# Heat Shock Protein 90 as a Drug Target: Some Like It Hot

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**Abstract** Heat shock protein 90 (HSP90) is a ubiquitously expressed chaperone that is involved in the posttranslational folding and stability of proteins. Inhibition at the $NH_2$-terminal ATP-binding site leads to the degradation of client proteins by the ubiquitin proteasome pathway. Inhibition of HSP90 leads to the degradation of known oncogenes, such as ERB-B2, BRAF, and BCR-ABL, leading to the combinatorial blockade of multiple signal transduction pathways, such as the RAS-RAF-mitogen-activated protein/extracellular signal-regulated kinase kinase-extracellular signal-regulated kinase and phosphatidylinositol 3-kinase pathways. Multiple structurally diverse HSP90 inhibitors are undergoing early clinical evaluation. The clinical focus of these drugs should be solid tumors, such as breast, prostate, and lung cancers, along with malignant melanoma, in addition to hematologic malignancies, such as chronic myeloid leukemia and multiple myeloma. HSP90 inhibitors can be used as single agents or in combination with other targeted treatments or conventional forms of treatment such as chemotherapy and radiotherapy. Clinical trials evaluating efficacy of these agents should include innovative designs to capture cytostasis evidenced by clinical nonprogression and enrichment of patient populations by molecular characterization. The results of clinical trials evaluating the efficacy of drugs targeting this exciting target are awaited.

## Background

***Biology.*** Heat shock protein 90 (HSP90) is an essential molecular chaperone that accounts for 1% to 2% of all proteins in the cell (1). It exists as multiple isoforms that includes HSP90α and HSP90β in the cytoplasm and GRP94 and Trap1 localized to the endoplasmic reticulum and mitochondria, respectively (2). Recent developments include identification of a new isoform, HSP90N. Transfection of RAS-deficient cell lines with this isoform has been shown to block differentiation and it has been hypothesized that HSP90N could activate RAF in a RAS-independent manner (3). There has also been interest in cell surface HSP90 (4). The HSP90 domain structure has three essential regions: an $NH_2$-terminal region (24-28 kDa), middle region (38-44 kDa), and a COOH-terminal region (11-15 kDa). The primary functions of the above-mentioned domains are ATP binding, client protein binding, and dimerization, respectively (5). The $NH_2$-terminal domain has been studied in most detail and consists of a highly twisted eight-stranded β sheet exposed to solvent on one side, whereas the other side is covered by α helices that pack to form a pocket, which is the ATP-binding site (6).

HSP90 functions as a part of a multichaperone complex, involving the dynamic association with various accessory cochaperones and client proteins. In an ATP-bound state, HSP90 adopts a closed conformation and becomes a mature complex that is essential for it to perform its function of client protein folding and stabilization. The chaperone cycle involves a complex series of loading and unloading events, which require a host of cochaperones, such as HSP70, HSP40, HOP, AHA1, and p23 (7). There is emerging evidence to suggest that a few of these cochaperones such as AHA1 and HSP70 could be independent molecular targets (8). In the event of client proteins not being chaperoned by the mature HSP90 complex, an E3 ubiquitin ligase such as CHIP is recruited and client proteins are degraded by the ubiquitin proteasome pathway (9).

***HSP90 as a cancer target.*** There are several important reasons why HSP90 should be considered an important molecular target relevant to cancer. HSP90 is key to the stability and function of a host of proteins that are important to the cancer cell, such as BCR-ABL, ERB-B2, epidermal growth factor receptor (EGFR), CRAF, BRAF, AKT, MET, VEGFR, FLT3, androgen and estrogen receptors, hypoxia-inducible factor (HIF)-1α, and telomerase, the list of which is being constantly updated.[1] These proteins influence the hallmark traits of cancer, such as growth factor independence, resistance to antigrowth signals, unlimited replicative potential, tissue invasion and metastasis, avoidance of apoptosis, and sustained angiogenesis (10). A cause of failure of therapies selectively targeting a single tyrosine kinase is attributed to activation of multiple parallel signal transduction pathways. Inhibiting HSP90 leads to combinatorial inhibition of multiple signal transduction pathways overcoming this problem (Fig. 1; ref. 11).

Therapeutic selectivity of HSP90 inhibitors, which accounts for a viable therapeutic index, could be explained by the

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[1] http://www.picard.ch/downloads/Hsp90interactors.pfd

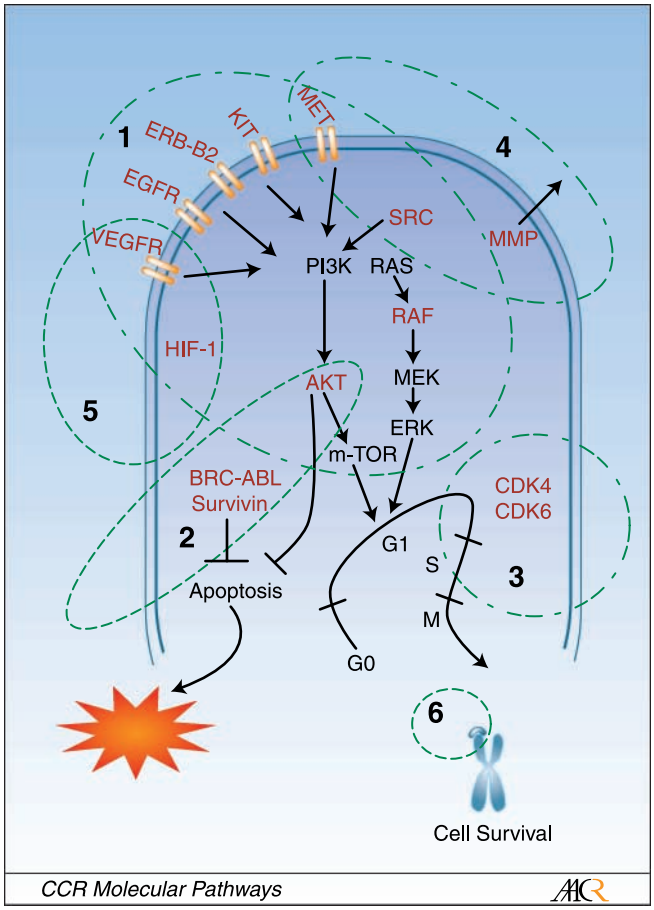


**Fig. 1.** Schematic representation of how HSP90 client proteins (*red*) contribute to key characteristics of a cancer cell: (*1*) growth factor independence (EGFR, ERB-B2, KIT, MET, VEGFR, and SRC), (*2*) invasion of apoptosis (AKT, BCR-ABL, SRC, and survivin), (*3*) resistance to antigrowth signals (CDK4 and cyclin D), (*4*) tissue invasion and metastasis (CMET, MMP-2, and SRC), (*5*) sustained angiogenesis (VEGF, MET, and HIF), and (*6*) unlimited replicative potential (h-TERT). HSP90 inhibition would lead to degradation of these client proteins by the ubiquitin proteasome pathway resulting in a combinatorial blockade of factors influencing these key characteristics of the cancer cell.

following factors. Firstly, cancer cells are addicted to oncoproteins (12) that drive malignant process; thus, depletion of oncoproteins affects cancer cell to a greater extent than normal cells. Secondly, oncoproteins are often expressed as mutant forms (e.g., BRAF and EGFR), which require HSP90 for stability and function to a greater extent than their wild-type counterparts (13, 14). Thirdly, cancer cells are subject to intratumoral acidosis, hypoxia, and deprivation of nutrients, creating an environment of cellular stress that requires the chaperone machinery to a greater extent than their normal counterparts (15). This argument could also be extended to cancer cells subject to stress by DNA damage due to chemotherapy or radiotherapy. Finally, there is evidence that some HSP90 inhibitors bind preferentially to HSP90 complexes in cancer cells compared with normal cells (16).

***HSP90 inhibitors.*** The antiproliferative activity of HSP90 inhibitors radicicol and geldanamycin was thought to be due to SRC inhibition; however, immunoprecipitation experiments showed immobilized geldanamycin bound to HSP90 in a stable and pharmacologically specific manner (17). Studies of the crystal structure of the $NH_2$-terminal domain of HSP90 showed that the benzoquinone ansamycin geldanamycin bound to the ATP-binding site, causing HSP90 inhibition (6). Since then, there have been a host of HSP90 inhibitors that have been evaluated in the clinic. Early attempts of drug development concentrated on geldanamycin and radicicol analogues, such as tanespimycin (17-allylamino-17-demethoxygeldanamycin), alvespimycin (17-dimethylamino-17-demethoxygeldanamycin; ref. 18), IPI-504 (19), and KF58333 (20). Synthetic small-molecule inhibitors such as AUY922A (21), BIIB021 (22), and SNX2112 (23) are more recent compounds being studied.

Although ATP binding at the $NH_2$-terminal domain of HSP90 has been the focus of drug development, other approaches are being considered and include inhibition of the COOH terminus of HSP90 (24) or the cochaperones AHA1 (25) and CDC37 (26). There are preliminary data to make a case for isoform-specific inhibitors (27, 28). Cochaperones such as HSP70 and HOP along with the E3 ubiquitin ligase CHIP interact with the chaperone complex and are possible anticancer targets (7).

## Clinical-Translational Advances

The discussion below is based on current understanding of biology of HSP90 and focuses on key clinical areas. Although early benefits of HSP90 inhibitors are likely in these areas, they are by no means exclusively confined to these disease subgroups. The evidence of clinical activity of HSP90 inhibitors is eagerly awaited.

***Breast cancer.*** Breast cancer is an indication where HSP90 inhibitors should be explored for a variety of reasons. Firstly, inhibition of HSP90 degrades ERB-B2, a client protein, and HSP90 inhibitors have shown activity in ERB-B2–driven xenograft models (29). Secondly, modulation of estrogen and progesterone receptor signaling has been a long-standing approach to treating breast cancer and both estrogen and progesterone receptors are clients of HSP90 (30). Thirdly, resistance of breast cancer cells to chemotherapy is known to involve the phosphatidylinositol 3-kinase pathway (31), which is modulated by HSP90 by virtue of one of its key signaling proteins (AKT) being a client protein of HSP90. Fourthly, inhibition of HSP90 has also been known to modulate angiogenesis of breast cancer xenografts (20). Finally, expression of HSP90 has been shown to correlate with adverse clinical outcomes, further validating HSP90 as a target in breast cancer (32). There have been reports of the HSP90 inhibitor tanespimycin having clinical activity when combined with trastuzumab in patients resistant to trastuzumab alone (33).

***Prostate cancer.*** Androgen deprivation has been the mainstay of the treatment of prostate cancer for more than half a century. The androgen receptor is a known client protein of HSP90 (34); thus, HSP90 inhibition can be used in hormone-sensitive prostate cancer. In addition, it is known that nuclear localization of the androgen receptors continues to play a role in hormone-independent prostate cancer and HSP90 inhibitors have been shown to disrupt ligand-independent nuclear localization of the androgen receptor (35). ERB-B2, a known client of HSP90, is also implicated in the pathogenesis of prostate cancer (36). In addition, HSP90 expression is

higher in prostate cancer compared with normal prostate epithelium, further validating it as a target in prostate cancer (37). Finally, radiation, another modality of treatment for prostate cancer, is potentiated by HSP90 inhibitors in prostate cancer cell lines (38).

***Melanoma.*** Malignant melanoma is relatively resistant to most conventional forms of cancer treatment (i.e., chemotherapy and radiotherapy). BRAF is a client protein of HSP90 and is mutated in 50% to 70% of all melanomas (39). HSP90 inhibition has been shown to preferentially degrade mutant BRAF over wild-type BRAF (13). In addition, HSP90 expression was positively associated with adverse histologic features such as with Clarke's level and was also found to be higher in metastatic melanomas (40). In uveal melanomas, HSP90 expression is associated with larger tumor size (41). There have been reports of prolonged stable disease in patients with malignant melanoma in early clinical trials of HSP90 inhibitors (42).

***Lung cancer.*** HSP90 is known to chaperone both wild-type and mutant EGFR and HSP90 inhibition preferentially inhibits mutant EGFR (14). Studies on resected non–small cell lung cancers have shown that a lower expression of genes encoding HSP90 in the specimens correlated with a better prognosis (43). Also of interest is that antiapoptotic pathways in small cell lung cancer require HSP90 and inhibition of HSP90 is shown to initiate apoptosis (44). There have been reports of prolonged stable disease in non–small cell lung cancer patients in early clinical trials of HSP90 inhibitors (45).

***Other solid tumors.*** Colon cancers are known to have a deregulated RAS-RAF-mitogen-activated protein/extracellular signal-regulated kinase kinase pathway by virtue of *KRAS* (46) and *BRAF* (39) mutations and HSP90 chaperones key client proteins in this pathway. In addition, EGFR is a client protein of HSP90 and modulation of EGFR signaling has previously been successful in treating colon cancer. Also of importance is that HSP90 inhibition blocks the phosphatidylinositol 3-kinase pathway, the activation of which is found to confer resistance to cetuximab, a monoclonal antibody to EGFR (47). Colon cancer cells are known to express DT-diaphorase, which causes resistance to the benzoquinone ansamycin class of HSP90 inhibitors (48); however, new small-molecule inhibitors of HSP90 are less susceptible to resistance by this mechanism. Ovarian and endometrial cancer has been shown to have mutations in the tumor suppressor gene *PTEN*, which can cause activation of the phosphatidylinositol 3-kinase pathway that signals through AKT, a client protein of HSP90 (49, 50). Renal cancers are associated with von Hippel Lindau gene mutations, leading to enhanced HIF-1α–regulated VEGF signaling. HIF-1α is a client of HSP90 (51) as is VEGFR (52), and although early clinical trials with HSP90 inhibitors as single agents have been disappointing (53), combinations of HSP90 inhibitors and tyrosine kinase inhibitors such as sorafenib have been initiated (54). Gastrointestinal stromal tumors are driven by CKIT mutations, and both CKIT and its downstream effector AKT are clients of HSP90 (55). Squamous cell cancers of the upper aerodigestive tract are EGFR driven (56), and both EGFR and multiple downstream signaling proteins, such as RAF and AKT, are client proteins of HSP90. Also of possible clinical significance is the fact that HSP90 inhibitors have been shown to potentiate radiotherapy in preclinical squamous cell cancer models (57).

**Table 1.** A summary of reported clinical trials of HSP90 inhibitors

| **Phase I (single agent)** | |
|---|---|
| 17-AAG* | 17-DMAG, 80 mg/m$^2$ once a week (75) |
| Once a week, RP2D 295-450 mg/m$^2$ (42, 76) | BII021, MTD 700 mg twice a week (22) |
| Twice a week, RP2D 200-220 mg/m$^2$ (77, 78) | IPI-504, MTD not reached (73) |
| Daily RP2D 40-56 mg/m$^2$ (77, 79) | SNX5422, MTD not reached (81) |
| **Phase I combination trials** | **Phase II trials** |
| 17-AAG + gemcitabine + cisplatin (81) | Melanoma (82) |
| 17-AAG + docetaxel (83) | Prostate (84) |
| 17-AAG + irinotecan (85) | Renal cancer (53) |
| 17-AAG + trastuzumab (33) | |
| 17-AAG + bortezomib (86) | |
| 17-AAG + sorafenib (54) | |
| 17-DMAG + trastuzumab (87) | |

NOTE: Comprehensive information about actively recruiting trials could be found on http://www.clinicaltrials.gov.
*Readers are directed to the references for details of schedules of drug administration in different trials.

***Chronic myeloid leukemia.*** BCR-ABL is a known client protein of HSP90 (58) and is known to drive chronic myeloid leukemia. Efforts are being concentrated on using HSP90 inhibitors in chronic myeloid leukemia resistant to kinase inhibitors such as imatinib (59).

***Multiple myeloma.*** There are multiple reasons why HSP90 inhibitors should be investigated in myeloma. Firstly, there is evidence that HSP90 is overexpressed in myeloma cells (60). HSP90 inhibition is shown to inhibit signaling pathways induced by cytokines, such as IL-6, by inhibition of multiple downstream signaling inhibitors, such as AKT (61). HSP90 is also associated with the activation of the unfolded protein response (62).

***Other hematologic malignancies.*** ZAP-70 is a known client protein of HSP90 and is associated with a subset of aggressive chronic lymphocytic leukemia (63). Inhibition of HSP90 is known to lead to abrogated ZAP-70 activity, and there have been suggestions of clinical activity in early-phase trials (22). Approximately one third of acute myeloid leukemias have activating mutations of FLT3, which activates downstream signal transduction pathways. FLT3 and downstream signaling proteins, such as CRAF and AKT, are known client proteins of HSP90 (64).

***Combination of HSP90 inhibitors and other treatment modalities.*** Both inherent and acquired resistance of cancer cells to tyrosine kinase inhibitors are thought to be in part due to activation of parallel signal transduction pathways. The combinatorial signal transduction blockade by HSP90 inhibitors makes them an ideal choice to explore combinations in this setting (54, 65, 66). Combination of HSP90 inhibitors and chemotherapy is additive (67) or synergistic (29), and careful thought should be given to the sequence of drug

administration (68). HSP90 inhibitors have been postulated to inhibit P-glycoprotein function, which may sensitize cancer cells to anticancer agents (69). The synergistic effects of the combination of HSP90 and radiation could be attributed to its effects on compromising DNA damage response (70) overcoming mechanisms of resistance modulated by the ERB family of oncoproteins (71) and its antiangiogenic effects on endothelial cells (72). HSP90 inhibitors could also be used in combination with antiangiogenic strategies, as VEGFRs are client proteins and HSP90 inhibitors are known to inhibit the production of angiogenic cytokines and responses of activated endothelial cells that contribute to tumor progression and metastasis (52).

***Clinical trial design.*** A list of reported early-phase trials of HSP90 inhibitors is summarized in Table 1. A significant proportion of the preclinical evidence related to HSP90 inhibitors suggest that HSP90 inhibitors are cytostatic when used as single agents. Phase I clinical trials have anecdotally observed prolonged periods of nonprogression of disease (42). Further, early clinical trials have shown [$^{18}$F]fluorodeoxyglucose (FDG) positron emission tomography responses but no evidence of disease response by Response Evaluation Criteria in Solid Tumors criteria (73). Clinical benefit from HSP90 inhibitors might need to be evaluated by clinical trial designs that measure nonprogression of disease, such as randomized discontinuation trials. In addition, early clinical trials should consider enriching patient populations with cancers that are likely to benefit from HSP90 inhibition rather than using the conventional paradigm of phase II trials testing cancers based on histologic phenotypes or anatomic site of origin. Efforts must be made to discover and apply predictive biomarkers that will predict for clinical response (45, 74). Indeed, some clinical trials that have observed early clinical benefit, such as the phase I trial of tanespimycin and trastuzumab, have inadvertently enriched patient populations (33). Sufficiently powered phase III clinical trials will be needed by treating ERB-B2–driven breast cancers to evaluate the combinations of HSP90 inhibitors with chemotherapy or radiotherapy.

***Conclusions.*** In summary, HSP90 is emerging as an exciting target in cancer. As a molecular chaperone, it contributes to the stability and folding of key proteins involved in the hallmark traits of cancer. Its potential to modulate multiple signaling pathways gives HSP90 inhibitors a potential to be used in a wide variety of cancers as single agents and in combination with targeted agents, conventional chemotherapy and radiotherapy. Evaluation of the efficacy of these agents should be driven by molecular characterization of tumor types and innovative trial design to fully realize their promise as anticancer agents.

## Disclosure of Potential Conflicts of Interest

AUY922 was developed by The Institute of Cancer Research in collaboration with Vernalis and Novartis. The Institute receives milestone payments from Vernalis. The Institute shares these payments with its staff through its rewards scheme. Dr. Udai Banerji is one of the Institute Staff that will benefit in this way.



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