. For now at least, the acquisition of high level, directly target-related resistance to HSP90 inhibitors does not seem to be a major contributor to the problem of their limited activity against recurrent, refractory cancers in most clinical trials to date.

2.2.4. Optimizing therapeutic efficacy

Therapeutic efficacy as reflected in clinical benefit is determined not just by direct anticancer activity but also by the speed and frequency with which resistance occurs. Activity and resistance are not independent variables (Fig. 1). The greater the log cell kill induced by a drug, the smaller the residual tumor burden in which resistance can develop. Likewise, even if a compound possesses relatively weak anticancer activity, if resistance does not occur, it can still be an effective agent. Historically, these considerations have been the basis for the design and use of combination therapies for the treatment of diseases ranging from microbial infection to cancer [82]. Ideally, by combining appropriate drugs, net activity can be increased and non-cross reactive mechanisms of action will make the development of resistance less likely. Counter intuitively, however, theoretical models and new experiments now suggest that antagonistic interactions between antibiotics, even at the expense of reducing net activity can actually counteract the evolution of drug resistance in bacteria [83,84]. Whether similar effects would be operative in cancers is unknown, but it does highlight the difference between optimizing activity and actually improving efficacy in the design of drug combinations. Driving a greater reduction in tumor volume by combining HSP90 inhibition with other agents may be less relevant to clinical efficacy than extending the duration of disease control that can be achieved.

3. Clinical experience

Over a dozen HSP90 inhibitors are undergoing clinical testing, either as single agents or in combination with cytotoxic or molecularly targeted drugs (Table 1). Pivotal trials to support approval of an FDA-licensed indication for tanespimycin, the most advanced agent, however, have recently been suspended by the sponsor for non-clinical reasons [85]. Although much progress is being made, no HSP90 inhibitor has yet achieved an approved indication in the treatment of cancer.
3.1. Benzoquinone ansamycins

Geldanamycin, a benzoquinone ansamycin, is the prototypical HSP90 ATPase inhibitor. Despite dramatic effects in cell culture, its activity in preclinical models was limited due to metabolic instability and dose-limiting hepatotoxicity. In an effort to improve tolerability and to address formulation issues, a number of semi-synthetic geldanamycin derivatives have been developed, including 17-AAG, 17-DMAG, IPI-504, and ABI-010.

3.1.1. 17-AAG (Tanespimycin)

17-AAG was the first HSP90 inhibitor to enter the clinic. In the initial phase I trials exploring weekly schedules, toxicities included fatigue, nausea, vomiting, diarrhea, and transaminase elevations [86–88]. The half-life was 4.1 h for 17-AAG and was 7.6 h for 17-AG, a major active metabolite. Consistent with its mechanism of action, induction of HSP72 in peripheral blood lymphocytes (PBL) occurred within 6 h of 17-AAG administration. At 24 h, concomitant depletion of client proteins was found in most tumor biopsy samples. However, at 96 h, the expected effects of 17-AAG were not reproducibly demonstrated, leading to the conclusion that at the studied dose and schedule, HSP90 inhibition persists between 24 and 96 h. Indeed, in a subsequent phase II trial in patients with metastatic melanoma, sustained inhibition of the MAPK pathway was not observed in post-treatment biopsies, and no patient achieved an objective response [89].

These and other data prompted exploration of a twice weekly schedule (given two weeks out of three), which was associated with consistent HSP72 induction in PBLs during the treatment weeks [90]. However, grade 3/4 reversible transaminase elevations were reported in nearly half of patients. Continuous twice-daily dosing was not feasible due to delayed hepatotoxicity [91]. Daily schedules (5 days every 3 weeks) have also been explored [91,92]; however, continuous IV daily dosing is not practical in the clinic, and breaks between cycles limit the goal of more sustained HSP90 inhibition.

An important limitation has 17-AAG has been formulation. 17-AAG is not water-soluble and requires a diluent including egg phospholipid and 4% DMSO. Hypersensitivity reactions were observed in the phase I trial. Furthermore, the amount of DMSO administered in a single infusion was as high as 40 mL, which may have contributed to the toxicity profile and led to a persistent odor that may have had a negative effect on quality of life. Despite improvements in formulation and promising evidence of activity for combination regimens incorporating the agent in breast cancer and multiple myeloma, the clinical development of 17-AAG has recently been discontinued by its sponsor, Bristol-Myers Squibb [93].

3.1.2. Water-soluble ansamycins: 17-DMAG and IPI-504

In attempts to overcome formulation problems, 17-DMAG and IPI-504 have been developed as water-soluble analogs of 17-AAG. In addition to its solubility, 17-DMAG is more active than 17-AAG in preclinical models and has the potential for oral bioavailability. 17-DMAG has been explored at a variety of doses, schedules, and methods of administration (i.e. oral or I.V.). On two daily intravenous schedules (3 consecutive or 5 consecutive days on a 21-day cycle), 9% of patients experienced unexpected grade 3/4 pneumonitis [94]. Furthermore, reliable depletion of client proteins in 24-hour biopsies was not observed. When given on a weekly schedule, DLT occurred in two patients treated at the 106 mg/m² dose, including one treatment-related death characterized by rapid (within 24 h) onset of grade 4 transaminitis and eventual hypotension, acidosis, and renal failure [95]. While better tolerated at the 80 mg/m² dose, grade 1/2 ocular toxicities were observed in four patients, including blurred vision, keratitis, and ocular surface disease. In contrast to the daily schedule, sustained induction of HSP72 was observed in PMBCs, and client protein depletion was observed in the tumor tissues. Two patients achieved an objective response (1 prostate, 1 melanoma) and 3 patients experienced prolonged stable disease (chondrosarcoma, renal cell cancer and prostate cancer). Ultimately, 17-DMAG development has been limited by uncertainty in the optimal trade-off between dose (higher doses seem, based on limited tissue samples, to be associated with more reliable target inhibition and expected downstream effects), frequency of administration, and toxicity [96].

IPI-504 (retasipimycin) is a water-soluble form of 17-AAG. In vivo, IPI-504 exists in equilibrium with 17-AAG. IPI-504 has reached phase II and III clinical trials. However, similar to 17-AAG and 17-DMAG, hepatotoxicity has been observed. For example, in a randomized, phase III trial of IPI-504 conducted in patients with gastrointestinal stromal tumors (GIST), four on-treatment deaths were observed, leading to early closure of the study after 47 of 195 planned patients were enrolled [97]. Three of the four patients had grade 3 or 4 transaminase elevations. Notably, in this study, about 20% of patients had had prior hepatic resections, which may have contributed to the excess toxicity observed. In contrast, in a phase II study of 76 heavily pre-treated patients with NSCLC at the same dose and schedule, IPI-504 had an acceptable safety profile, with infrequent (5–9%) grade 3 transaminase elevations [38]. In a phase II study among 26 patients with HER2-positive breast cancer treated at 300 mg/m² once weekly, grade 3 transaminase elevation was observed in only 1 patient, other grade 3 toxicities were rare, and no grade 4 toxicities were observed [98]. Thus, it appears that the toxicity profile of IPI-504 is dose and schedule dependent, and further dose escalations to improve the depth or duration of HSP90 inhibition may not be possible.

3.2. Second- and third-generation HSP90 inhibitors

Extensive efforts from both academic and industrial groups have resulted in the discovery and pre-clinical testing of an array of new synthetic inhibitor chemotypes [99]. Several of these new classes of compounds are being developed in an attempt to reduce the most prominent liabilities associated with first generation HSP90 inhibitors. These compounds share the ability to bind the N-terminal ATPase site of HSP90 with higher affinity than the natural nucleotides and prevent the chaperone from cycling between its ADP- and ATP-bound conformations. Of these, AUY922 (Novartis) and STA-9090 (Synta) are furthest in development.
3.2.1. AUY922

Common adverse effects of AUY922 have included diarrhea, nausea, fatigue, and vomiting [100]. Ocular toxicities, including blurred vision, darkening of vision, and delayed dark/light accommodation have also been observed, though they have usually been relatively mild and reversible. Although the mechanism remains unclear, such toxicity has been a recurrent issue for HSP90 inhibitors and has led to discontinuation of development for the Pfizer compound SNX-5422 [101]. In a Phase I trial of AUY922, stabilization of disease was observed, as were metabolic (FDG–PET) responses. Preliminary evidence of monotherapy activity has also been observed in a phase 2 trial of ER + or HER2 + metastatic breast cancer patients [102]. Numerous phase 2 monotherapy and combination trials are under way across a variety of malignancies.

3.2.2. STA-9090

STA-9090 (ganetespib) has reached phase III clinical trials. In phase I testing, the toxicity profile has been similar to AUY922, though ocular toxicity appears to be less frequently reported. Single agent clinical activity was observed in heavily pretreated NSCLC, breast cancer (both HER2-positive and triple-negative), gastric cancer, melanoma, and colon cancer. The most common adverse event has been transient diarrhea, which has been manageable with standard care. A Phase Ib/III trial in NSCLC has been recently initiated; Phase II trials in breast cancer, colon cancer, gastric cancer, melanoma and others are underway (see Table 1 and [37]).

3.2.3. Others

A range of additional chemotypes are in clinical development. Some such as MPC3100 (Myriad Pharmaceuticals) are orally bio-available, which could provide a real practical advantage given the issues of dose and schedule that have complicated the development of compounds requiring parenteral administration. Another advantage may be the potential for more continuous, sustained HSP90 inhibition, but this remains to be demonstrated. Several new agents show good central nervous system (CNS) penetration which could expand their range of potential indications to include primary brain tumors and CNS metastases.

In addition to small molecules that bind the N-terminal ATPase site of HSP90, several alternative approaches to HSP90 inhibition have been reported, but none of these are appropriate yet for clinical development. For example, the peptidomimetic compound shep-herdin was designed to disrupt the interaction of the anti-apoptotic protein survivin with Hsp90. Shep-herdin makes extensive contacts within the N-terminus of Hsps90 and can destabilize several Hsp90 client proteins as well as survivin. It is cell-permeable and selectively induces apoptosis in tumor cells [103]. An intriguing variation on the classical HSP90 inhibitor 17-AAG is a derivative designed to accumulate in mitochondria and inhibit the pool of Hsp90 found in this compartment only in cancer cells where it plays an essential anti-apoptotic role [104,105]. Novobiocin, a coumarin-based inhibitor of bacterial DNA gyrase binds to Hsp90’s putative C-terminal ATP binding site and alters Hsp90 conformation thereby impairing Hsp90-client interac-tions and possibly dimerization, albeit only at relatively high concentrations [106,107]. Novobiocin derivatives that possess better potency and HSP90 selectivity have been developed [108] and anti-cancer activity in a mouse model of head and neck cancer has been reported [109].

3.3. HSP90 inhibitors as monotherapy in molecularly defined cancer subsets

3.3.1. Non-small cell lung cancer

Non-small cell lung cancer is an attractive target for HSP90 inhibitors. Targeted inhibitors of EGFR and ALK have shown considerable activity in molecularly-defined populations. However, resistance almost invariably develops in the metastatic setting. Escape mechanisms include the T790M mutation in EGFR, but can also be related to cross-talk with other pathways or activation of downstream effectors. Hypothetically, HSP90 inhibitors may overcome these and other resistance mechanisms as discussed above in Section 2.2.

A phase II trial of IPI-504 was conducted in 76 patients with stage IIIB or stage IV NSCLC who had previously progressed on an EGFR tyrosine kinase inhibitor [38]. The study was designed to evaluate the primary endpoint of objective response rate in each of two arms: EGFR mutant and EGFR wild-type. In this heavily pre-treated cohort (median of 4 prior systemic regimens), responses were observed in 10% of EGFR wild-type patients and only 4% of patients with EGFR mutant tumors. In a post-hoc analysis, two of three patients with the EML4–ALK rearrangement achieved a PR and the third patient had prolonged stable disease. Findings in the EML4–ALK population have recently been confirmed in a trial of the fully synthetic HSP90 inhibitor STA-9090, where 4 out of 8 patients with the EML4–ALK rearrangement achieved an objective partial response, supporting a class effect [110]. Notably, preclinical models of acquired crizotinib resistance via amplification or secondary mutations of EML4–ALK still demonstrate sensitivity to 17-AAG [111].

Why might the activity of HSP90 inhibitors as single agents in NSCLC be apparently limited to patients with EML4–ALK rearrangements? The EML4–ALK fusion protein appears to be exquisitely sensitive to HSP90 inhibition and, in the setting of tumor addiction to the fusion protein, activity is seen [112]. Indeed, results of a recently reported phase II trial of the fully synthetic HSP90 inhibitor STA-9090 largely recapitulate the results of the IPI-504 trial. Alternatively, it has been suggested that the EML4–ALK fusion could simply be a favorable prognostic factor associated with longer progression-free survival in NSCLC patients, independent of specific treatment [113]. Another possibility is that mutant EGFR may require longer and more sustained depletion, and the “off weeks” required for IPI-504 and STA-9090 allow for resistance to occur. Finally, these trials tested HSP90 inhibitors in patients with EGFR-mutant tumors resistant to EGFR TKIs, and it is possible that HSP90 inhibitors as monotherapy are simply not sufficient to overcome the multiple resistance pathways that develop. This leads to the question of whether HSP90 inhibitors might more fruitfully be studied in combination with EGFR TKIs, where concurrent administration might delay the emergence of resistance, either by direct inhibition of resistance pathways, or by limiting the ability of tumor cells to adapt and respond as seen in the case of antifungal drug resistance (Fig. 2). In the case of EML4–ALK, (e.g. the subtype sensitive to HSP90 inhibitors), the next logical question is whether combined therapy with crizotinib plus an HSP90 inhibitor provides a better response rate and/or more prolonged disease control compared to either agent alone.

3.3.2. Melanoma and BRAF

Oncogenic BRAF, most commonly the V600E missense mutation, is present in 40–60% of melanomas [114,115] and leads to activation of the MAPK pathway. As RAF (both RAF1 and BRAF) and MAPK, among others, are HSP90 client proteins, it would seem logical that HSP90 inhibitors might have considerable activity against melanoma. Indeed, evidence of prolonged stable disease and a few scattered objective responses have been observed in phase I studies [86,85]. However, in a phase II trial of weekly 17-AAG in patients with metastatic melanoma, no objective responses were observed among 15 patients, including 9 with BRAF mutations. Notably, although post-treatment biopsies did show evidence of HSP70 induction, there was no significant effect of 17-AAG on BRAF kinases or phospho-ERK expression [89]. Based on these data, it is not possible to draw definitive conclusions about the status of HSP90 inhibitors for melanoma, and points to the importance of post-treatment tumor biopsies in understanding the adequacy of target inhibition and its downstream effects. It is still an open question whether more potent inhibitors, either alone, or more likely, given in combination with B-RAF kinase inhibitors might provide more robust evidence of clinical activity.
3.3.3. Renal cell carcinoma, prostate cancer

3.3.3.1. Disappointing single agent results. Renal cell carcinoma (RCC) would seem a logical malignancy in which to evaluate HSP90 inhibitors, given the efficacy of agents targeting VEGF/VEGFR (bevacizumab, sunitinib), mTOR (everolimus, temsirolimus) and multi-targeted agents (sorafenib). One might hypothesize that HSP90 inhibitors would be particularly effective “multi-kinase inhibitors”, given its mechanism of action, and have activity in RCC. However, a phase II trial of 17-AAG was negative, with no objective responses in 20 patients [116]. Whether this was due to insufficient depletion of target proteins or because HSP90 is not a valid target in RCC is difficult to know for certain.

HSP90 inhibitors are also associated with depletion of the androgen receptor and have anti-proliferative activity in mouse xenograft models of prostate cancer. A two-stage, single arm, phase II trial of 17-AAG (300 mg/m2 weekly for 3 of 4 weeks) was initiated in patients with castration-resistant prostate cancer [117]. No PSA responses were observed and the study was therefore terminated after the first stage of enrollment. As in RCC, whether the lack of activity truly reflects lack of validity of HSP90 as a target in prostate cancer, versus liabilities from first generation drug pharmacokinetics/pharmacodynamics is uncertain at this time. In addition, it is possible that combining HSP90 inhibitors with standard agents targeting the androgen receptor or with chemotherapy might provide efficacy not seen with single agent therapy.

3.4. Combination therapy

3.4.1. HER2-positive breast cancer

3.4.1.1. Targeting an oncoprotein and downstream pathways. Given that HER2 is one of the most HSP90-dependent client proteins known, HSP90 inhibitors are being actively studied in this tumor type, both alone and in combination with the HER2-targeted monoclonal antibody trastuzumab. HSP90 inhibitors also lead to depletion of many downstream members of the HER2 signaling pathway. Therefore, it has been hypothesized that HSP90 inhibition could potentially delay or reverse the development of trastuzumab resistance.

Promising activity in a phase I study combining 17-AAG with trastuzumab has led to several phase II studies in this patient population [118]. In a phase II trial of 17-AAG with trastuzumab, the objective response rate was 22% in patients with metastatic, HER2-positive breast cancer who had progressed on prior trastuzumab therapy [119]. Prolonged stable disease and minor responses were observed in a phase II study of IPI-504 with trastuzumab; however, that study was terminated early (after the first stage of accrual) because it did not reach the protocol-specified threshold of efficacy [98]. Notably, patients in this IPI-504 study were heavily pre-treated, with a median of 6 prior lines of chemotherapy for advanced disease. Based on these results, HER2-positive breast cancer remains an area of active clinical trials with the newer HSP90 inhibitors. A key question to be addressed is whether or not continued administration of trastuzumab in combination with HSP90 inhibitor is important for the activity that has been observed. It may be essential to maintain pressure on the signaling axis by continuing trastuzumab even though high level resistance to the antibody has been established. Precedent is provided by a recent study in which combination of the small molecule HER2 kinase inhibitor lapatinib and trastuzumab was superior to lapatinib alone in patients who had previously progressed on trastuzumab [120]. A related, very important question would be whether up-front combination therapy with trastuzumab and HSP90 inhibitor can prevent or delay the emergence of trastuzumab-resistance in naïve HER2-positive patients.

3.4.2. Multiple myeloma: targeting the proteasome

The proteasome has been demonstrated to be a valid and effective target in multiple myeloma. Thus, an attractive combination involves HSP90 inhibitors with the proteasome inhibitor bortezomib. Preclinical data indicate that combined therapy leads to synergistic suppression of the activity of the 20S proteasome and increased proteotoxic stress in tumor cells. Anti-tumor activity was seen in a phase I monotherapy trial of 17-AAG [121]. Notably, in a phase II combination study with bortezomib, conducted in heavily pre-treated refractory or relapsed patients, promising activity was observed [121]. Of relevance to this and other potential combinations, 17-AAG appeared to ameliorate the neuropathy often observed with bortezomib. An ongoing combination trial with AU922 will be of interest to follow as results become available.

3.4.3. Combinations with conventional chemotherapy

Combinations with several classes of cytotoxic agents as described in Section 2.1.1 have now reached clinical trial. Docetaxel is being combined with IPI-504 in a randomized phase II trial in NSCLC, and STA-9090 is currently in phase III testing in the same setting. Other combinations are considerably earlier in development and primarily involve first-generation HSP90 inhibitors. Examples include DNA-damaging agents such as the platinum which pre-clinical mechanistic insights suggest should enhance the activity of HSP90 inhibitors. Unfortunately, increased hematopoietic toxicity was seen for the combination of tanespimycin with cisplatin and no dose could be recommended for phase II testing [122]. A phase I study of the topoisomerase I inhibitor irinotecan in combination with tanespimycin found acceptable toxicity and some tumor shrinkage in patients with refractory solid tumors [12]. Phase I studies of tanespimycin and anti-metabolite nucleoside analogs such as gemcitabine and cytarabine have been reported in both hematopoietic malignancies and solid tumors [122,123]. As trials progress and efficacy data become available, it will be of great interest to determine whether benefits for cytotoxic combinations are seen in specific molecular subgroups as has been the experience with kinase inhibitors and whether molecular classification can be used to help guide patient selection.

4. Concluding thoughts

The concept of combining drugs to increase the likelihood of cure has a long history in the treatment of infectious diseases and cancer. With the exceptions of choriocarcinoma and Burkitt’s lymphoma, treatment with single agents has never been able to produce either significant remissions or to cure patients with disseminated cancer [124]. The principles of combining drugs with non-cross reactive modes of action and non-overlapping toxicities to prevent the emergence of resistance and consequent treatment failure were formalized in the quantitative mathematical modeling developed by Goldie and Coldman in the early 1980s [125]. They have guided the field ever since. In this sense, the idea of combining HSP90 inhibitors with other drugs to assemble more effective chemotherapy regimens is an obvious next step. The novelty of the work discussed in this review resides in the possibility that HSP90 inhibitors provide not only a new mode of anticancer action, but might for the first time provide a way to limit the intrinsic evolvability of cancers. While far from certain, if clinical trials are not designed to detect such an effect, it certainly won’t be found. Likewise, if development of HSP90 inhibitors is abandoned because they don’t shrink advanced cancers when given as single agents, a valuable opportunity to test the hypothesis that genetic heterogeneity and attendant evolvability can be controlled in clinical cancers.

Major limitations of first generation inhibitors have impaired the ability to test combinations due to dosing constraints and prominent off-target effects. Next generation compounds are now making it feasible in concept to critically address many key questions for the first
time. For example, oral dosing may allow for more sustained, low-level HS90 inhibition than IV dosing. Continuous but modest HS90 inhibition may not exhibit dramatic single agent activity, but it could alter the tumor landscape in ways sufficient to enhance the magnitude and duration of response to other agents without increasing systemic toxicity. Viewed in this light, induction of the heat shock response in systemic tissues might represent an indicator of acute toxicity. Rather than a target pharmacodynamic endpoint to confirm adequate drug exposure as employed in most clinical trials to date, the induction of HS72 in peripheral blood lymphocytes might actually be something to be avoided, at least in the development of continuous dosing regimens for oral HS90 inhibitors in combination with other drugs.

Many new and exciting concepts are ready to test, but practical issues will arise, especially in performing trials that involve combining agents that are proprietary to highly competitive pharmaceutical companies [126,127]. It is going to require a willingness on the part of agents that are proprietary to highly competitive pharmaceutical companies to address the issues that will arise, especially in performing trials that involve combining drugs.

Dosing regimens for oral HS90 inhibitors in combination with other drugs should be something to be avoided, at least in the development of continuous dosing regimens for oral HS90 inhibitors in combination with other drugs.

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