Ganetespib and HSP90: Translating Preclinical Hypotheses into Clinical Promise

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Abstract

As with many physiologic processes that become subverted during tumorigenesis, the chaperoning activity of heat shock protein 90 (HSP90) is often exploited by cancer cells to confer aberrant proliferative, survival, and/or metastatic potential. Functional inhibition of HSP90 results in the degradation of its client proteins, in turn providing a means to concomitantly disrupt multiple oncogenic signaling cascades through one molecular target. Pharmacologic blockade of HSP90 has, therefore, emerged as an innovative and multifaceted approach for the development of new antineoplastic agents. However, no HSP90 inhibitors are currently approved for cancer therapy and the full promise of this class of agents is yet to be realized. This review focuses on the preclinical activity profile of ganetespib, a potent small-molecule inhibitor of HSP90, the characterization of which has provided important frameworks for the optimal design and application of HSP90 inhibitor–based strategies in a variety of cancer types. Beyond client protein–driven tumors, ganetespib can also potentiate the effects of other molecularly targeted and standard-of-care therapeutics while simultaneously overcoming drug resistance in multiple tumor types, thereby positioning this compound as the leading HSP90 inhibitor currently under clinical development. Cancer Res; 74(5); 1294–300. ©2014 AACR.

Introduction

From an unlikely candidate to one of the most actively pursued drug targets in oncology, the molecular chaperone heat shock protein 90 (HSP90) represents a promising point for therapeutic intervention in cancer due to its essential role in regulating the maturation and functional stability of an extensive array of client proteins that contribute to nearly every aspect of the oncogenic process, including immortalization, pro-survival/antiapoptosis, metabolism, genomic instability, and dissemination (1). Unlike traditional targeted therapies (e.g., kinase inhibition) that selectively ablate one or a few oncogenes, inhibition of HSP90 activity results in the destabilization of hundreds of cellular clients (2), thus providing a means to simultaneously disrupt multiple oncogenic signaling cascades through a singular molecular target.

Originally, HSP90 was not considered an intuitive candidate for therapeutic targeting. The chaperoning function of the molecule is equally critical for normal physiologic and homeostatic processes and, indeed, genetic knockout is lethal (3). This profile would thus be expected to contribute to a high likelihood of systemic toxicities. However, during the identification and characterization of pharmacologic inhibitors of HSP90, it was discovered that as a class, these compounds displayed preferential and selective tumor retention (reviewed in ref. 4). Although the mechanisms remain to be fully elucidated, a model has emerged that HSP90 exists in tumor cells in a highly active, multichaperone complex that exhibits greater affinity for targeted inhibitors than that observed in normal cells (4).

The prototypical class of HSP90 inhibitors contains the natural product ansamycin, including geldanamycin and its derivatives 17-allylamino-17-demethoxygeldanamycin (17-AAG) and 17-dimethylaminopropylamino-17-demethoxygeldanamycin (17-DMAG). While characterization of these compounds delivered critical proof-of-concept evidence for targeted HSP90 inhibition as an effective strategy for cancer treatment, their clinical application was hampered by a number of pharmacologic and safety limitations. Since then, an increasing number of synthetic small-molecule inhibitors of HSP90 have been developed based on a diverse variety of chemical scaffolds (5). This review focuses on the preclinical activity profile of ganetespib, an investigational drug that has provided important frameworks for the design of new HSP90 inhibitor-based therapies in the clinical management of multiple human malignancies.

Ganetespib: An HSP90 Inhibitor with Favorable Potency, Activity, and Tolerability

Similar to the majority of small-molecule HSP90 inhibitors currently in development, ganetespib exhibits competitive binding with the N-domain ATP-binding pocket of HSP90, disrupting the chaperone cycle. Ganetespib belongs to the resorcinol-based group of synthetic, second-generation HSP90 inhibitors that include NVP-AUY922, AT-13387, and KW-2478; however, its chemical structure contains a unique triazolone moiety (6). With a molecular weight of 364.4, the compound is considerably smaller than the ansamycins and most second-
generation HSP90 inhibitors. This agent displayed a 20-fold superior potency to 17-AAG in a panel of 57 transformed cell lines of both hematologic and solid tumors (6) with \( \text{in vitro} \) activity typically in the low nanomolar range comparable to NVP-AUY922 (7). Ganetespib is preferentially retained in tumor tissues over normal tissues, with a half-life of more than 58 hours in non-small cell lung cancer (NSCLC) xenograft tumors compared with 3 hours in plasma and 5 to 6 hours in normal liver and lung (8). Similar drug accumulation has previously been demonstrated for 17-AAG and 17-DMAG in a rat model, no changes in the liver toxicity profile of ganetespib was directly compared with that of 17-DMAG in a rat model, no changes in the liver enzymes aspartate aminotransferase or alanine aminotransferase were observed following repeat dosing of ganetespib, even at levels above its efficacious dose range. In stark contrast, a dose-dependent and marked elevation of liver enzymes and histologic liver damage was seen in 17-DMAG-treated rats, at doses 12.5 times lower than ganetespib (6). In addition, drug-related ocular toxicity has emerged as an undesirable side effect of some newer HSP90-targeted agents, such as NVP-AUY922 and SNX-5422. Using a rodent model to assess the retinotoxicity potential of HSP90 inhibitors, we recently uncovered a relationship between photoreceptor degeneration with retinal drug distribution and retention (1). Unlike NVP-AUY922, ganetespib did not accumulate in the rat eye and was rapidly eliminated from retinal tissues. As a result, ganetespib did not induce extensive photoreceptor cell death as seen following NVP-AUY922 exposure (1). These findings are consistent with the clinical safety profile of ganetespib, which has shown only a minor incidence of visual disorders in more than 800 patients treated to date. For ganetespib, which shows that HER2-overexpressing (HER2\(^+\)) tumors are particularly susceptible to HSP90 inhibition (18). Moreover, clinical benefit was achieved in a phase II trial evaluating 17-AAG in combination with trastuzumab (an approved biologic inhibitor of HER2) in patients with metastatic HER2\(^+\) breast cancer who had progressed on prior trastuzumab therapy alone (19). The clinical development of 17-AAG has subsequently been halted; thus, it seems that a therapeutic opportunity exists within this patient group for an effective and tolerable alternate HSP90 inhibitor. In this regard, the preclinical activity profile of ganetespib in HER2\(^+\) breast cancer encompasses both potent oncogene inhibition and durable response properties, features that would support the use of intermittent dosing schedules in the clinical setting (20).

Gene rearrangements of ALK, most commonly with echinoderm microtubule-associated protein-like 4 (EML4), act as oncogenic drivers in a molecularly defined subset of NSCLC. Indeed, the clinical success of the ALK tyrosine kinase inhibitor (TKI) crizotinib in this patient population serves as a paradigm for molecularly targeted therapy in general. Because the EML4–ALK fusion protein is an established HSP90 client, we evaluated the possibility of targeting the chaperone function of HSP90 with ganetespib as an alternative approach to direct kinase inhibition in ALK-driven lung cancer (13). Not unexpectedly, ganetespib treatment resulted in the loss of EML4–ALK expression and depletion of multiple oncogenic signaling cascades in NSCLC cell lines harboring the rearrangement, consistent with previous reports for 17-AAG and its water-soluble derivate IPI-504 (14, 15). However, when directly compared with crizotinib, ganetespib exhibited significantly greater \( \text{in vitro} \) potency, superior antitumor efficacy, and prolonged animal survival in xenograft models (13). Thus, the multimodal effects of ganetespib on both ALK itself and other HSP90-modulated signaling cascades provided more complete and durable responses compared with kinase inhibition alone. The translational implications of these findings have already been established in clinical trials in which durable objective tumor responses were observed in patients with NSCLC who were undergoing IPI-504 or ganetespib monotherapy, whose tumors harbored ALK rearrangements (16, 17). Furthermore, oncogenic rearrangements of two additional kinases, ROS1 and RET, were also acutely sensitive to HSP90 inhibition by ganetespib (13). Similar to ALK, ROS1 and RET fusions are emerging as promising molecular targets in genomic subsets of NSCLC and, as the clinical significance of these putative oncogenic drivers becomes realized, pharmacologic blockade by ganetespib warrants further investigation as a therapeutic approach.

In breast cancer, considerable experimental evidence exists that shows that HER2-overexpressing (HER2\(^+\)) tumors are particularly susceptible to HSP90 inhibition (18). Moreover, clinical benefit was achieved in a phase II trial evaluating 17-AAG in combination with trastuzumab (an approved biologic inhibitor of HER2) in patients with metastatic HER2\(^+\) breast cancer who had progressed on prior trastuzumab therapy alone (19). The clinical development of 17-AAG has subsequently been halted; thus, it seems that a therapeutic opportunity exists within this patient group for an effective and tolerable alternate HSP90 inhibitor. In this regard, the preclinical activity profile of ganetespib in HER2\(^+\) breast cancer encompasses both potent oncogene inhibition and durable response properties, features that would support the use of intermittent dosing schedules in the clinical setting (20).
robust antineoplastic activity in *in vitro* and *in vivo* TNBC models (23). Furthermore, ganetespib exposure suppresses lung colonization and tumor growth in syngeneic mouse models of experimental and spontaneous metastasis (23). This antimetastatic feature of the compound has been strikingly reinforced by clinical observations of marked tumor shrinkage of lung and lymphatic lesions in patients with TNBC undergoing ganetespib monotherapy (23). These dramatic responses suggested that the metastatic tumors were acutely reliant on the chaperoning activity of HSP90, with one or more HSP90-dependent signaling pathways driving their growth and survival. A number of molecular alterations have been incompletely validated as therapeutic targets in triple-negative disease (24) and, among these, molecules such as EGFR, KIT, hypoxia-inducible factor-1α (HIF-1α), and members of the DNA-repair pathway are established HSP90 clients. While the challenge remains to identify which specific client proteins may ultimately serve as predictive biomarkers for those cancers likely to respond to HSP90 inhibitor treatment, our findings underscore the therapeutic potential of ganetespib for treatment of metastatic disease in patients with TNBC.

**Combinatorial Activity**

Despite the encouraging clinical activity seen in ALK-driven and HER2-amplified disease, similar translational benefit remains to be achieved with HSP90-inhibitor monotherapy in other client protein–driven patient populations. For example, GIST is a tumor type that may be considered particularly amenable to HSP90 inhibitor–directed therapy due to the high frequency of activating KIT mutations that drive tumorigenesis. However, prolonged inhibition of this client or its downstream pathways was not observed following targeted HSP90 blockade in either preclinical models or patient biopsies, and consequently only limited efficacy was seen in a phase II trial evaluating weekly administration of ganetespib to patients with GIST (25). HSP90 inhibitors are likely then to be most effective in such scenarios when used as part of combination treatments. In this regard, ganetespib has been found to potentiate the activity of standard-of-care chemotherapeutics and other molecularly targeted agents in a variety of human cancers, thus providing compelling rationales for the exploration of this compound as part of novel combinatorial treatment strategies.

Taxanes are antimitotic agents and represent one of the most widely used classes of chemotherapeutic drugs in the treatment of multiple human cancers, including lung, breast, and ovarian. A significant drawback to the use of these (and other) systemic cytotoxics, however, is that they manifest a variety of significant and dose-limiting adverse side effects in clinical practice. Synergistic combinatorial benefit between HSP90 inhibitors and taxanes has been described in different cancer models (26–29), suggesting that their nonoverlapping but complementary mechanisms of action are conserved across tumor types. Indeed, the combination of ganetespib with either paclitaxel or docetaxel promotes strong synergistic activity and enhanced antitumor efficacy in preclinical models of NSCLC, TNBC, and ovarian cancer (23, 30, 31). The mechanistic benefit afforded by the addition of ganetespib to the taxane regimens was found to be multifactorial and included loss of pro-survival signaling, direct impacts on the cell cycle machinery, and exacerbation of mitotic catastrophe (23). Thus, combined treatment with an efficacious HSP90 inhibitor such as ganetespib represents one potential strategy to improve therapeutic indices without additional toxicity and, importantly, is likely to benefit a higher percentage of patients with cancer, not just those with client protein–driven disease.

Studies of ganetespib in KRAS-mutant NSCLC have uncovered intuitively attractive therapeutic combinations that effectively counteract oncogenic KRAS activity (32). Activating KRAS mutations are one of the most prevalent oncogenic drivers in NSCLC and are associated with unfavorable clinical outcomes (33). However, KRAS has proven to be an intractable therapeutic drug target, and KRAS-mutant NSCLC tumors respond poorly to current therapies, highlighting an urgent need for new treatment strategies. While KRAS itself is not a client protein, many components of the downstream effector pathways aberrantly stimulated by constitutive mutant KRAS activity, including the canonical RAF/MEK/ERK and PI3K/AKT/mTOR signaling pathways, are acutely sensitive to HSP90 inhibition. Moreover, recent studies have provided a compelling rationale for the combinatorial use of targeted MAP–ERK kinase (MEK) and phosphoinositide 3-kinase (PI3K)/AKT/mTOR signaling pathways, are acutely sensitive to HSP90 inhibition. Moreover, recent studies have provided a compelling rationale for the combinatorial use of targeted MAP–ERK kinase (MEK) and phosphoinositide 3-kinase (PI3K)/AKT inhibitors in KRAS-driven lung tumors, with dual blockade of the mitogenic and survival signaling arms providing superior antitumor responses (34, 35). Because HSP90 inhibition conferred by ganetespib exposure can coordinate impact both tumorigenic pathways, combinations of ganetespib with a dual PI3K/mTOR inhibitor have been evaluated in KRAS-mutant NSCLC (32). As predicted, concomitant and potent blockade of the signaling cascades activated by KRAS mutation by this drug combination resulted in superior cytotoxic activity in *in vitro* and significant enhancement of antitumor efficacy in a xenograft model (32). At the molecular level, mTOR signaling represented an important mechanistic point of convergence between the...
two agents. Overall, these findings suggest that HSP90 blockade alongside mTOR inhibition warrants further investigation as a novel combinatorial approach for the treatment of KRAS-mutant lung cancer.

Mutational activation of BRAF, similarly resulting in dysregulation of the RAF/MEK/ERK signaling axis, is a feature of more than half of all malignant cutaneous melanomas (36). Unlike KRAS however, mutant BRAF has proved to be an actionable target for molecular therapeutic approaches and recently, vemurafenib, the first selective small-molecule inhibitor of BRAF\(^{V600E}\), was approved as first-line therapy for patients with metastatic melanoma whose tumors harbor this mutation. In addition, mutated BRAF is a highly sensitive HSP90 client protein, and we have shown that single-agent ganetespib is significantly more potent than vemurafenib or allosteric MEK inhibitors in BRAF-driven melanoma cell lines (37). A number of combination trials are currently underway in melanoma investigating the dual blockade of mutant BRAF and MEK, with early experimental evidence suggesting that this strategy may not only improve efficacy compared with single agents alone, but might also be an effective approach to prevent or delay the onset of acquired resistance (38). In preclinical models, strong synergistic activity was observed when ganetespib was used as a cotreatment with either vemurafenib or selective MEK inhibition, and the effects were robustly recapitulated in xenograft tumors (37). These data provide a novel framework for combining the modalities of HSP90 inhibition with either selective BRAF or MEK targeting as a promising avenue for therapeutic intervention in melanoma cells that display oncogenic addiction to mutant BRAF.

Ganetespib Treatment Overcomes Diverse Mechanisms of Drug Resistance

One of the most notable findings to emerge from the characterization studies of ganetespib was its capacity to overcome multiple forms of intrinsic and acquired resistance to other clinically relevant targeted agents, including TKIs (Fig. 1B). For example, in NSCLC, activating mutations in EGFR drive tumorigenesis and confer sensitivity to approved TKIs such as erlotinib and gefitinib. The acquisition of a secondary point mutation in the EGFR gatekeeper residue (T790M) represents the predominant mechanism by which NSCLC tumors develop acquired resistance to these drugs. Ganetespib can overcome the resistant phenotype in NSCLC cell lines, retaining full potency irrespective of EGFR mutational status (6, 8). In addition, resistance to EGFR-directed TKIs may also emerge through alternative oncogenic mechanisms. For example, hepatocyte growth factor, a ligand of the MET oncoprotein, can induce TKI resistance in lung tumors with EGFR-activating mutations by independently activating and restoring PI3K/AKT signaling via phosphorylation of MET (39). Ganetespib treatment was equally efficacious in overriding resistance induced in cell lines by this mechanism (6). Perhaps more importantly, ganetespib can also overcome acquired resistance to crizotinib in ALK\(^{++}\) NSCLC, as evidenced by multiple experimental models and tumor responses in the clinical setting (13). To date, a variety of specific ALK kinase domain mutations at different amino acid sites have been reported in patients who exhibit crizotinib resistance and a number of others have been identified through mutagenesis screens (40). It has been reported using a model of crizotinib-resistant NSCLC dependent on an L1196M gatekeeper mutation within the ALK domain that the cells were sensitive to HSP90 inhibition by 17-AAG (41). Preclinical experiments have revealed that ganetespib retains robust cytotoxic activity in mutated ALK-expressing cell lines irrespective of the mutational site or amino acid substitution present. Moreover, these findings were validated by marked tumor shrinkage of lung lesions observed in a patient with relapsed, crizotinib-resistant NSCLC upon commencing ganetespib treatment (13). Taken together, these data highlight the therapeutic potential of ganetespib within the crizotinib-refractory population.

In mutant BRAF melanoma, a high percentage of patients fail to initially respond to vemurafenib because of intrinsic resistance to BRAF inhibition; furthermore, the clinical experience with this drug has shown that the long-term efficacy of treatment is hampered by the invariable development of acquired resistance. A variety of mechanisms have been identified that contribute to the BRAF-inhibitor resistant phenotype that allow either bypass or reactivation of MAPK signaling (38). Similar to what was found for another next-generation HSP90 inhibitor, XL888 (42), ganetespib exposure can overcome both forms of resistance in \textit{in vitro} and \textit{in vivo} melanoma models (37). Taken together, it seems then that the complexity of resistance mechanisms to selective BRAF inhibitors may be overcome by the simultaneous targeting of multiple signaling pathways that is afforded by HSP90 blockade. In addition, combination treatment of vemurafenib-resistant xenograft tumors with ganetespib and the MEK inhibitor TAK-733 caused significant tumor regressions (37), providing a further compelling rationale for combining ganetespib with targeted MEK agents as a new approach for treating tumors with acquired resistance to BRAF inhibitors.

One particularly interesting observation to arise from the combinatorial studies performed in KRAS-mutant NSCLC was drug-induced activation of compensatory feedback signaling pathways in cell lines following exposure to either mTOR or MEK inhibitors (32). Such a response commonly occurs due to relief of negative feedback pathways required for cellular homeostasis and, importantly, this mechanism has been proposed to contribute to adaptive resistance to targeted therapeutic agents (43). Ganetespib potently suppressed this response in KRAS-mutant cell lines, further suggesting that HSP90 inhibition may provide a more universal means to overcome adaptive resistance in multiple oncologic settings.

Pathways to Achieving the Potential

Despite the enormous promise of HSP90 inhibitors as a class of therapeutic agents, none are currently approved for any cancer indication. The clinical development of the first generation of compounds was hampered by weak potency, modest clinical efficacy, and significant toxicities. To a large extent, second-generation agents have overcome many of these limitations, based on their higher relative potency and improved safety profiles. Key questions remain, however, including the identification of specific predictive biomarkers within cancer
types to stratify those individuals most likely to receive benefit from therapeutic HSP90 blockade. Moreover, continued exploration of biologically informed drug combinations is likely to provide a direct path to the most efficacious use of these agents (1). Indeed, it is not unreasonable to envision the eventual application of HSP90 inhibitors as adjuncts to systemic chemotherapy for a wide variety of human malignancies. On the basis of the extensive preclinical characterization and activity of the compound outlined here, it is perhaps not surprising that ganetespib is one of the most clinically advanced HSP90 inhibitors in development. The compound is presently undergoing evaluation in 14 human trials, including two related phase IIb/III and phase III studies in advanced NSCLC adenocarcinoma in combination with docetaxel (NCT01348126 and NCT01798485). Confirming preclinical predictions, the ganetespib + docetaxel combination has shown meaningful improvements in median overall survival compared with docetaxel treatment alone with no added toxicities (44). As our knowledge of HSP90 biology continues to grow, and appropriate patient populations likely to benefit from inhibitor-based therapies are identified, ganetespib is well positioned to become an important player in the application of new treatment strategies for the management of a variety of human malignancies.

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