Inhibition of HSP90 molecular chaperones: moving into the clinic

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Heat shock protein 90 (HSP90) is a molecular chaperone that is crucial for the stability and function of many proteins essential for cell survival. Many oncogenes, including tyrosine kinases, transcription factors, and cell-cycle regulatory proteins, are client proteins of HSP90. Inhibition of HSP90 causes client protein degradation via the ubiquitin–proteasome pathway, and is a mechanism that might simultaneously downregulate several redundant pathways crucial for cell viability and tumour development. HSP90 inhibitors are currently being developed as anticancer agents, and have shown early promising results in molecularly defined subgroups of solid tumours (eg, ALK-rearranged non-small-cell lung cancer and HER2-amplified breast cancer) and some haematological malignancies (eg, multiple myeloma). Here, we review the current status of HSP90 inhibitors in clinical development, including geldanamycin derivatives, resorcinol derivatives, purine analogues, and other synthetic inhibitors. We also discuss novel strategies and future perspectives on how to optimise the therapeutic potential of this exciting new class of drugs.

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Introduction: HSP function in physiological and pathological conditions

Protein synthesis is a highly controlled process essential for cellular growth, differentiation, and survival. Molecular chaperones, such as heat shock proteins (HSPs), are key elements in this process; they help the nascent polypeptide chain attain a functional conformation and facilitate protein stability, trafficking, and the proteolytic turnover necessary for protein intracellular localisation and function.[1]

HSPs are found in almost all living organisms, and their expression is increased in response to various cellular insults, including raised temperature, presence of heavy metals, and oxidative stress. Cellular stress causes protein denaturation, and denatured and aggregated proteins cannot function and must be rescued or eliminated with the help of chaperones. Increased HSP expression in response to stress is transcriptionally regulated mainly by heat shock factors (HSFs), as part of the heat shock response that leads to cellular protection from an aggression that would otherwise cause lethal damage.[1,2]

Defective chaperone function is noted in cellular senescence and in several diseases. If cellular stress proceeds unchecked by mechanisms such as the protein-refolding action of chaperones, intracellular proteins become denatured and insoluble, form aggregates, and precipitate. The development of inclusion bodies is a common pathological process in neurodegenerative disorders such as Parkinson's, Alzheimer's, Huntington's, and prion-related diseases, even in the absence of cellular stress;[1,2] drugs that can induce the heat shock response and promote HSP expression might have therapeutic value in these diseases. In neoplastic cells, by contrast, HSPs (particularly HSP90) are often overexpressed and present in activated multichaperone complexes, which are associated with a poorer prognosis. The oncogenic potential of cells is highly dependent on their ability to survive despite endogenous (hypoxia, pH changes, nutrient deprivation, dysregulated signalling pathways) and exogenous (radiation or chemotherapy) insults. Additionally, increased HSP expression can stabilise oncogenic proteins that are key drivers of the malignant phenotype. HSPs thereby promote independence of growth factors, tumour-cell survival, proliferation, immortalisation, neovascularisation, and metastasis, and indirectly modulate response to DNA damage and cell metabolism—ie, HSPs regulate most of the hallmarks of cancer.[2–4] Furthermore, cancer cells seem to be more dependent than normal cells on HSPs, and could therefore be more sensitive to HSP inhibition.

HSP inhibition in cancer therapy: who should we target?

HSPs are classified by molecular mass into the following categories: high-molecular-mass HSPs (≥100 kDa), HSP90 (81–99 kDa), HSP70 (65–80 kDa), HSP60 (55–64 kDa), HSP40 (35–54 kDa), and small HSPs (≤34 kDa).[1] So far, HSP90 has been the most widely tested target for cancer therapy. We focused this Review on HSP90 inhibition, and did not cover other chaperone targets, such as HSP27 or HSP70, since HSP90 inhibitors are the only ones in advanced stages of clinical development. HSP90 is an abundant, highly conserved chaperone that is crucial for the stability and function of many oncogenic proteins necessary for tumour development, including tyrosine-kinase receptors (ie, EGFR, HER2, c-KIT, MEK, VEGFR, FLT3, IGFR1), signal-transduction proteins (ie, BCR-ABL, ALK, BRAF, AKT), transcription factors (ie, androgen and oestrogen receptors, HIF1α, P53), cell-cycle regulatory proteins (ie, CDK4, RB, cyclin D), antiapoptotic proteins (ie, BCL2, survivin), and telomerase (hTERT; panel 1).[3–14] Five human isoforms of HSP90 have been identified, which differ in domain structure, cellular location, and substrate specificity. These include two cytosolic isoforms (HSP90α [inducible] and HSP90β [constitutively expressed]), an endoplasmic reticulum isoform (the

***Panel 1*: HSP90 client proteins associated with cancer**

- Tyrosine-kinase receptors:[3–8] HER2 (ERBB2), mutant EGFR, c-KIT, MAP2K1 (MEK), FLT3, VEGFR, IGF1R, EPHA2
- Signal-transduction proteins:[3–5,9,10] N-RAS, RAF1, mutant BRAF, BCR–ABL, AKT, NMP–ALK, IKK, SRC
- Transcription factors:[3–5,11] HIF1a, mutant P53, oestrogen-receptor α, androgen receptor
- Cell-cycle proteins:[3–5,12] CDK4, CDK6, PLK1, WEE1, PMYT1, cyclin D, mutant RB
- Antiapoptotic proteins:[3–5,13] APAF1, survivin, RIPK1, BCL2
- Others:[3–5,14] hTERT, FAK1, MMP2, MIF

glucose-regulated protein GRP94), a mitochondrial matrix isoform (HSP75/TRAP1), and isoform HSP90N.[1,2]

HSP90s are ATPases that exert their chaperone role through a complex cycle regulated by the binding and hydrolysis of ATP, and by several co-chaperones such as HSP70, HSP40, HOP, and P23 (figure 1, panel 2).[15–24] Inhibition of HSP90 causes client protein degradation via the ubiquitin–proteasome pathway (figure 2). Studies combining proteasome and HSP90 inhibitors showed a synergistic cytotoxic effect, with accumulation of ubiquitinated, misfolded oncogenic proteins.[25] This mechanism might simultaneously downregulate several pathways crucial for cell viability, which has resulted in substantial antitumour effects in preclinical models and could potentially prevent the emergence of tumour drug resistance.[2–5] The extent and duration of protein degradation with HSP90 inhibition might vary substantially for specific client proteins, with HER2 and ALK–EML4 showing the highest sensitivity.[4] In addition to the ATP regulatory cycle, HSP90 can be regulated post-translation by phosphorylation, nitrosylation, and acetylation.[26] Finally, induction of the heat shock response by cellular insults, such as cytotoxic drugs or radiotherapy, might counteract or reduce the lethal effects to the cell of many traditional antineoplastic agents or treatment strategies. In this context, HSP inhibitors could act as potential sensitisers for many anticancer drugs that would otherwise have limited therapeutic efficacy.

Clinically, HSP inhibition will probably have the greatest effect in tumours addicted to particular driver oncogene products that are sensitive HSP90 clients—ie, BCR–ABL in chronic myeloid leukaemia (CML), NPM–ALK in anaplastic large-cell lymphoma (ALL), FLT3 in acute myeloid leukaemia (AML), and ZAP70 in chronic lymphocytic leukaemia (CLL). Driver oncogenes to target in solid tumours might include HER2 in breast cancer, androgen receptors in prostate cancer, mutant EGFR or ALK translocations in some non-small-cell lung cancers (NSCLC), mutant KIT in gastrointestinal stromal tumours (GIST), and BRAF in some melanomas or colon carcinomas.[3–5] For some proteins, such as BRAF, HSP inhibitors have been shown to selectively induce mutant rather than wild-type protein degradation.[27] Moreover, studies have suggested that in tumours, HSP90 forms multichaperone, biochemically distinct complexes that specifically interact with oncogenic proteins and have higher affinity than HSP90 in normal tissues for specific small-molecule inhibitors.[28] Finally, malignancies where buffering of proteotoxic stress is crucial for cell survival (ie, multiple myeloma) might also be sensitive to HSP inhibition.

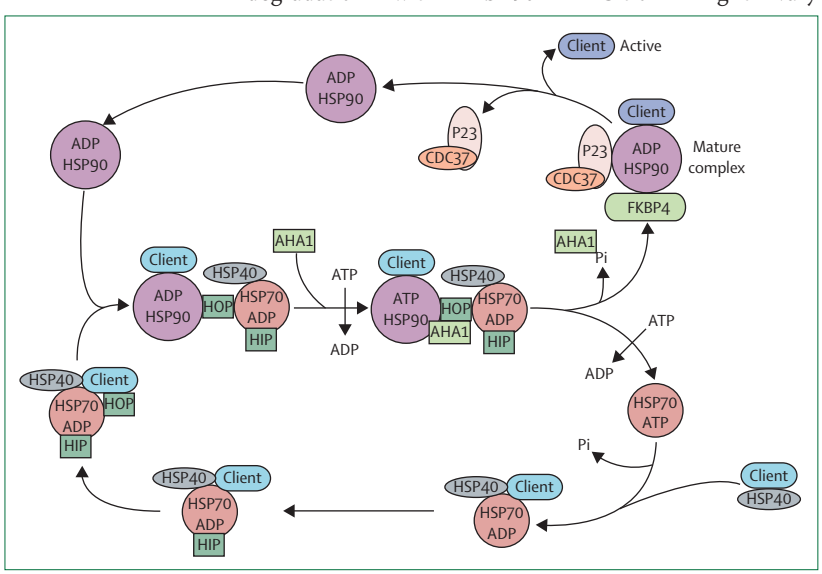

***Figure 1*: The HSP90 chaperone cycle**

HSP70, HSP40, and a client protein form an early complex. The client protein is transferred from HSP70 to HSP90 through the adaptor protein HOP (STI1). Binding of HOP is sufficient to stabilise the open conformation of HSP90. HSP90 adopts the ATPase-active (closed) conformation upon binding of ATP. P23 (SBA1) stabilises the closed state of HSP90, which weakens the binding of HOP and promotes its exit from the complex, hydrolysing ATP and liberating one phosphate molecule (Pi). Potentially, an immunophilin-type protein (FKBP4) associates to form a late complex, together with HSP90 and P23. After hydrolysis of ATP, P23 and the folded client are released from HSP90. The cofactors CDC37, HOP, AHA1, and P23 accelerate or slow specific steps of the cycle.

HSP90 inhibitors in clinical development

Geldanamycin derivatives

Geldanamycin is a naturally occurring benzoquinone ansamycin antibiotic first isolated in 1970 from *Streptomyces higroscopicus var geldanus*. Although soon found to have antiparasite and antineoplastic properties, it was not until the 1990s that HSP90 was identified as a molecular target.[29] Geldanamycin binds to the nucleotide-binding site of the N-terminal domain of HSP90 with higher affinity than ATP; this prevents ATP binding and hydrolysis, and eventually leads to enhanced depletion of oncogenic client proteins through ubiquitin-mediated proteasomal degradation. Despite potent antitumour activity of geldanamycin in more than 50 cell lines, clinical development was hampered because of poor solubility and stability, and unacceptable animal liver toxicity at therapeutic doses.[2,30] Geldanamycin analogues were then developed with improved pharmacological properties and toxicity profiles (table 1). Among them, 17-allylamino-17-demethoxygeldanamycin (17-AAG; tanespimycin) was the first HSP inhibitor to progress to clinical trials.