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HEAT SHOCK PROTEINS: A BETTER TARGET IN PREVENTION OF CANCER

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Abstract: Heat shock protein-90 is an energy dependent molecular chaperone involved in folding and stabilizing of various oncogenic client proteins to their active conformations that makes it promising anti-cancer target. Heat shock protein-90 inhibitors attacks at amino and carboxyl -terminal ATP pocket of molecule for the antitumor response. The clinical development of Heat shock protein-90 inhibitors from Geldanamycin and their analogues like Tanespimycin, Alveospimycin, Retaspimycin and newest agents till now has revealed miraculous encouraging results in field of acute myeloid leukemia, refractory prostate cancer, small cell lung carcinoma and breast cancer. Others derivatives of purine, resorcinol, indazolone have also a point of attraction. These categories of compounds will continuously driven forward by the optimization of pharmaceutical properties including pharmacokinetic, pharmacodynamic and toxicological in clinical studies with time. This class of compounds principally inhibits the ATPase activity of chaperon by to the N and C-terminal of chaperon. There are a multitude of second generation inhibitors currently under investigatory clinical trials. Till date, there is neither any FDA approved any heat shock protein-90 inhibitors nor standardized assay to ascertain inhibition. This review summarizes the current status of both first and second generation heat shock protein-90 inhibitors based on their chemical classification and stage of clinical development. Ultimately, these efforts will aid in maximizing the biological and pharmacological knowledge of potential of this class.

Keywords: ATPase activity, Chaperone, Client proteins, Geldanamycin, Heat shock protein 90 and Oncogenic



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INTRODUCTION

Summary of problem: Cancer, a disease due to division of cells which are aggressive, invasive, metastatic and normally resistant to apoptosis that makes them differs from benign tumors. Cancer may affect people at all ages but risk tends to increase with age.^[1] Now a day it has found that cancer is picking up speed again. Various factors like carcinogens, replication error, and genetic abnormality are potentially responsible. Treatment of cancer depends upon their type, location and severity stage. Combinational therapies of surgery with chemotherapy and radiotherapy are used normally. With the time newer drugs has been developed and still needs some more of novel drugs and therapeutic approach selective for cancer cell and lesser towards normal. In aim of specificity most suitable cancer or cellular targets has to be selected so the review found heat shock proteins most favorable and better suited target.

Overview of Heat Shock Proteins

Heat-shock proteins are molecular chaperones, also termed the 'cancer chaperone', are very important in process of generating and maintaining the stability and activity of various cancer involved proteins. HSP- expresses out in response to sudden stimulation to stabilize proteins in abnormal configuration. These is a group of proteins found in all type of cells and making sure that the proteins are in

the right shape at right place on right time by modulating conformation and function of proteins receptor. There are four major subclasses, HSP-60, 90, 70 and small heat shock proteins

HEAT SHOCK PROTIENS-90

This subclass is regulated by stress and heat; it is one of the most abundant eukaryotic dimer phosphoproteins.^[2] The suffix "90" means its weight in kilo Dalton. It is essential for viability both cytosol and endoplasmic reticulum. HSP-90 is highly conserved and expressed proteins starts from bacteria to mammals.

In mammalian cells, there are two or more genes encoding cytosolic HSP-90 homologues. HSP-90 α showing 85% sequence identity to HSP-90 β in human. The α - and β -forms are result of gene duplication phenomena that occurred long time ago in human genetic material.^[3] There are 5 functional and 12 non-functional genes responsible for encoding HSP-90 isoforms human genes encoding the HSP-90 isoforms (Table 1). Recently membrane associated variety of cytosolic HSP-90 has been identified, HSP-90 α - Δ -N. Coding sequence derived from the CD-47 gene and HSP-90AA1. However, gene encoding HSP-90N was later seen to be non-existent in human genome. It is possibly a cloning artifact or a product of chromosomal rearrangement occurring in single cell line.^[3,4]

Table 1: Isoforms of HSP-90

Family*	Location	Subfamily	Gene	Protein
HSP-90A	Cytosol	HSP-90AA (inducible)	HSP-90AA1	HSP-90- α_1
			HSP-90AA2	HSP-90- α_2
		HSP-90AB (constitutively expressed)	HSP-90AB1	HSP-90- β
HSP-90B	E.R.*		HSP-90B1	GRP-94*
TRAP	Mitochondria		TRAP-1	TNF* Receptor-Associated Protein

HSP:-Heat shock proteins, TNF: - Tumor necrosis factors, TRAP:-TNF receptor associated periodic GR: - Glucose-regulated protein, ER: - endoplasmic reticulum

The monomer of protein consists of four structural domains. N-terminal have a common binding pocket for ATP and exhibits ATPase activity which is essential for function of HSP-90 whether C-terminal have alternative binding site for ATP which becomes accessible when the N-terminal is occupied. The linker region helps in the dimerization and middle domain is involved in client protein binding, also important in modulating ATP hydrolysis. Removal of this drastically impairs the ATPase activity.

It has two major conformational states that are open ATP-bound (no intraprotein interaction) and closed ADP-bound state (contact with several residues). The ability of HSP-90 to clamp onto proteins allows it perform several functions including assisting folding, preventing aggregation,

and facilitating transport, signal transduction, protein degradation etc. HSP-90 forms several discrete sub-complexes, each containing different set of co-chaperones that function at different steps during the folding process of the client protein.^[5]

PHARMACOLOGY OF HEAT SHOCK PROTEINS

In unstressed cells HSP-90 plays a various important roles like folding of proteins, intracellular transportations, maintenance, and degradation of proteins along with improving cell signaling. It regulates the conformation and activity of a large variety of cell signalling molecules, transcription factors, cytoskeleton proteins etc (Table 2).

(A) Protein folding

HSP-90 not only assists the folding but also modulates the conformation of proteins (Fig 1). It is associated with many of structures of proteins which have led the discovery of the proposal that

HSP-90 is involved in protein folding in general and somewhat more selective than other chaperones. [6] HSP-90 has been shown to suppress the aggregation of a wide range of "client" or "substrate" proteins and hence acts as a general protective chaperone. [7-9]

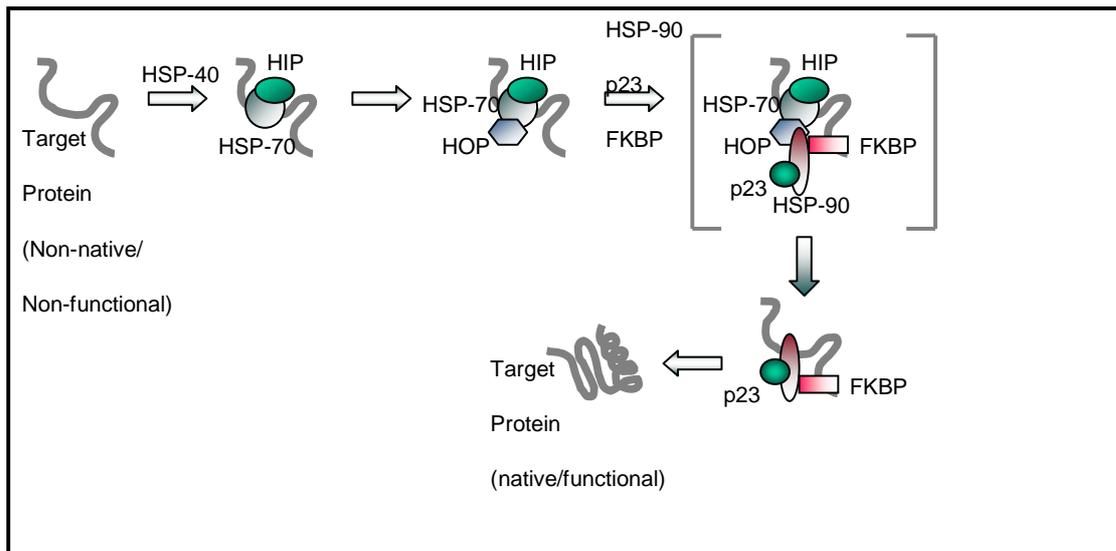


Fig: 1 Schematic representation of proteins folding

(B) Proteolysis

Biochemical degradation of protein occurs through hydrolysis by lysosomal autophagy and secretory vesicles but also by non-lysosomal path. Eukaryotic

proteins which are no longer needed are misfolded or damaged by the Ubiquitin-mediated degradation pathway. These ubiquitinated proteins are recognized and degraded by the 26S proteasome. [10]

Table 2: Physiological targets of HSP-90

Substrates	Notes*
Heat-shock factor	HSP-90 down regulates activity in conjugation with HSP-70 system
Other transcription factors	Receptors (steroid, glucocorticoid), hypoxia inducible factor-1 (HIF-1) etc.
Kinase	Tyrosine kinase (v-src, lck, insulin receptor, etc.) serine-threonine kinases (eIF-2 kinase, v-raf, c-raf, etc.), protein kinase CK-II (casein kinase-II)
Cytoskeleton	Actin, Tubulin (protection during heat stress)

*v-src:-viral sarcomplamic gene, lck: - lymphocyte specific tyrosine kinase, v-raf: - viral protein kinase, eIF-2:- eukaryotic inanition factor

Table 3: Cofactors proteins of HSP-90

Cofactor	Notes
HSP-70	HSP-90 activity dependent on HSP-70 system (incl. HSP-40)
HOP	HOP, Heat-shock Organizing Protein, brings HSP-70 and HSP-90 together via TPR interaction domains
p23	Modulates ATPase activity of HSP-90
HIP	Co-chaperone of HSP-70
PPases	Cyclophilin-40, FKBP51, FKBP52*
Others	Kinase-targeting co-chaperone Cdc37/p50

HSP: - heat shock protein, HIP: - heat shock interacting proteins, HOP; - hsp90 hsp 70

organizing proteins, TPR: - Tetratrieopeptido repeat, FKBP: - tacrolimus binding proteins, Cdc-37:-cell division chaperon-37

ROLE IN CANCEROUS CELLS

In cancer cells over expression of HSP-90 multi-chaperone complex “HSP-90 complexed with its client protein” having high ATPase activity and binding affinity to ligand that in result increase functionality and stability of many key signal proteins like **HER-2/Erb-B2, BCR-ABL, AKT/PKB, C-**

RAF, CDK4, PLK-1, MET, mutant p53, HIF-1 α , steroid hormone receptors (estrogen and androgen) and telomerase h-TERT. They are so much helpful for cancerous cells to prevent them selves from their own environment inherent toxicity, genetic instability and resist towards chemotherapy and promote their growth. That makes HSP-90 a particular hub for

the hallmark traits of cancer (Fig 2). List of HSP-90 client proteins with mechanism of action along with target type of cancer given below (Table 4). HSP-90 and client proteins complex maintains cancer cell in good against apoptosis and antigrowth signals and potentiates them for neoangiogenesis, growth replication and to invade surrounding tissue. [11, 12]

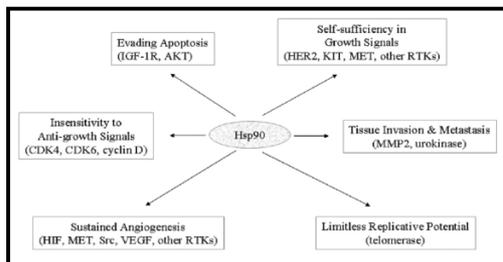


Figure 2: HSP-90 and the six hallmarks of a cancer cell.

(IGF-1R: Insulin like growth factor-1 receptor; RTK: Receptor tyrosine kinase; MMP: matrix metalloproteinases2; HIF:

Hypoxia-inducible factor; VEGF: - vascular endothelial growth factor)

The major cause of human cancer is not due to growth of the primary tumor itself but because of metastasis to distant tissues. Cancer metastasis is the result of a series of sequential and highly harmonic processes, including alteration of cancer cell adhesion and motility along with the ability to induce neoangiogenesis. HSP-90 and inhibitors play multiple roles in cancer metastasis (Table 4). Cancer cells must rely and highly dependent on this signaling proteins that regulate these events. [13, 14] Every event and step has different substrates or client proteins where the HSP-90 plays its role and that became the primary target for anticancer activity.

Table 4: List of HSP-90 client proteins, mechanism of action and potential target

Class	Client protein*	Mechanism of action	Potential target
Receptor tyrosine kinase	<ul style="list-style-type: none"> EGFR mutant ErbB2/HER-2 KIT 	Activation of downstream pro survival pathways, such as PI3-AKT and MAPK	<ul style="list-style-type: none"> Glioblastoma Breast cancer GIST
	<ul style="list-style-type: none"> AKT/PKB B-Raf mutant MET CDK4 Death domain kinase 	<ul style="list-style-type: none"> Activation of pro-survival and suppression of pro-apoptosis proteins Constitutively activates ERK signaling Involved in cellular proliferation, migration, invasion and morphogenesis Phosphorylates and inactivate Rib, allowing cell cycle to proceed Allows activation of NF-κB and its antiapoptotic signals 	<ul style="list-style-type: none"> Various cancers Melanoma Gastric Lungs Tumors over expression
Transcriptional Factors	<ul style="list-style-type: none"> HIF-1α ERα-receptors P53 mutant BCR-ABL 	<ul style="list-style-type: none"> Promoting angiogenesis Regulate genes for cell proliferation Transcription of genes for apoptosis Activates signal transduction pathways in leukaemogenesis 	<ul style="list-style-type: none"> Renal Breast Mutated in 50 % of type of cancer
	Chimeric fusion proteins	NPM-ALK	Induces cell transformation and proliferation
Others	<ul style="list-style-type: none"> Telomerase Apaf-1 Bcl-2 MMP2 	<ul style="list-style-type: none"> Prevents telomere shortening Crucial for apoptosome formation Regulates mitochondrial apoptotic pathway Facilitates invasion through cell adhesion, matrix digestion and cell migration 	<ul style="list-style-type: none"> Various cancers Small cell lung cancer Over expressed in cancer cell

*EGFR: - Epidermal growth factor receptor, MMP2:- Matrix metalloproteinase-2, NPM-ALK: - Nucleophosmin anaplastic lymphoma kinase, B-Raf; - viral sarcoplamic oncogene, KIT:-type of viral sarcoplama gene:-, BCR-ABL: - breakpoint cluster region leukemia viral gene, ErbB2:- Erythroblastosis oncogene B2.

Event 1- Cell Adhesion

Focal-adhesion kinase (FAK) is a substrate of the v-Src-oncogene. It interacts with integrin cytoplasmic tails, and stimulates the recruitment of other adaptor proteins, such as p130^{Cas}, paxillin and talin to form complexes known as focal adhesion complex and brought rearrangement of the actin cytoskeleton, cell migration and cell invasion. The function of FAK is regulated mainly by tyrosine phosphorylation.^[15, 16]

Integrin-linked kinase (ILK) is second client protein for cell adhesion. It is also called serine/threonine protein kinase. It is interacts with β 1, β 2 and β 3-integrin cytoplasmic tails. It also complexes with paxillin, parvin (α , β) and PINCH (1, 2). These association complexes are reported very much important for cell polarization and adhesion. HSP-90 inhibitors stimulates the proteasome-mediated degradation of FAK, reduces tyrosine phosphorylation of FAK. Drugs for targeting ILK client proteins are still need to be developed.^[17]

Event 2- Cell Motility

Receptor tyrosine kinase (RTK) and Erythroblastosis oncogene varieties like Erb-B, Erb-B2 and Erb-B1 which are also related to epidermal growth factors. Erb-B2 is of major importance in this, it causes depolymerization and repolymerization of the cytoskeletal proteins, actin and stimulating cell motility via classical signal transduction pathways. Breast and ovarian cancers are major outcomes of this rearrangement. HSP-90 inhibitors

interact with Erb-B2 using the ErbB2 kinase thus induces the dissociation of HSP-90 and client proteins complex, after which rapid proteasome-mediated degradation of ErbB2.^[18-20]

Hepatocyte growth factor (HGF) interacts with proto-oncogenic RTK and cellular-MET which activates tyrosine kinase signaling. This complex works as a potent mitogen, motogen and morphogen. It also induces complexation of FAK and cellular MET. HSP-90 inhibitors drugs destabilize both mature and immature MET in breast and lung cancer cells. They also inhibit and disrupt HGF-induced association of MET with FAK so in result cell motility was inhibited. Furthermore, inhibit HGF-induced cell scattering and MET-dependent transformations.^[21]

Insulin like growth factor (IGF) is also a subtype of receptor tyrosine kinase. Complex of HSP and substrate induces IGF-1R expression so in return the treatment reduces IGF-1R expression at the transcriptional level and induce IGF-1R protein degradation so diminishes the cell migration in pancreatic cancer cells.^[22]

c-SRC is small client protein which is transiently activated by multiple growth factors and RTK-mediated signaling pathways related to cell motility. Inhibition using HSP-90 drugs cause disruption of the c-SRC-HSP-90 complex and at the end results in its downregulation.

CDC-42 is another client protein associated with cell motility. It associates with respective activated kinase 1 and 2

(ACK-1 and ACK-2) that are members of the FAK family of nonreceptor tyrosine kinases. Ack1 mainly induces phosphorylation of p130^{Cas} and recruits the adaptor protein CRK, stimulating subsequent rearrangement of the actin cytoskeleton which is important in cell adherence and motility. HSP-90 inhibitors reduces the kinase activity of ACK-1 so suppresses induced tumor genesis, also inhibits contribution of ACK-2 kinase activity in cancer. [23]

Event 3- Neoangiogenesis [25]

Hypoxia-inducible factor (HIF)-1 when make a complex with HSP it becomes a transcriptional activator that regulates the transcription of a variety of pro-angiogenic genes including growth factors like VEGF. These factors are key mediators of angiogenesis and are specifically expressed in endothelial cells using VEGF receptors. HSP-90 inhibitors induce ubiquitination and proteasome-mediated degradation of PDGFR α in cancerous cells but not in normal cells so these can be suitable target for antitumor activity.

Event 4- Extracellular HSP-90 & Metastasis

Cell surface HSP-90/MMP2 is a substrate client protein for metastasis and extracellular HSP. It gets stimulation by environmental stresses and growth factors highly affected by phosphorylation and acetylation phenomena related to translation of proteins. CD-91 the cell surface receptor gets attacked by this and stimulates cell migration. It binds to the extracellular part of Erb-B2 and induces

phosphorylation, a decrease in kinase signaling so results in rearrangement of the actin cytoskeleton and subsequent cell migration. Only small molecules of HSP-90 inhibitors decrease MMP2 activity and cell invasion. [25-27]

ASSOCIATION OF INHIBITORS AND CLIENT PROTEINS

Normally HSP-90 association is important for maintaining the stability and functionality of numerous proteins named as client proteins which are frequently activated and mutated in cancer cells also include the oncogenic tyrosine kinase v-SRC, mutated oncogenes BCR/ABL, the receptor tyrosine kinase Erb-B2 and c-MET, and the serine/threonine kinase RAF-1. [28] They are regulated by the adenine nucleotide binding status of HSP-90. Nucleotide exchange and ATP hydrolysis occur at amino-terminal binding pocket (NTB) followed by the binding, chaperoning and release of client protein referred as the chaperone cycle. [29] HSP-90 in open state has higher affinity for client proteins than its closed state or ADP-binding counterpart. Although HSP-90 itself has weak ATPase activity, the ATPase activity is both positively and negatively regulated by co-chaperones, such as p23. HSP-90 inhibitors block cancer cell proliferation in vitro and cancer growth in vivo. These drugs works on the principle of competitive inhibition, they compete with ATP binding to the receptor at N terminal. It interrupt and stops chaperone cycle, which decreases the affinity of HSP-90 for oncogenic client and leads to their

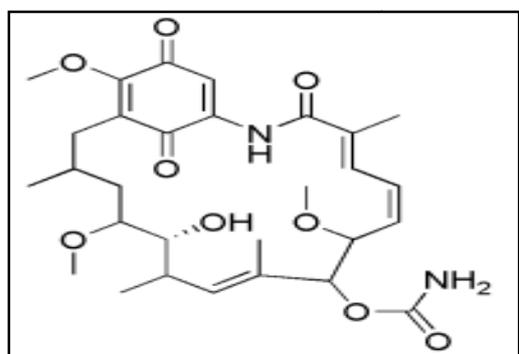
proteasome-mediated degradation and finally inhibition of HSP-90 function.^[30, 31]

HSP-90 INHIBITORS: DEVELOPMENT AND THEIR APPLICATION

Geldanamycin

Geldanamycin naturally obtained from *Streptomyces hygroscopicus*. GA binds with a high affinity to the ATP binding pocket of HSP-90. HSP-90 is a ubiquitous molecular chaperone critical for the folding, assembling and activity of multiple mutated and overexpressed signaling proteins that promote the growth and/or survival of tumor cells.

Geldanamycin (Fig 3) is chemically a benzoquinone ansamycin antibiotic, interferes with the action of the heat shock protein 90 (HSP-90) leading to the degradation of its client proteins complexes. Many of these client proteins are oncogenic in nature so it inhibits them and shows anticancer activity. HSP-90 client proteins include mutated p53, RAF-1, AKT, Erb-B2 and hypoxia-inducible



factor 1 α (HIF-1 α).^[32]

Fig 3: Geldanamycin

Mechanism of action: It has mainly three types of hypothetical concepts which are formation of free radicals, inhibition of tyrosine kinases and interference with the functions of group of HSP-90 proteins [Table 4].

a) Free radical formation

Structure of geldanamycin includes quinone ring as a part of molecule led to generation of intracellular free radicals through redox cycling. It was shown that the treatment of eukaryotic cells with it causes the formation of free radicals. However, this could only be observed at high concentrations of 100 μ M, at the other side the same was found cytotoxic effects in the nanomolar range. It can be reasonably concluded that the antitumor activity of GA cannot be explained by the formation of free radicals.^[33]

b) Inhibition of tyrosine kinase

It was classified as a tyrosine kinase inhibitor. The assumption was that benzoquinone ansamycins class (BA) directly act on sulfahydryl groups of v-SRC because Herbimycin-A, an analog of GA can make such effect also. A detailed study of pharmacodynamic showed that there is no correlation between its cytotoxic activity and the late onset of inhibition of kinases of the SRC-family.^[33, 34]

c) Binding and interference with the function of the HSP-90 proteins (Fig 4)

GA binds to HSP-90 client protein complex and down regulates all the hallmarks of

cancer Normally HSP-90 binds to many of the expressed kinase domains within the human genome. HSP-90 stabilizes the active conformations and mutant tyrosine kinase receptors, cytosolic serine-threonine and tyrosine kinases, transcription factors, structural proteins and other enzymes.^[31]

Client binding occurs through the middle domain of HSP-90 which leads to dimerization of HSP-90, binding of co-chaperones (HSP-70, HIP, HOP, cdc37) and binding of ATP with their hydrolysis. Many of these interactions are inhibited by small molecules that compete for the N-

terminal ATP binding pocket such as the benzoquinone ansamycins and their derivatives (geldanamycin, 17-AAG), radicols, and purine analogues (PU).

Thus, many signal transduction pathways require HSP-90 to perpetuate growth promoting signals. Inhibition of these signals by inhibition of HSP-90 ATPase activity leads indirectly to cell death. On the other hand, proteins that bind to the C terminus (green rectangle) of HSP-90, such as FKBP38 and IP6K2 (pro apoptotic protein kinase), are maintained in a constitutively inactive state by the interaction (red border).^[35, 36]

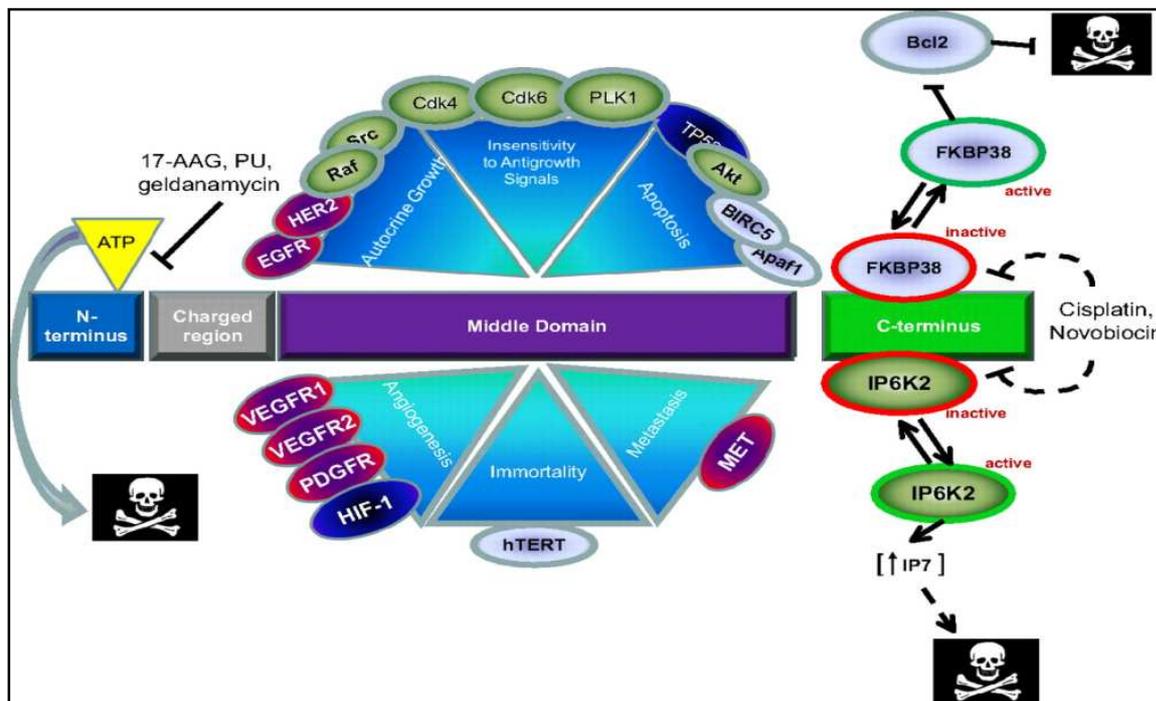


Fig: 4 Binding of HSP 90 complexes to client proteins

Resistance

Breast cancer cells are resistant to doxorubicin and cross-resistant to GA. For the same cytotoxic effect, higher concentrations of doxorubicin or GA had

to be used in resistant v/s wild type cells.^[32]

Since verapamil inhibit the multidrug resistance (MDR) pump should enhanced GA cytotoxicity but it was found wrong.

The conclusion was that GA is a substrate of the MDR pump and that overexpression of MDR may be a mechanism of GA resistance. In addition, GA-induced generation of free radicals was reduced in MCF-7/ADR^R (a breast cancer cell line) as opposed to the parental cells. Interestingly, HSP-90 β can be found in direct physical association with the MDR protein that is only presented in chemotherapy-resistant but not in wildtype cells.^[32, 36]

Contents and Storage

It is a yellow colored powder. All its analogues have same storage conditions. They should be stored at -20°C in the dark for long term stability. Geldanamycin and their derivatives are stable for 6 months if stored properly.

Geldanamycin derivatives (Fig 5)

17-AAG: - (17-Allylamino-17-desmethoxygeldanamycin):

It was synthesized by substitution of the methoxy group with an allylamine grouping base molecule. 17-AAG (Fig 5) is a less toxic and more stable purple colored analogue but with poor oral bioavailability and solubility so a better formulation like injectable suspension are of need.^[37] Even though 17-AAG binding to HSP-90 is weaker than GA, 17-AAG displays similar antitumor effects as GA and a better toxicity profile. Hepatotoxicity is prominent with daily administration whereas diarrhea and fatigue are others. 17-AAG is currently in phase II of clinical trial in several centers worldwide.

17-DMAG: - (17-(Dimethylaminoethylamino)-17-desmethoxygeldanamycin)

It is synthesized by substitution of the C-17 methoxy group of base molecule with N, N-dimethyl ethylamine resulted in 17-DMAG. It is the first water-soluble analogue having excellent bioavailability, volume of distribution and quantitatively metabolized much less than the 17-AAG due to having an ionizable amino group in it.^[38] It is useful in chemotherapy of prostate breast and ovarian cancer. Toxicities observed in clinical trials are neuropathy renal dysfunction, fatigue, cardio toxicity ocular adverse events and thrombocytopenia.

17-AEP-GA: - (17-[2-(Pyrrolidin-1-yl) ethyl] amino-17-desmethoxygeldanamycin)

This analogue has an alkyl amino group in place of the methoxy moiety at C17. It is less cytotoxic than GA and remains biologically active. It was found to induce similar tumor cell growth inhibition than 17-AAG and, unlike 17-AAG which is soluble in DMSO, to be water soluble.^[39]

17-DMAP-GA: - 17-(Dimethylaminopropylamino)-17-desmethoxygeldanamycin

It belongs to a new set of geldanamycin analogues that have been synthesized based on binding affinity to HSP-90 and water solubility. 17-DMAP-GA was shown to greatly inhibit the growth of cancer cells.^[40] Its binding affinity to HSP-90 was not significantly affected while its water

solubility was highly improved compared to 17-AAG.^[41]

IPI-504:- [17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride]

It is the water-soluble hydrochloride salt derivative of 17-AAG was developed names as Retaspimycin which is of purple color. It is synthesized by reduction of 17-AAG with sodium dithionite then

conversion to salt. It has hydroquinone ring whereas and 17-AAG consist of quinone ring and both remain in equilibrium.^[42] Since the quinone ring is primary reason for the hepatotoxicity of Geldanamycin so its reduction to a hydroquinone can results in lesser or zero toxicity. Various trials have been conducted in patients with multiple myeloma, small cell lung cancer, prostate cancer, breast cancer and gastrointestinal stomach tumor (GIST).^[43]

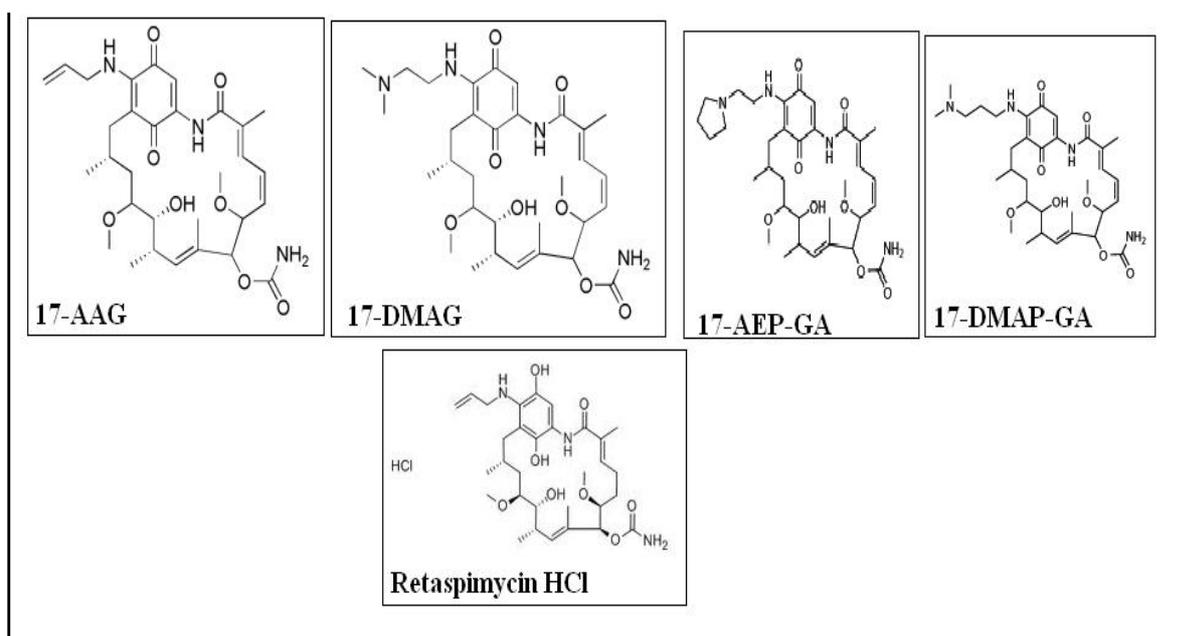


Fig 5: Structures of Geldanamycin derivatives

Purine and their derivatives

The molecule synthesized by incorporating a bent conformation based on purine-scaffold named as PU-3(Fig 6). It is prototype for the purine-scaffold category.^[44] Each of these molecules has in common amino (-NH₂) group attached to a ring and an aryl moiety separated by a distortion. If N₃ of the purine replaces

with a carbon to result in a purine like derivative called “Debio”- 0932 developed by company named Debiopharm.^[45]

Resorcinol derivatives

These are macrocyclic lactone antibiotic first isolated from the fungus *Monosporium bonorden*. Radicol is the first naturally obtained antibiotic which has showed anticancer activity. Resorcinol

moiety contains a reactive epoxide and α , β , γ and δ -unsaturated carbonyl groups the analogues of this were not discovered through direct modification in ring but they clearly resemble it by maintaining the resorcinol core as a critical element for binding of HSP

STA-9090 also named as Ganetespib is first in this class (Fig 6). This derivative contains a triazole ring has been tested for solid tumors in lungs, renal areas and

hematological malignancies. [46, 47] Novartis has developed a derivative of ixosazole containing in the resorcinol ring NVP-AUY-922. It has been tested for refractory multiple myeloma and metastatic breast cancer. In trial it was found to show arterial flutter, diarrhea, fatigue, darkening of vision and anorexia. Other adverse effects are events included nausea, vomiting, and night blindness. [67, 68]

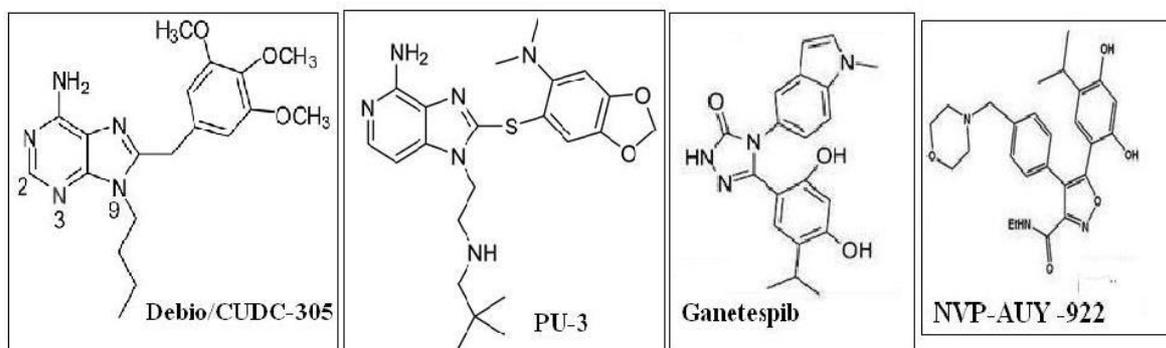


Fig 6: Structures of purine and resorcinol derivatives

Miscellaneous Drug and Molecules

Recent researches and trials over the globe have revealed some of other drugs effective drugs that are Novobiocin and Cisplatin.

- a) **Cisplatin:** - It binds to both the N and C terminal of HSP-90. Cisplatin blocks the aggregation prevention activity of HSP-90 at C terminus and induces a conformational change in HSP-90 at N terminus.
- b) **Novobiocin:** - Novobiocin binds to C-terminal domain and inhibits the

maturation of the heme-regulated eIF2 α kinase (HRI) in a concentration-dependent manner so it induced the dissociation of HSP-90 and Cdc37 receptors from immature HRI without affecting other binding partners, phosphoproteins and HSP-90 co-chaperones.

Agents and derivatives under clinical trials as described above with their solubility and the inhibitory concentration values are given below (Table 5 and 6).

Table 5: List HSP-90 inhibitor trials in cancer [49]

Name or Code of compound	Name of Sponsor	Category	Route*	Phase
17AAG as DMSO formulation	Kosan Biosciences	Geldanamycin	I/V	II
Tanespimycin (17AAG)	Kosan Biosciences	Geldanamycin	I/V	III
Alvespimycin (17-DMAG)	Kosan Biosciences	Geldanamycin	I/V/oral	I/II
Retaspimycin (IPI-504)	Infinity Pharma	Geldanamycin	I/V	II
CNF-1010 (17AAG)	Biogen Idec	Geldanamycin	I/V	III
CNF2024 (PU-3)	Biogen Idec	Purine	Oral	II/III
MPC-3100	Myriad Pharma	Purine	Oral	I/II
Debio 0932 (CUDC-305)	Debio Pharma	Purine	Oral	I/II
Ganetespib (STA-9090)	Synta Pharma	Resorcinol	I/V	II/III
NVP-AUY-922	Novartis	Resorcinol	I/V	II/III
SNX-5422	Serenex/Pfizer	Indazole	Oral	I

*I/V: - Intravenous, DMSO: - Dimethylsulfoxide, AAG: - alkylaminogeldanamycin, DMAG:- dimethylamino desmethoxy geldanamycin

Table 6: Solubility and IC₅₀ values of different Compounds [49]

Name or Code of compound	Solubility mg/ml at (25°C)			IC ₅₀
	Water	Ethanol	DMSO	
Geldanamycin (Natural)	<1 mg/ml	112 mg/ml	<1 mg/ml	K _d =1.2 μM
17-AAG (Tanespimycin)	<1 mg/ml	117 mg/ml	5 mg/ml	5 nM.
17-DMAG HCl (Alvespimycin)	<1 mg/ml	131 mg/ml	<1 mg/ml	62 nM.
Retaspimycin HCl (IPI-504)	<200 mg/ml	190 mg/ml	-----	-----
Ganetespib (STA-9090)	<1 mg/ml	40 mg/ml	9 mg/ml	4 nM.
AUY922 (NVP-AUY922)	<1 mg/ml	93 mg/ml	93 mg/ml	13 / 21 nM
SNX-2112	<1 mg/ml	93 mg/ml	1 mg/ml	-----

*IC: Half max inhibitory concentration; mg/ml: - milligram/milliliters; nM: -nanomolar

ADVANTAGES, LIMITATIONS AND FUTURE PROSPECTS

Advantages

- Combination therapies, applying low doses of these drugs together with convention chemotherapeutic agents, seem to be an effective way to target various cancers.

- In case of BCR/ABL-expressing leukemia, a low dose GA is sufficient to sensitize these cells to apoptosis.
- As most growth regulatory signals depend on HSP-90 for their function stability, HSP-90 is an ideal molecule to intervene in complex oncogenic pathways.

Limitations and future aspects

- 17-AAG has poor solubility and bioavailability so it needs new routes and dosage forms.
- It has relatively weak target potency, reduced activity in the presence of P-glycoprotein^[40] and low bioavailability and metabolism by cytochrome P450 CYP3A4.^[41]
- Recent studies have confirmed that increase in potency is due to the metabolism of 17-AAG to the more active hydroquinone form and future aspects can be more tolerable.
- CCT018159 (3, 4 diaryl pyrazole resorcinol) is able to inhibit human HSP-90 β with a similar potency to 17-AAG but with high degree of selectivity towards HSP-90 compared with topoisomerase II, HSP-72 and group of kinases.
- The cellular sensitivity to CCT018159 is not affected by P-glycoprotein and at micromolar concentrations it can cause degradation of client proteins and reduce tumour cell invasion and exhibit antiangiogenic activity.
- The diaryl pyrazole resorcinol series of novel HSP-90 inhibitors have similar cellular properties to 17-AAG, but have several possible advantages like aqueous solubility, independence from NQO1 enzymes and P-glycoprotein which may provide the basis for the future development of clinically superior HSP-90 modulators.

SUMMARY AND CONCLUSION

Cancer includes rapid and uncontrolled cell proliferation, mutations and deregulation of numerous genes. HSP-90 is a new and effective logical therapeutic target, inhibition of which delivers a combinatorial attack on multiple oncogenic targets and on all of the hallmark traits of malignancy. The development of HSP-90 inhibitors has moved forward rapidly alongside our growing understanding of the role of the chaperone in normal and malignant cells. Given their potential to degrade a number of different oncoproteins and affect multiple signaling pathways "HSP-90 inhibitors" to the date are of greatest clinical interest for objective of tumor regressions. The first and second generations of HSP-90 inhibitors act by blocking its intrinsic ATPase activity. Following on from the natural product-based agents, exemplified by 17-AAG and related analogues that have entered clinical trials, a variety of HSP-90-inhibitory chemo-types are now under development not going to developed from natural product geldanamycin. Clinical activity has been seen with 17-AAG in melanoma, resistant breast cancer, prostate cancer and endocrine-related cancers through effects on steroid hormone receptors, receptor tyrosine kinases and downstream signaling proteins. HSP-90 inhibitors have entered Phase II and III clinical trials, and have shown therapeutic activity in several types of cancer. So much miraculous and eye opening results have been observed with the geldanamycin analogue given in

combination with other antineoplastic drugs. While these results validate Hsp90 inhibition as a relevant anticancer strategy, the full potential of these inhibitors to be active in a broader spectrum of tumor types has yet to be achieved. A futuristic problem and drawback with this category is differential response and this could be because we are unable to select patients who might best benefit from therapy and perhaps have not successfully optimized the dosing by dosing and scheduling. Additionally, the current drug development scenario and observed effects in trials, this is possible to apply maximum tolerated dose in the case of these inhibitors. In order to optimally deliver HSP-90 inhibitor therapy tumor biopsies and novel targeted imaging studies must be incorporated into future clinical trials of these drugs and efforts at identifying robust biomarkers of response and resistance will be crucial next steps in rationalizing the clinical development of the growing list of Hsp90 inhibitors. This review further emphasizes the targeted approach of Hsp90 inhibition with newer dosage form for better solubility and bioavailability. Ultimately this directs the researchers into understanding the fundamentals of drug delivery and patient selection for these unique inhibitors and will allow the next and best generations of HSP-90 to realize their full potential and promise as anti-cancer therapeutics

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